

# Florida State Horticultural Society

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# Dracaenas

The genus *Dracaena* encompasses over 150 species (The World Flora Online, 2022), but only a few species are commonly cultivated as foliage plants. These are favored by interiorscapers because of the diverse shapes, colors, forms, and configurations that are available and because of the plants' ability to survive under low-light conditions with minimum care (Chen, et al., 2002). There is no organized program dedicated to dracaena breeding so the increase in cultivars is predominately due to growers selecting sports from cuttings or, in a few cases, new discoveries (Mwachala, et al., 2007; Wilkin et al., 2013). Production of dracaena hybrids has proven difficult since there is no practical method of synchronizing flowering among different species (Teng, 2007; Caple et al., 2014). Regardless of their origin, new tropical foliage plants need to be systematically evaluated (Henny and Chen, 2020).

*Dracaena* sp. should be grown in shade with a temperature of 70 to 90°F and a relative humidity of 60 to 100%. Temperatures above 90°F cause foliar chlorosis of *D. fragrans* 'Janet Craig' and notching of *D. fragrans* 'Warneckii'. Controlled-released or water-soluble fertilizers with micronutrients or a combination of both can be used for dracaena production. Fertilizers with low fluoride levels and an N:P:K ratio of 3:1:2 or 3:1:3 are best. The suggested application rate is 3 lb N per 1000 ft<sup>2</sup> per month, which should be reduced by 50% to 100% one month before shipment. Dracaena for interior use should be grown under shade. *D. fragrans*, and *D. marginata* can be grown under 63 to 73% shade (3250 to 4000 fc) and the other species under 73 to 80% shade (2500 to 3250 fc) (McConnell, et al., 2018).

The value of foliage plants, including dracaenas, in the interior environment is well documented. Studies have discussed how foliage plants can purify indoor air (Pegas, et al., 2012; Wolverton and Nelson, 2020) and improve human emotional health (Jang, et al., 2018). Dracaenas have been mentioned in the Proceedings of the Florida State Horticulture Society more than 40 times over the years.

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## 2021 PRESIDENTIAL ADDRESS



NORIS LEDESMA

*President of the Florida State Horticultural Society*

### *Greeting's members of the Florida State Horticulture Society!*

We would like to thank you for your participation in the society and for attending our annual meeting last October at Daytona Beach, Florida. I want to recognize the outstanding work of the Board head, Jeff Williamson, and all VPs, officers, and volunteers that managed despite the restrictions and challenges of COVID-19 to make a successful meeting possible. I want to express my sincere appreciation to those of you who volunteered your time and energy to participate in that capacity.

It has been a difficult year, but it's time to move forward. Horticultural production continues to be one the most important systems to meet the increasing demand of the competitive world market despite the environmental challenges faced.

The rise in demand is linked to an awareness among consumers for fruit and vegetables. It is good timing for Horticultural professionals to stand up.

The Florida State Horticulture Society, founded in 1888, is one of America's oldest horticultural societies. From our first meeting in the Ocala House, FSHS grew almost exponentially throughout the next few decades. Reading some of the articles of our devoted member, Ed Exteberria, reminds us of the evolution of our society. Our early roots were in agriculture and the Society's focus was on improving the quality of fruits, vegetables, ornamentals, and natural resources. The FSHS of today, despite all changes and challenges, comes together every year: researchers, extension educators, students, and progressive industry personnel.

The next FSHS annual meeting is scheduled for June 5, 2022, in Sarasota, and the Board is working to have different scenarios available for you. During the pandemic we have been doing more

zooming and less flying and this may continue. Perhaps this is a new way to expand the society and be able to expand our net for international researchers and students. Times are changing at an extraordinary speed; we must seriously think of expanding our horizons. We need to make some changes to move forward and become a force again in the state of Florida, and across the horticulture world. Evolution of our society must parallel the rapid changes in technology and provide the opportunity to expand our horizons. One initiative already in place is to offer a hybrid meeting for the upcoming meeting in Sarasota 2022. Conferences are increasingly being organized online, and people are getting more used to them. Even though, I believe there is truly nothing better than in person meetings to improve friendships and relationships.

There are new opportunities for partnerships where we can join with other groups of common interest. Our new alliance with the National Mango Board is just one example of many possibilities.

There are also new opportunities for financial supporters for our cause: new donors, members, corporations, and foundations are needed to keep our society functioning and to serve the horticultural sector in our state, our country, and the world.

I'm optimistic, so let's work together to promote our 135th Annual Meeting and share new initiatives. We are all privileged to work, shoulder to shoulder, in an industry whose mission is to bring people the sustenance and nutritional well-being that is embedded in what we do. After all, "horticulture" comes from the Roman words for "garden" and "cultivation." It is a noble tradition.

We are looking forward to meeting you in Sarasota!

A handwritten signature in black ink that reads "Noris Ledesma". The signature is written in a cursive, flowing style.

## **2021 MERITORIOUS SERVICE AWARDS**

### **For significant contributions to Florida horticulture**

#### **Presidential Gold Medal**

The presidential gold medal is awarded to the individual who has contributed most to Florida horticulture through work published in the Proceedings over the preceding time periods since the Handling and Processing Section was last eligible.



**Anne Plotto, (2nd from right)**

#### **Outstanding Commercial Horticulturist Award**

This award shall be presented annually to an individual who has made significant contribution to the commercial Florida horticultural industry and to the FSHS.



**Noris Ledesma**

### **President's Industry Award**

The award will be presented to the senior author of a single best paper given at the Florida State Horticultural Society annual meeting by an industry author.



**Jonathan Crane**

### **Florida Tomato Research Award**

This award shall be given to the individual or group who have done work with the most potential to further the fresh market tomato industry in Florida through advances reported in any single publication during the two (2) previous calendar years



**Guodong "David" Liu**

## **2021 Patron Members**

Members who annually pledge additional support for the student programs of the Society

**Anne Plotto**

**Ed Etxeberria**

**Craig Campbell**

**Gene McAvoy**

**Theodore Winsberg**

## **Friends of the Florida State Horticultural Society Award**

To recognize individuals for their longtime commitment and support of the Florida State Horticulture Society

not awarded

## SECTIONAL BEST PAPER AWARDS

From the Proceedings of the Florida State Horticultural Society Volume 133

Awarded to the senior author of the best and most meritorious paper as printed in the previous year's Proceedings in each of the six sections

### Agroecology and Natural Resources Section



**Lyn A. Gettys**

Evaluation of “Natural” Products for Water Hyacinth Control

Proc. Fla. State Hort. Soc. 133: 217–225. 2020

Authors: Lyn A. Gettys, Kyle L. Thayer, and Joseph W. Sigmon

University of Florida, IFAS, Fort Lauderdale Research and Education Center, Fort Lauderdale, FL

### Handling & Processing Section



**Lan-Yen Chang**

Recovery of Ripening by Mature Green Banana Fruit Following 1-Methylcyclopropene

(1-MCP) Application

Proc. Fla. State Hort. Soc. 133:148. 2020

Authors: Lan-Yen Chang and Jeffrey Brecht

Horticultural Sciences Department, University of Florida, IFAS, Gainesville, FL

## SECTIONAL BEST PAPER AWARDS (continued)

### Citrus Section



**Samuel Kwakye**

Evaluation of Varying Rates of Manganese on HLB-Affected Trees (Valencia) in Florida

Proc. Fla. State Hort. Soc.133:42–26. 2020

Authors: Samuel Kwakye, Davie Kadyampakeni, Tripti Vashisth  
University of Florida, IFAS Citrus Research and Education Center, Lake Alfred, FL

### Krome Memorial Institute



**Jonathan Denison** (right) with Guodong Liu

Artificial Chilling for Blackberry Production in Florida.

Proc. Fla. State Hort. Soc. 133:28. 2020

Authors: Jonathan Dennison<sup>1</sup>, Christianah Oladoye<sup>2</sup>, Jeffrey Williamson<sup>1</sup>, and Guodong Liu<sup>1</sup>

<sup>1</sup>Horticultural Sciences Department, University of Florida, IFAS, Gainesville, FL,

<sup>2</sup>Clemson University, Edisto Research Station, Blackville, SC

## SECTIONAL BEST PAPER AWARDS (continued)

### Ornamental, Garden & Landscape Section

**David Beleski**

(photo not available)

Micropropagation of a Novel Cordyline Hybrid

Proc. Fla. State Hort. Soc. 133:177. 2020

Authors: David Beleski , Wagner Vendrame, Jian Jian Xu, Damaris Hernandez,  
ane Nguyen, and Daniella Menendez

Environmental Horticulture Department, University of Florida, IFAS, Gainesville, FL

### Vegetable Section



**Yuqing Fu**

Developing Interspecific Bridge Lines between *Cucurbita pepo* and *C. moschata*.

Proc. Fla. State Hort. Soc. 133:126. 2020

Authors: Yuqing Fu, Vincent Michael, Pamela Moon, Christine Waddill,  
Shouan Zhang, and Geoffrey Meru

Tropical Research and Education Center, University of Florida, IFAS, Homestead, FL



## BEST STUDENT ORAL PRESENTATION AWARDS



From left to right: Xin Zhao, Professor of Horticultural Science, University of Florida, Gainesville (Chair of the Student paper judging committee); Isaac Vincent (first prize); Eric Resende (second prize); and Lis Natali Rodriguez Porto (third prize).

### **First Prize—Isaac Vincent**

Evaluating Sunn Hemp Biomass as a Carbon Source for Anaerobic Soil Disinfestation  
in Organic Strawberry Production

Authors: Isaac Vincent, Bodh Paudel, Jianyu Li, Erin Roskopf, and Xin Zhao  
Horticultural Sciences Department, University of Florida/IFAS, Gainesville, FL, 2USDA-ARS,  
US Horticultural Research Laboratory, Fort Pierce, FL [V-15].

### **Second Prize—Eric Resende**

Insecticide Lethal and Sublethal Effects on Corn Silk Flies (Diptera: Ulidiidae)  
following Topical Exposure

Authors: Eric Schwan Resende<sup>1</sup>, Julien Beuzelin<sup>1</sup>, Victoria Dunkley<sup>1</sup>,  
Silvana Paula-Moraes<sup>2</sup>, Dakshina Seal<sup>3</sup>, and Gregg Nuessly<sup>1</sup>

<sup>1</sup>Everglades Research and Education Center, University of Florida/IFAS, Belle Glade, FL; <sup>2</sup>West Florida Research  
and Education Center, University of Florida/IFAS, Jay, FL;

<sup>3</sup>Tropical Research and Education Center, University of Florida/IFAS, Homestead, FL [V-18]

### **Third Prize—Lis Natali R. Porto**

Lettuce Downy Mildew Differential Cultivars Are Resistant to Other Important Diseases

Authors: Lis Natali R. Porto, Richard N. Raid, Germán V. Sandoya-Miranda. Everglades Research and Education  
Center, University of Florida/IFAS, Belle Glade, FL [V-12].

## STUDENT SCHOLARSHIP RECIPIENTS



Xin Xao (left) congratulates Student Scholarship recipients.

**Victoria Adeleye**  
**Leigh Archer**  
**Tanyaradzwa Chinyukwi**  
**Blessing Chukwuaja**  
**Judyson de Matos Oliveira**  
**Dongjoo Kim**  
**Jin-Hee Kim**  
**Shannon McAmis**  
**Perservança Mungofa**  
**Dominick Padilla**  
**Eric Schwan Resende**  
**Lis Natali Rodrigues Porto**  
**Moonwon Soh**  
**Shufang Tian**  
**Shailaja Vemula**  
**Isaac Ryan Vincent**  
**William Cas Willborn**

# **BEST WRITTEN STUDENT PAPER AWARDS**

## **First Place - Syuan-You Lin**

Exogenous Gibberellic Acid and Cytokinin Effects on Budbreak, Flowering, and Yield  
of Blackberry Grown under Subtropical Climatic Conditions

Proc. Fla. State Hort. Soc. 133:34–31. 2020

Authors: Syuan-You Lin and Shinsuke Agehara  
Gulf Coast Research and Education Center, University of Florida, IFAS, Wimauma, FL

## **Second Place – Sudip Kunwar**

Field Performance of ‘Hamlin’ Orange Trees Grown on Various Rootstocks  
in Huanglongbing-endemic Conditions

Proc. Fla. State Hort. Soc. 133:63–72. 2020

Authors: Sudip Kunwar<sup>1</sup>, Jude Grosser<sup>2</sup>, Fred G. Gmitter Jr<sup>2</sup>, William S. Castle<sup>2</sup>, and Ute Abrecht<sup>1</sup>

<sup>1</sup>Southwest Florida Research and Education Center, University of Florida, IFAS, Immokalee, FL

<sup>2</sup>Citrus Research and Education Center, University of Florida, IFAS, Lake Alfred, FL

## **Third Place – Victoria O. Adeleye**

Vegetable Crops as Hosts of Thrips (Thysanoptera: Thripidae)

Vectoring Tomato Chlorotic Spot Virus in Tomatoes

Proc. Fla. State Hort. Soc. 133:110-115. 2020

Authors: Victoria O. Adeleye, Dakshina Seal, Oscar Liburd, Heather McAuslane and Hans Alborn  
Tropical Research and Education Center, University of Florida, IFAS, Homestead, FL

## Honorary Members (1988 to Present)\*

Albrigo, L. Gene	2012	Henricksen, H.C.	1939	Rolfs, Mrs. P.H.	1921
Anderson, J.B.	1922	Holland, Frank L.	1962	Ruehle, George D.	1958
Anderson, Shirley F.	2002	Holland, Spessard L.	1945	Sargent, Steven A.	2018
Beckenbach, J.R.	1967	Hoyt, Avery S.	1950	Saunders, Fred	1999
Berckmens, P.J.	1893	Hoyt, R.D.	1914	Sharpe, Ralph H.	1974
Berger, E.W.	1940	Hubbard, E.S.	1922	Shaw, Miss Eleanor G.	1927
Berry, Robert E.	1987	Hume, H. Harold	1927	Sherman, Wayne B.	2003
Blackmon, G.H.	1964	Ismail, Mohamed A.	1996	Showalter, Robert K.	1984
Bosanquet, L.P.	1924	Jackson, Larry K.	2000	Singleton, Gray	1962
Brecht, Jeffrey K.	2014	Jamison, F.S.	1962	Skinner, L.B.	1931
Brown, Arthur C.	1952	Johnson, Warren O.	1965	Sloan, G. Dexter	1964
Bryan, Herbert H.	2003	Jones, John Paul	1997	Smith, Paul F.	1972
Bullock, Robert C.	2008	Kender, Walter J.	2000	Smoot, John J.	1986
Burgis, Donald S.	1980	Knight, Robert J., Jr.	2006	Spalding, Donald H.	1987
Calvert, David V.	1997	Koo, R.C.J.	1978	Spencer, E.L.	1962
Camp, A.F.	1956	Krezdorn, A.H.	1979	Stamps, Robert	2010
Campbell, Beverly	2010	Krome, William H.	1973	Steffani, C.H.	1958
Campbell, C.W.	1988	Krome, William J.	1927	Stephens, James M.	1995
Cantliffe, Daniel J.	2006	Krome, Mrs. Isabelle B.	1960	Stevens, H.B.	1934
Carlton, R.A.	1962	Lawrence, Fred P.	1973	Swingle, W.T.	1941
Castle, William S.	2011	Lipsey, L.W.	1924	Syvetsen, James P	2013
Chase, J.C.	1939	Locascio, Salvatore J.	1996	Taber, George L.	1914
Chase, S.O.	1939	Logan, J.H.	1965	Tait, W.L.	1941
Childers, N.F.	1993	Lynch, S. John	1975	Talbott, George M.	1980
Clayton, H.G.	1956	MacDowell, Louis G.	1968	Tenny, Lloyd S.	1956
Colburn, Burt	1970	Magie, Robert O.	1977	Thompson, Ralph P.	1962
Commander, C.C.	1952	Mathias, A.F.	1972	Thompson, W.L.	1962
Cooper, W.C.	1981	Matthews, Richard F.	1992	Thullbery, Howard A.	1962
Dickey, R.D.	1968	Mayo, Nathan	1940	Todd, Norman	1991
Edsall, R.S.	1967	McCornack, A.A.	1986	Tucker, David P.	1999
Etzeberria, Ed	2016	Menninger, Edwin A.	1964	Veldhuis, M.K.	1972
Everett, Paul H.	1986	Miller, Leon W.	1972	Ward, W.F.	1962
Fairchild, David	1922	Miller, Ralph L.	1972	Waters, Will E.	1997
Fifield, Willard M.	1955	Montelaro, James	1985	Webber, H.J.	1941
Flagler, H.M.	1903	Morton, Julia F.	1989	Wedgworth, Ruth S.	1965
Floyd, Bayard F.	1944	Mounts, M.U.	1958	Wenzel, F.W.	1973
Floyd, W.L.	1939	Mowry, Harold	1950	Wheaton, T. Adair	1994
Ford, Harry	1985	Murdock, Del I.	1984	Wilfret, Gary J.	1998
Forsee, W.T., Jr.	1973	Newell, Wilmon	1940	Wilson, Lorenzo A.	1934
Gaitskill, S.H.	1909	Norman, Gerald G.	1967	Wiltbank, William J.	1987
Gardner, Frank E.	1967	Norris, Robert, E.	1962	Winsberg, Theodore W.	2007
Garrett, Charles A.	1951	O'Byrne, Frank M.	1962	Winston, J.R.	1960
Goldweber, Seymour	1984	Overman, A.J.	1988	Wolfe, H.S.	1964
Grierson, William	1979	Painter, E.O.	1909	Young, T.W.	1978
Guzman, Victor L.	1987	Peterson, J. Hardin	1950	Yothers, W.W.	1976
Haden, Mrs. Florence P.	1934	Pratt, J.B.	1980	Ziegler, L.W.	1976
Hall, David J.	2011	Redmond, D.	1893		
Harding, Paul L.	1968	Reed, R.R.	1970		
Hart, W.S.	1909	Reitz, Herman J.	1970		
Hastings, H.G.	1939	Reitz, J. Wayne	1955		
Hatton, Thurman T.	1987	Robinson, T. Ralph	1942		
Hayslip, Norman C.	1981	Rolfs, P.H.	1921		

*\*Date listed is the year in which the award was received.*

## Presidents of the Florida State Horticultural Society, 1888–Present

1888–96	Dudley W. Adams	1964	Willard M. Fifield	1993	Michael O. Taylor
1897–1904	George L. Taber	1965	Ernest L. Spencer	1994	Salvadore J. Locascio
1905–06	C.T. McCarty	1966	Arthur F. Mathias	1995	Mohamed A. Ismail
1907–08	P.H. Rolfs	1967	Ed H. Price, Jr.	1996	Walter J. Kender
1909	William C. Richardson	1968	J.R. Beckenbach	1997	Fred Saunders
1910–22	H.H. Hume	1969	G.M. Talbott	1998	Larry E. Beasley
1923–29	L.B. Skinner	1970	F.E. Gardner	1999	David W. Buchanan
1930–36	John S. Taylor	1971	O.R. Minton	2000	Will E. Waters
1937	C.W. Lyons	1972	R.A. Dennison	2001	Frederick S. Davies
1938–40	Charles I. Brooks	1973	B.E. Colburn	2002	William S. Castle
1941	T. Ralph Robinson	1974	G.G. Norman	2003	Jonathan H. Crane
1942	Henry C. Henricksen	1975	Leon Miller	2004	Craig Campbell
1943–47	Frank M. O’Byrne	1976	John W. Sites	2005	George J. Hochmuth III
1948	William F. Ward	1977	J.B. Pratt	2006	Jacqueline K. Burns
1949	Frank Stirling	1978	R.R. Reed	2007	Peter McClure
1950	Leo H. Wilson	1979	J.F. Morton	2008	James P. Syvertsen
1951	G. Dexter Sloan	1980	C. Wayne Hawkins	2009	Mary Lamberts
1952	Frank L. Holland	1981	W. Grierson	2010	Jeffrey K. Brecht
1953	R.S. Edsall	1982	Roger Young	2011	Richard Tyson
1954	M.U. Mounts	1983	Charles A. Conover	2012	Juanita Popenoe
1955	H.A. Thullbery	1984	Carl W. Campbell	2013	Nancy Roe
1956	R.A. Carlton	1985	Fred Bistline	2014	Ed Etxeberria
1957	R.E. Norris	1986	Al H. Krezdorn	2015	Steve Sargent
1958	A.F. Camp	1987	Richard F. Matthews	2016	Chris Oswalt
1959	S. John Lynch	1988	T.T. Hatton	2017	Mark Ritenour
1960	W.L. Thompson	1989	W.H. Krome	2018	Eric Simonne
1961	Ruth S. Wedgworth	1990	Tom J. Sheehan	2019	Gene McAvoy
1962	John H. Logan	1991	Larry K. Jackson	2020	Jeffrey Williamson
1963	Herman J. Reitz	1992	Daniel J. Cantliffe	2021	Noris Ledesma

## Outstanding Growers or Commercial Horticulturists

1997	Norman Todd	Grove Crafters, Labelle, FL	2010	Peter McClure	Evans Properties, Okeechobee, FL
1998	Ted Winsberg	Green Cay Farms, Boynton Beach, FL	2011	Michael Edenfield	Bayer CropScience, Windemere, FL
1999	Larry K. Jackson	Horticultural Consultant, Auburndale, FL	2012	Robert T. McMillan, Jr.	Kerry's Bromeliads Nursery Inc., Homestead, FL
2000	Gary E. Zill	Zill High Performance Plants, Boynton Beach, FL	2013	Alvin Cheng	JBT FoodTech, Lakeland, FL
2001	Murray J. Corman	Garden of Delights, Davie, FL	2014	<i>not awarded</i>	
2002	Craig Campbell	Valent Biosciences, Orlando, FL	2015	<i>not awarded</i>	
2003	Derek Burch	Masterworks, Plantation, FL	2016	Steve Rogers	Ecostat Inc., Highland City, FL
2004	Scott Emerson	Citrus & Vegetable Magazine, Tampa, FL	2017	Noris Ledsesma	Fairchild Tropical Bodanic Garden, Homestead, FL
2005	James "Buster" Pratt	Hanes City, FL	2018	Erin Harlow	Dubal County Extension, Jacksonville, FL
2008	David J. Hall	HDH Agri Products, Tavares, FL	2019	<i>not awarded</i>	
2009	Kenneth Shuler	Stephen's Produce, Jupiter, FL	2020	Noris Ledesma	Miami, FL
			2021	Noris Ledesma	Miami, FL

## FSHS Annual Meeting Host Cities

1888	Ocala	1922	Lakeland	1955	Clearwater	1990	Lake Buena Vista
1889	Orlando	1923	Orlando	1956	Orlando	1991	Miami Beach
1890	De Land	1924	Tampa	1957	Miami Beach	1992	Tampa
1891	Interlachen	1925	Eustis	1958	Clearwater	1993	Miami Beach
1892	Ormond Beach	1926	Cocoa Beach	1959	Miami	1994	Orlando
1893	Pensacola	1927	Bradenton	1960	Tampa	1995	Orlando
1894	Jacksonville	1928	Winter Haven	1961	Miami Beach	1996	Orlando
1895	Jacksonville	1929	Clearwater	1962	Miami Beach	1997	Orlando
1896	Jacksonville	1930	Sebring	1963	Miami Beach	1998	St. Petersburg
1897	Orlando	1931	Miami and Homestead (spring meeting)	1964	Miami Beach	1999	Stuart
1898	Orlando		Mariana (fall meeting)	1965	Miami Beach	2000	Lake Buena Vista
1899	Jacksonville	1932	Gainesville	1966	Miami Beach	2001	Stuart
1900	Jacksonville	1933	Lake Wales	1967	Miami Beach	2002	Marco Island
1901	St. Augustine	1934	Orlando	1968	Miami Beach	2003	Orlando
1902	Tampa	1935	Vero Beach	1969	Miami Beach	2004	Orlando
1903	Miami	1936	DeLand	1970	Miami Beach	2005	Tampa
1904	Jacksonville	1937	Ocala	1971	Miami Beach	2006	Tampa
1905	Jacksonville	1938	Winter Haven	1972	Miami Beach	2007	Palm Beach Gardens
1906	Jacksonville	1939	Hollywood	1973	Miami Beach	2008	Ft. Lauderdale
1907	St. Petersburg	1940	Tampa	1974	Miami Beach	2009	Jacksonville
1908	Gainesville	1941	Orlando	1975	Lake Buena Vista	2010	Crystal River
1909	Daytona	1942	West Palm Beach	1976	Miami Beach	2011	St. Petersburg
1910	Orlando	1943	Winter Haven	1977	Lake Buena Vista	2012	Delray Beach
1911	Jacksonville	1944	Winter Haven	1978	Miami Beach	2013	Sarasota
1912	Miami and Havana, Cuba	1945	Orlando	1979	Lake Buena Vista	2014	Clearwater
1913	DeLand	1946	Miami	1980	Miami Beach	2015	St. Augustine
1914	Palatka	1947	St. Petersburg	1981	Lake Buena Vista	2016	Stuart
1915	Tampa	1948	West Palm Beach	1982	Miami Beach	2017	Tampa
1916	Arcadia	1949	Tampa	1983	Daytona Beach	2018	Fort Lauderdale
1917	Arcadia	1950	Winter Haven	1984	Miami Beach	2019	Maitland
1918	Ft. Myers	1951	West Palm Beach	1985	Tampa	2020	Online via UFTEAMS
1919	Orlando	1952	St. Petersburg	1986	Miami Beach	2021	Daytona Beach
1920	Ocala	1953	Daytona Beach	1987	Orlando		
1921	Miami	1954	Miami Beach	1988	Miami Beach		
				1989	Tampa		

# 2021 Florida State Horticulture Society Keynote Address

## Publishing Horticultural Research: Impacts of Open Access

SUZANNE C. STAPLETON\*

*University of Florida, George A. Smathers Libraries, Gainesville, FL*

**Additional index words.** scholarly communications

**After more than a hundred years of general stability, the last two decades have seen enormous changes in scholarly publishing. These changes are due in large part to the ability to publish and share research online. The most recent shifts, and their moving parts, are being driven by open access initiatives and mandates for publicly funded research. The rise of free-to-read open access (OA) provides opportunities and challenges to researchers, as well as publishers. Opportunities include sharing research more equitably around the globe through OA journals, preprint repositories and other means while retaining copyright to one's own work. Challenges faced today by researchers include discerning where best to share one's work professionally and how to cover any publishing expenses. I'll provide an overview of recent publishing practices by University of Florida, Institute of Food and Agricultural Sciences scholars in light of these landscape changes. Along with greater choices and empowerment for authors, comes greater responsibility.**

Sharing credible information to advance knowledge of food production and related topics has been a consistent endeavor of mine throughout my careers as an extension agent, and now as a science librarian. The mechanisms for sharing new knowledge are changing and expanding yet the need to affirm trustworthiness continues. New knowledge is shared in a variety of formats, such as at conferences and through publication. In library jargon, publications fall into two categories: grey literature and peer-reviewed literature. Grey literature includes newsletters, social media, thesis and dissertations and University of Florida's Institute of Food and Agricultural Sciences (UF/IFAS) EDIS publications. Peer-reviewed literature includes journal articles, book chapters and [some] conference proceedings. Both have merit and are valuable means to disseminate research. Peer reviewed literature is the gold standard for academics in the sciences. Peer review provides a quality control process by which scientific rigor and reproducibility is evaluated.

Since 2009, the George A. Smathers Libraries (the Libraries) have partnered with the UF/IFAS Office of Research to prepare an annual report of peer reviewed publications for USDA capacity funding (Minson and Royster, 2015). These reports, from 2011 through 2020, are freely available in the University of Florida Digital Collection<sup>1</sup>. The libraries assist the process by aggregating citation data from each of the 28 UF/IFAS units, removing duplicates, and verifying the published version of record for the refereed journal articles. The scholarly output of UF/IFAS is impressive! Overall, there has been a 32% increase in the number of annual peer-reviewed journal articles published by UF/IFAS scholars since 2015. The COVID-19 pandemic is influencing publishing trends. There was a 17% increase from 2019 to 2020 in peer-reviewed articles published by UF/IFAS scholars, most likely the result of the pandemic-imposed work restrictions. This is consistent with sharp increases in submissions of scholarly manuscripts during the pandemic reported elsewhere (Else, 2020).

Scholarly communication is in the midst of big changes (O'Neill, 2016; Lavoie et al., 2014), driven by digital technology and online publishing. Building new knowledge in a traditional

manner involved limited editions and limited access to print publications. The modern digital means of knowledge creation are expanding the boundaries of scholarly communication through easier collaboration, real-time access, pre-and post-publication commentaries, as well as sharing of data, code, video, and other research practices. The pandemic highlights benefits and challenges of these changes. Benefits of the changes include the ability for rapid collaboration and dissemination of urgently-needed new information. Challenges include the risk of misinformation from poorly executed, poorly vetted, or misunderstood studies. Digital content can still be limited to subscribers, members, and licenses through paywalls and authentication services. However, there is a movement to make content free-to-read, accessible to all.

The Open Access (OA) movement began well before this term was first used in the early 2000s in three international declarations<sup>2</sup>. The research-sharing platform, arXiv, was launched by Paul Ginsparg in 1991 in the field of physics. Open access means that a publication is free to read. The OA movement was spurred on by the 2013 White House Public Access Mandate which requires publicly-funded research results to be available to the public within one year of publication. In Europe, both public and private funders have called for expanded public access to research results through cOAlition S. Effective this year, cOAlition S launched Plan S that requires recipients of research funding from cOAlition S members to provide immediate full text access to scholarly publications upon publication.

There are a variety of models of Open Access; the two most prevalent today are known as Green and Gold. Before discussing Gold versus Green OA, it may help to understand manuscript versions. A Preprint has not completed or undergone peer review. An Accepted Manuscript (also known as the Author Manuscript version) has completed peer review but is not in final format. The Version of Record is the official published journal article. Publishers add value in the final stages preparing the version of record by registering a DOI, indexing the article, adding metadata, and publicizing the issue to increase discovery and usage of the published work.

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<sup>1</sup> Annual reports of UF/IFAS peer reviewed publications are available at <https://ufdc.ufl.edu/IR00008243/00013/allvolumes>.

<sup>2</sup>The Budapest Open Access Initiative, 2002, the Bethesda Statement on Open Access Publishing, 2003, and the Berlin Declaration on Open Access to Knowledge in the Sciences and Humanities, 2003, together are known as the "Three Bs".

With Green OA, authors may share their accepted manuscript in an open repository or preprint server but the publisher's final version of record will only become free to read after an embargo period of time, determined by the publisher. Embargo periods typically range from 6 months to 2 years during which time full text access is restricted to subscribers. Green OA incurs no cost to authors and complies with the White House mandate.

With Gold OA, the version of record is free to read immediately upon publication at the publisher's website. Authors retain copyright and can select their preferred license for reuse of the article contents. Gold OA may refer to a journal or just to specific articles within a journal. Journals that publish both Gold OA articles and articles viewable by subscription only are known as hybrid journals. Publishing your work in a Gold OA journal or as a Gold OA article in a hybrid journal committed to becoming an OA journal (i.e. transformative journal) complies with Plan S.

It is becoming common for publishers to offer authors the option to publish their work as Gold OA after their manuscript is accepted. The current dominant business model for Gold OA relies on Article Processing Charges (also known as Article Publishing Charges or APCs). APCs vary, depending on the publication and, in some cases, the type of article. The highest APC I'm aware of is Springer's Nature, currently assessing \$11,140 APC. In plant sciences, APCs today are typically \$1,100 to \$3,500 per article. Some publishers have tiered APC, depending on the type of article. For example, Frontiers Media, publisher of Frontiers in Plant Science, has four different types of articles, each with a different APC. Society publications offer reduced APCs to authors who are society members. Always check with the publisher for current fees. Remember that publishing OA does not always incur a fee. Over 70% of the journals in the Directory of Open Access Journals do not charge APCs.

APCs shift publishing costs from the library and individual journal subscribers to authors (or their institutions or funders). There is growing recognition that this APC model, while making scholarly literature free to read, may limit what research is published to researchers with greater financial support, perpetuating similar biases toward Western researchers as in the subscription model (Chattopadhyay et al., 2017; Crawford, 2021). In addition to geographic bias, there is potential racial bias in scholarly publishing. Academics, publishers, and librarians, all of whom are vested in scholarly publishing, are overwhelmingly white. There is a need to critically evaluate the emerging models for scholarly publishing or we will implement systemic biases again, where the haves are heard and the have-nots are not. How can we assure open access to publish as well as open access to read? There are alternative funding models, such as "subscribe to open" where institutional subscriptions cover OA costs for all readers. The Research4Life program, launched in 2002 with support from the World Health Organization and the United Nations Food & Agriculture Organization, works with over 200 publishers to make some subscription content freely available to registered institutions in low and middle income countries<sup>3</sup>. Financial support to publishers can make a specific subset of publications OA, such as the invited Tansley Reviews & Insights published in the *New Phytologist*. Discussions are underway on viable alternatives to fund OA, perhaps through collectives (Crawford, 2021).

Questions arise: what are reasonable publishing fees and who should pay them? The move to OA squeezes society publishers, whose subscriptions help fund society business, including awards and grants. Dr. J. MacDonald, a past president of the American Phytopathological Society (APS) explains the competing needs

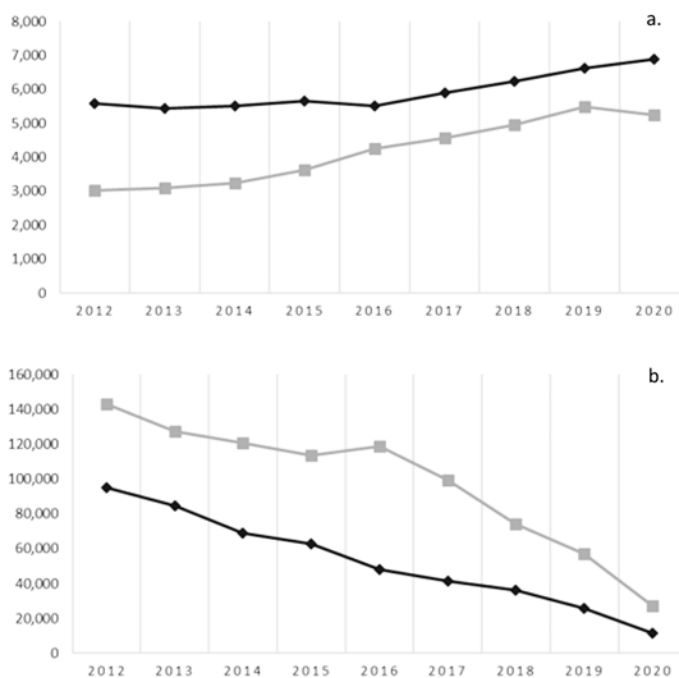


Fig. 1. Number of (a) publications and (b) citations to publications by UF authors published between 2012-2020 that are non-OA (access requires subscription; black line & diamonds) or OA (open access, free to read; grey line and squares). Data from InCites Benchmarking & Analytics, September 2021.

for sharing society research while maintaining financial health of the society and their journal:

*Our journals represent the center of our universe. They are critical to our future as a science and as a professional organization. It is essential that we make the research published in our journals widely accessible to the electronic audience of today, while at the same time protecting the financial health of those journals and ultimately of APS.*

—The American Phytopathological Society, 2021, para. 2

Small and mid-size publishers, especially non-profit society publishers that depend upon subscription and membership revenue, may never be able to offer Diamond OA, where no publishing fees are assessed.

Why do authors publish OA? Some are driven by philosophy and ethics. They may believe it is the best way to ensure that "today's research meets tomorrow's global challenges" (BioMedCentral, 2021) or regard the purpose of research to improve society universally. Others cite expectations and requirements of funders. There is on-going research into the impact of publishing OA (Piwowar et al., 2018; Wang et al., 2015). Springer, for example, reports a 60% increase in citations to OA articles compared to non-OA articles, four times as many downloads and 2.5 times more news or social media mentions within the first three years of publication<sup>4</sup>. Preliminary exploration of Clarivate Analytics' InCites Benchmarks and Analytics data suggests that although UF authors publish more often in subscription journals, there are consistently greater citations to OA articles by UF authors since 2012 (Fig. 1).

<sup>3</sup>Publishers select the content that will be made OA to institutions in lower and middle income countries. Researchers must be affiliated with these institutions to participate. Details on participating publishers, and eligible institutions and countries is available at <<https://www.research4life.org/>>.

<sup>4</sup>Benefits of publishing open access (OA), <<https://www.springer.com/gp/open-access/springer-open-choice>>.



Citations, today, are used to imply quality and impact of published work. A journal's reputation and ranking are heavily based upon citations. This can also be said for the reputations of institutions, research groups, and researchers. Two notable efforts, the Declaration on Research Assessment (DORA) and the Leiden Manifesto, call for responsible use of citation metrics. When using citation metrics, field-normalized data are preferred because publishing and citation patterns differ greatly between disciplines. Alternative metrics recognize that a publication's impact may extend beyond academia, beyond references in peer reviewed literature. Alternative metrics capture references to a publication in newspapers, social media, and policy documents, for example. Citation metrics for an article, book or book chapter, a journal, or an author are available through publishers, library subscriptions, and open source tools.

Unfortunately, the current predominant business model that relies on APCs for Gold OA publishing has attracted fraudulent entities. These entities abuse the academic publishing system to scam money. In 2019, the U. S. Federal Trade Commission fined one predatory publisher, the OMICS Publishing Group, \$50 million for false claims and hidden publishing fees<sup>5</sup>. Where you share your work will influence your future. You took great care to conduct your research, take care to share it wisely too. Assess the publisher and the publication before committing funds or losing rights to your work. Three guidelines are to assess the publication for quality control (editorial board composition, peer review policy), transparency of any fees, and registration and indexing (Leonard et al., 2021).

Smathers Libraries are testing out a number of ways to make OA publishing more affordable for UF authors. The UF Open Access Publishing Fund was available from 2010 through 2020. The fund is on hiatus<sup>6</sup>. The Libraries are now leveraging our subscriptions and memberships to negotiate discounts to UF authors that elect to publish OA<sup>7</sup>.

The Libraries are also conducting research to better understand UF researchers' attitudes and behaviors related to OA publishing. As part of this effort, we are participating in the Elsevier Gold OA Pilot where UF corresponding authors of manuscripts accepted into eligible Elsevier journals have the option to apply a 10-15% APC discount if they choose to publish OA. In the first year of this pilot, 72 articles were published OA, with an APC fee savings over \$30,000, and 413 eligible articles were published in a subscription model. Survey responses from 101 of these authors demonstrate familiarity with OA and fluidity in authors' decisions for OA publishing. Nearly half (48.8%) of the authors who used the subscription model and nearly two-thirds (64.7%) of those who published OA had published OA more than twice previously. Less than 18% of the survey participants had never published their work as OA before. When asked about their plans for the next year, only half of the respondents intended to use the same publishing model. (Fig. 2)

<sup>5</sup>The Federal Trade Commission case against OMICS Publishing Group, Inc. involved a three year investigation. Details of the case are available at <<https://www.ftc.gov/enforcement/cases-proceedings/152-3113/federal-trade-commission-v-omics-group-inc>>.

<sup>6</sup>This fund was managed by the George A. Smathers Libraries with financial support from the Office of the Provost. Each year, the funds were exhausted prior to fulfilling all requests, <<https://digitalpartnerships.uflib.ufl.edu/scholarly-communications/uf-open-access-publishing-fund/>>.

<sup>7</sup>A current list of discounts for University of Florida authors publishing Open Access is available at <<https://guides.uflib.ufl.edu/openaccess/>>.

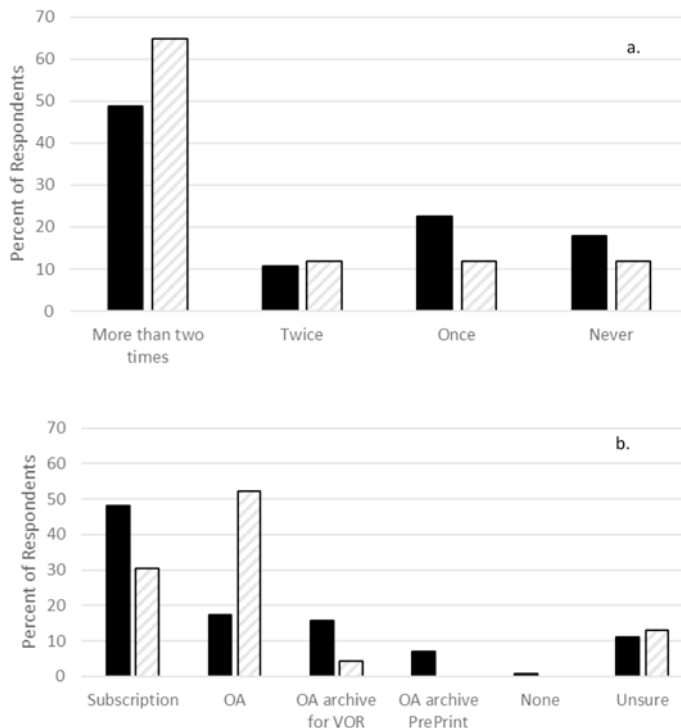


Fig. 2. Percent of survey respondents who (a) have previously published peer reviewed articles as OA (open access, free to read) more than two times, twice, once, or never (n = 101) and (b) intend to publish their research in the next 12 months as subscription, OA, OA archive for version of record (VOR), OA archive preprint, none, or unsure (n = 150). Survey respondents are identified as those who elected to publish an article with subscription (black bars) or OA (open access; grey striped bars) in the first year of the Elsevier Gold OA Pilot with the University of Florida George A. Smathers Libraries (Stapleton et al., 2021).

Comments shared by survey participants reveal challenges authors face when considering OA. One respondent reported “although open access is VERY expensive and prices are increasing, I feel that open access is critical for increasing readership of my scientific work.” Another expressed anxiety about staying competitive in their field, “I am frustrated because I know it is a helpful tool to get research out and ethically I think it is right to remove paywalls. I see other colleagues with institutional resources to support OA publishing getting more recognition for some of their work because of its broader reach. I wish UF had more resources to support this.”

Participants ranked the reasons for their publishing choice (OA or subscription). The top three reasons for publishing OA were: there is value in greater access to research, greater dissemination to a wider variety of audiences, and funding was available to pay the APC. The top three reasons for publishing with the subscription model were: funding was not available to pay the APC for OA, the APC is too expensive, and authors should not have to pay for their research to be published. Our research of OA publishing practices continues.

As OA increases access to scholarly information, the challenge will no longer be how to access needed information but how to ASSESS the information we can access. Publications during the COVID pandemic provide a prime example, where researchers and publishers alike freely shared scholarly publications during this time of great need. The World Health Organization identified this time as an “infodemic”, a period with “too much information including false or misleading information in digital and physi-

cal environments during a disease outbreak”<sup>8</sup>. We each need to continue to develop our information literacy skills to be able to identify high-quality information and avoid untrustworthy sources in our own research fields.

In the meantime, I recommend three actions. First, budget publishing costs into your grant proposals. For the time-being, OA is increasingly required by funders and OA is predominately managed by assessing authors APCs. Second, reflect and take action to minimize implicit bias in your work as researchers, authors, peer reviewers, and editors. Individual and collective action to reduce bias in scholarly publishing will broaden and enrich what is published. Third, stay informed of new models for scholarly publishing. For example, pre-print servers played a major role in responding to COVID-19. Preprint servers enable research to be shared quickly; however, keep in mind that content has not completed the peer review vetting process. Now there are discussions of adding a review aspect to preprint servers. Some publishers are exploring preprint servers as a source for invited publications.

Scholarly publishing, in all its forms, is an integral part of the development of new knowledge.

*It is a common assumption that publications are the output of research. This is a simplistic understanding of the role of publication in science. Publication can just as well be seen as a vital part of the research process itself. Publications and citations constitute the scientific discourse.*

—Larsen and von Ins, 2010, p.592

These current shifts in scholarly publishing create opportunities and challenges for researchers. As publishing options expand, authors can become more empowered to decide where and how to share their work. Simultaneously with this greater freedom, authors now have greater responsibility to understand the implications of their publishing decisions. I hope you will take care in deciding where and how you want to contribute your research to the scientific conversation.

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<sup>8</sup>The World Health organization determined that fighting misinformation is an important aspect for success in public health care <[https://www.who.int/health-topics/infodemic#tab=tab\\_1](https://www.who.int/health-topics/infodemic#tab=tab_1)>.

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# The National Mango Board and the United States Mango Market

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**Additional index words.** consumer demand, fresh-cut, mango, market penetration, market intensity, promotion, per capita consumption, return-on-investment

The National Mango Board (NMB) is a national agriculture promotion and research organization authorized by the United States Congress, approved and overseen by the U.S. Department of Agriculture (USDA), and supported by the fresh whole mango industry. The NMB is entirely funded by assessments from domestic and imported mangos and does not include any taxpayer dollars or government funding. The NMB's mission is to increase the consumption of fresh mango in the U.S. by inspiring consumers and educating them about the culture, flavor, and nutrition of mangos, while bringing the industry together. This mission is accomplished through four main programs: marketing, communications, research, and industry relations. Marketing and communications programs target consumers plus retailers, foodservice, nutritionists, and other key audiences with information about selection, ripening, cutting, varieties, nutrition, and great recipes. Research and industry relations programs help the entire mango supply chain deliver a quality product to the U.S. consumer through research and extension meetings to educate and inform growers, shippers, packers, importers, retailers and others. The research program also investigates the phytonutrient properties of mango and conducts studies on the potential health benefits of mango.

From 2005 to 2020, the mango import volume into the U.S. increased by 110% (from 260,842 MT to 549,211 MT) and per capita mango availability has increased by 193%, from 1.88 pounds to 3.63 pounds per person. Mexico, Peru, Ecuador, Brazil, Guatemala and Haiti are the countries with the highest volume. For retail sales per store per week, the mango category has increased from \$109 to \$262 and the volume of whole mango has increased from 132 to 261 units. From March 2013 to December 2020, NMB promotion programs generated a return on investment of 11.53 to 1.

The National Mango Board (NMB), based in the United States, is a promotion and research organization that was established in 2005 at the request of the mango industry. In 2001, U.S. mango importers, with the support of the Fresh Produce Association of the Americas, submitted a proposal to the U.S. Department of Agriculture (USDA) to request the formation of a national promotion, research, and information order specific for fresh mango, pursuant to the Commodity Promotion, Research, and Information Act of 1996 (AMS, 1996). This proposal was made to (1) develop and finance an effective and coordinated program of research, promotion and industry and consumer information regarding mango; (2) strengthen the position of the mango industry in U.S. markets; and (3) maintain, develop, and expand domestic markets for mangos.

In 2004, USDA finalized the review process of all information submitted including the mango industry's proposal, public rule making comments, a referendum conducted on mango first handlers and importers and concluded that a Mango Order should be established (CFR, 2004). The NMB was formally established the following year when USDA appointed the first group of board members, and the board met to create and approve the organization's bylaws and the initial mango promotion and research programs were set up.

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Thank you to Dr. Ronald Ward, Professor Emeritus of Agricultural Economics at the University of Florida for overseeing and analyzing the consumer research data and for his many years of supporting the NMB with his research. Also, thank you to Dr. Leonardo Ortega, NMB Director of Research and Industry Relations, for his leadership and for being a great ambassador for the mango industry. Finally, a sincere thank you to all members, staff, and partners for their dedication and support of the NMB mission and vision.

The Mango Order authorizes the collection of assessments from first handlers and importers that market 500,000 or more pounds of mangos per year to operate and cover the expenses of the NMB promotion and research programs. In 2020, the fresh whole mango assessment rate was three-quarters of a cent per pound of mango (\$0.0075/lb.) which generated approximately \$9 million in assessments (NMB, 2021).

Another requirement of the Mango Order is that a continuance referendum be conducted every five years to determine whether a majority of mango first handlers and importers are in favor of continuing the promotion and research programs. Failing to do so allows USDA to terminate the Mango Order. Mango continuance referendums have been conducted successfully in 2010, 2015, and 2020.

## National Mango Board (NMB) Structure, Priorities, and Programs

### NMB Structure

The NMB has eighteen (18) board seats that are assigned to mango industry members nominated by their peers and reviewed and appointed by the U.S. Secretary of Agriculture. The board seats are specifically defined in the Mango Order and include: ten (10) importers, eight (8) foreign producers, two (2) domestic producers, and one (1) first handler. Each board member serves a three-year term, with the possibility of serving a second consecutive term.

Board members are responsible for reviewing and approving NMB programs and budgets (which was set at approximately \$10 million at the start of 2020) and providing direction and guidance to NMB staff on the strategic direction and specific activities of the organization. NMB staff is responsible for executing the board's direction and managing the promotion and research programs. Each NMB member also serves on at least one of three committees: Marketing & Communications; Research & Industry Relations; and the Executive Committee.

### NMB Priorities

The NMB's short- and long-term priorities are guided by a strategic plan that is developed by NMB board members, staff, and partners. It is reviewed and updated every three years. The

current NMB Strategic Plan runs through the end of 2023 and includes the organization's mission, vision, objectives, and key strategic pillars. The NMB mission is "To increase the consumption of mango in the U.S. by inspiring consumers and educating them about the culture, flavor, and nutrition of mangos, while bringing the industry together". The vision is "For mangos to move from being an exotic fruit to a daily necessity in every U.S. household." The main strategic objective is for mango to "move toward becoming at least a top 15 whole fruit by value in the U.S. market by 2025." The NMB Strategic Plan also includes the following Five Key Strategic Pillars to achieve its mission, vision, and objectives:

1. **Deliver High-Quality & Flavorful Mangos.** Deliver consistently high-quality fresh products and improve the flavor profiles delivered to the consumer across the varieties of mangos.
2. **Market Positioning.** Continuously improve market positioning and adoption across all relevant consumer audiences for fresh mango, as well as products with mango ingredients
3. **Consumer Education.** Understand and analyze key consumption barriers and use strong research-based strategies to enhance consumer familiarity, understanding, and sentiment towards the purchase of fresh mango.
4. **Market Penetration.** Drive mango market penetration in retail, foodservice, and industrial uses of fresh mango products.
5. **Industry Service and Engagement.** Improve value-added and industry information for fresh mango to support greater efficiency. Build support for the program and participation by the assessment payers in the fresh sectors and throughout the supply chain.

Each of the five strategic pillars includes outlined plans to achieve specific objectives that are supported annually with business plans and budgets that contain further details and key performance indicators. The NMB Strategic Plan is the foundation for the NMB Programs which are responsible for fulfilling the mission, vision, objectives, and strategic priorities of the organization.

### **NMB Programs**

The NMB's overall mission is to increase the consumption of fresh mango in the U.S. by first, inspiring and educating consumers about the culture, flavor, and nutrition of mangos and second, bringing the industry together through programs in: Marketing, Communications, Research, Industry Relations, and Operations. Marketing and Communications programs target consumers, retailers, foodservice, nutritionists, and other key audiences with information on mango selection, ripening, cutting, varieties, and nutrition, plus food and beverage recipes. Research and Industry Relations programs focus on improving production and postharvest practices to deliver consistent high-quality mango to U.S. consumers and sharing this information throughout the supply chain through meetings and online webinars. Research programs also investigate the phytonutrient properties of mango and conduct studies on the potential health benefits of mango.

### **Marketing & Communications Programs**

Consumer Marketing and public relation campaigns are designed to increase mango consumption and awareness amongst

U.S. consumers as outlined in the NMB Strategic Plan. The program uses education primarily focused on nutrition, how to select and cut mangos, and prepare mango inspired recipes. In addition, consumer research identifies and generates key messaging, which is strategically applied to a combined omnichannel and multimedia approach including print, broadcast, and social media, influencers, and digital platforms.

The Retail Marketing and Promotion program increases mango awareness and overall purchases throughout the country. NMB works directly with retail and wholesale partners and supports whole and fresh-cut mango growth initiatives that include: training on mango quality and handling practices; establishing year-round mango category programs; improved merchandising and in-store display strategies; retail promotions; fostering ripe-and-ready-to-eat mango ripening programs; analyzing and sharing mango retail category data and consumer insights; motivating sustainability efforts; and highlighting mango nutrition messaging. There is a team of regional retail merchandisers who work directly with retailers throughout the U.S. and represent the NMB at industry tradeshows and events.

The Foodservice program positions mango as an on-trend ingredient and expands menu penetration by increasing awareness throughout the foodservice supply chain using education, training, and outreach to chefs, culinary influencers, and decision makers. The program also supports foodservice mango promotions and marketing campaigns that enhance consumer awareness. The NMB has worked with the Culinary Institute of America to establish an approved curriculum of 8.5 continuing education hours focused specifically on the use of mango in foodservice operations. The Foodservice program also works to expand the use and availability of fresh-cut mango and improve processing and handling practices.

The Communications program develops and manages year-round public relations communication and advertising campaigns with produce industry media and trade publications and provides communication support to all NMB programs. It also keeps the mango industry informed of NMB projects, activities, and available resources through the organization's website, newsletters, annual reports, social media, etc. The program maintains a crisis communication plan and collaborates with partners to provide crisis response training to key NMB staff and board members, and monitors and tracks industry issues that can affect the NMB or mango consumption.

### **NMB Research and Industry Relations Programs**

The Research program supports science-based investigation to improve the understanding of mango as a fruit and as a commodity. Research studies funded with universities and institutions around the world focus on the nutritional and health benefits of mango. The program supports the mango supply chain by supporting studies that improve production and postharvest best management practices that deliver more consistent and higher quality mangoes to consumers.

Completed NMB funded research studies can be found at <[www.mango.org/research-studies](http://www.mango.org/research-studies)>. They are presented to the industry through online webinars, social media, videos, and traditional media articles. Resources developed from research studies are included in existing resources, so the entire mango supply chain has access to the latest best practices. The NMB also supports research presentations at industry meetings, conferences, and symposia.

The Industry Relations program works with mango and produce industry organizations in the United States and countries

that supply the U.S. market with mangoes. The program also engages directly with stakeholders in the mango supply chain (growers, packers, shippers, importers, handlers, processors, retailers, manufacturers, and service providers). This outreach is an effective method of ensuring that NMB resources, research studies and related information reach industry members who can apply it directly to their operations. NMB stakeholders are interested in U.S. mango supply and demand dynamics and market development. NMB outreach efforts play a significant role in bridging the gap between the multiple layers of the supply chain by encouraging and generating communication among industry members.

A key resource by Industry Relations is the Mango Crop Report, which serves as a guide for both mango suppliers and marketers. This weekly report contains information and data on countries that are shipping or are about to start shipping mangos to the US. It compiles information from country mango organizations, industry contacts, econometric models, and USDA reports. The report includes shipping projections, volume shipped, volume that has arrived/is projected to arrive in the U.S., arrival volume comparisons to the prior year, details on varieties shipped from each country, status of mangos sent by ship, pricing information by port, variety, size, and Freight on Board (FOB) price comparisons to the previous year.

### Operations Program

The Operations program ensures the NMB is in compliance with all applicable legislation, regulations, standards, and policies set by USDA, and works closely with USDA on all oversight matters.

The annual NMB nominations process for selecting new board members is handled by the Operations program and involves educating and informing mango industry members of the duties and responsibilities of NMB members. The program organizes and manages all the nomination activities including submitting the information required by USDA for the Secretary of Agriculture to determine which nominees will be appointed as new board members.

Finally, each of the NMB programs attends mango industry events throughout the supply chain, starting on the production side and moving through every stage of the industry all the way to end users. NMB participates in produce industry events, and meetings with other commodity boards and with the USDA as necessary.

## U.S. Mango Market

### Mango Varieties Available in the U.S.

Mangos originated in Southeast Asia and India over 4,000 years ago. Their cultivation has since spread to other tropical and subtropical regions of the world (Campbell, et al., 2002). There are over a thousand mango varieties around the world, but only six varieties are commonly available year round in the United States: ‘Tommy Atkins’ (37%); ‘Kent’ (27.7%); ‘Ataulfo’ (19%); ‘Keitt’ (11.1%); ‘Haden’ (2.9%) and ‘Francis’ (1.8%) (Fig. 1). In addition, the following varieties have been reported in the U.S. marketplace in recent years: ‘Alphonso’, ‘Angie’, ‘Banilejo’, ‘Calypso’, ‘Edwards’, ‘Glenn’, ‘Julie’, ‘Kensington’, ‘Kesar’, ‘Mallika’, ‘Manila’, ‘Mingolo’, ‘Nam Doc Mai’, ‘Palmer’, ‘R2E2’, ‘Rapoza’ and ‘Smith’ among others.

### Mango Supply to the U.S. Market

Due to the potential risk of introducing non-native pests that could affect domestic agriculture, all mangoes produced outside

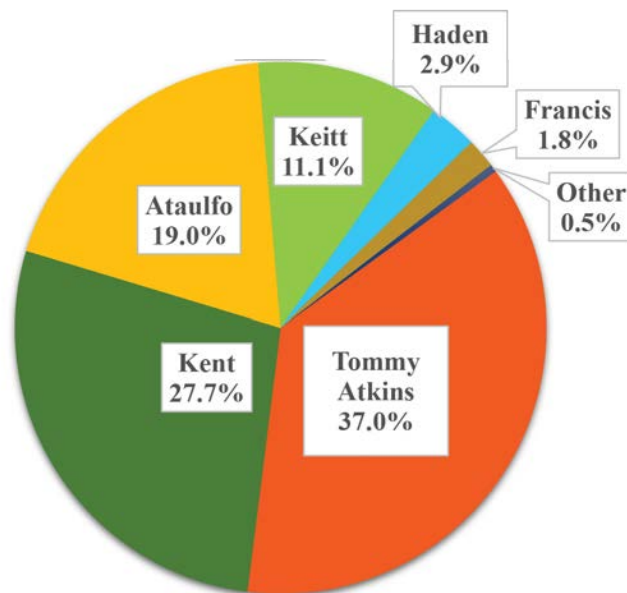


Fig. 1. Mango variety availability in the United States market. Source: USDA Market News and National Mango Board.

the United States mainland must have authorized import permits and comply with phytosanitary treatment protocols that have been reviewed and approved by USDA Animal Plant Health & Inspection Service (APHIS, 2018). These protocols are established are specific for each country or producing region. They currently include the following treatment methods: hot-water, irradiation, high temperature forced air, vapor heat, or the fruit is produced in a designated and recognized fruit fly-free area (APHIS, n.d.). As of 2021, 19 countries have APHIS-approved phytosanitary treatment protocols in place: Australia, Brazil, Chile, Costa Rica, Dominican Republic, Ecuador, Guatemala, Haiti, Honduras, India, Jamaica, Mexico, Nicaragua, Pakistan, Peru, Taiwan, Thailand, Venezuela, and Vietnam.

Nearly all mangoes supplied to the US market are from Latin American countries, accounting for ~97% of mango imports in 2020. Some domestic production exists in southern Florida, southern California, and Hawaii, but is mostly consumed by the local market or sold online. Puerto Rico also has commercial mango production, but it is mostly exported to the European market or consumed locally.

In 2020, most of the mangoes in the US market were supplied by Mexico (63%), Peru (14%), Ecuador (10%), Brazil (9%), Guatemala (2%), and Haiti (1.5%). (Fig. 2). Other countries exporting to the United States in recent years include the Dominican Republic, Costa Rica, Nicaragua, Jamaica, Australia, Thailand, India, and Pakistan.

### 2020 U.S. Import Data on Mango

In 2020, the total volume of mango imported into the US was 549,225 MT (FAS, n.d.). The standard container used by the mango industry is a 4-kg box. The 2020 import volume is roughly 137.3 million boxes, an increase of 11.4% compared to the previous year. From 2005 to 2020, the mango import volume into the U.S. increased by 110% (Fig. 3).

The 2020 volume from the five main exporting countries (Mexico, Peru, Ecuador, Brazil, and Guatemala) was 132.2 million boxes, which generated U.S. \$661 million FOB as measured at ports of entry. This represented an increase of 4.1% compared

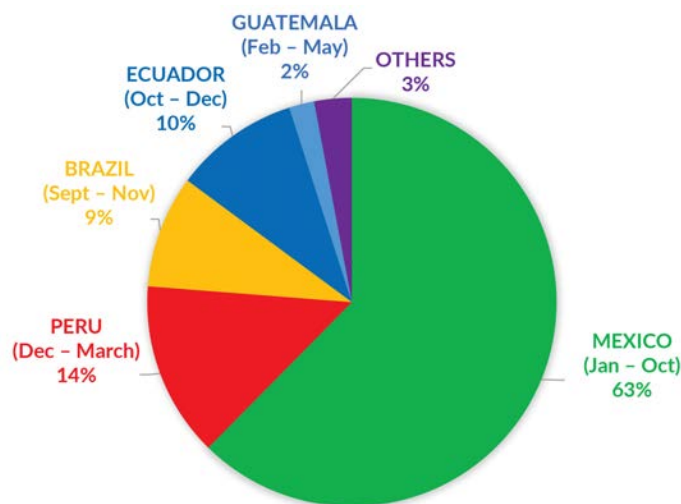


Fig. 2. Mango volume import percentage by country and months of export. Source: USDA Market News and National Mango Board.

to 2019. Since 2005, the increase in whole mango FOB has been 152% (Fig. 3). The average price per box of mangoes during 2020 was U.S. \$5.00, a decrease of 6.0% compared to \$5.32/box in 2019 (NMB, 2020a).

### U.S. Retail Data on Mango

Since 2007, the NMB working with the Nielsen Group's *Answers on Demand* (AOD), has been collecting and analyzing mango category retail sales data to provide a resource that maintains a historical reference and performance benchmarks to help retailers and mango industry members identify trends and opportunities in mango sales growth. The mango category performance benchmark in this report summarizes 2020 data for whole and fresh-cut mangos, including sales, volume, and pricing.

Mango category sales data are from U.S. grocery store chains with more than \$2 million in annual sales from roughly 18,000

stores. It is collected on a weekly basis by store and by item and represents fruit items sold in the produce department. Small independent chains and alternative format retailers such as Whole Foods and Trader Joe's are included in the data. Volume is measured by unit (1 whole mango = 1 unit), for those retailers who sell mangos by the pound, a conversion rate is applied. Price is in U.S. dollars (\$) per mango unit; per store sales are also in U.S. dollars (\$). A more detailed and descriptive analysis of mango performance (volume, price and sales) per store per week, per region, per quarter, and yearly is available through the NMB.

### US Retail Whole Mango Results

Whole mango dollars per store/week increased from \$220 to \$262 in 2020, an increase of 19%. (Fig. 4). This 12% growth was a result of an increase in mango volume per store/week from 233 to 261 units. The average retail price per mango increased by 6% from \$0.95 to \$1.01. (Figs. 5 and 6). Whole mango dollars per store/week increased in every quarter in 2020, while volume per store/week increased in every quarter except Q2, a result of the onset of the COVID-19 pandemic.

In 2020, the average retail price per unit per month dropped to \$0.85/unit in July, and peaked at \$1.28/unit in October. For 2020 overall fruit rankings at the retail level, whole mango advanced one spot to twelfth position in volume per store/week (Fig. 5) and moved up three spots from the prior year to seventeenth overall for dollars per store/week. (Fig. 4).

### U.S. Retail Fresh-Cut Mango Results

Fresh-cut mango retail performance is separated into two categories, "Mango Only" which is consumer packages that only contain fresh-cut mango, and "Mango Mix" which includes fresh-cut mango mixed with other fruits within the same package. Fresh-cut Mango Only dollars per store/week increased from \$100 to \$106 in 2020, an increase of 6%. While fresh-cut Mango Mix dollars per store/week decreased from \$108 to \$79, a decrease of 27%. (Fig. 7).

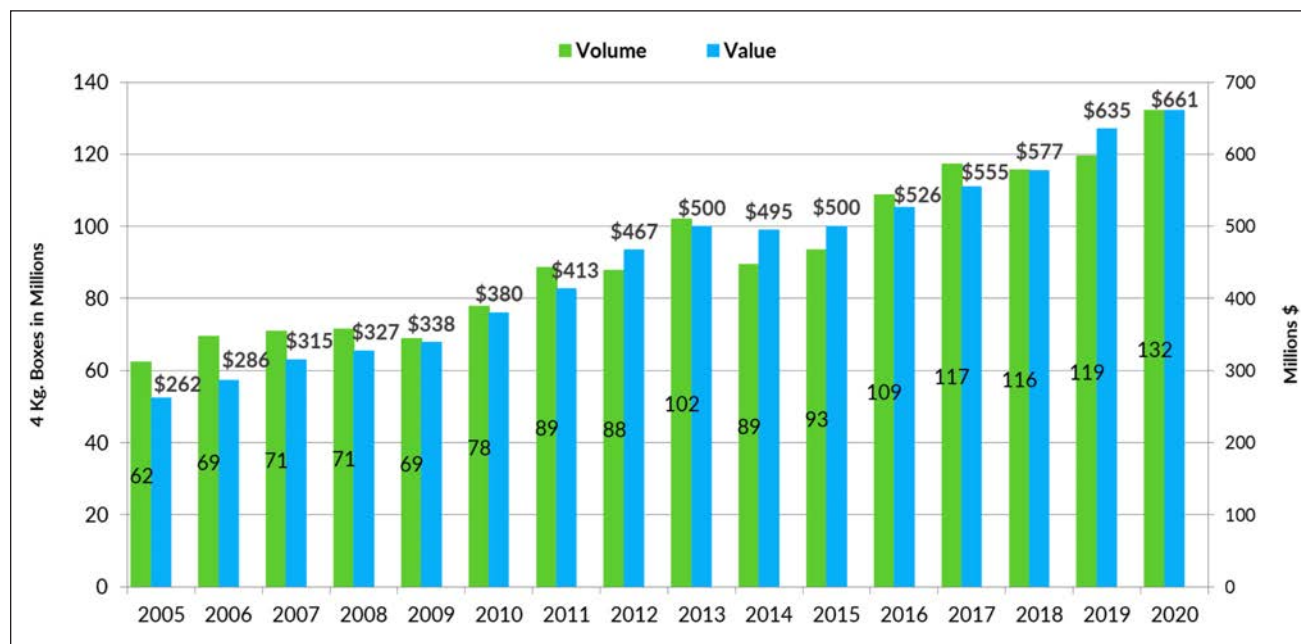


Fig. 3. Total Mango Import Volume & Value, for Five Main Exporting Countries (Mexico, Peru, Ecuador, Brazil, and Guatemala). Source: USDA Market News and National Mango Board.

FRUITS	Ranking	Dollars/Store/Week	% Change Year Over Year
<b>All Fruits</b>		<b>\$14,851</b>	<b>8.1%</b>
Apples	1	\$2,202	3.8%
Grapes	2	\$1,928	0.2%
Bananas	3	\$1,894	4.4%
Strawberries	4	\$1,866	16.0%
Avocados	5	\$1,618	7.7%
Blueberries	6	\$1,281	11.2%
Cherries	7	\$1,268	25.6%
Mandarins/Tangerines	8	\$930	10.2%
Watermelons	9	\$753	10.6%
Oranges	10	\$746	27.5%
Raspberries	11	\$722	9.2%
Lemons	12	\$526	22.0%
Peaches	13	\$444	-1.3%
Blackberries	14	\$443	14.2%
Limes	15	\$442	24.5%
Pears	16	\$273	-1.6%
<b>Mangos</b>	<b>17</b>	<b>\$262</b>	<b>19.4%</b>
Nectarines	18	\$255	1.3%
Pineapples	19	\$242	14.5%
Cantaloupe Melons	20	\$230	3.3%
Plums	21	\$177	8.5%
Dipped Fruit	22	\$170	15.3%
Grapefruits	23	\$144	15.9%

Fig. 4. 2020 Average Retail Value per Store/Week. Source: Nielsen Answers on Demand® (week 52 ended 12/26/2020) Total US × AOC.

FRUITS	Ranking	Volume/Store/Week	% Change Year Over Year
<b>All Fruits</b>		<b>9,505</b>	<b>6.6%</b>
Bananas	1	3,368	4.7%
Avocados	2	1,262	16.7%
Apples	3	980	1.7%
Limes	4	951	23.0%
Grapes	5	855	1.7%
Strawberries	6	567	13.2%
Lemons	7	541	17.7%
Oranges	8	433	10.0%
Blueberries	9	355	8.5%
Cherries	10	334	8.4%
Watermelons	11	313	-1.3%
<b>Mangos</b>	<b>12</b>	<b>261</b>	<b>11.9%</b>
Peaches	13	228	-7.2%
Raspberries	14	227	17.2%
Mandarins/Tangerines	15	212	11.6%
Blackberries	16	149	13.5%
Pears	17	149	-7.8%
Cantaloupe Melons	18	127	-4.6%
Pineapples	19	118	15.4%
Nectarines	20	116	-4.3%
Papayas	21	115	1.8%
Kiwis	22	95	3.3%
Grapefruits	23	81	11.2%

Fig. 5. 2020 Average retail volume per store/week. Source: Nielsen Answers on Demand® (week 52 ended 12/26/2020) Total US × AOC.

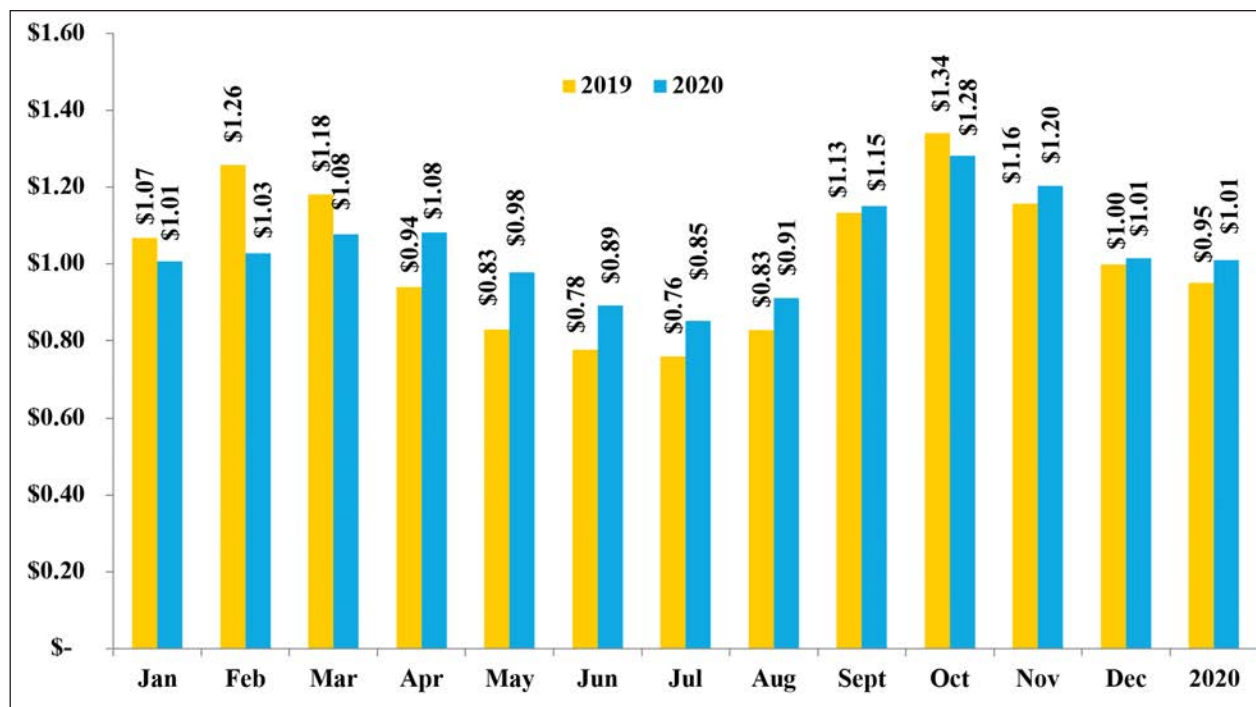


Fig. 6. 2020 Average monthly price of mango at retail. Source: Nielsen Answers on Demand® (week 52 ended 12/26/2020) Total US × AOC

At \$95,641,078, total fresh-cut Mango Only sales were up by 7.9%, while at \$40,810,723, total fresh-cut Mango Mix sales were down 4.6% in 2020. (Fig. 8). Total sales of fresh-cut mango increased in every month of 2020, except for February and April.

Fresh-cut mango sales per store/week peaked in August at \$124, but dipped to \$89 in December (Fig. 9). For 2020 fresh-cut fruit rankings, Mango Only ranked seventh and Mango Mix was ranked twelfth (NMB 2020b).

	Rank	Dollars/ store/week	% Change YOY	Dollar contribution to total fruit cut	% Change YOY
<b>Fresh cut fruit</b>		<b>\$1,553</b>	<b>0.1%</b>	<b>100%</b>	--
Watermelon	1	\$ 557	9.8%	27.2%	2.8%
Mixed fruit	2	\$ 394	-9.8%	20.7%	-2.6%
Pineapple	3	\$ 299	2.3%	15.9%	0.6%
Cantaloupe	4	\$ 168	2.8%	7.4%	0.3%
Specialty melons	5	\$ 168	-1.0%	2.8%	0.2%
Remaining berries	6	\$ 150	5.1%	0.2%	0.0%
Remaining fruit	7	\$ 148	-29.5%	1.4%	-0.8%
Fruit cocktail	8	\$ 121	-34.0%	0.0%	0.0%
Fruit salad	9	\$ 120	-12.5%	4.1%	-0.2%
Mixed berries	10	\$ 113	-3.8%	3.0%	-0.2%
Apples	11	\$ 108	-15.4%	4.3%	-1.0%
<b>Mango only</b>	<b>12</b>	<b>\$ 106</b>	<b>6.0%</b>	<b>3.6%</b>	<b>0.3%</b>
Strawberries	13	\$ 89	-1.8%	1.7%	0.1%
<b>Mango mix</b>	<b>14</b>	<b>\$ 79</b>	<b>-27.0%</b>	<b>1.5%</b>	<b>-0.1%</b>
Tangerines	15	\$ 66	13.8%	0.1%	0.0%

Fig. 7. 2020 Average fresh-cut mango dollars per store/week. YOY = year over year. Source: Nielsen Answers on Demand® (week 52 ended 12/26/2020) Total US × AOC.



	Rank	Dollars	% Change vs. YAGO	Dollar contribution to total fruit cut	% Change vs. YAGO
<b>Fresh cut fruit</b>		<b>\$2,659,014,773</b>	<b>-1.2%</b>	--	--
Watermelon	1	\$723,524,137	10.3%	27.2%	2.8%
Mixed fruit	2	\$549,659,312	-12.3%	20.7%	-2.6%
Pineapple	3	\$423,090,439	2.5%	15.9%	0.6%
Cantaloupe	4	\$197,765,747	2.5%	7.4%	0.3%
Apples	5	\$113,763,783	-19.8%	4.3%	-1.0%
Fruit salad	6	\$109,711,637	-6.1%	4.1%	-0.2%
<b>Mango only</b>	<b>7</b>	<b>\$95,641,078</b>	<b>7.9%</b>	<b>3.6%</b>	<b>0.3%</b>
Mixed berries	8	\$79,500,475	-8.1%	3.0%	-0.2%
Grapefruit	9	\$78,053,693	27.5%	2.9%	0.7%
Specialty melons	10	\$74,928,279	6.6%	2.8%	0.2%
Strawberries	11	\$43,904,131	6.8%	1.7%	0.1%
<b>Mango mix</b>	<b>12</b>	<b>\$40,810,723</b>	<b>-4.6%</b>	<b>1.5%</b>	<b>-0.1%</b>
Remaining fruit	13	\$36,128,572	-37.5%	1.4%	-0.8%
Pomegranate	14	\$31,727,380	-2.1%	1.2%	0.0%
Honeydew	15	\$29,916,420	-6.8%	1.1%	-0.1%

Fig. 8. 2020 Fresh-cut mango total dollars & contribution. Source: Nielsen Answers on Demand® (week 52 ended 12/26/2020) Total US × AOC.

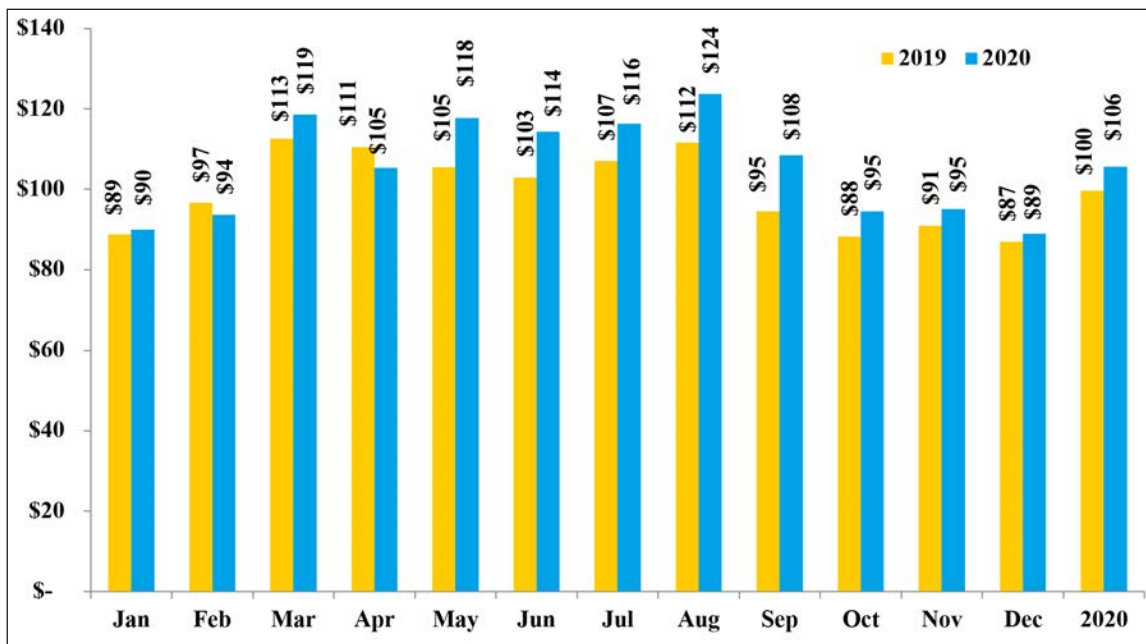


Fig. 9. 2020 Average fresh-cut mango monthly dollars per store/week. Source: Nielsen Answers on Demand® (week 52 ended 12/26/2020) Total US × AOC.

### U.S. Per Capita Consumption of Mango

Per capita consumption of mango in the US has been growing over the last fifteen years from 1.88 pounds in 2005 to an all-time high of 3.63 pounds in 2020, an increase of 193%. (Fig. 10).

In 2005 mango was ranked seventeenth in per capita fruit consumption, but may have moved up to twelfth place in 2020. Over the past fifteen years, per capita mango consumption has surpassed pears, peaches, honeydew melons, and grapefruit consumption. (Fig. 11).

### \*\*NMB Impact on Mango Demand and Return on Investment

Pursuant to the Mango Order, the NMB is required to conduct an independent evaluation on the effectiveness of its programs every five years. These reports are submitted to USDA and made available to the public. The purpose of the independent evaluation is to evaluate the impact of NMB programs on enhancing the U.S. demand for mangos. The NMB uses an extensive household for

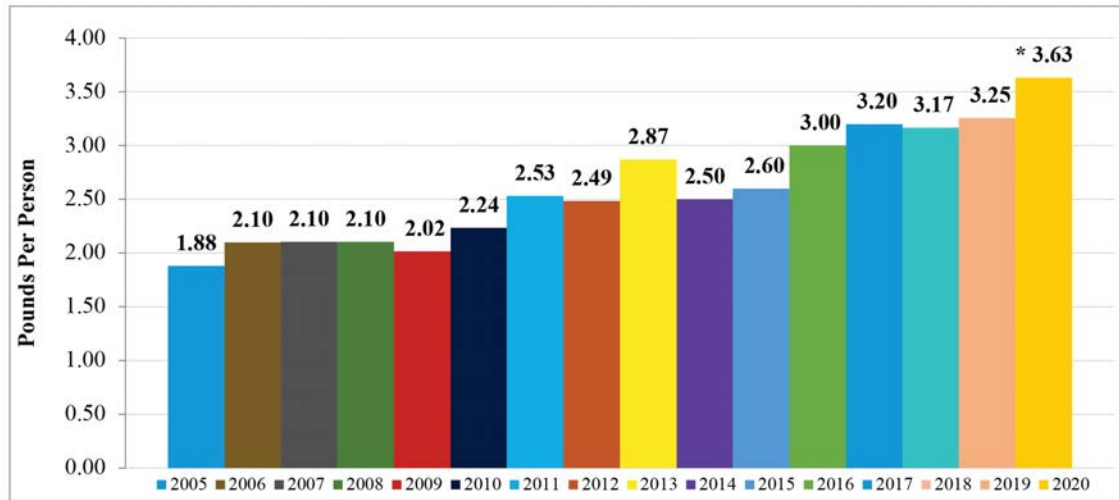


Fig. 10. U.S. per capita consumption of fresh whole mango. Source: USDA Economic Research Service. \*2020 Per Capita Consumption calculated by the NMB.

FRUITS	2005 Ranking Per Capita Consumption	2005 Per Capita Consumption in lbs.	2018 Ranking Per Capita Consumption	2018 Per Capita Consumption in lbs.	2020 Per Capita Consumption in lbs.
Bananas	1	25.2	1	28.3	
Apples	2	16.8	2	17.0	
Watermelons	3	13.5	3	15.7	
Oranges	4	11.4	4	8.2	
Grapes	6	8.7	5	8.1	
Avocados	10	3.5	6	8.1	
Pineapples	8	4.9	7	7.8	
Strawberries	7	5.8	8	7.1	
Cantaloupe Melons	5	9.6	9	7.0	
Mandarins/Tangerines	14	2.5	10	5.4	
Lemons	11	2.9	11	4.1	
Limes	15	2.0	12	3.5	
Mangos	17	1.88	13	3.17	3.63*
Pears	12	2.9	14	2.9	
Peaches	9	4.8	15	2.2	
Blueberries	21	0.4	16	2.0	
Honeydew Melons	16	1.9	17	2.0	
Grapefruits	13	2.6	18	1.6	
Papayas	18	0.9	19	1.3	
Cherries	19	0.9	20	1.3	
Raspberries	23	0.1	21	0.8	
Kiwis	20	0.5	22	0.6	
Apricots	22	0.1	23	0.1	

Fig. 11. U.S. fresh fruit per capita consumption. Source: USDA Economic Research Service. \*2020 Per Capita Consumption calculated by the NMB.

econometric demand models to show the probability of a consumer buying mangos and the number of mangos purchased. The success of NMB programs is measured based on each household's awareness of mango promotions. Both household awareness and NMB expenditure measures have had a statistically positive impact on U.S. mango demand.

For the five-year evaluation conducted in 2021, Dr. Ronald Ward, Emeritus Professor of Economics at the University of Florida, analyzed household surveys collected between March 2013 and December 2020. Approximately 1,000 household surveys are conducted each month. Care is taken to ensure the data are representative of the U.S. population. As of June 2021, the cumulative database includes nearly 170,000 entries dating back to 2008, but since household awareness questions only started in

2013, the models in the 2021 independent evaluation only cover the period from 2013 to 2020.

### Market Penetration, Market Intensity and Retail Mango Sales

Applying the household awareness model, both market penetration and market intensity were estimated with NMB programs in place and then assuming they did not exist. During the period of March 2013 to December 2020, both market penetration and market intensity increased with a portion of the growth attributed to NMB programs. Average market penetration with NMB promotions in place was 14.33% of households purchasing mangos during a two-week shopping period, compared to 13.55% without NMB promotions; this represents an increase of 0.78% for

Table 2. Results of the 2021 mango promotion awareness model.

Mango promotion Awareness model	Promotion awareness	Base 2013 Mar. to 2015 Dec.	2016 Jan.–Dec.	2017 Jan.–Dec.	2018 Jan.–Dec.	2019 Jan.–Dec.	2020 Jan.–Dec.
Market penetration	Yes	0.073	0.134	0.154	0.136	0.164	0.199
	No	0.069	0.125	0.146	0.129	0.155	0.189
Market intensity	Yes	3.224	3.960	3.608	3.389	3.636	3.635
	No	3.056	3.656	3.398	3.211	3.407	3.402
Average retail price (\$ per retail mango)		\$1.19	\$1.34	\$1.37	\$1.28	\$1.36	\$1.38
----- millions -----							
Household mangos	Yes	2199	1773	1880	1523	1993	2426
	No	1970	1530	1673	1735	1762	2160
Implied increase in mango demand		229	243	208	148	231	266
Household expenditures	Yes	\$2,559.72	\$2,380.71	\$2,571.83	\$1,930.86	\$2,708.60	\$3,330.71
	No	\$2,294.43	\$2,048.82	\$2,287.01	\$1,743.07	\$2,396.92	\$2,926.95
Gains		\$265.29	\$331.89	\$284.82	\$187.78	\$311.69	\$367.75
FOB equivalent (34.07% margin)	Yes	\$872.10	\$811.11	\$876.22	\$657.84	\$922.82	\$1,134.77
	No	\$781.71	\$698.03	\$779.18	\$593.87	\$816.63	\$1,009.48
FOB \$ Difference		\$90.38	\$113.07	\$97.04	\$63.98	\$106.19	\$125.29
NMB expenditures		\$17.57	\$6.59	\$6.12	\$6.87	\$7.90	\$6.63
<b>Implied ROI (starting with March 2013)</b>		<b>5.14</b>	<b>17.17</b>	<b>15.86</b>	<b>9.31</b>	<b>13.45</b>	<b>18.90</b>

Table 2. Estimated return on investment (ROI) of NMB awareness programs.

Rows	NMB Awareness Model (March 2013 to Dec. 2020)	With NMB (U.S. \$ millions)	Without NMB (U.S. \$ millions)	Difference (U.S. \$ millions)
1	Household Mango Expenditures	\$15,482.43	\$13,733.20	\$1,749.23
2	Retail/F.O.B. Factor	0.3407	0.3407	—
3	\$ Sales at F.O.B.	\$5,274.86	\$4,678.90	\$595.95
4	NMB Expenditures	\$51.68	\$0.00	\$51.68
5	ROI = (Row 3 / Row 4)	—	—	\$11.53

market penetration. Average market intensity was 3.575 mangos per purchase with NMB promotions in place, compared to 3.355 mangos per purchase without NMB promotions; an increase of 6.56%. (Table 1).

Using the studies above to the empirical models, it is possible to estimate the household mango expenditures directly attributable to NMB programs. Fig. 12 shows household mango expenditures with and without the NMB programs. The difference in demand times the average retail price is attributed to NMB mango promotion programs. During the 94-month period from March 2013 to December 2020, estimated household mango expenditures measured at FOB totaled U.S. \$5,274 million with the NMB programs in place and U.S. \$4,678 million without them, a difference of U.S. \$595 million. This represents an increase of 12.7% in FOB U.S. mango sales attributable directly to NMB programs.

### Return on Investment

The return on investment (ROI), a.k.a. the “Benefit Cost Ratio”, is a common formula used to measure the performance, of an investment. For commodity boards, such as the NMB, the ROI measures how much additional value has been created from the generic promotion programs relative to program expenditures. Using the analysis developed for measuring mango demand, it is possible to measure the ROI that the NMB promotion programs have generated for the mango industry (Ward, 2021).

Table 2 shows the differences between household mango purchases at retail from March 2013 to December 2020 with

and without NMB programs and expenditures. Since the NMB assessments are collected at the port-of-entry or FOB level, retail sales must be expressed at the same level. NMB research has determined that an adjustment factor of 0.3407 between the FOB and retail price is accurate. Using this adjustment factor allows the retail value to be expressed at the FOB level. The bottom line is that, for each dollar the mango industry has invested in NMB programs, an addition \$11.51 was generated in FOB sales.

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## Renewed Potential for Caimito (*Chrysophyllum caimito*) and Guanábana (*Annona muricata*) in South Florida?

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**Additional index words.** caimito, caimitier, graviola, soursop, star apple

Caimito and guanábana were first introduced into Florida in 1833 and sometime before 1879, respectively. Both species are cold and chill sensitive and were therefore recommended for only the warmest areas of south Florida (e.g., the Keys and along the extreme southeastern and southwestern coastal counties, Miami-Dade, Broward, Sarasota). Despite the environmental challenges, interest in these species has not declined over the years with re-assessments as to their potential periodically discussed and touted. Despite their intolerance to cold weather and windy conditions, small commercial plantings and scattered dooryard trees continued to be planted over the next 100+ years. The demand for caimito and guanábana fruit has grown along with the increase in immigrants from Latin America, the Caribbean, and S.E. Asia into the U.S. Until recently, the cool to freezing temperatures experienced in south Florida precluded commercial expansion of these fruit. However, as the climate has warmed, and the frequency of freeze events has decreased, renewed interest and planting of caimito and guanábana has increased. This paper will discuss the status, environmental conditions, and cultural practices that enhance chances for successful production of these fruits.

Caimito and guanábana have been grown in the warmest areas of Florida for over 100 years, mostly as specimen or dooryard trees due to their cold sensitivity and freeze intolerance (Grove, 1941; Lowe, 1937; Morton, 1987; Noonan, 1953; Wolfe, 1937). There is a demand for these fruits especially by people with a Latin[x] and Caribbean background or more recently by new generation of exotic fruit enthusiasts. Previously, planting caimito was recommended as a potential crop for south Florida and small plantings were established during the late 1980s and 1990s; however, chilling temperatures and periodic freezes precluded expansion of the acreage (Campbell, 1986). Recently, interest in planting these crops has increased and since about 2015, small groves have been established and trees appear to be growing and fruiting well (Campbell, 1986; Ledesma and Campbell, 2005).

This uptick in planting may be attributed to a lack of significant freeze events in south Florida during the last ten years and the general warming trend of the climate (Anonymous, 2021; USDA-Zones, 2021; AgroClimate, 2021; NOAA, 2021; IPCC, 2021). Especially during the last five years, monthly average temperatures have increased. This has been most notable during the fall and winter months. What is unknown is whether freezing events in south Florida will be a thing of the past or just less frequent, of less duration and/or of less intensity (cold). Thus, while there is enthusiasm for establishing additional acres, recommendations must be made with caution and freeze protection of these groves needs to be taken into consideration. Below the author discusses some of the basics for caimito and guanábana with basic parameters and recommendations.

Caimito (*Chrysophyllum caimito*) is in the Sapotaceae, originating in Mesoamerica (Central America and central to southern Mexico), is now distributed throughout the tropics (Morton, 1987; Petersen et al., 2012). Non-pruned trees are medium to large (25–100 ft tall) with a round canopy and weeping growth habit. The leaves are attractive, shiny green on the upper surface and golden-brown on the lower surface (Morton, 1987). The small

greenish-yellow bisexual flowers are held in clusters along leaf axils. Trees generally flower during late summer-early fall and fruit are harvested Feb. to June. The time from flowering to harvest ranges from 150–180 days and potential production ranges from ~130–250 lb/tree (Morton, 1987; Love and Paull, 2011). Fruit is round to oblate, two to four inches in dia. with either a purple or green peel (Fig. 2). The gelatinous pulp is sweet with a pleasant flavor, white, soft, and milky surrounding six to eleven seeds. Fruit size may increase with early fruit thinning. Trees may be planted from seed but there are named cultivars reproduced by vegetative propagation (Ledesma and Campbell, 2005).

Caimito is best adapted to hot tropical and warm-hot subtropical climates where freezing temperatures do not occur, temperatures average 65 °F or greater (Morton, 1987) and where temperatures remain above ~41 °F. Even chilling temperatures result in slowed plant and fruit development, defoliation, and sunburn damage on exposed fruit. Trees should be planted with wind protection to improve tree growth, potential fruit production, and reduce mechanical fruit injury (windscar). Suitable planting sites should not flood and should be surrounded by mature trees (or other suit-

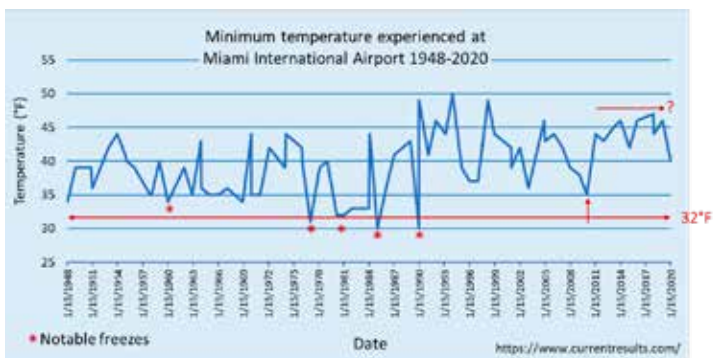


Fig. 1. Minimum temperatures experienced at the Miami International Airport from 1948–2020. In general, temperatures in the Miami-Dade agricultural area were colder. The last freeze event in the Homestead area was 2010 but since that time the low has been above ~39 °F.

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Fig. 2. Caimito fruit (purple type, top) and guanábana fruit (bottom). (Photo credits: J.H. Crane and I. Maguire, UF/IFAS TREC).

able plant material) for wind protection. Utilizing old established fruit groves where inner-grove trees are removed or planting adjacent to native habitat are ideal. An irrigation system for irrigation during dry periods and for freeze protection is critical.

Guanábana or soursop (*Annona muricata*) belongs to Annonaceae, originating in Central America, northern South America, and the Caribbean regions (Morton, 1987; Pinto et al., 2005). Non-pruned trees range from 15–35 ft tall with an erect oval to round canopy. The leaves are oblong to obovate and shiny dark green on the upper surface (lighter beneath); limbs and trunks are large (~1 1/4 to 1 1/2 inch dia) (Morton, 1987; Pinto et al. 2005). The bisexual fleshy flowers (usually single) emerge from stems and have three outer and inner petals surrounding a whirl of anthers and pistils on a common receptacle. Trees generally flower during late summer-early fall; fruit is harvested June to September, sometimes in spring. The time from flowering to harvest ranges from 105 to 180 days and is temperature dependent (Pinto et al., 2005; Worrell et al., 1994). The oval fruit is six to 14-inches long and weighs 34–140 oz or more. The pulp is off-white, granular, surrounding 100+ seeds scattered throughout the pulp. The flavor is sweet but more acid (sour) than sugar apple. Seedlings are common but there are named selections propagated by grafting and budding (Love and Paull, 2011; Pinto et al., 2005). Yields from mature trees range from 100–180 lb/tree with hand pollination, less without (Love and Paull, 2011; Koesriharti, 1991).

Guanábana is best adapted to tropical, humid to warm-hot subtropical climates where temperatures rarely dip below 60 °F (Pinto et al., 2005). Tree growth slows at or below 55 °F and chilling damage may occur at 41 °F or below. Chilling temperatures result in slow plant growth and fruit development and may result in defoliation. Tree establishment, growth and fruit production are greatly improved with wind protection. In general, well drained soils are preferred however guanábana does have some tolerance to excessively wet soils (Schaffer, 1998). Utilizing old established fruit groves where inner-grove trees are removed or adjacent to native habitat are ideal. An irrigation system for irrigation during dry periods and freeze protection is critical.

## Conclusions

Caimito and guanábana are cold sensitive fruit crops that have been in Florida for over 100 years but only recently are being grown on a small but commercial scale. This is due to the niche local and national market demand for the fruit and the lack of freezing temperatures and cold weather over the last five to ten years in extreme southern Florida. Planting of small plots may be recommended with the caveat that freezing temperature and/or cold temperatures below 41 °F may periodically occur, threatening the health and survival of these fruit crops. A strong recommendation is made for planting trees within a wind break and establishing a functional high volume irrigation system for cold (freeze) protection.

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## Passion Fruit: A Potential Alternative Fruit Crop for Florida

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Passion fruit can be grown in warm climates, which allows Florida growers to grow them throughout much of the state. Currently, Florida is estimated to have 50 acres in production, with most acreage located in the southern part of the state. Passion fruit has great potential for both value-added products as well as fresh fruit. Passion fruit can be used to produce juice, wine, jellies, candies, ice cream, and other value-added products. The vast majority of passion fruit is imported and valued at greater than \$1.35 per fruit, indicating high demand for the domestic market. Florida can become a major producer and meet domestic demand with high-quality passion fruit products. For passion fruit to become a significant crop in Florida, there are several key areas where additional research is needed. More information is needed to define the best management practices to successfully grow passion fruit in southern Florida in open field conditions as well as in central and north-central Florida in both open field and high tunnel conditions. Production costs currently remain unknown as there is no well-defined industry standards. Intermittent freezing temperatures in parts of Florida pose a significant threat to passion fruit production; methods for effective cold protection, such as high tunnel production, are needed. Productive cultivars with a wide range of desirable characteristics need to be determined. Harvest, postharvest handling, shelf-life, and processing methods will further help producers, processors, and retail establishments maximize and retain the value of the crop. Marketing aspects of passion fruit is an area that needs substantial investigation in order to attract potential producers to plant this crop.

### Origins and History

Passion fruit belong to the Passifloraceae family, which has worldwide distribution (Fig. 1). The *Passiflora* genus contains more than five-hundred species, most tendril-bearing vines. Several species have ornamental and agricultural value. The most widely grown and valuable species is *P. edulis*, passion fruit. Passion fruit is a short-lived evergreen perennial that produces an aromatic, potent tropical-tasting fruit. Its origins are warm climate regions of Brazil, Paraguay, and northern Argentina (Ulmer and MacDougal 2004). Purple passion fruit is the most commonly cultivated type in the United States. The name “passion fruit” dates back the 1500s when it was used by missionaries in Brazil to illustrate the wounds from Christ’s crucifixion (part of the “passion of Christ”) while trying to convert the indigenous population; the Portuguese name is “flor das cinco chagas” (“flower of five wounds”). The scientific name reflects this association. Passion flower (*P. incarnata*) is commonly mistaken for passion fruit (*P. edulis*) due to similar over-all appearances.

### Adaptation to Florida

Passion fruit is well-adapted to the tropical and semi-tropical conditions found throughout much of Florida. Specifically, recommendations are that passion fruit be planted in USDA hardiness zones 9b and higher. Central and South Florida are well within the recommended USDA hardiness zones which allows for a larger

area of potential production than is currently being utilized. The vines have limited drought and flood tolerance, thus they should be planted in well-drained soil and be provided with sufficient irrigation (Queensland Department of Agriculture and Fisheries, 2016). Passion fruit grow best in slightly acidic soil and a location that is in full sun throughout the day. Under suitable conditions, new plants grow quickly and may begin producing flowers and fruit within a year of planting. Rapid growth and subsequent fruit production may quickly return investment costs for entrepreneurial farmers. These characteristics make passion fruit an attractive alternative crop. Freezing temperatures, which can kill unprotected plants, present the most significant limiting factor for passion fruit production in



Fig. 1. Passion fruit flower. Photo credit: Mark Bailey.

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Florida. Established passion fruit vines can survive temperatures slightly below freezing, however becomes increasingly unlikely as temperatures decrease. Mature vines with dense foliage may tolerate temperatures slightly below freezing with some foliage loss, though exposure to temperatures in the mid-20s (°F) may result death of the aboveground vine (Campbell et al., 1977). In regions where cold temperatures or other weather conditions are a concern, high tunnel production may be a viable option. In situations where high tunnel production has not been implemented and cold is a concern, the base of the plants can be protected from lethal freeze events.

### Production in Florida

Passion fruit production in Florida is limited both in terms of acreage planted and region. Florida is estimated to have 50 acres in production, with most acreage located in the southern region of the state (USDA NASS, 2021). Acreage in production has remained limited for the past several decades. However, with adequate support and actionable information, production can be expanded beyond its current limited acreage and geographic location. Passion fruit is commonly sold as fresh fruit as well as used in the production of value-added products (Fig. 2). Passion fruit can be used to produce juice, wine, jellies, candies, ice cream, and other value-added products. Passion fruit juice is a source of dietary fiber, ascorbic acid, carotenoids, riboflavin, iron, potassium, and niacin (Percival and Findley 2007). The majority of fresh passion fruit sold in the United States is imported. It is valued at greater than \$1.35 per fruit, thus indicating high value and stable demand for this fruit (USDA AMS, 2021). The high value of the crop further provides incentives for growers to expand or begin production. Given concerns over reliability of



Fig. 2. 'Purple Possum' fruit. Photo credit Mark Bailey.

complex international supply chains and a range of other concerns associated with food imports, domestic production may have advantages that imports lack. With a comprehensive effort to develop this fruit, Florida can become a major producer and meet domestic demand with high-quality passion fruit products. Passion fruit as an alternative crop, while full of potential, will need to be supported by production and marketing information. Should substantial acreage be planted, it is possible that challenges may arise such as sourcing an adequate supply of starter plants in a timely manner. Passion fruit cultivars need to be evaluated for characteristics that include fruit size, appearance, disease resistance, quantity of pulp or juice, pulp-to-skin ratio, flavor, sugar and acid concentration, and aromatic qualities. Management practices for open field passion fruit production in southern Florida need study as do open field and high tunnel conditions in central and north-central Florida. It is difficult to determine production costs as there is no well-defined industry standards. Regions of Florida with intermittent freezing temperatures pose a significant threat to passion fruit production so reliable methods for effective cold protection, such as high tunnel production, are needed. Establishing harvest, postharvest handling, shelf-life, and processing standards will further help producers, processors, and retail establishments maximize and retain the value of the crop. Marketing aspects of passion fruit need significant investigation in order to attract potential producers to plant this crop. If current and prospective growers have access to marketing information, this may greatly reduce the potential risk associated with alternative crops.

### Conclusion

Passion fruit has great potential to become a significant alternative crop for Florida growers. In order to help current and prospective growers succeed, a comprehensive approach to identifying and responding to both production and marketing challenges is needed.

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## **Edible Fruit Coating Reduces Rate of Moisture Loss in Refrigerated Purple Pitanga (*Eugenia uniflora* L. 'Zill Dark') Fruits**

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**Additional index words.** Endura-Fresh 6100, Pitangueira, Sta-Fresh 2952, Surinam Cherry, food-grade wax

**Like numerous other crops, fresh purple pitanga fruits have a short shelf-life since the fruits quickly ripen and lose moisture, such that they become unattractive and unmarketable within a few days of harvest. Edible fruit coatings have been applied to a number of fruits and vegetables to enhance appearance and extend the shelf-life of the products. For these trials, we evaluated two readily available JBT fruit coatings often applied to tropical fruits. Sta-Fresh® 2952 and Endura-Fresh™ 6100 were diluted to two different concentrations as dips for ripe 'Zill Dark' pitanga fruits. After air drying, the fruits were placed in standard plastic berry clamshells; each clamshell containing twelve fruits was weighed and placed in refrigerated storage at 10 °C. Once every 24 hours, the clamshells were weighed to measure overall moisture loss of the fruits. Although the fruit weight decreases of the various treatments were not different after one or two days of refrigeration, after more than three days of refrigeration, fruits dipped in the Endura-Fresh™ 6100 at the 50/50 dilution rate retained moisture better than either fruits only dipped in deionized water or fruits dipped in any of the other treatments.**

Some edible fruits have relatively long shelf lives after they are harvested, but many others do not, quickly become over-ripe and unmarketable after a short period of storage. To increase the shelf life and to also improve the appearance of many fruits, edible fruit coatings of various types have been routinely applied to fruits such as apples, pineapples, mangos, oranges, pomegranates, and others (Olivas, et al., 2008). Some of these fruits, when coated and refrigerated, have a lengthy shelf life where they remain both palatable and marketable for weeks or months (Galus and Kadzińska, 2015). It is unlikely that the application of edible fruit coatings onto purple pitanga fruits would extend the shelf life of these soft, tropical fruits for months. However, just extending the marketable shelf life of the fruits from fewer than 7 days up to 10 days would greatly increase the ability of growers to get the fruits to market. There have not been many published research reports on either refrigerated storage (Griffis, et al., 2015; Mélo, et al., 2000; Santos, Silva and Alves, 2006; Santos, Silva, Mendonça, et al., 2006) or application of various edible food coatings to pitanga (Cerqueira, et al., 2009; Fritz, et al., 2019; Sanches et al., 2017). There does not appear to be any published information about applying the two JBT (John Bean Technologies Corp., Chicago, IL) edible food coatings that were used in this experiment to pitanga, although both Endura-Fresh™ 6100 and Sta-Fresh® 2952 are routinely applied to tropical fruits (Hu, et al., 2011; Nor and Ding, 2020; Razali, et al., 2016) both to reduce fruit weight loss and to inhibit fruit decay in low temperature storage. The objective of this study was to determine if various dilutions of either Endura-Fresh™ 6100 or Sta-Fresh® 2952 applied as a fruit dip would inhibit purple pitanga fruit weight loss during two weeks of low temperature storage.

### **Materials and Methods**

On 7 May 2021, ripe purple fruits were harvested from 'Zill Dark' pitanga plants located on the Florida Southern College campus in Lakeland, FL. The fruits were transported to the laboratory where they were placed on paper towels to check for juice leakage. They were evaluated individually to remove any unmarketable or damaged fruits. Two dilutions of both JBT Endura-Fresh™ 6100 and JBT Sta-Fresh® 2952 were prepared. Endura-Fresh™ 6100 is a dark brown liquid containing a proprietary blend of waxes. It was mixed with deionized (DI) water at two recommended rates: 50 mL Endura-Fresh™ 6100 was stirred into 50 mL of deionized water (50:50) and 25 mL of Endura-Fresh™ 6100 was stirred into 75 mL of deionized water (25:75). Sta-Fresh® 2952 is a gel-like paste containing a proprietary blend of fatty acids, fatty acid salts, and mono- and diglycerides derived from plants. It was weighed out and whisked into deionized water until blended. The two recommended rates were 10 g of Sta-Fresh® 2952 whisked into 110 mL of deionized water (10:110) and 10 g of Sta-Fresh® 2952 whisked into 190 mL of deionized water (10:190). Individual purple pitanga fruits were dipped into either deionized water alone or into one of the four edible fruit coating solutions. Thirty-six fruits were selected for each treatment. Fruits were completely submerged in one of the solutions; each fruit was removed from the solution with a plastic spoon and placed carefully on a plastic rack to air dry for 2 h. A small fan was used to speed up the drying process. The 36 fruits for each treatment were divided into three groups of 12 and were placed in plastic 4-oz berry clamshells so that there were three replicates of each treatment, 15 clamshells in all. The clamshells were then transported to Florida Gulf Coast University (FGCU) in Ft. Myers, FL. At FGCU, the individual clamshells, each containing 12 fruits, were weighed and placed

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in refrigerated storage at 10 °C. Every 24 h for 15 days, the clamshells were removed from the refrigerated storage and weighed. They were then returned to refrigerated storage, but their positions were changed each day to eliminate any effects of being placed on the top or the bottom of a stack of clamshells.

## Results

The weights of the ‘Zill Dark’ pitanga fruit dipped into either DI water or one of four different coatings (Endura-Fresh™ 6100 50:50, Endura-fresh™ 6100 25:75, Sta-Fresh® 2952 10:110, Sta-Fresh® 2952 10:190) were measured using three clamshell containers per treatment, yielding repeated measurements of the weights per treatment. Each weight variation for a treatment was observed over 16 days with Day 0 corresponding to the original weight of both the fruit and the container. The weight of every container decreased every 24-h period, indicating some water (with some volatile elements) was being lost from the fruits each day. The data with the replicates contained  $16 \times 5 \times 3 = 240$  observations corresponding to the number of days, the DI water control and four treatments, and three replicates respectively.

A repeated measures one-way analysis of variance [RM-ANOVA (Girden, 1992)] was used to determined differences between treatments and within treatments (time measurements); essentially not all fruits among the several treatments lost the same percentage of moisture weight at the same rate. Table 1 shows the results of RM-ANOVA. There were differences over time and between treatments at a 0.05 level of significance. A post-hoc analysis (Maxwell, 1980) was conducted to determine the treatments that showed significant differences. Table 2, shows a significant difference between Stay-Fresh 2952® 10:190 and Endura-Fresh™ 6100 25:75 and the control (DI Water). There were no significant differences among other comparisons. The *P*-values were adjusted to control familywise error rate (FWER) (Lehmann and Rojo, 2012) using Bonferroni correction.

A secondary analysis was performed to determine differences between treatments and within treatment (time measurements) without replicates. The replicates were summed to obtain a total weight per treatment. This analysis allowed us to determine if the variations in total weight among the containers, both between treatments and within treatments, caused any significant distortions in the data. A one-way analysis of variance (ANOVA) (Montgomery, 2017) was used with weight measurements over time starting with the initial total weight. The treatments were measured individually without any overlaps, satisfying the

Table 1. Results of repeated measures one-way analysis of variance (RM-ANOVA) comparing weights measured over time.

Effect	$df_1$	$df_2$	F-statistic	<i>P</i> -value	Effect Size
Time	15	30	35.74	< 0.0001	0.672
Treatment	4	8	4.85	0.0280	0.411
Treatment:Time (Interaction)	60	120	1.23	0.169	0.217

Table 2. Post-hoc analysis for RM-ANOVA.

Treatment-1	Treatment-2	<i>P</i> -value
2952 10:190	6100 25:75	0.0137
--	DI Water	0.0002

Table 3. Results of one-way ANOVA comparing treatment means and means over time.

Effect	df	SS	MS	F-statistic	<i>P</i> -value
Treatment	4	11975	2994	365.6	< 0.0001
Time	1	38915	38915	4572.7	
Residuals	74	606	8	--	

Table 4. Post-hoc analysis for ANOVA.

Treatment-1	Treatment-2	<i>P</i> -value (adjusted)
2952 10:190	6100 25:75	< 0.0001
--	DI Water	--
2952 10:110	DI Water	0.0029
6100 50:50	DI Water	0.0007

independence assumption of an ANOVA. A Shapiro-Wilks test (Shapiro, et al., 1968) for normality indicated that the normality assumption of an ANOVA was not violated for measurements in the treatment groups. The results of the ANOVA are summarized in Table 3 which shows significant differences between treatments at the 0.05 level. There were also significant differences for average weight measurements as weights decreased over time, an expected outcome. A post-hoc analysis was performed to identify differences in treatments. Differences among Stay-Fresh 2952® 10:190, Endura-Fresh™ 6100 25:75, and DI Water were consistent with the results in Table 2. The total weights (without repeated measurements) showed differences between Stay-Fresh 2952® 10:110, Endura-Fresh™ 6100 50:50 and DI Water (control) and these results are summarized in Table 4. Since the ANOVA analysis (Table 3) revealed there were significant differences between treatments, it seemed reasonable to display the results graphically so that they could more easily be visualized. This was done with the replicates for each treatment combined and the total percentage of weight remaining (after water loss in refrigerated storage) was tracked daily for 15 days (Fig. 1).

## Discussion

Although there were significant differences between various treatments in this experiment, it appears that Endura-fresh™ 6100 at a 50:50 dilution with DI water was better than any of the other treatments evaluated at slowing water loss from the purple pitanga fruits during refrigerated storage at 10°C. Reduced water loss is an important concern when the fruits are evaluated for extended marketability (Bai, et al., 2019), but there are other important concerns as well, including both appearance and taste of the fruits after refrigerated storage. Some fruits in our trials were relatively unchanged in appearance after the first week in refrigerated storage (Fig. 3a) when compared to Day 0 fruits (Fig. 2), whereas others did not appear to be marketable after 7 days in refrigerated storage (Fig. 3b). After 15 days in refrigerated storage at 10 °C, fruits from all treatments evaluated did not appear to be marketable. Additional experiments might test other dilutions of these or other edible fruit coatings for efficacy at reducing fruit water loss and other clamshell containers could be evaluated as well (Bai, et al., 2019). Other refrigerated storage temperatures could also be evaluated, and additional fruit selection efforts might reduce variability in the weights among fruit filled containers. Increasing the number of replicates for each treatment would allow some of the replicates to be terminated each day, so that the fruit color, juice color, acid, brix, and other measurements of stored fruits

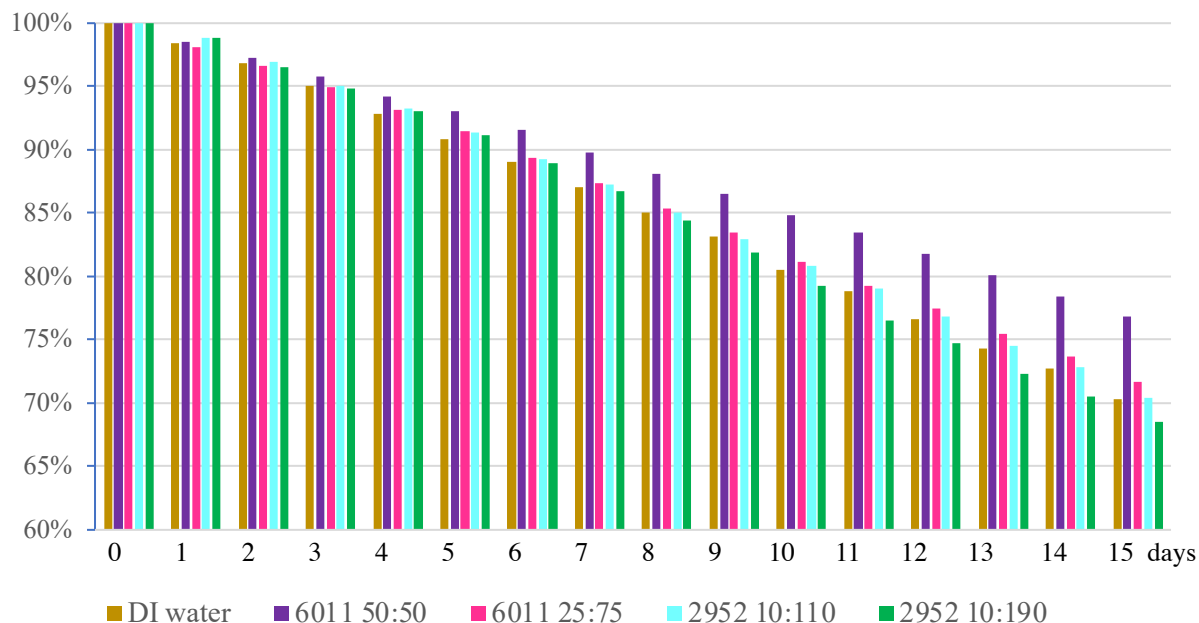


Fig. 1. Percentage of total weight remaining on a daily basis of ripe purple pitanga fruits in refrigerated storage (10 °C), three replicates per treatment combined.



Fig. 2. Ripe 'Zill Dark' pitanga fruits, dipped in deionized water, air dried, day of harvest.



Fig. 3 (a and b). Ripe 'Zill Dark' pitanga fruits after seven days in refrigerated storage at 10°C.

could be compared to those of fresh fruits harvested that same day to determine if or when measurable differences in parameters that might affect marketability of fruits occur.

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# The Lychee Erinose Mite: A New Serious Pest of Lychee in Florida

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**Additional index words.** eradicate LEM, litchi, lychee, mite, pest

**The Lychee Erinose Mite (LEM), *Aceria litchii*, is a specialist pest of lychee, *Litchi chinensis* Sonn. Vegetatively flushing mature and young lychee trees that frequently flush are more attractive to LEM infestations than dormant trees. LEM has the potential to severely weaken lychee trees and decrease fruit yield by 80%. Control of this pest is difficult because of the LEM-induced erineae (dense leaf hair proliferation on leaves, stems, flowers, and fruit) that protects the pest. The first detection of LEM in Florida was in 1955 in a lychee grove in Nokomis in Sarasota County. The second was an interception recorded on containerized plants, imported from China, in Coral Gables, in Miami-Dade County in 1993. Both times the pest was eradicated after being found on lychee trees. LEM was detected on Pine Island in Lee County in February 2018. Lychee fruit and plant parts are currently under quarantine in Lee County. Subsequently LEM infestations have been detected in 13 Florida counties. In February 2020, LEM was found in a backyard lychee tree in the northern portion of Miami-Dade County, FL. It has since been found in numerous locations in Miami-Dade County including several commercial lychee orchards. With approximately 70% of Florida's commercial lychee acreage being in Miami-Dade County, the spread of LEM within the county is troubling.**

## History

In February of 2018, there was a disturbing find on Pine Island, FL (Carrillo et al., 2019; Carrillo et al., 2020). A very damaging pest, the Lychee Erinose Mite (LEM), *Aceria litchii* was found on a few trees in a commercial lychee (*Litchi chinensis*), grove. This pest had been in Florida twice before: in 1955 in a lychee grove located in Nokomis in Sarasota County; and the second time was in 1993 as an interception recorded on plants, imported from China, in Coral Gables, in Miami-Dade County. The pest was localized and eradicated both times. This time eradication is still the goal, but it will be much more difficult because LEM has now spread to 13 counties within the State: Brevard, Broward, Charlotte, Collier, Hendry, Lee, Manatee, Martin, Miami-Dade, Palm Beach, Pinellas, Polk, and Sarasota. LEM has been successfully eradicated in Glades and Orange counties.

Florida is the leading producer of lychees in the country with an estimated 700 acres distributed across at least eight counties (Crane, et al., 2018). Miami-Dade County produces approximately 70% of those lychees. LEM will significantly damage the commercial lychee industry because it has the potential to seriously weaken trees and reduce fruit yield by up to 80% (Navia et al., 2013)

## Description

LEM is a microscopic mite that feeds on leaves, stems, flowers, and fruit of lychee. LEM attacks new growth and cannot be

killed by aracacides due to the protective erineae it induces (dense leaf hair proliferation on leaves, stems, flowers, and fruit). LEM disperses using air currents and honeybees. It can also be spread by fruit movement or contaminated pruning tools. It cannot be seen with the naked eye and is only visible with a high-powered microscope (Fornazier et al., 2021). LEM will only attack lychee trees and is not a danger to other related trees such as the longan.

## Eradication Efforts

The Florida Department of Agriculture and Consumer Services, Division of Plant Industry (FDACS-DPI) is currently engaging in an effort to eradicate LEM in Florida. Once LEM is found in a grove or home garden, FDACS-DPI enacts a protocol to remove LEM from that site. The treatment involves defoliating an infected tree. Because a large amount of canopy is lost in the pruning, the trunks of the trees are painted before the tree is pruned with a 50/50 mixture of white latex paint and water to protect the trunk from sun scalding. The removed branches are disposed of through burning, burying, or chipping. Sulfur is then applied to the tree using a foliar spray at the time of branch removal and again when there is new growth to protect. Sulfur is then sprayed every 7–15 d from bud break until leaves harden, up to 3 months and eight applications in total (Carrillo et al., 2021; Crane et al., 2019). If possible, the sprays should occur from the beginning of new growth emergence until the new growth is mature (hardened-off) (Nishida, et al., 1955). The sulfur spray does not kill the mite, rather it protects the tree from re-infestation prophylactically. The sulfur registered for use is labeled organic and is limited to a specific brand, Microthiol Dispers®<sup>®</sup>, which has a Special Local Needs (SLN) emergency label specifically for LEM.

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Lee County, where LEM was first found in 2018, is currently under quarantine and cannot sell lychee fruit in Florida unless it is treated postharvest with a paraffinic oil dip. The paraffinic oil dip postharvest treatment was first shown to be effective by (UF/IFAS) faculty (Revynthi et al., 2021). Fruit inside the quarantine area may be sold outside of Florida without the postharvest treatment.

### Extension Efforts

When the pest was confirmed in Florida in 2018, the UF/IFAS tropical fruit specialist, tropical fruit entomologist, a commercial entomologist, and tropical fruit Extension agent developed a plan to educate the public about LEM. A series of workshops were given, and multiple factsheets were written. The workshops were in conjunction with FDACS-DPI. FDACS-DPI is tasked with the job of eradicating LEM in Florida. All LEM finds should be reported to FDACS. In 2020 and 2021, six workshops were given to homeowners and commercial growers (271 attendees), along with one in-service training designed for UF faculty and Extension agents (54 attendees). Nine factsheets were written and published, along with two blogs, one article in a popular magazine, multiple scientific papers, and several webpages. The publications covered LEM identification, history, biology, control methods, FAQs, pruning methods, and what to do if the pest is found. This information was sorely needed but was not available before the UF team produced it. The Miami-Dade Extension tropical fruit agent was contacted 90 times by homeowners and commercial growers from multiple counties within Florida that believed they had the pest. Over 95% of the contacts had correctly identified LEM based on the literature produced by UF/IFAS.

### Reporting LEM

It is recommended that you scout for LEM by looking at the leaves of your lychee tree for the characteristic leaf blisters or a rusty colored hairy mass. If you feel you have LEM on your trees, please contact FDACS at 1-888-397-1517 and report the find. They will need your name, address, email, phone number and the number of trees suspected to have LEM.

Once they are alerted, FDACS will contact you, confirm you have LEM, and then prune the sections of your tree that have LEM and treat your trees for the pest. At the current time, this service is free, as they are trying to eradicate the pest throughout the state of Florida.

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## Physiological Responses of Diploid Banana Accessions to In Vitro Chilling Stress

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Banana (*Musa* spp.) is a tropical fruit crop characterized by optimal growth and fruiting at average temperatures in excess of 80 °F, whereas cold stress has been established to have detrimental effects on growth, yield, and fruit quality. In this study, we accordingly sought to identify diploid banana germplasms with cold tolerance potential. Twelve in vitro growing accessions [*Musa acuminata* ssp. *zebrina*, *M. acuminata* ssp. *zebrina* ('Zebrina GF'), *M. balbisiana*, *M. acuminata* ssp. *burmannica* ('Calcutta 4'), *M. lolodensis*, *M. barioensis*, *M. coccinea*, *M. mannii*, *M. acuminata* ssp. *malacconsis*, *M. acuminata* ('Meleng'), and *M. velutina*] were subjected to temperature stress at 39 °F (4 °C) for 48 hours with the cold-tolerant *M. itinerans* serving as the positive control. Following cold treatment, we detected increases in total chlorophyll content in *M. barioensis*, *M. mannii*, and *M. velutina*. High levels of malondialdehyde (MDA) were recorded in the *M. coccinea*, 'Calcutta 4', *M. velutina*, and *zebrina* accessions, whereas in contrast, *M. balbisiana*, *M. acuminata* ssp. *malacconsis*, and *M. itinerans* showed negligible increases in MDA content. Furthermore, *M. balbisiana* and *M. mannii* showed slight difference in radical scavenging capacity and levels of total phenolic compounds when subjected to control and chilling conditions. Collectively, our rapid screening results revealed that *M. coccinea*, 'Calcutta 4', *zebrina*, and *M. velutina* bananas are sensitive to cold stress. Diploid *Musa* cultivars, such as those assessed in this study, can be utilized for banana improvement programs using somatic fusion techniques. Screening for cold stress in the field is currently ongoing to validate the in vitro results.

Bananas and plantain (*Musa* spp.) are important sources of valuable nutrients and energy (D'hont et al., 2012; Mahmoud et al., 2020). Commercially grown cultivars of these species are derived primarily from *Musa acuminata* and/or *Musa balbisiana*. Seedless triploid varieties, which are the most commonly cultivated bananas and plantains, are produced from crosses between two diploid wild species to generate tetraploids, which are in turn crossed with diploids (Simmonds and Weatherup, 1990; Wu et al., 2016).

Bananas are typically cold-sensitive plants and low temperatures are among the most significant environmental stressors threatening global banana production (Yang et al., 2015). Temperature fluctuations are a key factor in successful banana production. Cold stress has been established to have a number of detrimental effects on plant growth, impairing photosynthetic performance, and causing the wilting of leaves, chlorosis, growth reduction, changes in membrane integrity, and loss of cell compartmentalization (Bo et al., 2017; Cao et al., 2015). Additionally, chilling stress inhibits enzymatic activities and protein synthesis (Cheng and Song, 2006; Mahajan and Tuteja, 2005) and induces an excessive accumulation of reactive oxygen species (ROS), which may ultimately cause plant death (Suzuki et al., 2012). Frost injury can severely damage the banana pseudostem, killing it in most cases.

Bananas show a high degree of genetic variability with respect to cold tolerance. Cavendish (*Musa* spp.; AAA Group) bananas that include a number of commercially important cultivars, are, however, more cold sensitive than are plantains (*Musa* spp. Dajiao; ABB Group), which can tolerate temperatures of between 0

and 4 °C. Accordingly, these plantains have been proposed as a potential germplasm resource for cold tolerance-related breeding in banana (Yang et al., 2012). Recently, a number of researchers have focused on sequences in the *Musa itinerans* genome, with a view toward assessing the potential utilization of this species in breeding programs. *M. itinerans* var. *itinerans* (Yunnan banana) is a close relative of banana progenitors distributed in across wide areas of subtropical China (Hajjar and Hodgkin, 2007; Häkkinen et al., 2008), and Li et al. (2015) have reported that *M. itinerans* is both cold tolerant and Foc-TR4 resistant, and thus represents a promising resource for enhancing biotic and abiotic stress tolerance in banana.

In this study, we aimed to identify diploid banana germplasm with cold tolerance potential, and to this end, we evaluated 12 *Musa* accessions growing in vitro [*M. acuminata* ssp. *zebrina*, *M. acuminata* ssp. *zebrina*, ('Zebrina GF'), *M. balbisiana*, *M. acuminata* ssp. *burmannica* ('Calcutta 4'), *M. lolodensis*, *M. barioensis*, *M. coccinea*, *M. mannii*, *M. acuminata* ssp. *malacconsis*, *M. acuminata* cv. 'Meleng', and *M. velutina*], which were subjected to cold stress at a temperature of 39 °F (4 °C) for 48 h. The cold-tolerant species *M. itinerans* served as the positive control. We examined physiological and biochemical changes induced in response to exposure to cold stress.

### Materials and Methods

**PLANT MATERIAL AND CHILLING TREATMENTS.** Aseptic cultures of the aforementioned accessions (obtained from the Bioversity International *Musa* Germplasm Transit Centre) were subcultured as described by Mahmoud et al. (2020) in a modified 3/4-strength MS (Murashige and Skoog, 1962) basal medium supplemented

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with 13.4  $\mu$ M 6-benzylaminopurine, 1.25 mM  $\text{KH}_2\text{PO}_4$ , and 3% (w/v) sucrose, with the pH of the medium being adjusted to 5.7 prior to autoclaving. Cultures were maintained at  $26 \pm 1$  °C under a 16-h/8-h photoperiod. After two rounds of sub-culturing, well-developed shoots were selected for chilling stress treatments. Uniform shoots of the 12 accessions were subjected to cold stress at a temperature of 39 °F (4 °C) for 48 h, and thereafter selected parameters in the treated plants were compared with those of the same accessions maintained under normal growth (control) conditions [25 °C (77 °F)].

**PHYSIOLOGICAL AND BIOCHEMICAL VARIABLES.** All chemicals used in this study were purchased from Sigma–Aldrich (St. Louis, MO).

Total chlorophyll was extracted from fresh leaves (100 mg fresh weight) using 1 mL of absolute methanol. Following centrifugation at 10,000 rpm for 15 min at 4 °C, the extract thus obtained was further diluted 10-fold with fresh methanol in accordance with the method described by Lichtenthaler and Wellburn (1983).

Malondialdehyde (MDA) content was measured as described by Heath and Packer (1968). A 100 mg sample of frozen leaves was suspended in 0.5 mL of 0.1% (w/v) trichloroacetic acid (TCA), and the resulting homogenate was centrifuged at 14000 rpm for 10 min at 4 °C. The supernatant thus obtained (0.5 mL) was mixed with 1.5 mL of 2-thiobarbituric acid in 20% TCA and then heated at 95 °C for 25 min. The reaction was stopped by placing tubes on ice for 25 min, after which the absorption of the supernatant was monitored at wavelengths of 532 and 600 nm.

Radical scavenging capacity was measured using a 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) assay, as described by Blois (1958). An equal amount of DPPH solution (1 mM) and plant extract were mixed and incubated for 30 min at 4 °C, after which absorbance was monitored spectrophotometrically at 517 nm, using methanol as a blank solution. The control solution was DPPH added to methanol instead of leaf extract. Percentage DPPH inhibition was calculated as [(control absorbance–sample absorbance)/control absorbance]  $\times$  100

Total phenolic compounds (TPCs) in fresh leaf tissues were extracted in 1 mL of 100% methanol, followed by centrifugation at 10000 rpm for 20 min. The extract thus obtained was incubated overnight at room temperature until complete evaporation and dryness. The resulting dried gel was dissolved in 5 mL distilled water and diluted 75-fold in distilled water. TPC contents were determined based on reacting the extract preparation with 0.5 mL Folin reagent for 3 min, followed by the addition of 2 mL of 20%  $\text{Na}_2\text{CO}_3$  solution. The change in color of the reaction mixture was determined at 650 nm after standing at room temperature for 1 h, and TPC contents were estimated from a standard curve of gallic acid according to Singleton and Rossi (1965), with values being expressed as mg gallic acid/100 g fresh weight tissue

Proline was extracted according to the method described by Bates et al. (1973) in aqueous sulfo-salicylic acid (3% w/v). The reaction mixture (2 mL of supernatant extraction, 2 mL of glacial acetic acid, and ninhydrin reagent) was incubated for 1 h at 100 °C in a water bath, followed by incubation in an ice bath to stop the reaction. The reaction mixture was then mixed vigorously with 4 mL of toluene in glass tubes, and after warming at 25 °C, the color change was monitored at 515 nm using a UV/Vis spectrophotometer for proline content determination.

Soluble protein content was estimated according to the procedures described by Desjardins et al. (2009). Frozen leaf powder (100 mg) was extracted in 1 mL of 0.15 M phosphate buffer (pH 7.8) containing 1 mM EDTA and then centrifuged at 14000 rpm

for 20 min at 4 °C. The resulting supernatant was collected, and absorbance was measured at 280 nm using a NanoDrop UV-Vis spectrophotometer (Thermo Scientific, Waltham, MA). The concentration of proteins in samples were determined with reference to a bovine serum albumin standard curve, with soluble protein contents being expressed in terms of  $\mu$ g/mL.

**EXPERIMENTAL DESIGN AND STATISTICAL ANALYSIS.** The experiment was of a randomized complete block factorial design, with two factors [accessions (12 accessions) and treatments (control and cold)] and 10 replicates per treatment. Mean separation between accessions for a particular treatment was assessed using Tukey's honestly significant difference test ( $P \leq 0.05$ ). *P*-values were used to compare mean separation between treatments for a particular accession, with significant differences being indicated by different lower-case letters in figures. Data were analyzed using JMP Pro 15 software.

## Results and Discussion

**EFFECTS OF CHILLING STRESS ON THE PHYSIOLOGY OF BANANA ACCESSIONS.** Cold stress induces physiological changes in banana, including those in chlorophyll content, MDA accumulation, and the antioxidant defense system. To address the various challenges associated with banana cultivation, in recent decades breeders have exploited the potential of wild relatives in their breeding programs, and in doing so, have made a number of significant advances (Heslop-Harrison and Schwarzacher, 2007). Wild relatives constitute a rich source that can be mined for multiple desirable traits, including tolerance to biotic and abiotic stresses. In this regard, we evaluated 12 diploid banana accessions with respect to their tolerance to chilling stress.

**CHLOROPHYLL CONTENTS.** We observed that in most of the assessed lines, there were significant reductions in total chlorophyll content in response to exposure to chilling conditions. Conversely, subjecting *M. barionensis*, *M. mannii*, *M. acuminata* cv. 'Meleng', and *M. velutina* to chilling stress was found to promote increases in chlorophyll contents. However, no significant difference in total chlorophyll content were detected for *M. lolodensis* plants of the same accession maintained under control and chilling conditions (Fig. 1).

Chilling stress has an immediate effect on chloroplast function, as reflected in lower rates of photosynthesis and reductions in chlorophyll pigment biosynthesis (Adam and Murthy, 2014). Additionally, exposure to low temperatures can cause an imbalance in chlorophyll fluorescence-based parameters (Ensminger et al., 2006). However, under certain circumstances, compared with chlorophyll *a*, increases in chlorophyll *b* and carotenoid pigments are generally induced in response to chilling stress, in order to enhance photon capture (Adam and Murthy, 2014).

**LIPID PEROXIDATION.** Malondialdehyde (MDA) is a final product of cell membrane damage and is routinely used as a marker to assess lipid peroxidation. In the present study, MDA was found to accumulate at high levels in banana following chilling treatment (Fig. 2). Among the assessed accessions, *M. balbisiana*, *M. acuminata* ssp. *malacconsis*, and *M. itinerans* were shown to be the most cold-tolerant, characterized by the lowest contents of MDA (0.85, 0.54, and 0.69 nmol·g<sup>-1</sup> FW, respectively) (Fig. 2). Moreover, in comparisons of plants grown under control and low-temperature conditions, *M. balbisiana* and *M. acuminata* ssp. *malacconsis* were characterized by *P*-values of 0.0095 and 0.0025, respectively, whereas for other accessions,



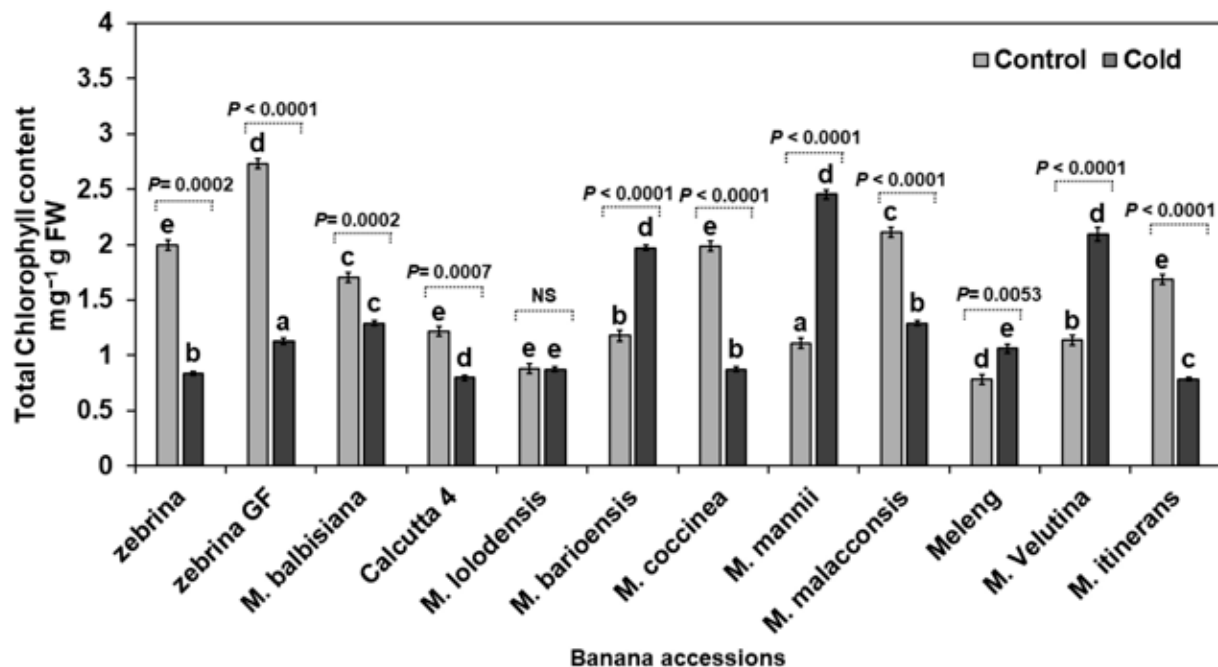


Fig. 1. Effect of chilling treatment on total chlorophyll content in the leaves of banana accessions. Mean separation between accessions at a particular treatment is indicated by differing lowercase letters by Tukey's honestly significant difference test ( $P \leq 0.05$ ).  $P$ - values were used to compare mean separation between treatments in a particular accession.

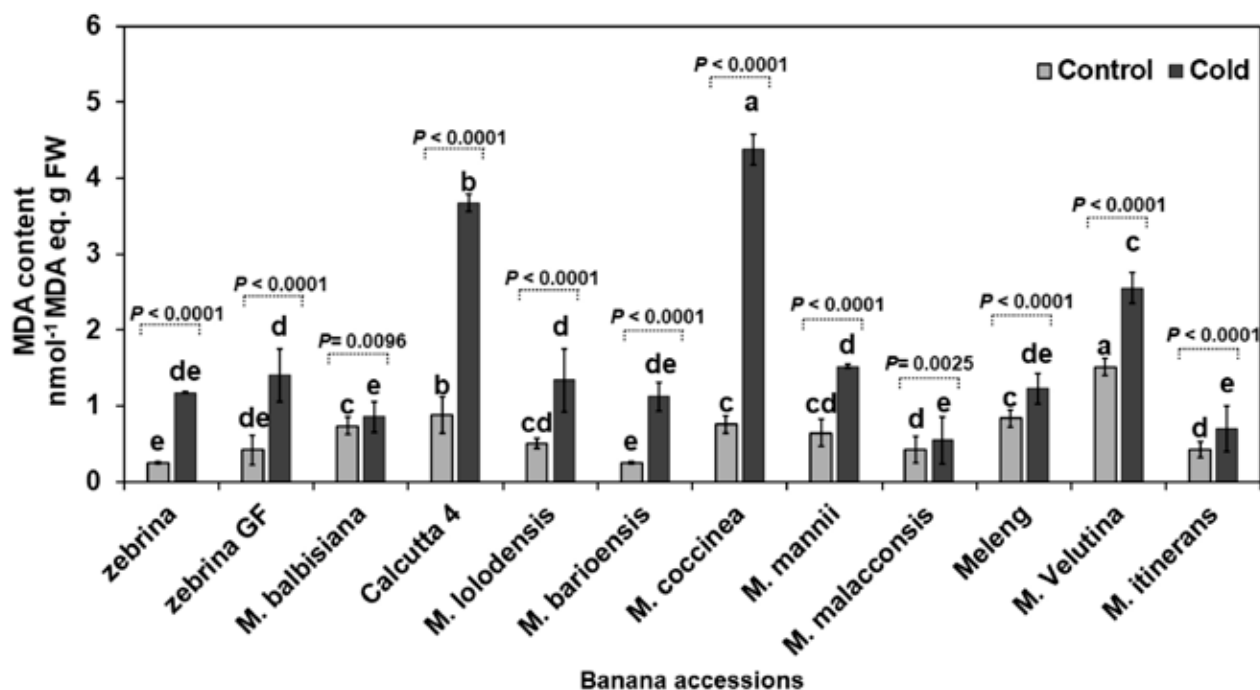


Fig. 2. Effect of chilling treatment on malondialdehyde (MDA) in the leaves of banana accessions. Mean separation between accessions at a particular treatment is indicated by differing lowercase letters by Tukey's honestly significant difference test ( $P \leq 0.05$ ).  $P$ - values were used to compare mean separation between treatments in a particular accession.

we recorded  $P$ -values  $< 0.0001$ . The highest accumulations of MDA were detected in *M. coccinea*, followed by 'Calcutta 4', and *M. velutina* (3.66, 4.37, and 2.55  $\text{nmol}\cdot\text{g}^{-1}$  FW, respectively). In this context, Uemura and Steponkus (1999) have reported a close relationship between alterations in plasma membrane lipid composition and freeze-induced membrane lesions under chill-

ing stress conditions. Cold-sensitive plants tend to generate high levels of MDA in response to chilling stress (Moore and Roberts, 1998; Zhang et al., 2009), and on the basis of measured MDA contents in the evaluated banana accessions, we identified 'Calcutta 4', *M. coccinea*, and *M. velutina* as the most cold sensitive.

#### FREE RADICAL SCAVENGING ACTIVITY AND TOTAL PHENOLIC

**COMPOUNDS.** We found that chilling stress promoted a significant reduction in DPPH-radical scavenging activity in Zebrina, ‘Zebrina GF’, ‘Calcutta 4’, *M. lolodensis*, *M. acuminata* ssp. *malaconsis*, *M. acuminata* cv. ‘Meleng’, and *M. velutina*, recording  $P$ -values  $< 0.0001$  when compared with the same accessions maintained under control conditions. In contrast, we detected no significant differences in percentage DPPH-radical scavenging activity under control and chilling conditions for the accessions *M. balbisiana* (13.30% and 14.39%, respectively), *M. mannii* (29.05% and 25.70%, respectively), and *M. itinerans* (33.31% and 29.61%, respectively) (Fig. 3A). The TPC contents in leaves following chilling stress were estimated to evaluate changes in cold-induced oxidation in banana accessions. Highest TPC values were recorded in ‘Zebrina GF’ and *M. coccinea* (46.73 and 46.33 mg·g<sup>-1</sup> gallic acid FW, respectively) (Fig. 3B), whereas significant differences in the TPC contents of accessions exposed to control and chilling

conditions were recorded for *M. balbisiana* (30.57 and 34.98 mg·g<sup>-1</sup> gallic acid FW, respectively), *M. lolodensis* (20.55 and 26.56 mg·g<sup>-1</sup> gallic acid FW, respectively), *M. mannii* (30.57 and 34.58 mg·g<sup>-1</sup> gallic acid FW, respectively), and *M. acuminata* cv. ‘Meleng’ (25.09 and 28.43 mg·g<sup>-1</sup> gallic acid FW, respectively).

The findings of the DPPH assay revealed that, compared with the control accessions, banana accessions differ in their response to chilling conditions. Some accessions showed a similar response under both control and cold temperature conditions, thereby indicating lower levels of ROS generation in cells compared with those detected in other accessions. Observed differences may also reflect the activities of ROS scavengers in different accessions, which contribute to enhancing cold stress tolerance. In this regard, phenolic compounds, a widespread group of secondary metabolites produced by plants, can, given their strong ability to donate electrons and hydrogen atoms, play important roles

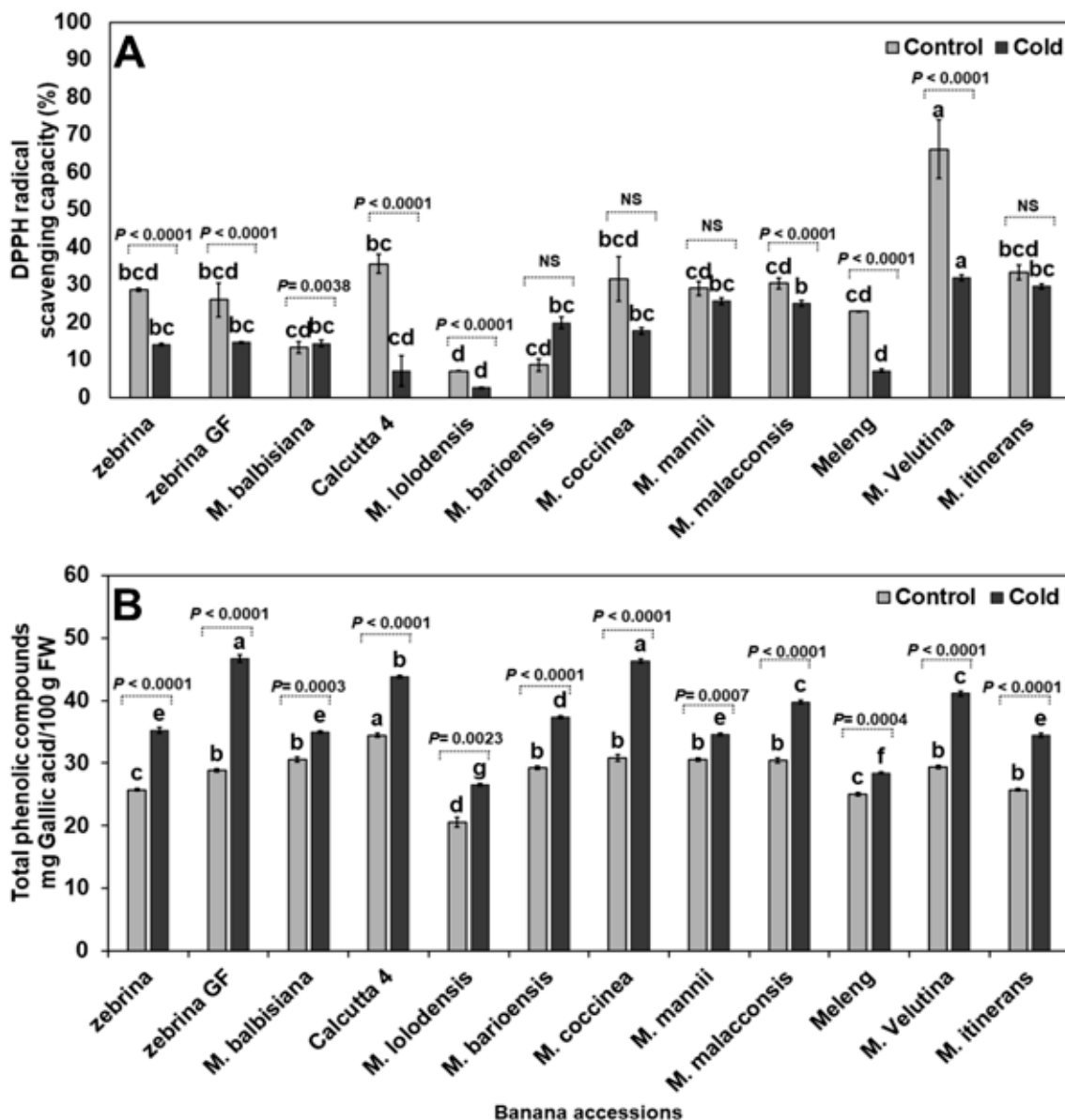


Fig. 3. Effect of chilling treatment on 2-diphenyl-1-picryl-hydrazyl-hydrat (DPPH) radical scavenging capacity percentage (A) and total phenolic compounds (TPC) (B) in the leaves of banana accessions. Mean separation between accessions at a particular treatment is indicated by differing lowercase letters by Tukey's honestly significant difference test ( $P \leq 0.05$ ).  $P$ -values were used to compare mean separation between treatments in a particular accession.

in eliminating ROS and preventing lipid peroxidation, (Sivaci et al., 2014).

**PROLINE AND SOLUBLE PROTEIN CONTENTS.** All assessed banana accessions were observed to undergo a significant increase in proline content in response to chilling condition when compared with the same accessions maintained under control conditions (Fig. 4A). In plants exposed to chilling stress, the highest contents of proline were recorded in ‘Zebrina GF’ (20.90  $\mu\text{mol}\cdot\text{g}^{-1}$  FW), followed by *M. coccinea* and ‘Calcutta 4’ (18.98 and 18.08  $\mu\text{mol}\cdot\text{g}^{-1}$  FW, respectively). In contrast, the lowest recorded proline contents were those in *M. lolodensis* and *M. acuminata* cv. ‘Meleng’ (5.95 and 6.76  $\mu\text{mol}\cdot\text{g}^{-1}$  FW, respectively). Similarly, we observed reductions in soluble protein contents in Zebrina and *M. itinerans* leaves in response chilling conditions; however, all other accessions were found to have elevated levels of soluble proteins. The highest contents of soluble proteins were recorded

in *M. balbisiana* (4.16  $\mu\text{g}\cdot\text{mL}^{-1}$ ) followed by ‘Zebrina GF’, *M. coccinea*, and *M. acuminata* ssp. *malacconsis*. In contrast, we detected no significant differences in soluble protein contents in the *M. acuminata* cv. ‘Meleng’ and *M. velutina* accessions subjected to control and chilling conditions (Fig. 4B).

It has been demonstrated that osmolytes, highly soluble low molecular weight compounds, such as proline, accumulate in plants in response to chilling stress (Ashraf and Foolad, 2007; Serraj and Sinclair, 2002). Proline plays several important roles protecting plants against abiotic stresses by contributing to the modification of cellular osmosis, detoxification of ROS, maintenance of membrane integrity, and the stabilization of enzymes and other proteins. Additionally, osmolytes play roles in protecting cellular organelles from dehydration and injury (Ashraf and Foolad, 2007; Dar et al., 2016; El-Mahdy et al., 2018; Sharma and Dietz, 2006). In the present study, we found that in all assessed banana accessions, foliar proline contents

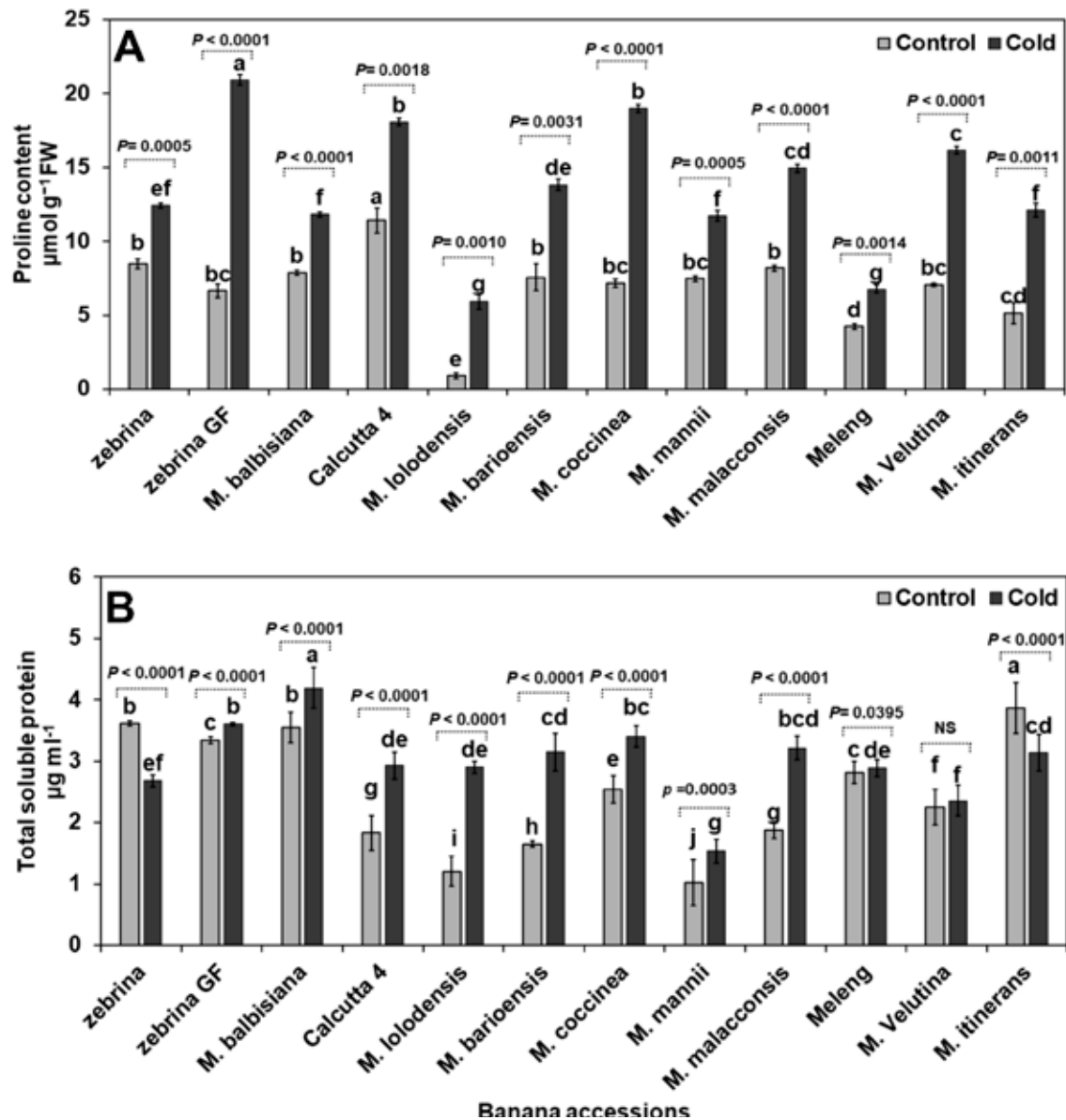


Fig. 4. Effect of chilling treatment on proline content (A) and total soluble protein (B) in the leaves of banana accessions. Mean separation between accessions at a particular treatment is indicated by differing lowercase letters by Tukey's honestly significant difference test ( $P \leq 0.05$ ).  $P$ -values were used to compare mean separation between treatments in a particular accession.

increased in response to chilling stress, which is consistent with the findings of Wang et al. (2013), who also recorded high levels of proline in banana subjected to cold stress. Furthermore, El-Mahdy et al. (2018) have described the physiological and biochemical responses in in vitro-cultured banana cv. Williams and Grand Nain that contribute to enhancing chilling tolerance via different mechanisms, including an enhancement of proline biosynthesis. Furthermore, it has been demonstrated that plant cells produce a characteristic spectrum of proteins associated with the adaptation of growth under chilling conditions (Graham and Patterson, 1982). Plants generate different types of proteins, such as antifreeze proteins and heat shock proteins, to counter the adverse effects of cold stress (Lin et al., 2005; Liu et al., 2018; Wei et al., 2017), and in response to chilling stress, we detected increases in the total soluble protein contents of most of the assessed banana accessions.

### Conclusion

In terms of banana breeding, cold-resistant wild diploid material is considered a valuable resource for genetic breeding programs that aim to enhance banana cold resistance. Based on our observations in this study, we established that the accessions *M. coccinea*, 'Calcutta 4', Zebrina, and *M. velutina* are highly sensitive to cold stress, whereas *M. balbisiana*, *M. mannii*, *M. acuminata* ssp. *malacconsis*, and *M. itinerans* tended to show higher tolerance to chilling stress than the other accessions. High levels of malondialdehyde were recorded in *M. coccinea*, 'Calcutta 4', *M. velutina*, and Zebrina, whereas in contrast, *M. balbisiana*, *M. acuminata* ssp. *malacconsis*, and *M. itinerans* are characterized by negligible increases in malondialdehyde content and *M. balbisiana* and *M. mannii* showed slight differences with respect to radical scavenging capacity. A large field trial with this germplasm will provide conclusive evidence on their ability to withstand cold in central and north Florida. These cold tolerant germplasm will provide valuable genetic resources to produce new edible banana germplasm that can be successfully utilized to extend the cultivation range of banana into more temperate zones.

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## Metepantle: Traditional Agroforestry System of the High Valleys of Mexico

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**Additional index words.** adaptable, maguey (*Agave atrovirens* Karw. ex Salm-Dyck), resilient

The name metepantle comes from the Náhuatl metl (maguey) and pantle (between), which literally means between magueys (agaves). It is an agroforestry system in the central highlands of Mexico that has lasted for more than a thousand years. We felt it was important to record how this system combined functions that have allowed it to last for 54 years in the community of La Reforma, Tlaxcala. The main component of the metepantle is the maguey (*Agave atrovirens*), which is established in a rows, interspersed with prickly pear cactus, fruit and timber trees. Alley crops, mainly small grain cereals, vegetables, or maize are established between the lines of maguey. Maize is managed under the traditional milpa system, which combines beans (*Phaseolus vulgaris*), ayocote (*P. coccineus*, [runner beans]), broad beans [*Vicia faba*, (fava beans)] and squash or pumpkin (*Cucurbita pepo*). This agroforestry system produces multiple products such as grains, legumes, fruits, vegetables, construction materials, firewood, fodder, aguamiel [fermented maguey sap (pulque)], maguey stalks, and mixiote (parchment or film-like membrane of maguey used to wrap meat dishes). The metepantle provides habitat for wildlife, bees, and livestock. It helps retain water and soil on steep slopes, helps conserve agrobiodiversity, and supplies ingredients for the traditional cuisine of the central highlands. The metepantle allows farmers to manage different products which provide economic resources and different foods throughout the year. It has been able to combine multiple components, be resilient and adaptable to climate change, and remain productive for 54 years.

The Food and Agriculture Organization of the United Nations FAO (2013) indicates that in recent years, global problems in the agriculture–food binomial have been emerging: shortages; malnutrition (hunger, obesity, and related diseases); surplus supply and additional costs; food contamination; poverty; climate change; and a financial crisis.

Creating sustainable food production systems which do not adversely affect the environment is a challenge that today's society must face. It requires the transformation of conventional farming systems into agroecological systems which are productive (Hernández-Mansilla et al., 2013).

The metepantle agroforestry system in the Mexican central highlands is a traditional agroecosystem that generates multiple products and benefits and is closely linked to family dairy production. It is an agroecological approach which can improve milk production, respect animal welfare and use biotechnologi-

cal innovations, while helping producers adapt to and mitigate climate change.

The present work was carried out from 2018 to 2021 in the highlands of Mexico. We worked with small farmers in the community of La Reforma, municipality of Españita, Tlaxcala state on a metepantle-agroforestry system, where milpa (maize) and forage crops are interspersed with maguey plants (*Agave atrovirens* Karw. ex Salm-Dyck), nopales [*Opuntia ficus-indica* (prickly pear)], and fruit trees. We used agroecological management with a participatory methodology to improve the production of family dairies which use a system that has lasted for 54 years. The objective of this research was to record how the components of this system have worked to ensure its continuance.

### Materials and Methods

The work was carried out on 6 ha of an agroecosystem managed with milpa and forage belonging to the Montes de Oca family. This system is located in the central Mexican Altiplano at an altitude of 2640 meters (INAFED, 2012). The vegetation

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is pine–oak forest, with a cold temperate climate and an average annual rainfall of 1195.2 mm from July to September.

The participatory methodology of diagnosis, planning, monitoring and evaluation, proposed by Geifus (2002), was used. Evaluations were carried out using participatory observations and follow-up during the period from 2018 to 2020. We obtained data on the production of perennial and annual crops in the metepantle.

## Results and Discussion

### CHARACTERIZATION OF THE TRADITIONAL METEPANTLE SYSTEM.

In the case study, the central element of the metepantle was maguey, planted in cambisol soil in lines across the 20% slope at a 4 m spacing. Fruit trees [peach, plum, quince, tejocote (Mexican hawthorn), or capulin (wild or black cherry, *Prunus salicifolia*)] and prickly pear cactus were interspersed among the maguey. A traditional milpa is developed (a mixture of maize, squash, beans, ayocote, and fava beans) along with maize or fodder oats in alleys 40 to 50 m wide between the lines of maguey. (Table 1).

Two local types of maize are used, “cañuela,” which is early with two color variations, white and light brown “aguadientado,” with crystalline starch, an 8-row cob of eight (a very thin ear) and high hectolitre weight [100 L, used for flour quality]. The other maize is late, yellow, with crystalline starch. Early local varieties of beans, late ayocote (runner bean) and intermediate fava beans are planted. Squash/pumpkin is a late local variety. Finally, intermediate open pollinated oats are used.

Agroecological management in this system consists of solid biofertilization (bokashi) and liquids made from cattle manure

and molasses, enriched with micronutrients. Mineral broths [sulfocalcic broth (lime sulfur)] are used for fungal diseases. Manure is used as a fertilizer after being given several months to mature. This manure is distributed and incorporated into alleys or used on magueys, nopales and fruit trees.

**PRODUCTS FROM THE TRADITIONAL METEPANTLE SYSTEM.** Most of the products from the metepantle are eaten on the ranch with the surplus sold locally. In the milpa, maize, beans, broad beans, and ayocote are grown for human consumption. Pumpkin and pumpkin flowers are eaten as vegetables, while the pumpkin seed is sold as a snack (Table 2). Grain yields are higher than that reported by Rosas et. al (2016), who indicate that on average the yield is 1.77 t·ha<sup>-1</sup> for maize produced between fruit trees, in nearby Vicente Guerrero. This highlights the advantages of the metepantle system.

Stubble maize, fava bean, bean, ayocote, and prickly pear cactus stubble are a source of food for the cattle, whether supplied by cutting and hauling or by direct grazing. This grazing has a dual function: to feed eight adult cattle and to fertilize with the manure generated by the cattle. The maize that is planted is either yellow maize (late) or cañuela maize (early) varieties. This practice concurs with Cesín (2010) who said it was common to plant yellow, red or blue creole maize in the metepantle, suggesting early types since the crop depends entirely on rainwater. It should be noted that generally maize grown in these systems do not produce grain since producers are interested in obtaining as much forage with fiber as possible.

Maguey is the main component of the metepantle system. It provides multiple products, including stalks for cooking barbacoa

Table 1. Species of the traditional metepantle system.

Name	Species	(Plants/ha)	Cycle
Maguey	<i>Agave atrovirens</i> Karw. ex Salm-Dyck	100	Perennial
Nopal (prickly pear)	<i>Opuntia ficus-indica</i>	200	Perennial
Yellow maize	<i>Zea mays</i> L.	50,000	Late
Cañuela maize	<i>Zea mays</i> L.	50,000	Early
Bean	<i>Phaseolus vulgaris</i> L.	10,000	Early
Ayocote (runner bean)	<i>Phaseolus coccinus</i> L.	10,000	Late
Fava	<i>Vicia faba</i> L.	500	Early
Pumpkin	<i>Cucurbita pepo</i> L.	1000	Late
Peach	<i>Prunus persica</i> L. Batsch.	100	Perennial
Tejocote (Mexican hawthorn)	<i>Crataegus mexicana</i> Moc. Sessé	50	Perennial
Plum	<i>Prunus domestica</i> L.	100	Perennial
Capulin (black cherry)	<i>Prunus serotina</i> subsp. <i>capuli</i> (Cav.) McVaugh	100	Perennial
Membrillo (quince)	<i>Cydonia vulgaris</i> Pers.	100	Perennial

Table 2. Availability of fodder in the family production unit.

Species	Yield (ton/ha)	Self-consumption (%)	Sale (%)
Metepantle with oats			
Oats (fodder)	4.5	100	0
Metepantle with simultaneous cultivation			
Oats (grain)	1	100	0
Maguey	0.5	50	50
Nopal	0.5	100	0
Maize	3.5	90	10
Maize stubble	9	100	0
Ayocote	0.15	100	0
Beans	0.1	100	0
Pumpkin	0.1	0	100

or as cattle fodder, the cuticle (mixiote) used in cooking, the production of aguamiel (tequila) and pulque (fermented sap). The floral tayo is used as poles, the flowers are edible and when dried, the whole plant can be used as biofuel. This means that 50% of the various products are destined for local sale (Table 2). These uses agree with Narváez (2016), and provides evidence that the metepantle helps supply local demand and contributes to the conservation of traditional gastronomy.

The maguey lines produce tender nopales (prickly pear cactus leaves), which are used fresh, as are the fruits. A small portion of the fruit is made into preserves and the firewood generated by pruning is used as biofuel. SAGARPA (2017) indicates that the living barriers established from the association of these species provide benefits such as pH improvement, maintenance of microelement retention levels, increase in phosphorus, potassium, and available organic matter, decrease in soil loss, and greater water retention, in addition to generating income.

In the metepantle there are also several other types of plants that are of great importance because they have food uses such as quelites (Mexican wild greens) (*Amaranth* spp, *Chenopodium berlandieri*, *Portulaca oleracea*, etc.) and medicinal uses (*Eryngium heterophyllum*, *Argemone mexicana*, *Chenopodium graveolens*, etc.). All these species flower which makes the metepantle an important foraging area for bees, hummingbirds, and bats, along with the flowering maguey, nopal, and fruit trees. They are traditionally used as an alternative source of fodder as is *Heterotheca inuloides* (Mexican arnica). These species are abundant in the region and are valuable for traditional uses and local consumption. Cesín et. al (2010), indicate that the use of these plants (sometimes called weeds) is part of traditional knowledge.

In addition, the metepantle conserves plant agrobiodiversity with an average of 20 species. This allows the management of wildlife and livestock, which contributes to the productive diversity of the system. Narváez (2016) indicates that the metepantle coexists with diverse animals and insects that are beneficial to crops and that among the animals it attracts are bees, hummingbirds, wasps, moths, and bats, which play an important role in pollination. On the adjacent ground you can find small mice, cacomixtles (cacomistle), hares, armadillos, badgers, gophers, vipers, lizards, and ants. Each of these animals play a role in the food chain and are important for agriculture because they regulate the insect population and help with biological pest control.

### **Resilience of the traditional metepantle system**

The metepantle we studied has been managed under an agroecological approach for 54 years, three generations, which has preserved and maintained its productive stability due to the following:

**DIVERSIFICATION OF SPECIES.** The principle of planting maize simultaneously with beans, ayocote, and broad beans, based on the association of leguminous plants with nitrogen-fixing bacteria which benefits all the crops. Pumpkin is part of the milpa and benefits from the interaction of the various crops (Sánchez and Romero, 2017). Its leaves help shade the soil, thus conserving moisture for a longer time. This helps the milpa withstand prolonged periods of drought or erratic rainfall and reduces the presence of weeds. Ebel et. al (2017) said that the milpa is characterized by a synergy between crops and integrates them to favor their performance as a whole which generates resilience to

external disturbances. This is seen by the fact that the harvests of maize + squash and bean + squash polycultures exceeds the respective monocultures of each species.

**THE MANAGEMENT OF MAIZE VARIETIES.** The importance of planting two maize varieties of different growth cycles is based on how the rains occur. When the rainy season begins early, the late yellow maize is planted, but when the rains are delayed, the early “cañuela” is planted. Generally, both varieties are planted because, in the event of early frosts, the yellow maize can be lost, but the “cañuela” has matured and is not damaged. This strategy is based on the reinterpretation of traditional knowledge for the production of creole maize. It is a combination of new knowledge and beliefs, expressed in agricultural decisions and actions, some of which are quite effective. Moving the sowing date, using early varieties, not sowing dry, and experimenting ways increase moisture retention are examples. Improving the availability of nutrients in the soil and performing rituals of petition and thanksgiving for rain and good harvest also play roles (Munguía et. al 2015).

**CROP ROTATION USING FORAGE SPECIES.** Oats are sown to obtain forage for cattle feed. In some cases, its cultivation is preferred to the milpa when rains are delayed. The milpa may be lost due to adverse climatic events such as delayed rains or late frosts. In this sense Gonzálves et. al (2018) suggest that successive sowing of crops is an adaptation strategy to climate change since it breaks the biological cycle of pests and diseases, provides nutrients and raises the level of organic matter.

**THE USE OF NATIVE SPECIES OF ARID ZONES.** Maguey and nopal, being species adapted to arid zones, have the virtue that they continue to produce even when there are prolonged periods of drought. Their arrangement in the metepantle assists with soil conservation and rainwater capture and retention. These species are considered to have multiple environmental benefits, as described by Narváez (2016). They are useful for soil and water conservation, favor biodiversity which is beneficial for crops and the environment, provide carbon credits, allow aquifer recharge and the practice of sustainable agriculture, even when combined with the construction of terraces.

**USE OF BIO INPUTS.** Using biofertilizers and organic fertilizer and pest management without pesticides provide a healthier environment for human health. Since most of the inputs are produced on the ranch helps reduce production costs. The metepantle system provides a good management that maintains productivity and generates profitability.

**AGROECOLOGICAL PRINCIPLES.** The diversification of species and using specific varieties at specific times, can ensure harvests despite extreme climatic events such as too much or too little rain and late or early frosts. This follows Cesín et. al (2010), who says that the knowledge and management of the agricultural cycle shows a capacity to adapt to changing scenarios and the great resilience of agroecosystems.

### **Conclusion**

Given the attributes of metepantle and its ability to combine multiple components, it is more resilient and adaptable to the onslaught of climate change. This ensures the productivity of the system and allows the farmers who manage the system to make a profit as well as being more food secure.



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## Aggregate Distribution and CO<sub>2</sub> Emission in the Soil of Agroforestry System

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Coffee agroforestry systems in Mexico are characterized by their high species diversity and their potential to mitigate CO<sub>2</sub> emissions. Currently, these systems are being modified with the introduction of avocado as a way to address sanitation and price problems. This modification could alter organic matter concentrations, change soil aggregation, as well as influence soil biological activity and CO<sub>2</sub> emissions. The objective of the research was to evaluate the variation of aggregate size and CO<sub>2</sub> emissions produced by soil respiration with the changes generated by the introduction of avocado into a coffee agroforestry system, to demonstrate its potential to reduce CO<sub>2</sub> emissions and maintain carbon stores in the soil. Five agroforestry systems were selected: 1) traditional coffee polyculture with renovation; 2) traditional coffee polyculture with severe pruning and cleaning; 3) traditional coffee polyculture abandoned; 4) coffee and avocado agroforestry system (ACS); and 5) avocado monoculture. In each system, soil samples were collected at three depths: 0–10, 10–20, and 20–30 cm, for triplicate, during the 2019–20 production cycle, to determine bulk density, aggregate distribution, minimum weight diameter (MWD), chemical characteristics, soil respiration, soil organic carbon (SOC) and degradation rate constant (k<sub>d</sub>). In the ACS a lower range (0.6–0.8 mm) of MWD at three depths, lower soil respiration (150 kg CO<sub>2</sub>-C ha<sup>-1</sup>·h<sup>-1</sup>), positive SOC balance and negative k<sub>d</sub> were found. Therefore, the introduction of avocado contributes to the reduction of CO<sub>2</sub> emissions from soil respiration and to the conservation of carbon stores.

Agroforestry systems are recognized for their ability to mitigate carbon dioxide (CO<sub>2</sub>) emissions from soil biological activity (Yago et al., 2019). In Veracruz, Mexico, coffee agroforestry systems dominate and are characterized by a high diversity of species that fulfill the functions of providing shade for coffee production, food, and raw materials for self-consumption. Currently, coffee agroforestry systems are being displaced by avocado monoculture, due to price variations in the coffee market (Jaffe et al., 2008) and low production caused by sanitary damage attributed to climatic variations (Villers et al., 2009).

Coffee agroforestry systems modified with avocado plantings generate variation in floristic composition and this could have a negative impact on biodiversity and environmental services (Escamilla, 2016). This change in floristic composition could influence the variation of CO<sub>2</sub> emissions generated by soil respiration (SR) because the quantity and quality of organic residues entering the soil could be altered. These alterations could change the microenvironments where microorganisms release CO<sub>2</sub> to the atmosphere, affecting concentration as well as the potential to store carbon (C) in the soil (Iqbal et al., 2010). Variation in the quantity and quality of organic residues could alter soil organic matter concentrations and change the soil aggregation state. This

aggregation can influence the development of soil biological activity and CO<sub>2</sub> emissions (Chatterjee et al., 2020).

The objective of this research was to evaluate the variation of aggregate size and CO<sub>2</sub> emissions produced by soil respiration with the changes generated by the introduction of avocado plants in the coffee agroforestry system in the region of Huatusco, Veracruz, Mexico, to demonstrate the potential of modified agroforestry systems to reduce CO<sub>2</sub> emissions and carbon storage in the soil.

### Materials and Methods

The experiment was conducted in the community of Tlaxopa, Municipality of Huatusco de Chicuellar, Veracruz (19°10'25" N Lat.; 96°57'30" W Long., México) at an average altitude of 1300 m. In study area, the climate is semi-warm and humid with an average annual temperature and precipitation of 16.4°C and 2018 mm/year, respectively. The soils are Andosols with a dark color, fine texture, strong acidity, and high organic matter content.

Five agroforestry systems were selected: 1) traditional coffee polyculture with renovation (CPR); 2) traditional coffee polyculture with severe pruning and cleaning (CPC); 3) traditional coffee polyculture–abandoned (CPA) (systems 1–3 are 21 years old); 4) coffee and avocado agroforestry system (ACS); and 5) avocado monoculture (MCA) (systems 4–5 are eight years old). Soil sampling points were randomly located in each systems.

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For the 2019–20 production cycle, soil samples were collected at three depths: 0–10, 10–20, and 20–30 cm, in triplicate, to determine bulk density, aggregate distribution, minimum weight diameter (MWD), chemical characteristics, soil respiration, soil organic carbon (SOC) and the degradation rate constant ( $k_d$ ). The determination of aggregates in the soil was carried out with the methodology of Le Bissonnais (1996), using the following sieve sizes: 6.35, 4.76, 3.36, 2.0, 1.0, 0.5, 0.25, and < 0.25 mm. The resulting information was used to determine the minimum weight diameter (MWD) and bulk density with the cylinder method ( $\text{Mg}\cdot\text{m}^{-3}$ ). Soil  $\text{CO}_2$  emissions were assessed using soil respiration (SR). EGM-4 equipment was used to measure the  $\text{CO}_2$  emissions flux generated by SR. It is based on the dynamic chamber method proposed by Parkinson (1981). SOC and inorganic carbon of soil (ICS) were determined before and after incubation. All determinations were performed in a TOC-V CPN Shimadzu C analyzer. These determinations were used to perform C balance using the methodology proposed by Rahman (2013). The SOC degradation rate constant ( $k_d$ ) was calculated according to the kinetic model proposed by Tanvea et al. (2008). Statistical analysis consisted of analysis of variance (ANOVA,  $P < 0.05$ ) and Tukey's HSD mean comparison to analyze  $\text{CO}_2$  emissions generated by soil respiration. The minimum weight diameter was compared with a dual-axis plot to analyze interactions.

### Result and Discussion

Soil pH ranged from 3.6 to 4.8 in all systems and at all depths, a range that corresponds to highly acidic soils (Table 1). The low bulk density values correspond to typical Andosols soils; the values ranged from 0.48 to  $0.70 \text{ Mg}\cdot\text{m}^{-3}$  and increased with depth. These results are in agreement with those reported by Rosas et al. (2008) for coffee plantations. The CPA had the highest bulk density at the 0–10 and 20–30 cm depths, however at the intermediate depth (10–20 cm), the CPR and CPA had values similar to ACS. Bulk density results showed that traditional coffee systems have lower soil porosity (73.2 to 75.4%) for the development of biological activity than systems associated with avocado (73.9 to 82.3%) at the three depths (a real density of  $2.65 \text{ g}\cdot\text{cm}^{-3}$  was used). This confirms that the agronomic management in systems associated with avocado produce soil conditions similar to intensively managed ones (Siavosh et al., 2010).

The CPA had an average of 72.8% aggregates with diameters > 1mm, at the 0–10 cm depth (Fig. 1A); 69.9% at 10–20 cm (Fig. 1B) and 62.7% at 20–30 cm (Fig. 1C). For systems associated with avocado, an average of 74.2% aggregates with diameters

< 1 mm was reported for the 0–10 cm depth, 69.4% at 10–20 cm and 62.5% at 20–30 cm. These distributions showed that the aggregates are 250% higher in the traditional coffee systems compared to the agroforestry system modified with avocado. The distribution of aggregates < 1 mm in ACS is attributed to the low bulk density values generated by the introduction of avocados into the system. Bulk density values are lower than the average ( $0.82 \text{ Mg}\cdot\text{m}^{-3}$ ) reported by Siavoch et al. (2010) for traditional coffee systems with minimum weight diameters varying between 0.6 to 0.8 mm, a range that confirms the high potential to store carbon in the long term but deficient to generate soil activity, i.e., generate lower  $\text{CO}_2$  emissions (Chatterjee et al., 2020).

Cumulative respiration ( $\text{kg}\cdot\text{ha}^{-1}\cdot\text{h}^{-1} \text{ CO}_2\text{-C}$ ) ranged from 46.7 to 62.6 in all systems and at all depths (Table 2). CPC system with pruning and severe cleaning had the highest  $\text{CO}_2$  emissions by microbial respiration in the shallowest depth (0–10 cm); the same was seen at the lowest depth (20–30 cm) while at intermediate depth (10–20 cm) no significant differences were found between systems. ACS had 18.8% less emissions than CPC but emitted 9.9% more  $\text{CO}_2$  than the traditional coffee–abandoned (CPA) at the 0–10 cm depth (Fig. 1E). At 10–20 cm there were no statistically significant differences and at 20–30 cm depth (Fig. 1F) it resembles the system with lower soil respiration (CPR). This system corresponds to coffee renewed, i.e., with a high density of young coffee which has not yet created conditions which promote soil microbial activity at deeper soil layers (Handa et al., 2014).

The MCA with intensively managed had MDW 50% higher than ACS and so exceeds the 50% soil respiration values (Fig 2A). Similarly, CPC reported higher SR than traditional coffee. This may be attributed to the presence of aggregates that exceeded the CPR by 44.8% and CPS by 28.2%. However intensive pruning management contributes to a MWD 3.7 times higher than that for MCA. This relationship is also observed at the other depths (Figs. 2B and 2C). All systems not associated with avocado had an increase in SOC >100% (Fig. 2D), CPR (132%), and CPC (207%) had the highest values of minimum weight diameter and SOC. Since CPA had a higher MWD than the CPR (132%), it generated a smaller increase in SOC. At depths of 10–20 and 20–30 cm (Fig. 2E) the variation in SOC had the same tendency as at the shallowest depth for systems associated with avocado, CPC had a lower SOC increase; CPR and CPA were the ones with the largest increases in SOC by soil respiration, both systems having a greater than 400% increase. The modification of the systems generated less soil activity which can be attributed to the fact that ACS and MCA systems are less diversified than traditional coffee systems (Xi et al., 2012). Cumulative emissions,

Table 1. General characteristics of the soils of each studied system.

System	pH			BD ( $\text{Mg}\cdot\text{m}^{-3}$ )		
	0–10 cm	10–20 cm	20–30 cm	0–10 cm	10–20 cm	20–30 cm
ACS	4.8 ± 0.31 a	4.7 ± 0.24 a	4.6 ± 0.27 a	0.49 ± 0.03 b	0.55 ± 0.04 ab	0.55 ± 0.04 ab
MCA	4.8 ± 0.31 a	4.4 ± 0.24 a	4.4 ± 0.27 a	0.48 ± 0.03 b	0.49 ± 0.04 b	0.47 ± 0.04 b
CPR	3.7 ± 0.31 a	3.7 ± 0.24 a	3.8 ± 0.27 a	0.66 ± 0.03 a	0.67 ± 0.04 ab	0.69 ± 0.04 a
CPC	4.0 ± 0.31 a	3.7 ± 0.24 a	3.7 ± 0.27 a	0.65 ± 0.03 a	0.71 ± 0.04 a	0.70 ± 0.04 a
CPA	3.7 ± 0.31 a	3.6 ± 0.24 a	3.7 ± 0.27 a	0.67 ± 0.03 a	0.67 ± 0.04 ab	0.66 ± 0.04 a
<i>P-Value</i>	0.0676	0.0331	0.1179	0.0012	0.0155	0.0053
HSD	1.437	1.119	1.264	0.137	0.192	0.172

ACS = Avocado-coffee system. MCA = Monoculture of avocados. CPR = Coffee traditional polyculture with renewal. CPC = Coffee traditional polyculture with pruning and severe cleaning. CPA = Coffee traditional polyculture abandoned.

Tukey test ( $P \leq 0.05$ ); different letters indicate statistically significant differences.

*P-Value*: Probability value; MSD: Minimal significant difference.

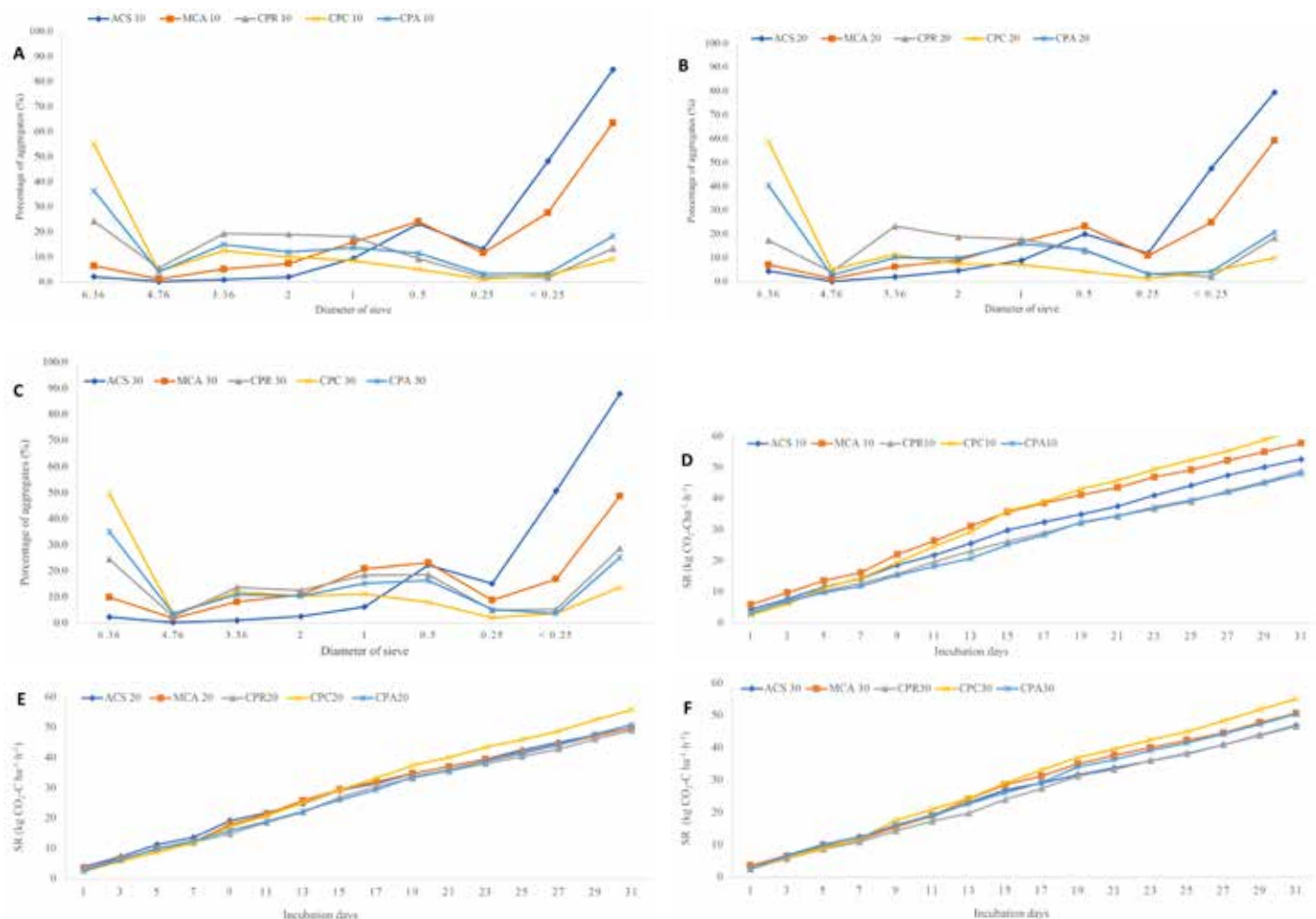


Fig. 1. Average distribution of soil aggregates. (A) Percentage of aggregates by sieve size at the depth of 0–10 cm; (B) Percentage of aggregates by sieve size at the depth of 10–20 cm; (C) Percentage of aggregates by sieve size at the depth of 20–30 cm; (D) Accumulated SR at the depth of 0–10 cm; (E) Accumulated SR at the depth of 10–20 cm; (F) Accumulated SR at the depth of 20–30 cm.

however, are similar the average ( $147 \text{ kg} \cdot \text{ha}^{-1} \cdot \text{h}^{-1} \text{CO}_2\text{-C}$ ) reported by Hergoualc’h et al. (2008) in coffee system with Andosols soils. For traditional coffee systems, the greater diversity of composition species contributes to soil biological activity and thus there is an increase in  $\text{CO}_2$  emissions (Chen & Chen, 2019). Sheng et al. (2010) found that the availability of diversified substrates promotes soil respiration and Siavosh et al. (2010) showed that

Table 2. Carbon dioxide ( $\text{CO}_2$ ) emission generated from soil respiration.

System	$\text{CO}_2$ emission ( $\text{kg} \cdot \text{ha}^{-1} \cdot \text{h}^{-1} \text{CO}_2\text{-C}$ )		
	0–10 cm	10–20 cm	20–30 cm
ACS	$53 \pm 1.95 \text{ bc}$	$50 \pm 2.32 \text{ a}$	$47 \pm 1.25 \text{ b}$
MCA	$58 \pm 1.95 \text{ ab}$	$50 \pm 2.32 \text{ a}$	$51 \pm 1.25 \text{ ab}$
CPR	$49 \pm 1.95 \text{ bc}$	$49 \pm 2.32 \text{ a}$	$47 \pm 1.25 \text{ b}$
CPC	$63 \pm 1.95 \text{ a}$	$56 \pm 2.32 \text{ a}$	$55 \pm 1.25 \text{ a}$
CPA	$48 \pm 1.95 \text{ c}$	$51 \pm 2.32 \text{ a}$	$51 \pm 1.25 \text{ ab}$
<i>P-Value</i>	< 0.05	0.31	< 0.05
HSD	9.09	10.79	5.82

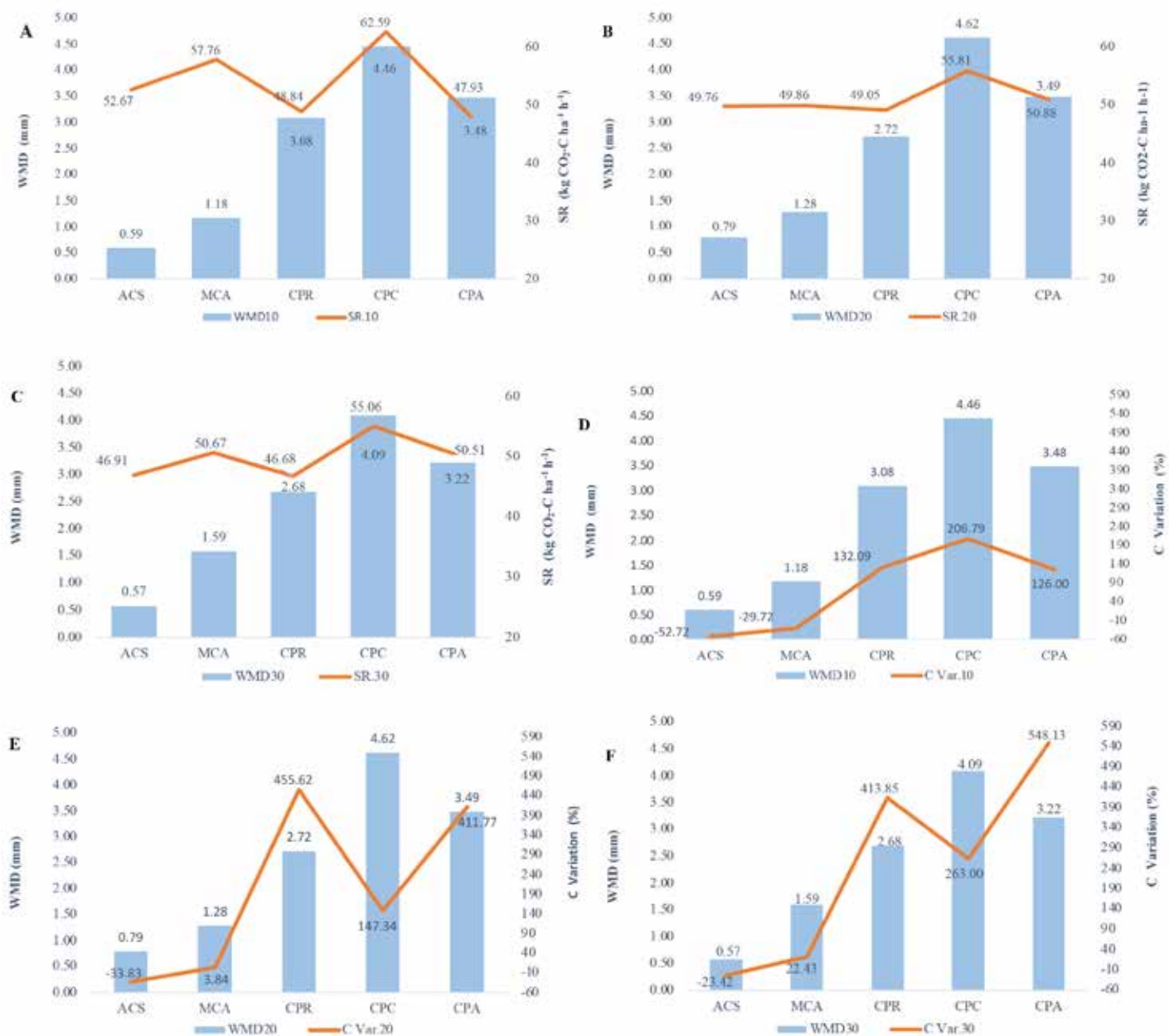
ACS = Avocado-coffee system. MCA = monoculture avocado. CPR = Coffee traditional polyculture with renewal. CPC = Coffee traditional polyculture with pruning and severe cleaning. CPA = Coffee traditional polyculture abandoned. DMS = Minimal significant difference *P-Value*: Probability value; Tukey test ( $P \leq 0.05$ ).

soil respiration in traditional coffee systems is higher than in systems with intensive management.

The soil organic carbon balance was positive for ACS and reported the highest average range ( $9\text{--}26 \text{ Mg} \cdot \text{ha}^{-1} \text{C}$ ) at all depths. MCA showed a positive balance in the shallowest depth (0–10 cm) (Table 3) while the other systems had a negative balance. Measurements at the three depths had statistical similarities with ACS. In traditional coffee systems, a negative balance was generated to store SOC at all depths.

The SOC degradation rate constant ( $K_d$ ) was negative for the ACS system at all depths and positive for all other systems. CPA had the highest cumulative  $K_d$  at all depths, allowing for the following order: CPA = CPR > CPC > MCA > SAC. The positive SOC balance in coffee agroforestry systems modified with avocado indicates the ability of the systems to generate conditions that inhibit soil biological activity and, therefore have lower  $\text{CO}_2$  emissions. The output of ACS was the lowest showing that the modification of traditional coffee systems influences the mineralization processes of labile SOM (Le Noë et al., 2019). In traditional coffee systems, a negative balance was generated producing higher residual C concentrations due to soil biological activity.

The ability of the systems to promote C pools and decreases emissions generated by soil activity is related to their ability to



**Fig. 2.** Relationship between the weighted minimum diameter (WMD) and the variation of C (Var.). (A) WMD and accumulated soil respiration at depth of 0–10 cm. (B) WMD and accumulated soil respiration at depth of 10–20 cm. (C) WMD and accumulated soil respiration at depth of 20–30 cm. (D) WMD and Var. in depth of 0–10 cm. (E) WMD and Var. in depth of 10–20 cm. (F) WMD and Var. in depth of 20–30 cm.

**Table 3.** Soil organic carbon (SOC) balance and degradation rate constant ( $K_d$ ).

System	SOC balance (Mg·ha <sup>-1</sup> C)			Degradation rate constant ( $K_d$ )/day		
	0–10 cm	10–20 cm	20–30 cm	0–10 cm	10–20 cm	20–30 cm
ACS	26 ± 4.6 a	15 ± 5.1 a	9 ± 3.1 a	-2.3 ± 0.3 b	-1.24 ± 0.39 c	-0.77 ± 0.29 d
MCA	9 ± 4.6 a	-1 ± 5.1 a	-5 ± 3.1 a	-1.03 ± 0.3 b	0.15 ± 0.39 c	0.76 ± 0.29 c
CPR	-38 ± 4.6 b	-78 ± 5.1 b	-63 ± 3.1 c	2.8 ± 0.3 a	5.56 ± 0.39 a	5.32 ± 0.29 ab
CPC	-61 ± 4.6 c	-37 ± 5.1 c	-44 ± 3.1 b	3.6 ± 0.3 a	2.91 ± 0.39 b	4.19 ± 0.29 b
CPA	-37 ± 4.6 b	-87 ± 5.1 c	-81 ± 3.1 d	2.7 ± 0.3 a	5.31 ± 0.39 a	6.08 ± 0.29 a
<i>P</i> -Value	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
HSD	21.193	23.641	14.202	1.38	1.8	1.36

ACS = Avocado-coffee system. MCA = Monoculture avocado. CPR = Coffee traditional polyculture with renewal. CPC = Coffee traditional polyculture with pruning and severe cleaning. CPA = Coffee traditional polyculture abandoned.

Tukey test ( $P \leq 0.05$ ), different letters indicate statistically significant differences.

*P*-Value = Probability value, DMS = Minimal significant difference.

degrade SOM which was negative for ACS according to  $K_f$  results, suggesting that the modified systems have low soil activity. The low values of accumulated CO<sub>2</sub> emissions during incubation contributed to maintaining the soil C pool. This response shows that the low degradation capacity of ACS contributes to decreased soil respiration (Zimmermann et al., 2009).

### Conclusion

In conclusion, the lowest minimum weight diameter was generated in the two systems associated with avocados. These systems had lower microbial activity with a positive SOC balance and negative SOC aggregation constant. The ACS system generated the lowest concentration of cumulative CO<sub>2</sub> emissions from soil respiration. The introduction of avocado in the traditional coffee systems contributes to the reduction of CO<sub>2</sub> emissions and SOC storage.

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## Constraints to Rootstocks for Growing Low-chill Peaches in Florida's Changing Climate

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**Florida's peninsular shape and close proximity to the equator creates a humid, subtropical environment, with more than half of the year characterized by warm temperatures (> 32.2°C) and high relative humidity (> 50% RH) (Black, 1993). Over two-thirds of the state is surrounded by the Gulf of Mexico or the Atlantic Ocean, which absorb the sun's rays and radiate heat into the atmosphere facilitating the warm air temperatures for which Florida is known. These warm, tropical waters also bring about heavy rain fall and extreme seasonal weather events that impact both coastal and inland areas.**

From 1988 to the present day, statewide precipitation in Florida has shown a steady increase [National Oceanic and Atmospheric Administration (NOAA), undated]. The Conservation Biology Institute asserts that Florida has latitudinal variation in precipitation trends with southern Florida predicted to experience a decrease, the northern portion of the state an increase, and the central region experiencing virtually no change in precipitation (Ward, 2008). Conversely, Martinez et al. (2012) found that precipitation is increasing in all regions of the state; however, the northern region of Florida was predicted to experience a greater increase in precipitation. These latitudinal differences in precipitation may coincide with variations in air temperatures throughout the state. When looking at air temperature trends in Florida, Martinez et al. (2012) found that temperatures in the northern panhandle and central region tended to decrease over time, whereas in the southern region temperatures tended to increase. Statewide air temperatures in Florida have been increasing since about 1977 (NOAA, undated).

This range allows growers to produce a variety of crops, ranging from tropical to temperate. Nevertheless, Florida faces global competition for many of its exports including berries, citrus, and sugar cane. This has motivated efforts to diversify crops grown in Florida to allow growers the opportunity to stake claim to a variety of markets. One of these markets is low-chill peaches. Compared to national leaders in peach production, Florida has a short winter season which quickly becomes an early spring season. This allows growers to provide fresh peaches to consumers while other states do not have ripe fruit. Despite this market advantage, biotic and abiotic obstacles such as pest and pathogen pressure, flooding, high temperatures, and salinity stress pose risks to successful cultivation of peaches in Florida.

### Available Rootstocks for Florida Peach Production

Beginning in the 1950s, the University of Florida, IFAS (UF/IFAS) in collaboration with the USDA-Agricultural Research Station (USDA-ARS) Peach Rootstock Breeding Program in Byron, GA, has made efforts to establish peach as a viable crop for Florida. Through careful breeding and appropriate cultural practices, the UF/IFAS Stone Fruit Breeding program, currently led by Dr. José X. Chaparro, has sought to develop cultivars that exhibit disease resistance, low chilling requirements, and large, high-quality yields (Sarkhosh, 2019b). In commercial production of many fruit crops, grafting of scion cultivars onto rootstock cultivars is a recommended practice to increase tree resistance to biotic and abiotic stresses. *Prunus* rootstock cultivars, 'Sharpe', 'Flordaguard', and 'MP-29'; have been released for commercial production and at-home gardening in Florida (Table 1).

Despite the plum rootstock 'Sharpe' having resistance to the biotic stressors listed in Table 1, it has decreased yield and fruit size compared to 'Flordaguard' rendering it impractical for commercial use (Beckman et al., 2008; Sarkhosh, 2019a). Currently, 'Flordaguard' is the recommended rootstock cultivar for peach production in Florida because of its low-chilling requirement and resistance to *Meloidogyne incognita*, *M. javanica*, *M. floridensis*, three damaging root-knot nematodes. The plum × peach rootstock cultivar 'MP-29' has similar resistance to pathogens as 'Sharpe' with even greater resistance to *Armillaria* root rot (Beckman et al., 2012) (Fig. 1). 'MP-29' has reduced vigor compared to 'Flordaguard', and can be used as a semi-dwarfing rootstock to increase planting density (Beckman et al., 2012). These adaptations have aided in optimizing peaches for production in Florida, but there are still environmental obstacles facing the economic viability of peach as a crop in Florida.

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Table 1. Available rootstock cultivars for cultivation in Florida<sup>a</sup>.

Cultivar	Date Released	Chilling Units	Genetic Components	<i>Meloidogyne incognita</i>	<i>Meloidogyne javanica</i>	<i>Meloidogyne floridensis</i>	<i>Armillaria</i> Root rot	Peach Tree Short Life
'Sharpe'	2007	500	<i>Prunus angustifolia</i> (Marsh.) 'Chickasaw' plum × unknown plum species	Resistant	Unknown	Resistant	Resistant	Resistant
'Flordaguard'	1991	300	Six generations from Chico 11 × <i>P. davidiana</i> (Carr.) Franch, C26712	Resistant	Resistant	Resistant	Susceptible	Susceptible
'MP-29'	2012	750	'Edible Sloe' plum × SL0014 ( <i>P. persica</i> )	Resistant	Resistant	Resistant	Resistant	Resistant

<sup>a</sup>Data from Sherman et al., 1991; Beckman et al., 2008; Beckman et al., 2012; Horticultural Sciences Department (HOS), 2012; Sarkhosh et al., 2019a; Sarkhosh et al., 2019b; and Sarkhosh et al., 2020.



Fig. 1. Effects of *Armillaria* root rot in a Florida peach orchard. Photo credit T. Beckman.

### Where Do We Go from Here?

In addition to appropriate rootstock selection, it is critical to understand potential environmental stressors. Florida's long rainy season, coupled with warm soil temperatures, provides a breeding ground for pathogens to thrive. Developing disease resistant rootstocks is imperative to combatting Florida's moist soils. While 'Flordaguard' is the only recommended rootstock for Florida peach production, it is susceptible to *Armillaria* brown rot, peach tree short life, and *Botryosphaeria dothidea* (peach gummosis) (Sarkhosh, 2019a). These pathogens wreak havoc on established and newly planted orchards alike, causing dieback and reducing tree longevity and fruit quality. Peach tree short life (PTSL) is defined as the unexpected death of young peach trees, typically during the spring season (Beckman and Nyczepir, 2004). Researchers conclude PTSL is the result of an interaction of several factors, including susceptibility to *Macroposthonia xenoplax* (ring nematode), cold damage, bacterial canker, and variation in late winter and early spring temperatures (Ritchie and Clayton, 1981; Nyczepir et al., 1983; Beckman and Nyczepir, 2004). The proliferation of these pathogens can be facilitated by extreme environmental conditions, such as waterlogged soils after heavy rainfall.

Regardless of the location, flooding is regarded as a persistent threat to the state of Florida. The state's low elevation puts agricultural operations at risk of wind and flood damage following severe weather events such as tropical storms and hurricanes. Researchers believe the observed increase in the frequency and

magnitude of tropical storms is likely caused by climate change (Mann and Emanuel, 2006; Kossin et al., 2007). Since this trend is likely to continue, it is vital that *Prunus* rootstocks selected for Florida peach production be tolerant to the increased risk of flooding.

Florida's hurricane (June-Nov.) and rainy seasons (June-Oct.) occur mostly during the warmest months of the year (May-Sept) (Martinez et al., 2012; Zhang et al., 2017; NOAA, 2021). The overlap between high temperatures and heavy rain creates the potential for the simultaneous exposure of crops to flooding and heat stress. Reighard et al. (2001) hypothesized that the shallow nature of *Prunus* root systems led to greater damage to roots than scions, following a hurricane. The close proximity of *Prunus* root systems to the soil surface increases their risk of damage from high soil temperatures and/or flooding. Increased precipitation in soils having a hardpan, can cause a perched water table, effectively waterlogging the soils (Fig. 2). Excess rainwater in the rootzone can displace soil oxygen, resulting in low soil oxygen concentrations, or hypoxia. Hypoxia decreases the plant's ability to absorb CO<sub>2</sub> through stomata in the leaves, and subsequently photosynthesize (Len et al., 2020; Salvatierra et al., 2020). The overall growth and development of the plant is affected, which can stunt or even kill the plant entirely (Fig. 2).

Hypoxia also inhibits root respiration resulting in insufficient energy production that can hinder the plant's ability to recover from possible mechanical injuries leaving wounds susceptible to pathogens and high light intensity (Crane et al., 1999). In the likely event of severe weather occurring during a period of high temperatures in Florida, plant rootzones may be subjected to greater stress. High temperatures in the rootzone can lead to



Fig. 2. Post-flooded Florida peach orchard following Hurricane Irma (2017). Photo credit: A.Sarkhosh.



decreased photosynthetic activity, oxidative stress, and cellular damage and death (Kotak et al., 2007; Fahad et al., 2017). Data from the Florida Automatic Weather Network (<http://fawn.ifas.ufl.edu>) indicates that several cities from south to north central Florida have experienced soil temperatures ranging from 32 to 49 °C in the past 20 years. The potential for these stresses to occur simultaneously provides justification for further development of a hypoxia and high temperature tolerant rootstock.

While peach is considered intolerant of flooding, wide variation within the *Prunus* genus has revealed other species that exhibit tolerance to flooding (Ranney et al., 1994; Pimentel et al., 2014; Ranney et al., 2016; Pérez-Jiménez et al., 2017; Rubio-Cabetas et al., 2018; Toro et al., 2018; Klumb et al., 2020). Recently, researchers have determined that the medium-chill plum rootstock, 'MP-29', has increased tolerance to flooding compared to the low-chill peach rootstock, 'Flordaguard' (McGee et al., 2021) (Fig. 3). This wide variation has led researchers to investigate tolerance to rootzone temperatures that exceed averages in specific production areas (Bonomelli, et al., 2009; Hao et al., 2011; Bonomelli et al., 2012; Gainza et al., 2015). Further research is needed to determine if low-chill *Prunus* rootstocks exhibit tolerance to high soil temperatures occurring in Florida.

Salinity stress in the rootzone of glycophytes (plants intolerant to salinity stress) can result in a reduction in photosynthetic activity, disrupting plant development as well as cell death prompted by the accumulation of harmful reactive oxygen species (Massai et al., 2004; Ranjbarfordoei et al., 2006; Aazami et al., 2012; Papadakis et al., 2018; Shahid et al., 2020). Generally, plums exhibit lower mortality and fewer acute symptoms when exposed to salinity stress in the rootzone compared to peaches (Nasr et al., 1977; El-Motaium et al., 1994). The risk of salinity stress for crops in Florida is exacerbated by heavy precipitation, rising sea levels, and storm surge (Papacek et al., 2020; Wdowinski et al., 2020). These events can lead to a rise in the water table, which can prevent the downward flow of salts in the soil, leaving them in the rhizosphere (Boman and Stover, 2018).

A study involving salinity stress in citrus trees found that even root-knot nematode resistant rootstocks became susceptible to the nematodes when irrigated with highly saline water (Syvertsen and Levy, 2005). Operations located in coastal areas will need to mitigate the risk of high salinity with careful attention to production factors such as proper irrigation, drainage, and nutrient supply and balance. Operations located inland are not exempt

from the risk of salinity stress as periods of high temperatures without adequate irrigation or precipitation can lead to increased evaporation leaving behind excess salts in plant rootzones. While there is limited research concerning tolerance to high salt levels in irrigation water used for low-chill *Prunus* rootstocks, the increased risk of flooding in Florida due to severe weather events and heavy rainfall may motivate future researchers to investigate this.

## Conclusion

As climate conditions continue to deteriorate, researchers race to predict and understand the impact of our changing climate on agricultural crops, including peaches. The diverse nature of the *Prunus* genus has facilitated global efforts to optimize peach production to various environments. Florida's short winter season is abruptly met with an early spring season allowing growers to provide fresh peaches to the consumer, while other domestic competitors do not have ripe fruit. Despite the progress made to perfect peach production in Florida's environment, there is still much work to be done to ensure the economic viability of commercial peach production in Florida. Environmental constraints to peach production such as pathogen pressure, salinity stress, flooding, and heat stress complicate the successful cultivation of peaches in Florida. The UF/IFAS Stone Fruit Program is considering these obstacles and working towards expanding our knowledge of how low-chill *Prunus* spp. respond to these stresses, with the ultimate goal of developing low-chill peach rootstocks that are tolerant to one or a combination of the aforementioned biotic and environmental stresses.

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Fig. 3. Rootstock cultivar, 'Flordaguard', response to waterlogged soil. Photo credit: T. McGee.

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## ‘O17-19-1’, An Early Ripening Self-fertile Hybrid for Muscadine Table Grape

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**Additional index words.** fruit size, ripening diversity, yield, scar

‘O17-19-1’ is an early ripening muscadine breeding line developed in 2011 at the Center for Viticulture, Florida A&M University, by crossing ‘Majesty’ × ‘Ison’. Its fruits ripen in the end of July to early August in Tallahassee, Florida, about 2–3 weeks earlier than most muscadine grapes. This breeding selection is self-fertile, productive, uniform in fruit ripening and fruit size, moderate in vine vigor, and disease resistance. The fruits of ‘O17-19-1’ are attractive with an elongated shape (1.14 L/D) and smooth skins. The fruits average 11.1g, with a sweet, crunchy taste.

Muscadine (*Vitis rotundifolia* Michx.) grape is a multimillion-dollar industry in the southeastern United States. The grapes are grown for processing and the fresh grape or table fruit market. With the steady growth of the industry and market expansion over the past decades, the diversity of muscadine grapes has become more urgent than ever. This is special true for table muscadine grapes, due to more diversified consumers today and the short postharvest shelf life of muscadine grapes. Unlike the cluster harvesting of bunch grapes, muscadine grapes are harvested individually without a stem by detaching the fruit from their stems or rachis. This separation results in either a wet or dry scar on the skin of every fruit. The dry scar fruit used for fresh market have minimal damage to the skin with no apparent juice leaking. Since skin integrity is compromised, the scar could provide an entrance into the fruit for mold pathogens, which could cause fruit to spoil or rot and make storage very difficult. Current table muscadine grapes are overwhelmingly are only a few mid-season ripening cultivars such as ‘Fry’ and ‘Ison’, due to their large fruits, satisfactory yields, and good fruit tasting. This mid-season concentration of production could easily create problems during harvesting season, since it is difficult to use the storage system used with bunch grapes. Because of the short postharvest shelf life nature of muscadine grapes, the muscadine grape industry has been trying to solve this problem through cultivar diversity with different ripening or harvest dates. Unfortunately, the effort has not been successful due to the lack of commercially available cultivars.

To provide the industry with these cultivars, in recent years FAMU’s grape breeding program has been working to develop table muscadine grapes with different ripening dates. ‘O17-19-1’, is a breeding line we developed recently. It possesses four key traits as a table grape: early ripening, self-fertile, large fruit, and high yield. Together with other preferred horticultural characteristics, this breeding line has shown good potential for table muscadine grape industry.

### Origin

‘O17-19-1’ originated from the grape breeding program at the Center for Viticulture and Small Fruit Research (CVSFR),

Florida A&M University (FAMU), Tallahassee, FL (30°28’42”N; 84°10’21”W). It is a hybrid of ‘Majesty’ × ‘Ison’ in 2009, which was selected in 2015. ‘Majesty’ is a newly patented (2011) muscadine cultivar developed by the CVSFR which produces very large black-red colored fruits weighing about 3–4 grams more than the largest muscadine varieties. ‘Majesty’ bears female (pistillate) flowers and requires pollinators to set fruits. ‘Majesty’ is a hybrid between ‘Supreme’ (US Plant Pat. No. 7,267) and ‘Triumph’ (unpatented). ‘Ison’ was released by Ison’s Nursery and Vineyard (Brooks, GA) in 1986. It is self-fertile, uniform in ripening, and a leading cultivar for the table muscadine grape industry. It can ripen a few days earlier than other midseason cultivars.

The early ripening trait with larger fruits of ‘O17-19-1’ was first observed in 2014. Its self-fertile flowers were confirmed in 2015 and further evaluation started then. Two leading table cultivars, ‘Fry’ and ‘Ison’, have been compared with ‘O17-19-1’ for major horticultural characteristics.

Evaluations were conducted at the vineyard in the CVSFR. Vines were planted at density of 10 ft in rows and 12 ft between rows, trained into single-wire bilateral cordon system. Vines have been annually pruned with 3~4 buds spur-pruning technics. Commercial vineyard management have been applied to the vines.

### Major Characteristics

**EARLY UNIFORM RIPENING.** ‘O17-19-1’ fruits ripen from late July to mid-August in Tallahassee, FL, which makes it one of the earliest muscadine grapes, if not the earliest. Its harvest period is about 10 days, which is short compared with other muscadine grapes. In comparison, the harvest dates of ‘Fry’ and ‘Ison’ start from late August to early September, which are about two to three weeks later than ‘O17-19-1’ (Fig. 1, Table 1).

**FLOWERS.** ‘O17-19-1’ bears hermaphroditic (perfect, self-fertile) flowers (Fig. 2). Inflorescences typically grow at the 3rd and 4th nodes; there are about 75 individual flowers in a flower cluster.

**FRUIT CHARACTERISTICS.** *Larger fruit.* ‘O17-19-1’ fruit averaged 11.1g, which is larger than ‘Ison’ (Fig. 3, Table 2), though the fruits are smaller than those of the gynoecious cultivar ‘Fry’. On the other hand, the self-fertile flower trait may make ‘O17-19-1’ more attractive to the grape industry.

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Table 1. Horticultural characteristics of O17-19-1 and leading table muscadine cultivars in Tallahassee, FL.

Cultivar	Flower	Fruit ripening	Uniform ripening	Vine vigor	Pruning wt <sup>†</sup> (lb/vine)	Internode length (cm)	Internode diam. (cm)	PD score (0–5)	Ripe rot rate (%)
Fry	f <sup>‡</sup>	early Sept.	even	m	7.4	3.8	1.6	0	18.9
Ison	p	end Aug.	even	m–h	10.7	4.1	1.7	0	5.2
O17-19-1	p	end Jul. ~ mid Aug.	even	m	7.1	4.0	1.6	0	5.5

<sup>‡</sup>f = gynoecious (female) flowers; p = perfect flowers.

<sup>†</sup>10-ft canopy vines with single-wire bilateral cordon training system.



Fig 1. Fruit veraison of 'Ison' (left) and fruit ripening of 'O17-19-1' (right) around 1 Aug. 2021.



Fig 2. Self-fertile flowers of 'O17-19-1'.



Fig 3. Fruit of 'O17-19-1' (L), 'Fry' (M), and 'Ison' (R).



Fig 4. Semi-compact clusters and fruits of 'O17-19-1' at harvest.

**Fruit SSC.** The fruit soluble solid content (SSC) of 'O17-19-1' was 15.5%, which is close to 'Ison' but somewhat lower than 'Fry' (Table 2).

**Pleasant taste.** 'O17-19-1' fruits have a moderate aroma and a neutral sweet taste with semi-firm flesh texture, edible skin. This produces an overall pleasant taste.

**Fruit appearance.** The fruits of 'O17-19-1' are attractive with their obtuse-ovate shape (1.14 L/D, Table 2) and smooth skin. The color of 'O17-19-1' fruits is dark purple to near black at peak ripeness with inconspicuous lenticels (Fig. 4).

**Fruit dry scar.** Average dry scar rate of 'O17-19-1' was 88% from 2018 to 2021, which is similar to 'Ison', but higher than 'Fry' (Table 2).

**Ripe rot.** The ripe rot rate of 'O17-19-1' was 5.5%, which is close to 'Ison', but lower than 'Fry' (Table 1).

**Storage quality.** Studies with 'O17-19-1' during a 2-week storage at 75 °F showed that marketable fruits after 1 and 2 weeks in storage were 79% and 34% respectively. This was virtually the same as 'Ison', and much higher than the 48% and 11% marketable yields of 'Fry' (Table 2).

**MEDIUM SIZED AND SEMI-COMPACT CLUSTERS.** The semi-compact clusters of 'O17-19-1' are midsized with eight fruits on average (Table 2, Fig. 4). The semi-compact clusters may allow for better air flow inside the clusters than the very compact ones of most muscadine grapes, which may reduce disease.

Table 2. Productivity and fruit characteristics of 'O17-19-1' and leading table muscadine cultivars in Tallahassee, FL.

Cultivar	Fruits/cluster	Dry scar rate (%)	Yield <sup>‡</sup> (lb/vine)	Fruit shape (L/D)	Fruit size (g)	SSC (%)	TA (%)	Commercial fruit % at 75 °F	
								1 week	2 week
Fry	7.3	81.3	32	0.99	11.8	16.3	0.40	48	11
Ison	10.0	86.0	37	1.10	9.8	15.8	0.45	77	35
O17-19-1	8.0	88.8	31	1.14	11.1	15.5	0.41	79	34

<sup>‡</sup>10-ft canopy vines with single-wire bilateral cordon training system

**PRODUCTIVITY.** from 10-ft of 'O17-19-1' vines on a single-wire bilateral cordon training system. This is similar to 'Fry', but lower than 'Ison' (Table 2).

**GROWTH HABIT.** 'O17-19-1' is a moderately vigorous vine. Its shoot internode length and diameter are 4.0 cm and 1.6 cm respectively, which is similar to 'Fry' and 'Ison' (Table 1). The pruning weight from a 10-ft canopy vine of 'O17-19-1' 7.1 lb, which is close to 'Fry', but lower than 'Ison' (Table 1). The shoots of 'O17-19-1' tend to grow horizontally with a somewhat semi-erect growth.

**ANNUAL GROWTH CIRCLE.** In Tallahassee FL, 'O17-19-1' bud breaks from late March to early April. It blooms in late May, fruit

veraison starts in early July, fruits ripen from late July to early August, and leaves fall in late December.

**SYMPTOMS OF DISEASE.** Pierce's disease (PD) symptoms have not been observed on 'O17-19-1' vines during the evaluation period, while a few black rot symptoms on leaves could be observed during or after harvest.

In brief, the primary advantage of 'O17-19-1' is early ripening, together with its self-fertile flower, large fruit, and satisfactory productivity that industry always looking for. This breeding line has demonstrated good potential to produce early ripening table muscadine grapes.



—Scientific Note—

## Genotyping-by-sequencing of Florida Passion Fruit Germplasm Reveals Accession Relationships

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**Additional index words.** diversity, *Passiflora*, phylogenetics

The passion fruit genus, *Passiflora*, comprises more than 500 species that are admired for their ornate flowers and tropical-tasting fruit. The yellow or purple colored *P. edulis* Sims. is the most commercially important. Passion fruit vines are challenged by both abiotic and biotic stresses. Understanding the available genetic diversity allows breeders to combine favorable traits into elite cultivars.

Understanding diversity within a breeding germplasm is necessary for knowing how and when to use specific accessions. Molecular markers assist in breeding by enabling informed decisions related to parent selection, confirming parentage of potential hybrids, identifying mislabeled accessions and in resolving species assignments. The purpose of this study was to develop single nucleotide polymorphism (SNP) markers to characterize a passion fruit germplasm collection with an emphasis on native passion fruit species.

### Materials and Methods

Plant DNA was extracted from 59 *Passiflora* accessions from 20 different species using a CTAB protocol. DNA samples were sequenced by the University of Minnesota Genomics Center with the restriction enzymes PstI and MspI. The resulting 78 million sequenced reads went through a bioinformatics pipeline to perform quality filtering, genome alignment, variant calling, and variant filtering, resulting in around ~5000 SNPs (single nucleotide polymorphism) available for downstream analyses.

### Results and Discussion

The ~5k SNPs had uneven coverage across the nine chromosomes (Fig. 1). The average polymorphic information content i.e. the information value for SNPs, was 0.25. When considering *P. edulis* alone ~1300 SNPs were found to be polymorphic and could be used further in breeding and genomic studies to understand the genetic control of phenotypes. The average heterozygosity was 0.09 for *P. edulis* yellow fruited types (n = 15), 0.11 for purple-fruited types (n = 7), and 0.11 overall (n =

26, includes types with unknown fruit color). Lower values of heterozygosity indicate a smaller amount of diversity within the population analyzed.

A phylogenetic analysis based on identity by state resulted a dendrogram of groups of similar species and accessions. In the dendrogram a mislabeled accession was identified, all *P. edulis* grouped together both yellow and purple-fruited types. Eight passion fruit species native or invasive to Florida (including *P. biflora*, *P. foetida*, *P. incarnata*, *P. lutea*, *P. multiflora*, *P. pallens*, *P. sexflora*, and *P. pallida*) were well-separated as expected into subgenus (*Passiflora* and *Decaloba*) groupings. Four *P. laurifolia* seedlings showed patterns likely the resulting of a self-pollination. Interspecific hybrids between *P. edulis* and *P. incarnata* were confirmed. The resulting data can be applied to future passion fruit research and breeding both between species and within the commercial species.

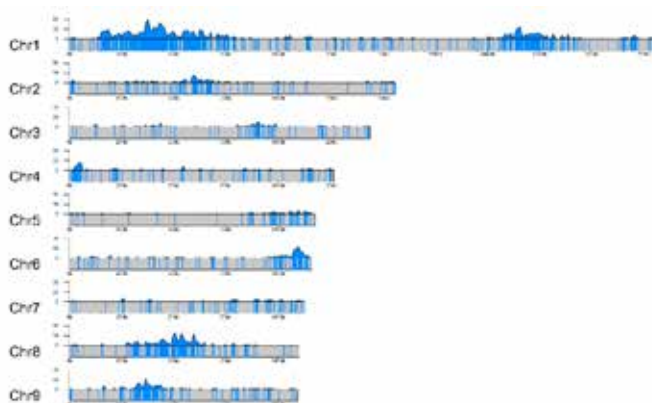


Fig. 1. SNP marker density mapped onto the nine chromosomes of the *P. edulis* genome.

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—Scientific Note—

## Nitrate and Electrical Conductivity Monitoring for Blueberry Production with Five Nitrogen Rates

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**Additional index words.** blueberry production, nitrogen management, soil sensors

Production of blueberry is rapidly expanding in Florida due to its profitability and nutritional value. Nitrogen is the most crucial nutrient element for blueberry production. The goal of this study was to monitor soil nitrogen changes with soil chemical analyses and soil sensors after nitrogen fertilizer application. Two southern highbush blueberry varieties were used in this study: ‘Emerald’ and ‘Farthing.’ The trial was carried out at the University of Florida, IFAS Plant Science Research and Education Unit in Citra, FL. Five nitrogen rates were used: 50, 87, 125, 200, and 300 lb/acre N. Nitrogen fertilizer as urea was applied in even applications on 15 Mar., 5 Apr., 26 Apr., 17 May, 7 June, 28 June, 19 July, 9 Aug. 9, and 20 Sept. 2021. Soil samples were collected from the five treatments four times, approximately 14 days after each split nitrogen application. The nitrate analysis was done by a commercial lab. The soil sensors were installed in two treatments: 125 and 300 lb/acre N at three depths: 6, 12, and 30 inches. Soil moisture and EC were monitored and recorded with dataloggers from March to July. The results show that: 1) soil nitrate content increased from 0.1 mg/kg in early growth stage to 8 mg/kg in late growth stages; 2) soil nitrate levels also increased significantly with N application rate; and 3) soil nitrate levels were relatively similar with soil depth for the first soil sampling, but decreased significantly with soil depth for the other soil samplings except 23 Apr. (Fig. 1). There was not much difference between the two varieties except for the first sampling, where ‘Emerald’ had significantly lower nitrate level than ‘Farthing’. The soil sensor data was noisy but did show some dynamic changes in EC with 125 lb/acre N (Fig. 2). The top 6-inches of soil always had the greatest nitrate content. When N fertilizer was applied, there was an increase; however, there was no pronounced increase for the 19 July application because there was heavy rain (6.5 in.) just two days after the N application. These data show that over fertilization of N results in significant leaching away from the root zones. The EC monitoring data suggest that growers need to check the weather report to minimize N loss through leaching before making any fertilizer applications.

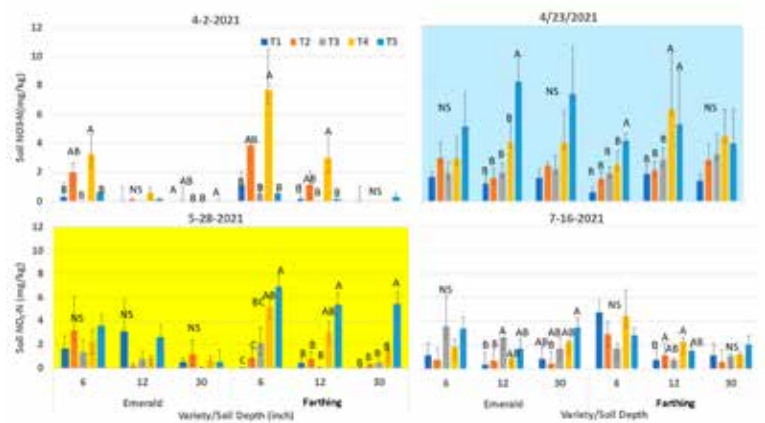


Fig. 1. Soil nitrate contents between ‘Emerald’ and ‘Farthing’ with five nitrogen rates (50, 87, 125, 200, and 300 lb/acre N) at three depths (6, 12, and 30 inches).



Fig. 2. Soil EC monitoring for ‘Emerald’ with a nitrogen rate of 125 lb/acre N at three depths (6, 12, and 30 inches). The arrows indicate the dates of N application.

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—Scientific Note—

## A Look at Vigor Management Options for Growing Peaches in Florida's Subtropical Climate

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**Additional index words.** postharvest management, stone fruit

The postharvest growing season for peaches in Florida can last up to 200 days, requiring that growers manage pest and disease pressures to maintain a healthy tree canopy. A larger canopy reduces air movement, reduces spray penetration, and increases shading. If left unmanaged, the management of the bearing surface of vigorous trees require ladders to prune or harvest fruit effectively. Hand pruning can become cost-prohibitive, thus alternative methods like root pruning could be of use pending the development of an effective management strategy.

Plants generally maintain a shoot:root ratio. Altering either the shoots or the roots could lead to the manipulation of the other as the plant regains the original balance. Summer pruning should keep trees at a manageable size as energy goes towards regrowing shoots to achieve the shoot:root balance. It is thought that root pruning could cause the shoots to have reduced growth as energy is shifted to root growth in an attempt to regain the shoot:root balance. Root pruning is a more common practice with apple than with other orchards. Root pruning done as early as the 1980s, and has been found to be most effective when done during the late dormant part of the season. It was also found that the degree of growth control was greater the closer to the trunk of the tree the pruning was done. However, not many studies have been conducted on root pruning in *Prunus* species. Thus, the effects of root pruning should be evaluated as it might be an effective means of vigor control for other cultivars of peach, pending appropriate trials. Some of the other management techniques that we evaluated consisted of dwarfing rootstocks and the use of prohexadione-calcium to block gibberellin biosynthesis. Our evaluation of rootstocks was brief as only one rootstock is cur-

rently recommended for use in Florida due to its resistance to rootknot nematode *Meloidyne floridensis*. Additional rootstocks are currently being trialed for use in Florida, however none have been tested across the entire state and so cannot be recommended for commercial use. 'MP-29' is a standout among those being trialed as it has promise for being tolerant to flooding.

The current lack of compact scion varieties and/or dwarfing rootstocks recommended for commercial use in Florida means that growers must use alternatives such as chemical or cultural techniques to control canopy size and shape. The use of PGRs that block GA biosynthesis could result in shorter internode length and ultimately reduced overall vegetative growth. Kudos 27.5 WDG (Fine Agrochemicals, Worcester, UK), which contains the active ingredient prohexadione-calcium, is a product labeled for use in apples and sweet cherries. It inhibits gibberellin production resulting in reduced internode elongation, ultimately reducing tree growth. Its use as a plant growth regulator is more common in apple orchards than in stonefruit. Prohexadione calcium could be used to reduce stem length in peach for growers trying to manage vigorous growth during Florida summers.

In review, Florida growers need a toolkit of cultural and chemical techniques as they continue to explore the opportunities for subtropical stone fruit production. Summer pruning techniques have been developed, alternative rootstock options are being trialed, treatments such as root pruning and prohexadione calcium are all being tested. In the future we hope to provide recommendations for growth control of peach trees under Florida growing conditions.

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—Scientific Note—

## Establishing a Domestic Vanilla Industry

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**Additional index words.** extension, genomics, plant breeding, *Vanilla planifolia*

Vanilla has potential to be among the highest grossing agricultural commodities in Florida. Spices like vanilla comprise a small portion of our diet, but have major impacts on the sensory quality of our food. Vanilla extract comes from the cured beans of either *Vanilla planifolia* or *V. x tahitensis* as legally defined by the FDA. *V. planifolia* is native to North and Central America, but Madagascar is today's leading vanilla grower. Domestic vanilla production is becoming increasingly attractive as international supplies are perennially strained and demand for vanilla extract increases as global food companies pledge to remove artificial ingredients from their products. Most vanillin, the primary flavor component of vanilla extract, is chemically synthesized, but vanilla extract has the potential to support growers in Florida, Hawaii, and Puerto Rico striving to meet an evolving consumer base favoring local, organic, and natural products. Vanilla is somewhat unique in that the species not been domesticated through plant improvement, and today's industry relies on cultivated, wild clones. Since 2016, we have collected around 300 vanilla accessions for trialing in Florida. The primary objective of the vanilla collection was to identify adapted types that are virus-free and that grow well in Florida. This presentation will describe our 1) preliminary results from characterizing the vanilla collection, 2) efforts to connect with growers, and 3) genomics and breeding work to develop superior vanilla cultivars.

### Materials and Methods

The living vanilla collection of almost 300 accessions has been characterized using genotyping-by-sequencing with ~5,000 single nucleotide polymorphism DNA markers. Additionally, hundreds of vanilla samples from other countries were also analyzed. Vanillin content was analyzed using high performance liquid chromatography. Grower communication through public talks and a symposium were used to disseminate information. The full genome of *V. planifolia* 'Daphna' was generated and is now being used to identify gene candidates for priority traits.

### Results and Discussion

Genomics has greatly improved our understanding of vanilla diversity, resolved species assignments, and identified hybrids. Our recent work has divided *V. planifolia*, the commercial species, into three distinct types based on heterozygosity levels and SNP patterns. Future work will include deep phenotypic characterization of these types. Vanillin content of Florida-grown vanilla

beans averaged 3.5% vanillin (dry weight) for the 2021 harvest. This exceeds current industry standards and shows promise for domestic vanilla production of exceptional quality vanilla beans.

We continue our efforts to provide scientifically-validated information for vanilla growers in southern Florida and beyond (Fig. 1). Our Vanilla Orchid Symposium in 2021 attracted ~100 participants. We are currently supporting mass propagation efforts to generate commercial quantities of vanilla planting material as we design best practices for vanilla bean curing that will exceed domestic food safety requirements.

The publication of the complete vanilla genome supported discovery research that could lead to increased vanillin content in improved vanilla cultivars. This is a critical tool that will make all breeding activities more efficient. We anticipate continuing our research to develop vanilla cultivars with improved traits to support domestic production.

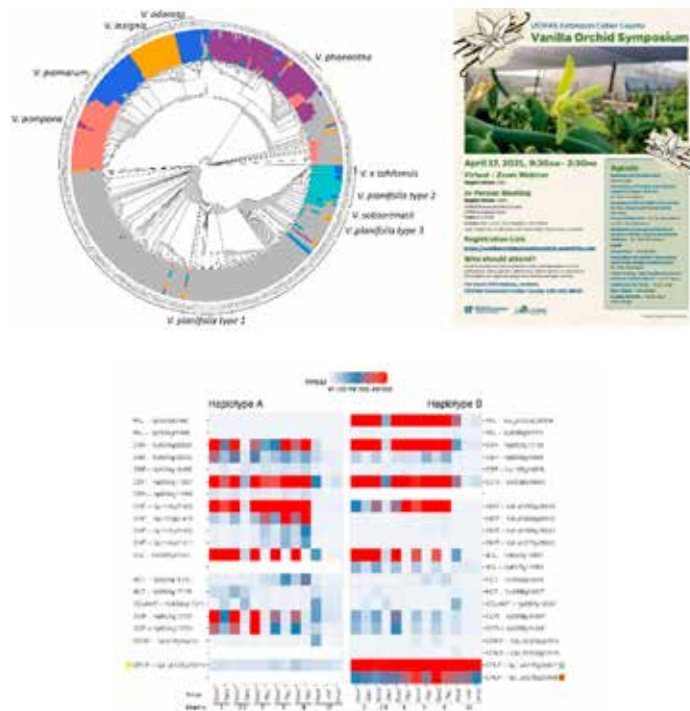


Fig. 1. Vanilla diversity (top left), Vanilla Orchid Symposium flier (top right), and vanillin biosynthetic pathway (bottom).

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—Scientific Note—

## An Introduction to a Breadfruit Grove in Big Pine Key, Florida

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Breadfruit (*Artocarpus altilis*), whose origin may be from the Malay Archipelago, but is best known from the South Pacific, is frost intolerant and grows only in truly tropical regions (70 to 100 °F), an annual rainfall of 80 to 100 inches and 70 to 80% relative humidity.

In 2013, Patrick Garvey purchased Grimal Grove in Big Pine Key, FL (USDA hardiness zones 11a to 11b), the southernmost tropical fruit park in the United States. He began an intense volunteer program to restore the abandoned property that had become a dump site (Garvey, 2016) working with The Growing Hope Initiative, a non-profit organization which engages and transforms sustainable communities. Grimal Grove is in the lower Keys, about 97 miles southwest of mainland Florida. Elevation is 6–7 ft; mean annual precipitation ~50 in, most occurring between May and October. (Gato, et. al. 1991, CoCoRaHS, 1998). The soil in this area is a very gravelly loam formed from oolitic limestone, with depths from 0–4 inches a pH of 7.4–8.4 (Hurt, et. al., 1995). Permeability is moderately rapid, though flooding can occur during tidal fluctuations and spring tides.

Large raised beds, originally created by the grove's original owner Adolf Grimal, help support the tropical fruit forest that was re-planted by Garvey after years of neglect. In 2017, just as the grove was beginning to thrive, Hurricane Irma, a powerful category 4 hurricane, made landfall on Cudjoe Key, just 10 miles to the south, leveling almost all the trees on the property; a 3-ft saltwater storm surge engulfed the property. One of the few trees that survived, was a 'Ma'afala' breadfruit gifted to Garvey in 2014. Irma topped the tree to just 7 ft from its previous height of 20 ft. Within 18 months, the tree was fruiting again. In 2021, Garvey harvested approximately 200 breadfruit from this single tree.

### Current Production

Today, there are approximately 30 breadfruit trees on the 2.5-acre property with plans to expand. Most of the trees are the coastal variety 'Ma'afala', which is more drought and salt tolerant, but also include five 'White' and one each of 'Ulu fiti', 'Puaa', and 'Mei'. 'Ma'afala' outperforms the other varieties. Propagation can be challenging with air layering being most successful. Depending on the time of year and rainfall, air layers are ready to be removed from the parent tree in 6 weeks to 2.5 months.

Trees receive very little supplemental water except for 10 min on a drip irrigation system twice a day, which is essential the first year after planting. Trees are fertilized twice a year with an 8–3–9 proprietary fruit blend and are mulched regularly with a utility mulch that is a mix of tropical hardwoods. Pest pressures have been minimal to non-existent.

Interest in the breadfruit grove continues to grow. Garvey has received requests from three different wholesale nurseries in South and Southwest Florida for finished air layers and 3-gal trees. Currently, he has 150 potted trees ranging from 3- to 15-gal and 150 air layers. Garvey is also the South Florida distributor for Mutiny Island Vodka, a breadfruit-based vodka company based in St. Croix, USVI. Mutiny Island Vodka uses breadfruit to help reduce world hunger and build food secure communities in collaboration with The Breadfruit Institute and Trees that Feed Foundation. Discussions underway for a potential breadfruit distillery in Key West.

### Future Potential

Grimal Grove is a case study of potential future breadfruit production in south Florida as climate change contributes to rising temperatures and disruption of food availability. Garvey is encouraging people to plant more breadfruit trees with the hope of generating more local interest in the fruit and creating economic opportunities. A study is underway in eight South Florida counties with 32 backyard breadfruit growers.

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—Scientific Note—

## **UF–IFAS Farmworker Safety Training Program During Global Pandemic: Lessons Learned on How to Reduce Risks and Protect Your People**

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**Additional index words.** farm labor, health risk management, pandemic, worker safety

Faced with the onset of the human health pandemic due to COVID-19 virus in early 2020, faculty and staff at the University of Florida, Institute of Food and Agricultural Sciences (UF/IFAS) Southwest Florida Research and Education Center (SWFREC) and the Farm Labor Supervisor (FLS) team rapidly collated and conveyed information to farm labor supervisors about how best to ensure the safety and well-being of the 150,000+ migrant and seasonal farmworkers and their families who travel annually to work on Florida farms and ranches. Florida Department of Health (FDOH) and the U.S. Bureau of Labor Statistics data indicated that four of the ten Florida counties with the highest number of COVID-19 cases were also home to the highest numbers of domestic and/or H2A crop workers. According to the Florida Department of Agriculture, in 2012 the seven counties with the highest number of these workers sold 3.179 billion dollars of agricultural products.

The UF/IFAS FLS COVID-19 Farmworker Safety program provides science-based information and best management practices to farm labor supervisors that ensure farmworkers are aware of, and take measures to adopt, Center for Disease Control (CDC) recommendations on COVID-19 monitoring and safety measures, including hand-washing, face-coverings and social-distancing both on and off-farm. The program goal is to protect farmworker health and livelihood while providing essential services to Florida's agribusinesses. The authors intend to share our findings of the impact and significance of these COVID-19 trainings with academic and industry colleagues with the goals of raising awareness of the value and importance of research and educational efforts aimed at protecting the health and wellbeing of farm employees to the long-term viability of the US food supply chain.

### **Materials and Methods**

The FLS team developed content and hosted several 90-minute Zoom webinars conducted concurrently in English (n = 600) and Spanish (n = 200) from August through October 2020. Our target audience included farm labor supervisors, farm owners, and farm managers. Initially one webinar was offered, however, in response to demand from the Florida agricultural industry, the team offered an additional five training dates to accommodate farm owners and farm labor supervisors, as farmworkers returned for the fall/winter seasons. Given the high level of importance of reaching as many supervisors with this critical information needed to protect the health and safety of the farmworkers, the FLS-funded program offered the webinars at no cost to participants. Live session participants each receive a certificate of attendance, and a list of references to help them source supporting materials and resources specific to protecting farm workers from COVID-19 infection and limiting transmission through best management practices recommended by the CDC.

An evaluation was conducted using Qualtrics to measure changes in participant knowledge levels regarding COVID-19 pandemic and farmworker health and safety protocols and to collect demographic information. A link was provided in the chat box and attendees were encouraged throughout the webinar, and in follow-up emails, to share their feedback with the FLS team.

### **Results and Discussion**

A total of 83 (10.4%) responses were recorded and generated evidence-based data indicating the impact of the program. Sixty percent (60%) of attendees were male; 48% indicated they were of Spanish, Hispanic, or Latino origin; 3% were Black/African-American; and 49% White/Caucasian. Evaluation responses

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generated the following evidence-based data indicating the impact of the program:

- 98% agree/strongly agree with the statement: “As a result of this workshop, I know of additional resources I can access about COVID-19 exposure, symptoms, and prevention while working on farm.”
- 100% agree/strongly agree with the statement: “As a result of this workshop, I know of people I can go to for further support about protecting and caring for my health while on the job, at home, and in public places, during the COVID-19 pandemic.”
- 93% indicated they already practice social distancing (> 6 feet).
- 72% indicated they already wear a face covering while working, and an additional 28% indicated they intend to wear a face covering while working as a result of attending this training.

Attendees were asked to rate their level of knowledge “before” and “after” the training on six training topics. On average, knowledge levels improved for each topic after attending the training (Fig. 1).

Updated COVID-19 Farmworker Safety webinars were offered in May 2021, shared at the 2021 SW Florida Citrus and Vegetable Expo, and are included in current UF/IFAS-SWFREC FLS Basic Training 101: Administration and Safety program as the pandemic continues to unfold into the 2021–22 harvesting season. We continue to post daily Frequently Asked Questions (FAQs) responses to industry questions received during and after the trainings, along with the latest information on farmworker COVID-19 testing sites offered statewide and any UF/IFAS, CDC, Florida Departments of Health, Labor and Agriculture and Consumer Services updates, via our Facebook site <<https://www.facebook.com/FLSTraining15>> and posted on the UF/IFAS-SWFREC YouTube channel <<https://www.youtube.com/user/TheSWFREC>>.

Interestingly, nearly 78% indicated they had never attended previous UF/IFAS Farm Labor Supervisor Training, which began in 2005 and has had more than 1300 participants earn Certificates of Completion. This finding suggested to the authors that we are able to attract our farm labor supervisor target audience when the program content is aimed at current worker health and wellbeing needs. Participant comments noted that the program helped them feel more confident about ways to provide and maintain safe on- and off-farm environments, better understand how COVID-19 affects agricultural businesses, and receive access to best practices from a trusted authority that would help reduce negative impacts of the virus in the workplace and local communities. Our evaluation feedback motivated our team to recommend farm owners labor supervisors to establish a written, public-facing COVID-19 Prevention Strategy, to include at a minimum: screening (temperature/wellness) procedures; cohort groupings to minimize spread and ease contact tracing; quarantine procedures and facilities; provision of masks, personal protection equipment (PPE) supplies, and equipment; document COVID-19-related expenses; and access to equitable vaccination and/or testing incentives.

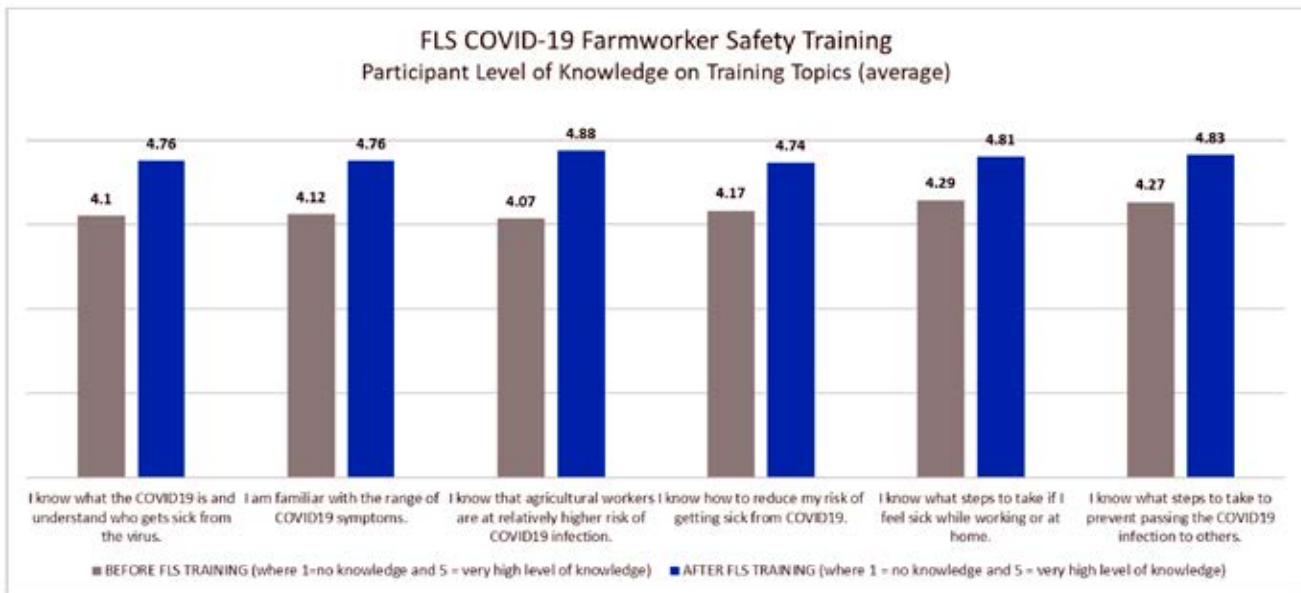


Fig. 1. Average participant level of knowledge on training topics.



## **Ground Cover Growing Systems for Tea Production in Florida**

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### **Abstract**

Tea, the second most consumed beverage in the world after water, is produced by brewing leaves of *Camellia sinensis*. Florida has a suitable climate and soils for tea production. As the Citrus industry in Florida is affected by greening disease, tea is proposed as an emerging new alternative crop. Our research aims to find sustainable production methods for growing tea under Florida field conditions. To understand potential impacts of cover crops on tea production, in 2018 three tea accessions ('Large leaf,' 'Red leaf,' and 'Fairhope') were installed at two sites in north central Florida to test three groundcover types: perennial peanut, a crimson clover rotation, and weed-barrier cloth. Plant growth, survival, insect damage, and disease incidence and severity were observed over the following two years. The effects of the plant variety and groundcover type were examined. As of Summer 2020, 'Large leaf' had the highest survival rate, while the weed barrier cloth treatment experienced lowest survival rate among groundcovers. In Spring 2021, the weed barrier cloth treatment had the lowest incidence of insect damage and disease. The 'Fairhope' accession experienced the highest incidence of insect damage, but lower incidence of disease compared to 'Large leaf.' Two shade tree species—persimmon and mulberry—were established during this experiment, so that future work could explore the performance and impact of different shade trees as well as other components of the production system.

The abstract was presented at the 2021 FSHS Annual Meeting.

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## National Mango Board's Tools for the Mango Industry

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The Research & Industry Relations program of the National Mango Board helps the entire mango supply chain deliver a quality product to the U.S. consumer through research and extension meetings to help educate growers, shippers, packers, importers, retailers, and others. This program is divided into sub-programs that address key concerns of the mango industry. One such sub-program is Food Safety and Sustainability, which aims to provide science-based information to the mango industry using various tools to enhance the safety of mangos. It engages producers, packers, and other members of the mango industry in reducing their environmental and social footprints with easy-to-implement sustainability practices. These tools are versatile and are encompassed into both the mango food safety and mango sustainability websites. What is food safety? How can one deal with physical, chemical, and biological risk factors within the facility or farm? How sustainable is the farm and/or packinghouse? How can one become more sustainable? How can one create and implement an action plan that will lead to better sustainable practices? Both holistic and detailed answers are provided within the website and are available for download on smartphone, tablets, and computer devices at no cost. These tools are didactic and can be used to train and educate all personnel on the good manufacture and agricultural practices as well as the value and benefits of adopting sustainable practices.

The National Mango Board (NMB) is a national promotion and research organization supported by assessments from both domestic and imported mangos with oversight by the United States Department of Agriculture (USDA). The mission of the NMB is to increase the consumption of mangos in the US by uniting the industry and strengthening the mango market through the implementation of different programs such as Industry Relations, Marketing, and Research. Through the Research and Industry Relations (RIR) program the NMB creates tools that are intended for the mango industry. These tools allow industry members to not only connect with one another but also, to get quality information about the industry, which will in turn help them make educated decision regarding their respective business.

- How many boxes of mango reached the different US ports of entry?
- Where did those mangos come from?
- What varieties are in season?
- How to connect with members of the industry?
- What are the prices given at the ports of entry?

These are but few of the questions that the tools created by the NMB. They are available on its website <[www.mango.org](http://www.mango.org)>. The NMB however, does not have the authority to control mango volumes or mandate quality standards. It does not interfere in any way with the mango market and is not directly able to impact price. The NMB does not advocate for or against any quarantine treatments. The role of the NMB is to support the industry in its efforts to provide quality mango and educate U.S. consumers.

This paper intends to provide insight into the tools available for the benefit of the mango industry members. A brief description of the most used tools will be presented as well as “how-to” properly navigate them. For any subsequent question and inquiry, it is highly recommended to reach out to the NMB for further assistance.

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### Supplier Database

Levin (2016) in his final literature review report for the NMB found that mangos are produced in over 90 countries worldwide. Between 1996 and 2005, production grew at an average annual rate of 2.6%. The ten leading mango producers during the years 2003–05 include Mexico (5.5%) in fourth place and Brazil (4.3%) in seventh place. In terms of distribution, Mexico, Brazil, Peru, Ecuador, Haiti, and Guatemala supply the majority of mango imports to the North American market.

The mango industry, therefore, presents a heterogeneity that allows for versatility within the industry itself. This versatility can also create challenges when it comes to pinpointing certain members based on their role in the supply chain. The mango supply chain is made up of thousands of hard-working individuals from hundreds of companies both in the US and abroad. How the different members relate and connect to one another, is an important factor in influencing the demand for mango in the US. How can demand be increased? Brecht et al. (2020) say that it is by providing outstanding-quality mangos in the market that consumers will want to purchase again and requires a commitment to quality by each stakeholder involved in mango production and handling. To achieve outstanding quality mangos, all members must work in together. This is the main objective of the supplier database (Table 1) which represents the current members of the mango industry. However, as this is a very dynamic industry, should a role within the supply chain not appear in the categories listed above, a generic option is also provided.

### NMB Mango Crop Report

In 2000 the U.S. imported approximately 518 million pounds of whole mangos for an FOB value of \$140.7 million. In 2015, mango imports reached nearly 861 million pounds and were valued at \$401.1 million FOB. That is a 1.66-fold increase in volume and a 2.83-fold increase in economic value. On a per pound value basis, average FOB prices increased from 27.4 cents to nearly 46.6 cents through 2015. Since both prices and

Table 1. Categories in the supplier database form.

Growers outside the United States	Growers in the United States
Exporters	Importers
First Handlers	Wholesalers
Brokers	Custom Brokers
Educational institutions	--
Packers <sup>2</sup>	Fresh-cut Processors
Processor	--
Transportation	--
Nurseries	--

<sup>2</sup>Includes forced air ripening/conditioning.

volumes have increased over these years, positive shifts in U.S. demand for mangos have taken place (Ward, 2016). The weekly NMB Crop Report, sent via email subscription, provides an overview of the state of the mango market for the specific week being reported including: total volume; price/variety; counties in season and both seasonal and weekly projections. This allows the stakeholder to make informed decision regarding supply and demand. The email gives a snapshot of the entire report and a web link to the full report which is usually no more than 15 pages (Fig. 1). Mango availability throughout the year is divided into two sub-seasons, Spring/Summer and Fall/Winter with specific countries which are in season. For each country in season, about two pages are allocated explaining the status of export for that country and how much weight does it carry when compared to the overall information.

To receive the weekly Crop Report, subscribe on the NMB website. It will take less than two minutes.

### Digital Crop Report

The Digital Crop Report will launch later this year. It will have the same information as the current Crop Report provides but will also allow for the retrieval of retroactive data for past weeks and past seasons. The user can make comparisons and get a better understanding of both the volume and price on any given week and season. It also allows for looking at the volume by variety and size, a feature lacking in the current Crop Report. In Fig. 2

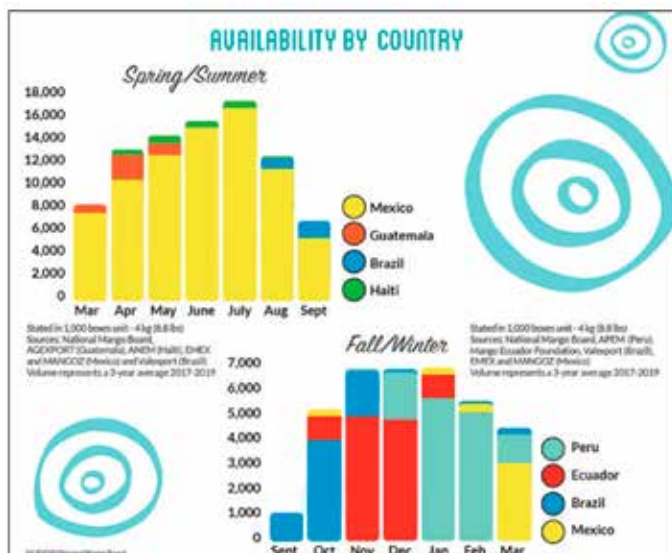


Fig. 1. Availability by country—NMB Crop Report. ©2020 National Mango Board and used by permission of the National Mango Board. All rights reserved.

we see an interactive graph available in the Digital Crop Report. By clicking on one of the legends, the corresponding line in the graph will disappear, giving way to the specific information requested. The graphs also can be downloaded as images for use in PowerPoint presentations.

The information in both versions of the Crop Report is always a week behind. The Report assigns numbers to the weeks.

### Research Section—NMB

The NMB strongly believes in the power of research for better decision making and boosting organizational credibility. The research committee, comprised of 11 members from the mango industry, directs and prioritizes the research projects to be conducted on behalf of the NMB. The research program at the NMB includes: Nutrition and Health, Production and Post-harvest, Industry Relations, Food Safety and Sustainability. All these programs can be found on the NMB website www.mango.org. For this paper, the area of Food Safety and Sustainability are explained in another section.

The Nutrition and Health program is designed to provide information about the phytochemical compounds and nutritive value of the main mango varieties consumed in the U.S. and their relation to human health.

The Postharvest Practices Research program will generate new knowledge to minimize postharvest losses through the mango supply chain in the U.S.

The Industry Relations program is designed to enhance industry communications and preparedness for a unified mango industry. The expected outcome of this program is to guide communications outreach efforts and initiatives to increase awareness and support for the NMB, and its programs. It also educates the mango industry and encourages better sharing of information.

Since 2005 the NMB has funded over 100 different projects in the abovementioned areas. The final reports of these projects have been submitted by researchers. Most of these projects are available in both Spanish and English for the convenience of industry members.

### Mango Sustainability Program

The production of some agricultural commodities, including the mangos, depends on natural and social resources. These include clean water, wildlife habitats, carbon sequestration, flood

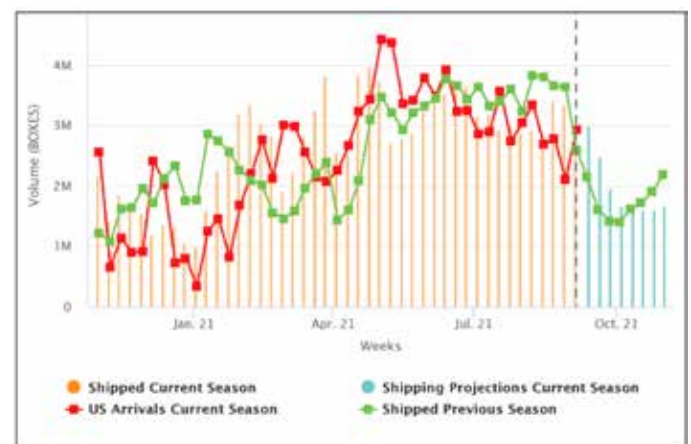


Fig. 2. Shipment, projected arrivals, and season projections. From: The Digital Crop Report, NMB website. ©2020 National Mango Board and used by permission of the National Mango Board. All rights reserved.



protection, groundwater recharge, landscape amenity value, and social well-being, which not only ensure a bright future for mango production and for people and the environment. As the needs of the mango industry change, the NMB is committed to supporting supply chain sustainability to ensure long-term growth.

“Sustainability is defined as the successful management of critical business risks for a business to continue to be profitable today and for the foreseeable future. It sees business opportunity in efficiency, adaptability and planning for current and future resource availability changes, as well as in improving the lives of its employees and nearby communities. Sustainability is imperative to economic success in today’s world and will become more so in the decades to come, as global supplies of nonrenewable resources dwindle, and demand grows” (NMB, 2017)

The Mango Sustainability Program (MSP) is a NMB initiative that engages mango producers and packers in reducing their environmental and social footprint with easy-to-implement sustainability practices. It is designed to ensure our industry has a positive impact on both the people and the environment.

The MSP provides an evaluation tool to industry members which enables them to gauge the internal practices and/or operations at the farm or the packinghouse. The tool does an analysis of current and, based on established parameters of sustainability, provides recommendations. At the end of the evaluation a score is provided along with recommendations either to maintain current activities and/or to encourage the adoption of new ones.

The Mango Sustainability Manual provides great insight into the sustainability approach. It is downloadable and easy to navigate.

#### Mango Food Safety Program

“In 2011, the Food Safety Modernization Act (FSMA) was signed into law and two rules will impact mango growers, packers, and those who import mangoes into the US. The Produce Safety Rule will impact growers and some packers. [...] Some mango packers, importers, and anyone involved in fresh cut or value-added mango products will fall under the Preventive Controls (PC) for Human Foods Rule, under FSMA. The PC rule requires all food handling companies have a Food Safety Plan, including a hazard analysis”. (Danyluk, 2016)

The Mango Food Safety website <[www.mangofoodsafety.org](http://www.mangofoodsafety.org)> is a tool that was created by the NMB to provide to the industry insight into the FSMA rule. It contains a Food Safety Training Kit, basic activities to help prevent mango contamination and a harmonized GAPs Food Safety plan template. This tool condensed the FSMA rule in a way that makes it easier to train farm and packinghouse employees.

The kit includes PowerPoint presentations which can be used in different formats. There is also an e-learning section which a short voice-video are explaining the “Do’s and Don’ts” when it comes to mango food safety as it relates to personal hygiene, washing hands, injuries etc.

Table 2. Useful tools from the National Mango Board.

- 
- **Register a company in the Supplier Database**  
<<https://www.mango.org/supplier-database-form/>>
  - **Subscribe to the NMB Crop Report**  
<<https://www.mango.org/professionals/industry/crop-information/mango-crop-report/>>
  - **Mango sustainability evaluation**  
<<https://www.mangosustainability.org/>>
  - **Download the manual**  
<<https://www.mango.org/wp-content/uploads/2018/04/NMB-2017-Workbook-English.pdf>>
  - **Food safety for mango growers and packers**  
<<https://www.mangofoodsafety.org/index.html>>
- 

### Conclusion

The tools created by the National Mango Board for the benefit of the mango industry contribute to fulfilling its mission: to increase awareness and consumption of mango in the U.S. (Table 2)

These tools connect the industry members, inform and educate consumers and members alike, provide crucial information for the mango market, and help keep the industry apprised on topics such as: FDA rulings; USDA requirements; sustainability, food safety and more.

The NMB is just a click away. Research studies, marketing strategies, finding a specific mango industry member, training materials, marketing materials, mango market information are all available to you.

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## ‘Rapoza’ A Potential Mango Cultivar for the Americas

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**Additional index words.** mango cultivars, *Mangifera indica*, Sechura desert

‘Rapoza’ is a large, high-quality mango cultivar (*Mangifera indica* L.) selected by Dick Hamilton of the University of Hawaii in the 1970s. A progeny of ‘Irwin’, it produces large, attractive, and excellent quality large red fruits weighing 600–800 g with desirable characteristics for a commercial mango. It is generally late bearing under South Florida conditions, where the fruit matures over a long period, (late July to October). It has good flavor, excellent disease resistance and good appearance. Trees are vigorous and productive with a rounded canopy. ‘Rapoza’ has gained attention in the past decade as a red mango alternative in the Americas with particular interest in Peru. ‘Rapoza’ was introduced to Peru in 2010 and trees have been evaluated in different regions of the Sechura desert, located south of Piura, Peru. It bears regularly and sets well. Preliminary data of the performance of ‘Rapoza’ under Sechura desert conditions is provided, including phenological stages of orchards and yield.

Growing, producing, and marketing the right mango cultivar or cultivars has a critical impact on establishing, maintaining, and expanding the fresh fruit business in the western hemisphere. Although the mango trade is still dominated by the major cultivars ‘Tommy Atkins’, ‘Keitt’, ‘Kent’, ‘Haden’ yet they all have their issues. Currently, new orchards of ‘Tommy Atkins’ are increasing, but the mango industry has expressed the need for new alternatives. The establishment and development of new cultivars can be a difficult task. There are questions that must be resolved. Answers about production, postharvest handling, transport, and marketing considerations are just some areas that require evaluation if new mango cultivars are to be introduced successfully.

There has been some effort from the private sector in Peru, where novelty mango cultivars have been introduced over the past decade. The objective is to review a range of potential cultivars that have the attributes necessary for export. The purpose of this report is to offer preliminary results of the cultivar ‘Rapoza’ growing in different regions of the Sechura desert, south of Piura, Peru.

Peru is currently the main source of mango imports into the United States which accounts for about 43% of their production. The total amount of fresh mangos in 2019 was 204,000 tons with 90% of being ‘Kent’ reported by Produce Blue Book (2020).

The mango production in Peru is concentrated in the northern coastal valleys, principally in the areas of Olmos, Motupe in Lambayeque, the valley of San Lorenzo, Chulucanas, Tambogrande and Sullana in Piura, Casma and Ancash. They are all have a dry tropical climate that allows mangos to be grown with few problems from fungal diseases.

‘Rapoza’ is also commercially produced in South Florida on a small scale, where trees are well adapted to the humid conditions of the region.

The authors want to thank Dan Lyons Farms and For the Love of Mangos for their support of this project and for contributing to the survey.

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### Origin

‘Rapoza’ was selected from an open-pollination population of ‘Irwin’ seedlings grown at the Poamoho Research Station, Oahu County, Hawaii. The original selection was made in 1985, and the selected tree was designated FR6T6 Scions were distributed to growers in Hawaii and propagated in South Florida. The tree was introduced to South Florida in the past decade by Franky Sekiya Nursery, Hawaii. Today about 1000 trees are grown for the local market in South Florida.

**TREE DESCRIPTION.** Fig.1 shows the tree with a round canopy. Trees in South Florida reach a height of 10–15 ft with a spread of 8 ft, but they can be kept smaller with annual pruning. The tree flushes twice a year, with moderate vigor than can be controlled with proper pruning. Trees are mechanically pruned, as well as by hand.

**FLOWERS.** The flowers are produced on terminal inflorescences with thousands of individual flowers that typically set less than 1.4% with natural pollination. Flowers of ‘Rapoza’ are shown



Fig. 1. ‘Rapoza’ tree.

in Fig. 2. They have a high percentage of hermaphrodite flowers (50% to 70%) compared with other cultivars, under South Florida conditions (Ledesma, et al. 2018). Male flowers are a pale cream color with yellow in the center while the hermaphrodite flowers are pink. Flowers have good tolerance to anthracnose (*Colletotricum gloeosporioides*) and moderate susceptibility to powdery mildew (*Oidium mangiferae*).

**FRUIT.** The tree produces large fruits weighing 600–800 g, with a rounded ovate shape and a moderate flattened base with small lateral beak (Fig. 3). The fruit has a slightly undulating finish with an intense dark-red blush that covers almost the entire surface with a natural wax. The fruit is firm, with a soft texture and few yellow lenticels. The flesh has a tangerine orange color and is firm and melting, with almost no fiber. The flavor of ‘Rapoza’ has been recognized as an appealing flavor for South Florida residents (Ledesma, et al. 2019). The fruit has a pleasant cantaloupe aroma, with an enjoyable sweet taste with hints of



Fig. 2. ‘Rapoza’ flowers.



Fig. 3. ‘Rapoza’ fruit.

orange peel, spondias, honey, and other citrus. °Brix falls between 19–21, with excellent eating quality. It is a polyembryonic cultivar. The seed is 3.9 in long, 2.3 in. wide, and 1.6 in. thick.

**HARVEST AND POSTHARVEST.** In South Florida there are few commercial orchards of ‘Rapoza’ destined for local market. The fruit has fair to good anthracnose tolerance and low incidence of internal breakdown issues. Sap burn is not a problem. The fruit is late season, between August to October. Local farmers harvest by hand at 70% to 80% maturity stage for the ripe market. The fruit is sold online or at roadside markets. Trees are easy to handle and highly productive, with an average yield of 50 kilos per tree for five-year-old trees under South Florida conditions.

### Trial Status in Peru

**LOCATION.** ‘Rapoza’ was introduced to Peru in 2010 and trees have been evaluated in different regions (Piura and Lambayeque) of the Sechura Desert. ‘Rapoza’ trees were evaluated for five years.

**ENVIRONMENTAL CONDITIONS.** The Sechura Desert is a coastal desert located south of the Piura Region of Peru along the Pacific coast and inland to the foothills of the Andes. Its extreme aridity is caused by the upwelling of cold coastal waters and subtropical atmospheric subsidence, but it is also subject to occasional flooding during El Niño years.

In the Sechura, the summers are short, oppressively hot, and mostly cloudy; the winters are long, comfortable, windy, and mostly clear. It is dry year-round. Over the course of the year, the temperature typically varies from 62 °F to 87 °F and is rarely below 59 °F or above 90 °F. “The Sechura desert has mostly sandy soils, but there are also soils formed by remains of gastropod and bivalve shells, as well as rock; the latter formed by the ‘Illescas massif’, which is a remnant of the western Andes in middle of the desert.” (Galves, et al 2006).

‘Kent’ is Peru’s most important commercial cultivar grown for export. It is a red skinned variety weighing between 600 and 750 g, which was selected in Florida in 1932. The ‘Kent’ produced in Peru has an attractive color and excellent quality. It has captivated the international market and has managed to position the Peruvian mango as one with a high reputation. Normally the ‘Kent’ variety has some red flush, however in other climates it retains a green color even when it is completely ripe.

‘Kent’ is a mango with high quality and flavor. The tree has a manageable size. It has good economic performance, and excellent quality. ‘Kent’ is versatile, valuable in the industry as fresh, frozen, and dehydrated fruit as well as for juice and pulp. ‘Kent’ is imported into the United States mainly from central and northern Mexico and from Peru. However, the main disadvantage of ‘Kent’ is its lack of adaptability to humid tropical climates, where it has poor flowering and low economic yields

Peru reports an increase in new orchards in the Piura region with ‘Kent’ variety where both small and large producers are investing. It is estimated that in the last three years they have gone from 32,000 ha to 34,000 ha of mango destined for export. Peru continues to have new plantings of ‘Kent’, but producers are looking for variety alternatives with characteristics similar to ‘Kent’ in quality and color and more adaptability to climate change and perhaps more tropical.

**TRIAL SPECIFICATIONS.** The orchard consists of 1.5 acres. The planting for the current study compared ‘Rapoza’ with ‘Kent’. The trees were planted a spacing of 7 m between the rows and

**Table 1.** Average yield ‘Rapoza’ vs ‘Kent’.

Cultivar	Yield per tree (kg)		
	Year 3	Year 4	Year 5
Rapoza	25	28	62
Kent	15	25	38

1 m within the row. Trees have been hand pruned every year for the first three years to develop good tree architecture. The trees flowered and bore fruit three years after planting.

Both ‘Rapoza’ and ‘Kent’ were grafted on ‘Chulucana’ rootstock; one-year-old grafted trees were planted in the field.

For management, the trees were irrigated an average of 10 liter per tree per day for the first year and up to 50 liters per tree/day by year five. ‘Rapoza’ trees were treated with the same protocols for commercial mango production in the region with ‘Kent’.

No bloom induction has been used during the evaluation period. ‘Rapoza’ mangos were evaluated as they compared with ‘Kent’.

### Phenological Observations

**PRUNING AND NEW GROWTH RATE.** Pruning is done in Piura in April (week 15). Observations showed that ‘Rapoza’ took 90 days to regenerate new growth, mature and start blooming. ‘Kent’ took longer, about 112 days.

**BLOOMING.** Blooming starts at almost the same time as ‘Kent’, in September (week 37). Panicle formation takes few days longer in ‘Kent’ compared with ‘Rapoza’. Preliminary observations show that ‘Rapoza’ does not need low temperatures to bloom unlike ‘Kent’. Young ‘Rapoza’ trees start blooming early when trees were one year old so blossoms had to be removed to allow better growth.

**FRUIT SET.** From flower to fruit set, ‘Rapoza’ takes an average of 49 days, compared with ‘Kent’ which takes an average of 42 days.

**YIELD.** Trees start producing three years after planting. The three-year evaluation recorded phenological stages from Feb. 2019 to Apr. 2021 (Table 1). The number of fruits per tree was recorded, finding a maximum fruit weight of 600 g.

**SUNBURN SENSITIVITY.** One of the regular activities in commercial orchards of ‘Kent’ is sunburn protection. With temperatures increasing, farmers raised the issue of sun protection for fruit. Current options include everything from spray-on sun protection products to shade netting, to overhead misting and sprinkler systems to bring orchard temperatures down. Preliminary observations show that ‘Rapoza’ fruit had minimal sunburn compared with ‘Kent’.

**COLOR DEVELOPMENT.** ‘Kent’ usually turns a greenish-yellow color with some red blush as it matures. Color development is often achieved by allowing light into shaded parts of trees to enhance blush development, but this must be done carefully to avoid sudden exposure of fruit to direct sunlight. Even a relatively short period of exposure to intense direct sunlight could cause significant damage especially in ‘Kent’. ‘Rapoza’ is showing better color, and its red skin seems to be covering up to 90% of the surface of the fruit.

### Conclusions and Recommendations

Results were significant for both appearance and yield. Yield parameters were highest in ‘Rapoza’ with an average of 25 kg per tree by the first year of production compared with ‘Kent’ with 15 kg per tree. The yield rate continued same pattern for the following two years of evaluation with 62 kg per tree for ‘Rapoza’ and 38 kg per tree for ‘Kent’ by year five.

Spacing 7 m between rows and 1 m between trees must be evaluated further. Using regular pruning has shown good results through the end of this trial, however this information must be reviewed using the same parameters as the trees get older. Color is an important criteria for commercial purposes. Preliminary observations show that ‘Rapoza’ has better color than ‘Kent’ under similar conditions. ‘Rapoza’ fruit has good color even inside the canopy, and fruits exposed to the sun are less sensitive to sunburn. Sunburn is a serious problem in the Peruvian Sechura Desert. It requires additional management expenses. Having a cultivar which is less sensitive to sunburn is advantageous. ‘Rapoza’ has a natural red color flush and a higher percentage of red skin compared with ‘Kent’. Further analysis of these data have to be evaluated for further recommendations.

We recommend continuing this trial for at least three more years. Future trials are recommended using different spacings and observing yield per tree, vegetative variables, and reproductive variables that can be affected by planting density. Further analysis of harvest and postharvest procedure are necessary using a wide range of descriptors both qualitative and quantitative, including characteristics of the tree and the fruit.

The adaptations of a variety to environmental pressures such as drought, wet weather during flowering, temperature, pests, and diseases are also important selection criteria, because they determine the consistency of the crop and fruit quality. Future analysis should include the management activities applied to the orchards and the response on growth.

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## Genomics-enabled Plant Breeding of Mango

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Mango is a species that is rich in diversity, but biological constraints limit efficient plant improvement through breeding. Most mango cultivars today have been the result of chance seedlings grown by mango enthusiasts in contrast to most crop cultivars that result from intentional plant breeding. Mango flower biology is one factor impacting this outcome. Thousands of mango flowers are borne on individual panicles, but few pollinated flowers will result in a mango fruit. As a result, controlled hybridization using elite parents has been challenging as most flowers abort prior to embryo formation. Alternative strategies to hand pollination include selecting half-sibling populations where only the maternal parent is known, or caging mature trees with insect pollinators to achieve a higher proportion of progeny with known parentage. The recent publication of two mango genomes and an efficient genotyping platform have enabled a new discovery tool for selecting mango hybrids. In total, 140 parental cultivars and 575 mango seedlings from 11 maternal parents were genotyped using 365 single nucleotide polymorphism (SNP) markers. The 11 maternal parents included ‘Duncan’, ‘Edward’, ‘Glenn’, ‘Haden’, ‘Lemon Zest’, ‘Palmer’, ‘Pruter’, ‘Ruby’, ‘Tommy Atkins’, ‘Van Dyke’, and ‘Young’. Seedling genotypes were sorted using the known, maternal parent and “impossible SNPs” were used to identify the most probable paternal parent. Selfing rates from 2% (‘Duncan’) to 40% (‘Tommy Atkins’) were recorded. The planting design of the mango collection also enabled the localization of paternal trees in relation to the maternal parent. For ‘Glenn’ mango, for example, 91 seedlings were genotyped successfully and pollination did not appear to be correlated with proximity to the maternal tree. This might be informative for identifying insect pollinators of mango. Comparing and contrasting trends from all populations can inform cultivar-level differences for selfing rate and paternal pollinator preferences. This could inform mango planting strategy in the future. Finally, the genotyping results enabled the selection of mango seedlings with known parentage and are leading to the development of new mango cultivars with novel trait combinations.

### Materials, Methods, Results, and Conclusion

The University of Florida Tropical Research and Education Center (UF TREC) mango collection and open-pollinated seedlings were genotyped in collaboration with the USDA Subtropical Horticultural Research Station (SHRS). Estimation of genetic diversity and relatedness in a mango germplasm collection using SNP markers and a simplified visual analysis method). Quality traits like mesocarp thickness and aroma identified large differences among mango cultivars.

Applying genomics-assisted selection enabled the selection of hybrid mango seedlings based on potential quality. These include hybrids like ‘Tommy Atkins’ × ‘Glenn’, ‘Glenn × Mallika’, and ‘Haden’ × ‘Cushman’. Future work will correlate SNPs with quality traits like aroma to enable seedling selection. We anticipate that this will allow the selection of seedlings with higher probabilities of being large fruited, aromatic, and disease resistant. This is especially valuable for a long-lived, perennial tree.

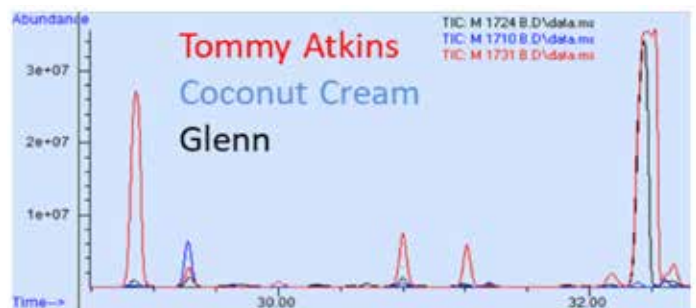
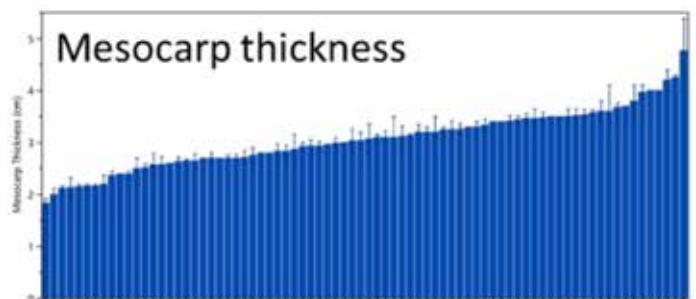


Fig. 1. Mango diversity at the University of Florida, Tropical Research and Education Center (UF TREC) (top), mesocarp thickness distribution among the UF TREC mango cultivars (center), and an example of differing aroma profiles for three cultivars (bottom).

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## A Review of Asam Kumbang (*Mangifera quadrifida* Jack.)

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**Additional index words.** Rancha-rancha, Asam Rarawa, Asem Rawa, Sepam, Lekub, Damaran, Asam Rawawa, Rawa-Rawa, Ubab. conservation, *Mangifera* species.

The mango, *Mangifera indica* L. (Anacardiaceae), is the best known and most widely cultivated species in the genus *Mangifera*. *M. quadrifida* is a species endemic to Malaysia and commonly known as a Rancha-rancha or Asam Kumbang. It is ultra-tropical and grows in undisturbed lowland forests, often in inundated land or along riversides. Tribal and local people use it for food (either unripe or ripe) as well as for medicinal purposes. There is increasing interest for ex situ and in situ conservation, but currently this is an International Union for Conservation of Nature (IUCN) red listed species. *M. quadrifida* has the potential for use as part of breeding programs to improve mangos and to produce new hybrids with reduced susceptibility to disease that may flower naturally in the tropics with no cold induction. A general review, recording experiences with local communities in Borneo, and horticultural remarks includes its adaptability to modern cultivation and its potential as a commercial crop.

Although *Mangifera indica*, is the most common commercially grown species, there are many other species of this genus that are used for food. Borneo, the third largest island in the world, has a vast tropical rainforest and is an indispensable source for food for the local people. Street markets in Borneo, Malaysia, and Indonesia seasonally display wild mangos for sale. Most have edible fruit with the potential for breeding and as rootstock. For over a decade, the author has traveled to these remote areas to identify their potential for use in breeding (Ledesma et al., 2014).

Surveys on *M. quadrifida* were carried out by visiting markets, and home gardens in Sarawak (Malaysia), where indigenous people have planted trees of their favorite fruit around their traditional longhouses for generations. Edible wild mangos are in critical danger of extinction and represent an important resource for the future of mangos. However, there is still lot of confusion of their taxonomic descriptions as little has been done to advance those goals. There is a possibility of wild hybridization between species, which may be detected with genetic analysis.

Asam Kumbang (Sarawak) is a fruit appreciated throughout Borneo with markets having different phenotypes. Fruit characteristics can vary in size and shape. Fruit is deep purple.

### Origin

The species is endemic to Brunei, Malaysia (Sabah and Sarawak), and Indonesia (Sumatra, Kalimantan, and Java) (Lim, 2012). There are different common names according to region/country of origin. In Malaysia, it is called: Asam Kumbang, Sepam, Lekub, or Damaran; in Brunei: Rancha-rancha; while in Indonesia: Asam Rawawa, Rawa-Rawa, Ubab, or Balangan. It is a tropical species. Its range is from sea level to 1000 m. The tree grows naturally in lowland forests, often inundated, along riverbanks or in undisturbed forest.

*M. quadrifida* has been reported in official and private collections, as well referenced as herbarium specimens. *M. quadrifida* was introduced to south Florida in 2004, and it is also in Hawaii and Puerto Rico.

**TREE DESCRIPTION.** The tree is large and can reach up to 25 m tall and 112 cm trunk in diameter with a dense canopy. Juvenile leaves are bronze to purple (Fig. 1); and soften in color as they age to a glossy deep green with woody twigs (Fig. 2); The trees grow in acidic soils in swamps.

**FLOWERS.** Flowers are white to light cream very fragrant and pollinated by honeybees (Ledesma et al., 2015) (Fig. 2).

**FRUIT.** The fruit averages 150 g and deep is purple with a rubbery skin and yellowish lenticels. The pulp is bright orange, acidic and fibrous. Seeds are hard and reddish. The fruit is used immature as pickles and ripe to prepare “Sambal belacan” [chile sauce]. There are fruits of different shapes and sizes commonly found for sale in the markets in south Kalimantan (Fig. 1).

### Status of *Mangifera quadrifida* in South Kalimantan, Indonesia

The Borneo rainforest is the oldest rainforest in the world, and one of the most biodiverse (Saw, 2010). There have not been many efforts to save wild mangos.



Fig 1. Characterization *M. quadrifida* (A): fruit, (B) seed, and (C) juvenile leaves.

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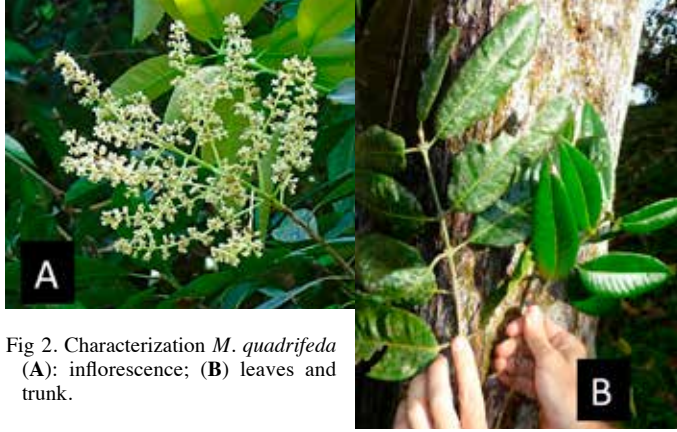


Fig 2. Characterization *M. quadrifeda* (A): inflorescence; (B) leaves and trunk.

Tunas Meratus is a small nonprofit institution in south Kalimantan which collects material for propagation and distribution. Their objective is to incentivize forest gardens as part of a broader land-use spectrum that contains farms and home gardens, to protect the forests (Shaffiq et al, 2013). Tunas Meratus works with several fruit species in the region including *Artocarpus*, *Durian*, and *Mangifera* species. As part of the project, a survey was conducted over the past five years to identify *M. quadrifeda* trees in Marajai village, Halong district, Kalimantan, Indonesia.

The results of the survey showed only few mature trees left in the village. They reported no more than 15 *M. quadrifeda* growing in the lowlands forest, about 20–30 m tall and more than 60 cm diameter. Working with the community, seeds and cuttings are collected from the trees for propagation.

### Horticultural Remarks

**PROPAGATION.** *M. quadrifeda* (Asam kumbang) seeds are used in south Kelamatan, but often *M. casturi* is used as a rootstock for Asam kumbang trees. Other reports state that *M. quadrifeda* is propagated by seed and it is used as a rootstock for *M. casturi*. In-situ conservation programs in the south Kalimantan, Indone-

sia are using *M. quadrifeda* trees are grafted on ‘Hampalam’ (*M. casturi*) (Nove Arisandi, 2020, personal communication).

In south Florida *M. quadrifeda* has been propagated on to *M. rubropatela*, *M. casturi* and *M. indica* ‘Turpentine,’ the latter was not successful.

**Breeding.** Asam Kumbang has apotential for breeding to improve mangos and produce new hybrids with reduced susceptibility to disease, that will naturally flower in the tropics without cold induction (vernalization) and produce good quality, marketable fruit.

**TREE SIZE AND FRUIT PRODUCTION.** *M. quadrifeda* can be a huge tree reaching up to 25 m high, with a trunk diameter over 100 cm and a dense and spreading canopy. Compared to *M. indica*, *M. quadrifeda* is adapted to higher humidity and wetter soils.

For planting under south Florida conditions, soils should be made as fertile as possible, and the young trees benefit from mulching. Such amendments improve water-holding capacity, nutrient retention and availability, and soil structure. Low humidity is detrimental to the health of young trees. The tree grows well in south Florida, but it has not bloomed for the past six years.

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## The Mango Collection at the Preston B. Byrd/ Mary Heinlein Fruit & Spice Park

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**Additional index words.** *ethnobotanic, genetic collection, Miami-Dade County*

**The Preston B. Byrd/Mary Heinlein Fruit & Spice Park is a 38-acre botanical garden in Homestead, Florida, that is operated by the Miami-Dade County Parks, Recreation and Open Spaces Department. The Park is the only ethnobotanical park in the United States and contains more than 500 species of economically and culturally significant plants from five tropical and subtropical regions around the world. It showcases rare plants and educates the public on the connection to and reliance on the plant world. It also serves as a repository of cultivars for area growers and residents. Within the Park, there are several large collections of cultivars of species with commercial interest: annona, avocado, banana, bamboo, lychee, and mango. The Park's mango collection contains more than 170 different cultivars, some of whose introduction to the park dates to the 1950s. This valuable collection is a delight to visitors each summer, and it serves as a valuable source of germplasm for the research community.**

The Fruit & Spice Park is operated by the Miami-Dade Parks, Recreation and Open Spaces Department. It is situated on 38 acres in the southwest farming area of Miami-Dade County among commercial tropical fruit groves, plant nurseries, and winter vegetable fields. The Park displays over 2500 specimens of 500 species of tropical fruits, nuts, spices, and trees of economic and cultural importance. The Fruit & Spice Park is a botanical garden that strives to expand and enrich people's understanding of their reliance on the plant kingdom in the past, in the present, and future.

The Fruit & Spice Park was established on 20 acres in 1944 as a tropical fruit park, a display for the newly relocated northerners to introduce them to the tropical fruit trees that could grow in the climatic and soil conditions unique to the area. Most new residents to south Florida, were familiar with temperate crops, such as apples, peaches, and grapes, but were unfamiliar with the many options of tropical fruit trees available locally, such as mangos, avocados, jackfruits, and limes. The Park provided the opportunity to observe these fruits growing in the landscape along with activities including weekly tours of the Park and fruit displays at area events.

The 1980s was a period of significant development in the Park as well as involvement and support from the community. The Park plant collection grew, educational and outreach activities expanded, and the Park increased in size to 38 acres. On 24 Aug. 1992, Hurricane Andrew caused massive damage to the Fruit & Spice Park. Over 750 canopy trees were destroyed along with Park infrastructure. After the hurricane, bond referendums funded repairs, improvements, expansion to the Park, and a new Master Plan redesigned the layout of the collection into geographical regions and redirected the focus of the park to an ethnobotanical approach, which continues today.

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The author would like to recognize the the staff of the Fruit & Spice Park for their dedication and support for the Park, and the impact made by Park managers Chris Rollins and Jim Stribling, as well as Maurice Kong for his many mango introductions, and all other plant enthusiasts who have contributed to the Park's plant collections over the years.

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The Park recently received recognition as a Level III accredited botanical garden from ArbNet, an interactive, collaborative, international community of arboreta and tree-focused professionals, and accreditation from Botanical Gardens Conservation International (BGCI,) a plant conservation charity dedicated to global plant conservation and environmental education.

The Fruit & Spice Park was created with the vision of educating south Florida residents and visitors on the varieties of trees they could grow in the soils and climate of South Florida. Since that time, the botanical collection has greatly expanded. The Park has increased the number and breadth of educational and outreach programs and made a lasting connection to the local community and visitors, working to instill in the people their connection to and dependence on the plant world.

### The Collections

The Fruit & Spice Park comprises 38 acres of planted ground. The climate of the area is classified as a tropical monsoon climate with hot, humid, wet summers, and warm, dry winters. These weather conditions are favorable to support the growth of an amazing variety of tropical plants. The plant collection is divided into five tropical regions of the world each featuring ethnobotanically significant plants: Africa, Asia, Australia, and the Pacific Islands, Mediterranean zone, and Tropical America. Plants in each area are selected for the collection based on the economic, religious, and/or cultural impact they have made in people's lives. The stories of these plants are shared with the public in order to educate them and assist in their recognition and appreciation of their connection to the plant world.

Interspersed among the five geographical regions of the park, are cultivar and species collections of several commercial agricultural crops. These collections are of interest and importance to area growers as they display the growth habits of the various cultivars and provide a source of bud wood for the nursery trade and tropical fruit enthusiasts. These collections include: Annona, *Annona* spp. (77 accessions, 13 species, 14 cultivars); Avocado, *Persea americana* (76 accessions, 47 cultivars); Banana *Musa* spp. (118 accessions, 6 species, 68 cultivars); Bamboo, various genera



(135 accessions, 4 genera, 37 species) and Mango, *Mangifera* spp. [217 accessions, 7 species, 170 cultivars of *M. indica* (Table 1)].

**MANGO COLLECTION.** The Fruit & Park houses the only mango, *Mangifera indica*, collection that is open to the public in the continental United States. Of all the fruits in the park, the mango collection is the most popular and well known to the visitors.

In 1981 there were six mango trees of five varieties in the Park: ‘Kent’, ‘Anderson’, ‘Haden’, ‘Zill’, and ‘Brooks Late’. Today the mango collection houses 217 accessions of 170 cultivars, donations of plants and/or budwood from the Parks horticultural support club, Tropical Fruit & Vegetable Society of the Redland (TFVSR) private collectors, local nurseries, collecting trips, and the mango collections at the USDA Agricultural Research Service –Subtropical Horticultural Research Station (Chapman Field), in Miami, FL, and the University of Florida, Institute of Food and Agricultural Sciences’s Tropical Research and Education Center (UF/IFAS TREC), in Homestead, FL. The Park’s mango collection serves an educational exhibit for the visitors and also as a germplasm bank for local nurseries, enthusiasts, researchers, and growers for the further development of the industry.

The Park features its mango collection at two events: The Summer Fruit Festival and Mango Mania, both in June. The Summer Fruit Festival highlights the variety of tropical fruits that are grown in south Florida in the summer months, and features a mango display of around 200 mango cultivars. Volunteers harvest mangos from the collections at the Park, Chapman Field, TREC, and private collectors, and are displayed and labeled for the event. Lectures, plant sales, and mango tasting are offered to the festival attendees.

The following weekend a similar event called Mango Mania is sponsored by the Tropical Fruit and Vegetable Society of the Redland. Tickets are sold to attend this much-loved fund-raising, educational event. The volunteers return to the same collection sites to harvest additional mangos. The fruits are labeled and displayed on plates on tables under a tent at the Park. The event begins with a two-hour lecture from an area expert discussing mango cultivation, propagation, harvesting, the history of the mango industry in Florida, prominent mango cultivars, etc. and then moves to the display table where attendees move around the display table and sample ripe mangos. It is a memorable experience and a wonderful opportunity for visitors to try new mangos, compare different cultivars, and share the discovery of new delicious mango flavors.

#### Additional Activities

The Fruit & Spice Park offers visitors many ways to learn about and enjoy the Park: guided tours, fruit sampling, horticultural classes, volunteers, internships, and festivals. Visitors can taste fallen fruit in season providing an enjoyable opportunity for education.

**HORTICULTURAL SOCIETIES.** The Tropical Fruit & Vegetable Society of the Redland (TFVSR) and the South Dade Garden Club (SDGC) have supported the Park for many years. SDGC has met at the Park since the 1944 and helps sponsor the Parks’ summer camp programs and memorial tree planting program. TFVSR formed in 1981 to increase public interest in the cultivation and use of tropical fruit and vegetables and to support the Park. It has supplied the Park with tree labels, a greenhouse, nursery, irrigation systems, farm equipment, numerous trees, etc., and has hosted 13 international tropical fruit conferences with speakers and participants from around the world.

**Table 1.** List of *Mangifera indica* cultivars L. currently growing at the Preston B. Byrd and Mary Heinlein Fruit & Spice Park, Homestead, FL.

Cultivar	Origin
Ah Ping	Hawaii
Alampur Baneshan	India
Alphonso	India
Anderson	Florida
Angie	Florida
Aroemanis	Indonesia
Ataulfo	Mexico
Bailey’s Marvel	Florida
Beverly	Florida
Bombay	Jamaica
Brahm Kai Meu	Hawaii
Brooks Late	Hawaii
Bullock’s Heart	Hawaii
Burd	Florida
Butter Cream	Florida
Cambodiana	Vietnam
Carrie	Florida
Cecil	Florida
Chok Anon	Thailand
Cobiella	Florida
Coconut Crème	Florida
Cogshall	Florida
Cotton Candy	Florida
Cushman	Florida
Daung	Thailand
Davis-Haden	Florida
Diab	Egypt
Diamond	Florida
Dolce de Leita	Cuba
Donkey Killer	Philippines
Dot	Florida
Du Dus	Trinidad
Duncan	Florida
Dupuis	Florida
DuPuis Saigon	Florida
Dwarf Hawaiian	Hawaii
Early Gold	Florida
East Indian	Jamaica
Edward	Florida
Eulogio	Tropical Asia
Ewais	Egypt
Excalibur	Hawaii
Extrema	Brazil
Fairchild	Panama
Fascell	Florida
Fernando	Florida
Fiji Long	Pakistan
Florigon	Florida
Ford	Florida
Fruit Punch	Florida
Galaxy	Florida
Gary	Florida
Gaylour	Egypt
Glenn	Florida
Golden Nugget	Florida
Goldens Lippens	Florida
Golek	Indonesia
Gopher	Florida

**Table 1.** (continued).

Cultivar	Origin
Graham	Trinidad
Haden	Florida
Harvest Moon	Florida
Hatcher	Florida
Hindi Besanara	Egypt
Hodson	Florida
Hong Sa	Thailand
Ice Cream	Trinidad
Imani Pasand	Trinidad
Irwin	Florida
Ivory	Thailand
Jakarta	Florida
Jaqueline	Florida
JB2	Florida
Jehangir	India
Julie	Caribbean
Juliette	Florida
Justin	Florida
Kaeow Savoy	Thailand
Karti Clombra	Tropical Asia
Keitt	Florida
Kensington	Australia
Kent	Florida
Kesar	India
Lancetilla	Honduras
Lemon Zest	Florida
Lippens	Florida
M4	Florida
Mabruka	Egypt
Madame Francis	Haiti
Mahachanook	Thailand
Mallika	India
Mandalay Yin Guay	Myanmar
Manga Blanca	Cuba
Mangga Pari	Tropical Asia
Martin	Florida
Maya	Israel
Mendoza	Tropical Asia
Mesk	Egypt
Montong	Thailand
Mulgoba	India
Mun Kun Si	Thailand
N-5	Florida
N-6	Florida
Naomi	Israel
Neelum	India
Nelson	Jamaica
Number 11	Jamaica
Okrong	Thailand
Okrong Tong	Thailand
Orange Sherbet	Florida
Osteen	Florida
Page	Florida
Pettigrew	Florida
Philippine	Philippine

**Table 1.** (continued).

Cultivar	Origin
Phimsen Mun	Thailand
Pickering	Florida
Po Pyu Kalay	Myanmar
Pohn Sawadee	Indonesia
Prieto	Cuba
Rapoza	Hawaii
Rataul	Pakistan
Ritah	Jamaica
Rosa	Brazil
Rosigold	Florida
Royal Special	Florida
Ruby	Florida
Ryan	Florida
Saint Maui	Hawaii
Sammy	Florida
San Felipe	Cuba
Sandersha	India
Sein Ta Lone	Myanmar
Sensation	Florida
Sheila	India
Shew Ya Za Ma	Myanmar
Sia Siam	Thailand
Sia Tong	Thailand
Sindhri	Pakistan
Smathers	Florida
Southern Blush	Florida
Spirit of '76	Florida
Springfels	Florida
Starch	Trinidad
Step	Hawaii
Sucari	Tropical Asia
Sugar Loaf	Florida
Sunset	Florida
Super Julie	Florida
Sweet Tart	Florida
Tahar	Israel
Tommy Atkins	Florida
Tong Dom	Florida
Tong Pi Kahn	Thailand
Torbert	Florida
Triple Sec	Florida
TT	Florida
Turpentine	Cuba
Tyler Premier	Kenya
Ugly Betty	Florida
Val x Carrie	Florida
Valencia Pride	Florida
Vallenato	Colombia
Van Dyke	Florida
White Alphonse	India
White Pirie	Hawaii
Wise	Florida
Yai Grom	Thailand
Zeylanica	Sri Lanka
Zill	Florida
Zillate	Florida

**VOLUNTEER PROGRAMS.** The Park operates an educational program for schools and summer camps with classes on pollinators in the food system, proper nutrition, and good eating habits. An extensive volunteer program has grown over the years that attracts area gardeners, students, fruit enthusiasts, area youth groups, and students participating in community service programs.

**FESTIVALS.** Highlights of the year are the seven themed festivals held at the park: Redland Heritage Festival, Asian Culture Festival, Blues and BBQ, Orchid Festival, Summer Fruit Festival, Grow Fest!, and Mango Mania. These festivals are presented by the Park or in partnership with community groups, and each focuses on a specific horticultural or cultural theme associated with the Park. They have become much-anticipated events in the community. At every event, the Park presents a tropical fruit display of the fruits, nuts, and spices that are harvested from the Park at that time of year. It is created and staffed by knowledgeable park volunteers.

### **Research/Collaboration**

The Fruit & Spice Park works closely with and strongly supports the nearby research institutions at Chapman Field and the UF IFAS TREC. The Park has shared plant material with these organizations, sought out their expertise on various topics over the years, and provided them with a separate location and different growing conditions in which to conduct research. It has been a valuable partnership and we look forward to continuing for years to come.

The Florida Division of Plant Industry maintains early detection traps for pests in the Park. The Fruit & Spice Park is also used as a place of study and living laboratory for classes offered in the curricula at the University of Miami, and Florida International University, and homeschools and summer camp programs.



# **Expanding Mango Consumption Phase 1: Mango (*Mangifera indica*) Cultivar Evaluation for the National Mango Board**

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**Additional index words.** mango cultivar evaluation, mango germplasm, mango varieties

**Growing, producing, and marketing the right cultivar or cultivars has a critical impact establishing, maintaining, and expanding the fresh fruit business in the western hemisphere. The international fresh mango fruit business is no different. Although the major cultivars in the trade, i.e., ‘Tommy Atkins’, ‘Keitt’, ‘Kent’, ‘Haden’ ‘Madame Francis’ and ‘Ataulfo’ possess many of the attributes of successful commercial cultivars, they all have their drawbacks. The fresh fruit mango trade in the western hemisphere is expanding and the market seems poised to accept new peel colors, shapes, sizes, and flavors. However, establishing sufficient production and marketing of new cultivars can be a daunting task. There are major considerations such as production, handling–postharvest, transport, and marketing, which need to be worked out as much as possible if a new cultivar is to be successfully introduced. An initial step is for the mango industry to review a range of potential cultivars that have the attributes necessary for commercialization. The purpose of this report is to offer the results of a panel of nine international mango experts assembled as a part of a National Mango Board sponsored project to identify mango cultivars with potential for commercialization to enhance the international mango industry on this continent.**

Worldwide fresh mango production has increased about 44% during the past 20 years from 24.7 million metric tons in 2000 to 57.4 million metric tons in 2021 (FAO-STAT, 2021). Fresh mango imports to the U.S. have risen from 1.1 billion pounds in 2017 to over 1.2 billion pounds (lb) in 2020 valued at \$493.0 million (USDA-ERS, 2021). U.S. per capita mango consumption has increased from 1.75 lb in 2000 to 3.21 lb by 2019 (Statista, 2021).

The National Mango Board (NMB) is a national promotion and research organization formed in 2004 under U.S. federal marketing order legislation and is under the auspices of the USDA–Agricultural Marketing Service (CFR, 2003). The board’s mission is to increase U.S. consumption of fresh mango through marketing and promotion, research, and industry development. The NMB is funded through assessments on imported and domestic whole fresh mango fruit. The board is composed of fresh mango importers, handlers (an entity which handles or markets mangos), and domestic and foreign producers. The NMB develops and executes strategic promotion, research, and industry development plans to further the best interests of the mango industry and ensure funds are invested wisely.

Currently in the Western Hemisphere export trade, six mango cultivars predominate: ‘Tommy Atkins’, ‘Keitt’, ‘Kent’, ‘Ataulfo’ (‘Honey’), ‘Haden’, and ‘Madame Francis’ (‘Francis’). While all these cultivars have many positive attributes, the industry is

actively investigating alternative cultivars to expand the pallet of mangos for consumers and producers. The NMB is searching for mango cultivars with excellent handling characteristics, superior pulp flavors and textures, peel colors, shapes, and seasons of availability.

Stemming from the NMB mission and vision, a first step was to evaluate potential mango cultivars for wider commercialization. This paper reports primarily on Phase-1 of this effort. The Mango Cultivar Evaluation Project—Phase-1 was designed to garner the expert advice of numerous mango researchers who were intimately familiar with mango cultivars, their performance and fruit quality characteristics. The purpose of this report is to offer the results from a panel of scientists intimately familiar with mango cultivars and mango growing and handling to further develop the international mango industry of this continent.

The criteria for a successful mango cultivar vary somewhat by which part of the mango business is considered. Producers require that cultivars come into bearing fruit as soon as possible and have reliable crop yields, moderate to excellent resistance to diseases, insects, and pests, possess acceptable fruit quality, and can tolerate postharvest handling and shipping. In contrast, packers and handlers are more interested in cultivars that tolerate picking and postharvest quarantine treatments, sorting and packing, storage, and shipping. Marketers require a year-round fruit supply of blemish-free fruit of acceptable internal and external color, taste, and texture, that ship and store well and that ripen to acceptable eating quality.

The project began in 2017. The first step in this process was to form a panel of experts (Table 1) from Australia, Brazil, Israel, Mexico, Peru, South Africa, Spain, Central America, and the United States. Many on the panel were previously or are currently active in mango cultivar selection for their national industries.

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Table 1. Mango Cultivar Expert Panel members for the National Mango Board project to identify mango cultivars with potential for commercialization.

Country	Name and title	Institution
Australia	Ian S.E. Bally, Horticulturist/Breeder	Dept. of Primary Industries, Queensland
Israel	Yuval Cohen, Plant Breeder	The Volcani Institute, Bet-Dagan
United States	Jonathan H. Crane, Tropical Fruit Crop Specialist	Tropical Research and Education Center, Univ. of Florida, IFAS, Homestead, FL
Honduras and Perú	Dr. Odilo Duarte, Prof.	PanAmerican School of the Americas, (retired), currently, Consultator en Agronegocios
Mexico	Samuel Salazar-Garcia, Dir.,	INIFAP (Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias), Tepic-Mazatlan
United States	Noris Ledesma, Curator	Tropical Fruit Program, Fairchild Tropical Botanical Garden, Miami, FL
Brazil	Francisco Pinheiro, National Leader	Mango Breeding Program, Empresa Brasileira de Pesquisa Agropecuária (EMBRAPA)
South Africa	Johann du Preez, Manager/Horticulturist	Bavaria Estate, Westfalia, So. Africa
Spain	Victor Galán Saúco, Research Prof. (retired)	Instituto Canario de Investgaciones Agrarias, Canary Islands, Spain

Jonathan Crane was the Panel Coordinator. The experts agreed to participate by offering their input and assessment of a very wide range of mango cultivars. The assessment was conducted through an extensive survey where each expert was asked to list, based on their data and expertise, their top five to ten cultivars. For each cultivar evaluated, background information on the origin, location of the evaluation, germplasm availability, current commercial status, environmental adaptability (e.g., climate, soil types, cold and salinity tolerance), tree growth habit (e.g., tree vigor, canopy structure), production attributes (e.g., kg fruit produced/tree and season), disease tolerance (e.g., mango malformation and anthracnose), and detailed fruit characteristics (e.g., dimensions, weight, peel and pulp color, pulp texture) was recorded. Additional information requested was intended market (e.g., fresh and fresh-cut), postharvest handling characteristics (e.g., sap burn, chilling injury, and internal breakdown), and tolerance to quarantine treatments and cooling (e.g., hydrocooling and forced-air cooling).

### Summary of the Mango Cultivar Panel Findings

Panel members (PM) recommended 37 cultivars. Previously mentioned criteria were used to select the top cultivars for further evaluation (Table 2). Part of these criteria included the most frequently selected cultivar by PM and fruit and tree characteristics described from the cultivar surveys. We asked the PM to provide their “best” selections. This information was tallied and used as the criteria for further evaluation. The author then compiled the information and ranked each cultivar by the number of experts naming a specific cultivar in their top mango cultivar list. In some cases, PM top selections were not readily available for further evaluation or there was too little data to form the basis for further evaluation at this time. Based on the PM survey information, cultivar ranking and available literature, the Panel Coordinator selected the top six cultivars for further recommendation. These six were ‘Agam’, ‘Angie’, ‘Calypso’, ‘Mallika’, ‘Rapoza’, and ‘Shelly’. Others for further consideration included ‘Cogshall’, ‘Maha Chinook’, ‘Noa’ and ‘Osteen’ (Table 3).

Table 2. List of mango cultivars recommended by the panel members.

Cultivar	Cultivar
Agam	NMBP1243
Ah Ping	NMBP4069
Angie	NMBP1201
Ataulfo Diamante	Noa
Ataulfo Zafiro	Omer
Calypso	Orli
Cavallini	Osteen
Cogshall	Otts
Duncan	Papo de Peru
Edward	R2E2
Espada Ouro	Rapoza
Favo de Mel	Rosa 2
Gouveia	Rosigold
Harders	Shelly
Isis	Southern Blush
Kensington Pride	Tali
Maha Chanook	Valencia Pride
Mallika	Young
Nam Doc Mai	

The next step in the NMB mango cultivar evaluation process (Phase 2) is underway. Phase 2 consists of evaluating these top cultivars and other selected candidates for postharvest and quarantine treatment handling characteristics. This will provide further evidence of commercial suitability for export and import markets. This project is currently underway by the postharvest lab (Jeffrey Brecht) in the Horticultural Sciences Department at the University of Florida/IFAS in Gainesville, FL. Once Phase 2 is complete, an evaluation of the top cultivars from Phases 1 and 2 may be planted in replicated trials in selected mango producing regions to collect in-field tree performance, fruit production and

Table 3. Brief description of selected mango cultivars for further evaluation and potential commercial expansion.

**Agam**



Medium- to large sized fruit (330-520 g; average 450 g), roundish (broad-shaped) with a dark red to scarlet color and numerous yellow dots (lenticels). The pulp is dark orange, firm texture with very low fiber. The fruit has a pleasant aroma and a rich sweet flavor with good to excellent eating quality. Anthracnose tolerance unknown. Tolerant to postharvest handling but unknown tolerance to postharvest quarantined treatment (e.g., hot water treatment). Trees harvested early season.

*Photo credit: Volcani Research Center.*

**Angie**



Medium size fruit (350-480 g), oval to oblong-shaped fruit with a yellow pink to reddish blush that turns yellow at ripening. The pulp is yellow to orange colored with very low fiber, has a pleasant aroma, is sweet (18-22°Brix) with excellent eating quality. Generally harvested in late spring (May in U.S.).

*Photo credit: Noris Ledesma.*

**Calypso**



Medium- to large sized fruit (350-570 g), elliptic to round shape with a slight beak; a yellow-pink peel. The pulp is orange, firm with medium-low fiber, and has a sweet rich flavor and mild aroma, good eating quality. Mid- to late season harvest period.

*Photo credit: <https://twitter.com/calypsomangoes>.*

**Cogshall**



Medium size fruit (280-500 g), 11-14 cm long, 6.2-8.5 cm dia., oblong with rounded base, yellow-to-yellow-orange peel with crimson blush. The pulp is yellow-orange, low in fiber, with a pleasant aroma and spicy, sweet rich flavor, with good to excellent eating quality. 'Cogshall' is an early to midseason cultivar.

*Photo credit: J.H. Crane.*

**Maha Chinook**



Small to medium sized fruit (262-435 g), ellipsoid shaped with a slight beak and a yellow-pink peel. The pulp is light orange, firm with very low fiber, and has a sweet distinct flavor and mild aroma; very good eating quality.

*Photo credit: Ian Bally.*

**Mallika**



Medium size fruit (280-510 g), oblong-sigmoid shaped fruit with a bright yellow-to-yellow-orange peel. The pulp is orange colored with very low fiber, has a pleasant strong aroma, and is sweet (20-22°Brix) with excellent eating quality. Harvest season June-July (Florida)

*Photo credit: Ian Maguire.*

**Noa**



Large fruit (483-812 g; average 650 g), 13-14 cm long, 9-10 cm dia., broad elliptic-oval shaped with a yellow ground color and large-area with a blend of red, orange, yellow and green color blush, numerous small yellow dots (lenticels). The pulp is medium-orange, firm with low fiber. The fruit has a pleasant aroma, and a rich sweet flavor with good eating quality.

*Photo credit: Volcani Research Center.*

Table 3. (Continued)

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**Osteen**



Large fruit (500-760 g), 12-15.5 cm long, 8.5-10.5 cm dia., oblong shaped with yellow-orange color and purple or lavender blush with numerous small white dots (lenticels). The pulp is yellow colored with low fiber, firm, has a mild pleasant aroma, and is sweet with good eating quality. Harvested July to early September (Florida).

*Photo credit: Mark Nickum.*

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**Rapoza**



Large fruit (650-800 g), oblong shaped with a red blush extending over half the fruit peel. The pulp is yellow, yellow orange colored with very low fiber, has a pleasant aroma, and is sweet (19-21°Brix) with excellent eating quality. Harvested July-August (Florida).

*Photo credit: J.H. Crane.*

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**Shelly**



Medium sized fruit (300-700 g), roundish (apple-like) shaped with a yellow ground color and large-area red blush, numerous small yellow dots (lenticels). The pulp is medium to deep yellow, yellow orange, very firm with low fiber. The fruit has a mild, pleasant aroma, and is sweet with good eating quality. Harvest mid- to late season.

*Photo credit: The Volcani Research Institute.*

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quality information along with further harvest and postharvest handling characteristics.

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## **Mango Internal Discoloration “Cutting Black” or “Corte Negro”**

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### **Abstract**

Internal discoloration (“cutting black” or “*corte negro*”) is a physiological disorder of mango fruit that may be induced preharvest but is expressed postharvest. *Corte negro* is characterized by diffuse brown or gray-to-black flesh discoloration in partially ripe to ripe fruit. It has been observed in all common mango cultivars imported to the United States from Mexico, Central America, and South America. We conducted trials over three or four seasons depending on location at two farms in Ecuador with ‘Tommy Atkins’ and ‘Ataulfo’ (‘Honey’) and two farms in Peru with ‘Kent’ mangos; a single trial with ‘Honey’ mangos at a third farm in Peru with a history of *corte negro* was included in the fourth year. We tested the hypothesis that mangos become predisposed to develop *corte negro* symptoms at some critical period of fruit development due to low calcium (Ca) and/or high nitrogen (N). We further tested the hypothesis that *corte negro* symptoms develop in response to stress from either the United States Department of Agriculture, Animal and Plant Inspection Service (USDA APHIS) phytosanitary hot water treatment or postharvest chilling temperature (storage at 10 °C versus 24 °C). Our attempts to either induce or prevent induction of *corte negro* by manipulating N and Ca application rates had no effect on the incidence of *corte negro*. A possible role for quarantine hot water treatment in development of *corte negro* was disproven in the first two seasons of research. However, we established that *corte negro* occurred almost exclusively in fruit from refrigerated storage (three weeks at 10°C plus one week at 24 °C) with no difference among fertilizer treatments.

The abstract was presented at the 2021 FSHS Annual Meeting.

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## A Review of the Effect of Magnesium on Performance of Huanglongbing (HLB)-affected and Non-HLB-affected Citrus Trees

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Additional index words. *Citrus sinensis*, huanglongbing, macronutrient

**Citrus production is one of the most important and valuable fruit industries worldwide, although production and area harvested have been declining in some of the biggest producing countries, mainly because of Huanglongbing (HLB), also known as citrus greening. Nutrients play an important role in defense mechanisms and have the potential to extend the productive life of HLB-affected citrus trees. Ensuring adequate magnesium (Mg) nutrition could be important in this context because of its role in photosynthesis and carbohydrate partitioning. This paper reviews how Mg affects citrus trees with and without HLB. Results from past research are inconsistent, most likely because those studies aimed to raise soil pH and overcome Mg deficiencies by using low-solubility Mg sources (e.g., dolomitic lime). In contrast, recent data using water-soluble Mg fertilizers show that Mg may affect vegetative growth (greater canopy volume and leaf Mg), fruit yield and fruit quality (greater juice acidity). While Mg applications can have positive effects on vegetative growth (e.g., canopy volume) and leaf Mg concentration, and either positive or negative effects on yield and juice quality, grove management including a balanced nutrition program seems to be optimal to address HLB. Adequate management of Mg and its interaction with other nutrients may help keep citrus trees in production despite HLB and a well-designed fertilization program may allow growers to partially overcome the effects of HLB on citrus growth and productivity.**

Citrus (oranges, tangerines, lemons, limes and grapefruit) are the fruit trees with the highest global production, with more than 130 million tonnes (t) of fruit produced per year (FAO, 2019). In the US, Florida is the state with the highest citrus production, accounting for 57% of bearing citrus acreage and 45% of total production in 2016-2017 (FDACS and USDA, 2021). Citrus production in Florida occupied around 170,000 ha in 2020, a 52% reduction from about 350,000 ha in 1996 (USDA, 2020). Production in Florida was less than 3 million t in 2019-2020, down from a peak of 12.4 million t during the pre-HLB 1997-1998 season (FDACS and USDA, 2021). In just 5 years, from 2012 to 2017, the value of orange production in Florida declined by \$330 million (FDACS and USDA, 2021). This reduction that occurred in the past 20 years has been ascribed to the devastating effects of citrus greening that is also called huanglongbing (HLB), hurricane damage and increasing urbanization (Ferrarezi et al., 2019; Kadyampakeni, 2012). A similar decline in area has been observed in Brazil whereas acreage increased in both China and India. Consequently, China and India have both increased orange production whereas US production has declined, although Brazil production has been stable in the last 15 years despite the severe loss in acreage. It is likely that HLB has played a major role in both production and area harvested dynamics in Florida and in other citrus producing countries.

Nutrients play an important role in plant development and defense mechanisms against diseases (García-Mina, 2012; Huber and Jones, 2013; Schumann et al., 2010) and nutrient applications can help citrus trees mitigate HLB symptoms (Handique et al., 2012; Morgan et al., 2016; Pustika et al., 2008; Rouse et al., 2012). Magnesium applications may increase or decrease disease severity, depending on plant species, disease type, environmental conditions, and the rate of Mg application (Datnoff et al., 2007; Spann and Schumann, 2009). Magnesium plays a very important role in phloem transport of photosynthesis products and its imbalance could lead to excessive accumulation of sugar, starch and amino acids in source tissues like leaves (Hermans et al., 2005; Cakmak and Kirkby, 2008; Huber and Jones, 2013). As HLB is a phloem-restrictive disease, a balanced nutritional approach including Mg could benefit the vegetative and productive performance of HLB-infected trees.

The objective of this paper is to review the effects of Mg applications on tree development, productivity and fruit quality variables in citrus trees infected and non-affected with HLB. The role of this divalent cation has been studied and documented, but there is limited information about its effects on HLB-affected citrus trees and most importantly, there is little information about which Mg rates are optimal in the HLB context.

### Description of HLB and its interaction with citrus

One of the major reasons for the decrease in citrus production and cultivated area in Florida is HLB (Alvarez et al., 2016; Court et al., 2018; Kadyampakeni et al., 2015). Huanglongbing, “yellow dragon disease” in Chinese, was first reported in China in 1919 (Bové, 2006). The disease is now found in more than 40 countries in Asia, Africa, and America. HLB was first detected

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in Florida in 2005 although the vector was likely present as early as 1998 (Alvarez et al., 2010).

HLB is caused by the phloem-restrictive, gram-negative bacteria *Candidatus Liberibacter* spp. that is spread by its vector, the Asian citrus psyllid (ACP) (*Diaphorina citri*, Hemiptera: Liviidae). There are three known *Candidatus Liberibacter* species: *Candidatus* (*Ca.*) *Liberibacter* (*L.*) *africanus*, which affects citrus trees in Africa; *Ca. L. asiaticus*, also known as CLAs, which affects citrus trees in Asia and America; and *Ca. L. americanus*, which affects citrus trees mainly in Brazil (Bové, 2006). The vector, ACP, is an insect that sucks phloem sap with a proboscis and infects citrus plants with the causal pathogen during the process. A young plant flush is needed for ACP eggs to be laid and to further develop into nymphs (Stansly et al., 2018).

Huanglongbing causes lower vegetative growth, smaller fruits with no symmetric size and poor color (greening) (Bové, 2006), root mortality, stunted branches, fruit drop, severe leaf defoliation, and finally plant mortality (Bassanezi et al., 2011; Graham et al., 2013; Kadyampakeni et al., 2014). According to Graham et al. (2013), four-year-old symptomatic HLB-affected *Citrus sinensis* 'Valencia' trees may lose up to 38% of root mass density (mg/cm<sup>3</sup> soil) compared to non-symptomatic trees. Furthermore, Johnson et al. (2019) reported that HLB may cause up to 50% root loss early in disease development and 70% when canopy decline starts in citrus. Regarding productivity, HLB had more severe effects on yields in late maturing varieties, like 'Valencia' compared to early and mid-season maturing varieties, like 'Hamlin', 'Westin', and 'Pera' in Brazil (Bassanezi et al., 2011). In addition to yields and vegetative growth, HLB may also affect fruit quality by decreasing sweetness (°Brix) and increasing acidity, similar to immature fruits (Bassanezi et al., 2009; Dagulo et al., 2010).

Currently, HLB has no cure, but important research efforts are under way to develop disease tolerant/resistant cultivars, formulate a chemical cure and establish alternative production practices that would optimize irrigation, plant nutrition and pest management to maintain productivity in affected groves. As recent evidence suggests that HLB symptoms in citrus can be reduced with an enhanced nutritional program (Handique et al., 2012; Kadyampakeni et al., 2016; Morgan et al., 2016; Rouse et al., 2012; Zambon et al., 2019), it is important to continue investigating and understanding the role of nutrients in this plant-disease relationship.

### The role of Mg in citrus nutrition and HLB management

Nutrition plays an important role in the development of citrus plants. Nitrogen (N), phosphorus (P), and potassium (K) are very important components of plant structure and metabolism and micronutrients such as iron (Fe), zinc (Zn) and manganese (Mn) are key parts of enzymatic activities and, photosynthesis (Kadyampakeni et al., 2015; Obreza et al., 2008; Ramírez-godoy et al., 2018). Normally, nutrient management focuses on primary macronutrients, although micronutrients have received increasing attention lately, especially in HLB-affected citrus (Atta et al., 2018, 2020a; Uthman, 2019; Zambon et al., 2019). However, less attention is given to secondary macronutrients like calcium (Ca), sulfur (S), and magnesium (Mg). Magnesium is an important component of the chlorophyll molecule and it is linked to cell division and metabolism (Chen et al., 2018; Morton et al., 2010; Schumann et al., 2010). Magnesium is a bridging element for the aggregation of ribosome subunits, a necessary process for protein synthesis, and under deficiency or excessive concentrations, the ribosome subunits dissociate and protein synthesis stops (Chen

et al., 2018). Besides enzyme regulation, Mg also regulates the cation-anion balance and cellular pH (Hawkesford et al., 2012). There are many interactions among Mg and nutrients uptake in the soil and plant, including synergisms and antagonisms that must be considered in a balanced nutritional approach (Anderson and Albrigo, 1971; Havlin et al., 2013; Quaggio et al., 1992; Zekri, 2016).

Nutrition also plays an important role in disease resistance, as pathogens affect plant physiology after infection. Pathogens can interfere with water and nutrient transport inside the plant, which can cause deficiencies induced by nutrient immobilization and root starvation (Schumann and Schumann, 2009), especially for secondary macronutrients like Mg.

### Magnesium role in the soil-plant interface and in citrus with HLB

Magnesium is absorbed by plants as exchangeable Mg in the clay-organic matter complex (Weil and Brady, 2017) and roots take it up mainly by mass flow (Havlin et al., 2013). Magnesium root absorption takes place in the apical root zone, as opposed to K whose absorption takes place primarily in the basal zone (Morton et al., 2010; Hawkesford et al., 2012). Once the plant absorbs Mg, it is distributed into different organs. In Tarocco orange of southern Italy, Rocuzzo et al. (2012) found that 22% of the annual citrus Mg uptake goes to fruit production, 51% to abscised leaves and 19% to pruning material. This partitioning may be variety-dependent however, as Morton et al. (2010) observed rootstocks that absorb more Mg than others. Seedy citrus varieties may have higher Mg requirements than seedless ones (Zekri, 2016) as seeds represent a tenth of fruit dry biomass but a fifth of Mg fruit content (Camp, 1947). Mineralization of organic residues and composting may help recover Mg from abscised leaves and pruned biomass by making it available again for the plant, but the availability of this recycled Mg will depend on many factors like climate, soil, and management.

Magnesium is involved in starch decomposition and sucrose formation in citrus fruits (Zhou et al., 2018) and Mg-deficient citrus trees have poor fruit quality (Smith, 1966), i.e., smaller fruits with lower acidity and soluble solids content (Quiñones et al., 2012). Magnesium deficiency promotes the accumulation of starch in citrus leaves because carbohydrate export via the phloem is inhibited. Also, Mg deficiency impairs the lignification of vascular organs in roots, affecting water and nutrient flow from roots to aboveground biomass, while also leading to cell wall lignification of vascular cambium and spongy parenchyma cells (Huang et al., 2019). This generates a lower concentration of carbohydrates in sink organs such as roots and higher concentration of carbohydrates in the leaves (Arbona and Gómez-Cadenas, 2012; Hawkesford et al., 2012). Cakmak and Kirkby (2008) suggest that the role of Mg in phloem-loading process seems to be cultivar-specific and the accumulation of carbohydrates in Mg-deficient leaves is caused directly by Mg deficiency stress. As HLB restricts phloem movement and promotes accumulation of carbohydrates in leaves and depletion in roots (Etxeberria et al., 2009; Hawkesford et al., 2012; Huber and Jones, 2013), this high carbohydrate concentration in leaves could provide a favorable environment for pathogens and pests and even dilute the concentration of other nutrients used for plant defense, like Ca (Huber and Jones, 2013). In fact, Mg and K are used to improve phloem movement of carbohydrates, Zn, B, Mn, and other nutrients that are applied foliar in HLB-affected citrus (Rouse et al., 2012).

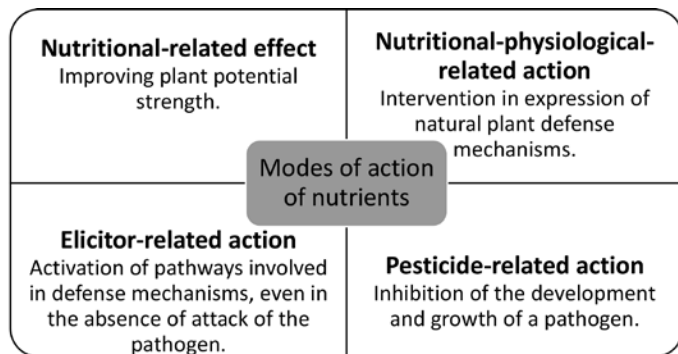


Fig. 1. Different levels of action of nutrients in plant defense mechanisms. Adapted from García-Mina (2012).

Interestingly, HLB-infected citrus trees have less Mg in leaves and roots compared to non-infected trees (Morgan et al., 2016; Zambon et al., 2019; da Silva et al., 2020; Shahzad et al., 2020). Zambon et al. (2019) found that HLB-affected ‘Valencia’ grafted on ‘Carrizo’ rootstock had around 20% less Mg leaf concentration and almost 50% less Mg root concentration compared to non-affected trees in a greenhouse experiment, whereas ‘Valencia’ grafted on ‘Swingle’ rootstock had almost 40% less Mg root concentration compared to HLB-free trees in a field trial. Da Silva et al. (2020) found that HLB-affected citrus plants had less Mg concentration in the sap extract compared to healthy plants. Reduced root growth, either due to HLB or Mg deficiency, can affect Mg uptake, exacerbate Mg deficiency and decrease the uptake of other nutrients (Camp, 1947; Hawkesford et al., 2012; Morgan et al., 2016; Shahzad et al., 2020). Therefore, both the upward and downward movement of nutrients inside the plant are affected by Mg levels, which could potentially accelerate physiological damage, especially for trees affected by HLB.

In contrast, increasing Mg concentrations beyond the optimal level may cause accumulation in vacuoles, which could cause negative effects under drought stress, e.g., photosynthesis inhibition (Hawkesford et al., 2012). A balanced and constant nutrition of Mg is crucial to prevent deficiencies or excess and allow for appropriate plant development and performance.

As Mg affects the expression of plant defense mechanisms, adequate Mg nutrition may help the plant battle against the disease by promoting carbohydrate movement and restoring phloem function in citrus trees affected with HLB (García-Mina, 2012; Rouse et al., 2012; Huber and Jones, 2013) García-Mina (2012) defines and differentiates levels of action for nutrients in plant defense mechanisms (Fig. 1). He emphasizes the importance of understanding how, when and why nutrients are helpful to fight against pathogens and diseases, although the mechanisms responsible for these benefits remain unclear in many studies. According to García-Mina (2012), the role of Mg in citrus is mainly nutritional-physiological-related, i.e., it affects the expression of natural plant defense mechanisms when the plant is attacked by the pathogen. However, Mg may have deeper interactions with disease response in citrus, as Mg can complement or antagonize other minerals and its exchangeable content in soils or tissue may affect the incidence of plant diseases (Huber and Jones, 2013).

### Application of Mg in citrus

In the pre-HLB period, Mg was mainly applied in citrus to correct deficiencies (Koo and Calvert, 1965; Smith, 1966; Koo, 1971; Lavon et al., 1999). A Mg deficiency may appear when soil

exchangeable Mg is low, when soil K and/or Ca exchangeable content are high or when soil pH is low (Obreza and Morgan, 2008). For a Mehlich 3 extraction, less than 25 mg/kg of exchangeable soil Mg is considered to be low in Florida (Obreza and Morgan, 2008). Values considered as low, medium or high for Mg among different soil extractants in Florida can be found in Table 1.

Applications of dolomite can correct Mg deficiencies while also increasing soil pH in acidic soils of the United States, Africa and Australia (Smith, 1966). When soil pH is about 4.5–5, the use of dolomite to raise pH to 6.0–6.5 should increase the availability of Mg. Although this practice was recommended in the past, growers are now opting to acidify soils instead of raising soil pH in the HLB context. Morgan and Graham (2019) found that bicarbonates and high soil pH may be exacerbating the negative effects of HLB in Florida citrus, so they achieved higher yields and higher juice quality by acidifying irrigation water and decreasing soil pH. Therefore, growers should carefully choose their Mg material to avoid raising soil pH to a level that aggravates HLB symptoms. If soil pH is in the optimum range, soil applications of magnesium sulfate/Epsom salt ( $MgSO_4$ ), langbeinite or Sul-Po-Mag ( $K_2SO_4 \cdot 2MgSO_4$ ) and chelates may correct Mg deficiencies (Havlin et al., 2013; Zekri, 2016) and may be better options than dolomite. Soil applications of soluble Mg may require about six months to increase citrus leaf Mg concentrations (Esteves, 2022), whereas less soluble forms like dolomite may require two years (Koo, 1971; Smith, 1966). In addition, maintaining an adequate soil Ca:Mg ratio when fertilizing with Mg is critical, as this ratio should be lower than 10:1 for most crops according to Havlin et al. (2013).

In addition to selecting the right Mg source, using the right Mg application rate is key to correct soil and/or plant deficiencies, which will depend on many factors such as plant and soil Mg concentrations as well as soil pH. Koo (1971) tested three different Mg rates to correct Mg deficiency in citrus: after 3 years, the highest rate (269  $kg \cdot ha^{-1}$  MgO equivalent) corrected visual deficiencies whereas deficiency symptoms were still visible in the two other rates (67.2  $kg \cdot ha^{-1}$  and 168  $kg \cdot ha^{-1}$  MgO equivalent). Atta et al. (2020) and Esteves (2022) tested 45  $kg \cdot ha^{-1}$  Mg and 101  $kg \cdot ha^{-1}$  Mg, respectively, in similar studies with different cultivars and different sites and found significantly higher soil Mg and leaf Mg concentrations with HLB-affected trees. For Florida citrus, Zekri (2016) and Obreza et al. (2008) recommend applying Mg at a rate equal to 20% of the N rate (the recommended N rate is between 135 and 224  $kg \cdot ha^{-1}$ ) when the soil levels of this nutrient are medium to low. When leaves already show deficiencies, foliar applications of magnesium nitrate [ $Mg(NO_3)_2$ ] may correct the nutrient deficit (Zekri, 2016).

Interactions between Mg and other elements may also lead to deficiencies due to interactions and competition for uptake and exchange sites by other cations. High salinity, the use of fertilizers high in potassium salts and manure may aggravate Mg deficiency (Zekri, 2016). In apples, Vang-Petersen (1980) concluded that symptoms of Mg deficiencies were more dependent on the leaf

Table 1. Soil test interpretations used in Florida for three extraction methods (Obreza and Morgan, 2008).

Extractant	Nutrient	Soil test interpretation		
		Low	Medium	High
Mehlich 1	Mg	< 15	15–30	> 30
Mehlich 3	mg/kg	< 25	25–33	> 33
Ammonium acetate (pH 4.8)		< 14	14–26	> 26

K:Mg ratio than leaf Mg concentration alone, as K competes with Mg and the concentration of K in the plant depends on the K:Mg in the growth medium. As a result, recommendations for K:Mg ratios are higher for fruit crops like citrus (2:1) as opposed to field crops (< 5:1) or vegetables (3:1) (Havlin et al., 2013).

### The effects of Mg inputs on citrus growth, yield and fruit quality

Past research conducted on Mg application in citrus evaluated different variables like yield and fruit quality, mostly before HLB. Now that HLB has become widespread, new research is needed to determine if guidelines on Mg nutritional requirements must be adjusted.

**YIELD.** Citrus yield response to Mg applications is inconsistent (Table 2), although many experiments that did not find significant results with Mg application used dolomitic limestone and low-solubility Mg sources. Calvert (1970) did not find any response on dolomitic limestone applications in ‘Cleopatra’ mandarin because soil Mg levels were already adequate, although the low solubility of dolomitic limestone might have also played an important role. In central Florida, Koo (1971) obtained a higher yield (36.8 t of fruit/ha) with a 168 kg·ha<sup>-1</sup> MgO equivalent treatment compared to 67 and 269 kg·ha<sup>-1</sup> MgO equivalent, but soil Mg concentration was not correlated to fruit production or quality. Although he did not find statistically significant differences in yield response due to Mg sources, there was a trend of higher average values with the most soluble sources. Koo (1971) concluded that the optimal Mg rate could be lower than 168 kg·ha<sup>-1</sup> MgO equivalent on soils not severely depleted in Mg, but this experiment was done before HLB was reported in Florida.

In a commercial grove where HLB was detected during the 2005–06 season, Rouse et al. (2012) monitored orange tree (*C. sinensis*) varieties ‘Hamlin’ and ‘Valencia’ that were managed with soil and foliar application of many nutrients, including a combination of foliar (0.84 kg·ha<sup>-1</sup>) and soil (9.6 kg·ha<sup>-1</sup>) Mg applications. Yields slightly increased through time and varied between 45.7 to 73.1 t·ha<sup>-1</sup> for ‘Hamlin’ sweet orange and 34.2 to 62.2 t·ha<sup>-1</sup> for ‘Valencia’ sweet orange between 1999 and 2012, while Florida’s average tended to decline and ranged from 27.9 to 43.3 t·ha<sup>-1</sup> during the same period (Fig. 2). Yields seemed to be sustained by the application of nutrients described by Rouse et al., (2012) and Singerman (2016), although the total annual cost per hectare of this nutritional program (including soil and foliar fertilizations) was around \$1121. This would become an additional expense that growers have to make to maintain grove productivity (Rouse et al., 2012).

Esteves et al. (2021) established a split-plot experiment in central Florida comparing the effects of a 101 kg·ha<sup>-1</sup> Mg treatment to a grower standard treatment receiving 56 kg·ha<sup>-1</sup> Mg across three N levels (168, 224, 280 kg·ha<sup>-1</sup> N), in triplicate plots per combination of N and Mg fertilization. They found no significant difference between the Mg and grower standard treatments at the higher N rates (224 and 280 kg·ha<sup>-1</sup> N), although Mg fertilization increased yields relative to the grower standard at the lowest N rate of 168 kg·ha<sup>-1</sup> (Esteves et al., 2021). This suggests that N could also be playing a role and that a single nutrient may not address a complex disease, although a more balanced and holistic approach may be effective. These results are based on two years of data and may become more evident with a longer experiment.

Table 2. Compilation of results obtained in different experiments with application of magnesium (Mg) in citrus.

Authors	Scion and rootstock	Mg source and MgO (%)	MgO equivalent rate	Yield	CAV <sup>z</sup>	TC <sup>y</sup>	LAI <sup>x</sup>	Leaf Mg concentration	Brix	Acidity
Esteves et al., 2021	Valencia on Swingle citrumelo	MgSO <sub>4</sub> (16%)	101 kg·ha <sup>-1</sup> Mg/year	NS <sup>w</sup>	+ <sup>v</sup>	NS	-- <sup>u</sup>	+	NS	+
Atta, 2019	Hamlin on Cleopatra and Hamlin on Swingle	MgO <sub>3</sub> S <sub>2</sub> (7%)	75 kg·ha <sup>-1</sup> /year	--	NS	--	+	+	--	--
Quaggio et al., 1992	Valencia on Rangpur lime	calcitic (6%) and dolomitic (20%) limestones	0, 3, 6, and 9 t·ha <sup>-1</sup>	--	--	--	--	--	+	+
Koo 1971	Valencia orange on rough lemon	MgSO <sub>4</sub> (27%) MgO (91%) MgCO <sub>3</sub> (20%)	67, 168, and 269 kg·ha <sup>-1</sup> /year	+	--	--	--	+	NS	NS
Calvert, 1970	Temple on Cleopatra mandarin	dolomitic limestone	0–51 kg·ha <sup>-1</sup> at 1st, 4th, 6th, and 10th year	NS	NS	NS	--	NS	NS	NS
Weir, 1969	Valencia on sour orange	kieserite (MgSO <sub>4</sub> )	1.81 and 3.63 kg/tree/year	NS	--	--	--	+	--	NS
Koo & Calvert, 1965	Marsh grapefruit on rough lemon rootstock	magox-90 (91.5%), seawater magnesia (93%), magnesia-65 (66%), magnesium sulphate (27.5%), and langebeinite (18%)	0.27, 0.91, 1.54 kg/tree/year	--	--	--	--	+	--	--

<sup>z</sup>CAV = canopy area/volume.

<sup>y</sup>TC = trunk circumference.

<sup>x</sup>LAI = leaf area index.

<sup>w</sup>NS = no significant results.

<sup>v</sup>+ = increased

<sup>u</sup>Blank cells (--) = either no data available or not measured.

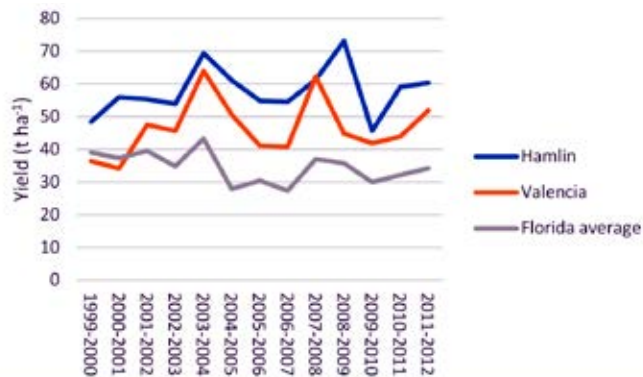


Fig. 2. Total yield by season and citrus (*Citrus sinensis*) variety from the Orange Hammock Grove in Hendry County, FL compared to Florida's average yield. Adapted from Rouse et al. (2012).

**VEGETATIVE GROWTH.** Variables like trunk measurements, tree height and canopy values are used as indicators of vegetative growth in citrus (Calvert, 1970; Obreza et al., 1993). Calvert (1970) reported a non-significant increase in trunk circumference, tree height, tree width and tree canopy area with Mg applied as dolomitic limestone on pre-HLB Temple oranges on 'Cleopatra' mandarin rootstock (Table 2).

Esteves et al. (2021) documented the effect of Mg on canopy volume and TCSA on HLB-affected citrus trees and found a greater canopy volume with the Mg treatment relative to the grower standard when N was supplied at a rate of 168 kg·ha<sup>-1</sup> N. Atta et al. (2020b) found significantly higher leaf area index (40%) in the Mg treatment compared to the control (Table 2).

As HLB restricts phloem movement (Hawkesford et al., 2012; Huber and Jones, 2013), supplying Mg may promote phloem movement (Rouse et al., 2012) and improve vegetative growth. Despite the benefits of Mg fertilization, the seven-year old trees from Esteves et al. (2021) had similar canopy volumes to two-year old trees measured before HLB became widespread (Obreza et al., 1993). Smaller canopy volumes could be due to HLB-driven increases in defoliation and branch stunting that reduce vegetative growth drastically (Bové, 2006; Graham et al., 2013; Kadyampakeni et al., 2014) or because the planting density of the experiment was three times higher compared to commercial groves.

### Leaf Mg concentration

The response of citrus tree leaf nutrient concentration to Mg application depends on many factors, including soil exchangeable Mg content, prior leaf Mg concentration, soil and plant interactions with other nutrients and Mg source and rates. Weir (1969) found a significant increase in leaf Mg concentration with kieserite application, a magnesium sulfate mineral with about 25% MgO and 20% S (Table 2). Koo and Calvert (1965) reported a significant

increase in grapefruit (*Citrus ×paradisi*) leaf Mg concentration with Mg application of oxides and sulfates and no effect on other plant or soil variables, although none of the tested sources had a low solubility (e.g., dolomite, carbonate). Koo (1971) observed a significant increase in leaf Mg concentration among sources and rates for trees that were initially Mg-deficient, with MgCO<sub>3</sub> being slower in correcting the deficiency compared to MgSO<sub>4</sub> and MgO (Table 2). The highest rate (269 kg·ha<sup>-1</sup>) was also faster at correcting Mg deficiency compared to the other two rates tested (67 and 168 kg·ha<sup>-1</sup>). In contrast, Calvert (1970) found no significant difference in leaf Mg concentration when dolomite was applied.

Some of these early Mg experiments used dolomite as a Mg source to raise soil pH and control leaf Mg deficiencies. Dolomite is less soluble than other Mg sources and citrus trees may take several years to respond to fertilization treatments (Camp, 1947; Koo, 1971). Interestingly, Koo and Calvert (1965) observed that Mg may become less available at high pH, regardless of source and rate, highlighting the critical role soil pH and its management play for Mg uptake in citrus trees.

The effect of Mg nutrition may also be influenced by the scion and rootstock combination. Leaf Mg was significantly increased by Mg nutrition in 'Hamlin' grafted on 'Swingle', but it was not clearly influenced in 'Hamlin' grafted on 'Cleopatra' (Atta, 2019). Esteves et al. (2021) found higher leaf Mg with Mg fertilization in 'Valencia' grafted on 'Swingle', regardless of N application rates (Table 2), although yields increased with Mg fertilization only at the lowest N rate, suggesting that the link between leaf Mg and yield is not straightforward, at least not at recommended N rates or above.

Leaf Mg reference levels vary according to location and may also differ depending on variety, rootstock and tree age (Table 3; Menino, 2012). The reference levels given by Rodriguez et al. (1961) and Jorgensen et al. (1978) are based on previous work done by Reuther et al. (1954) and Chapman (1960), more than 50 years ago. More recent studies report lower values, with critical levels of Mg equal to 0.3% (Liu et al., 1984) or 0.13% (Shimizu et al., 1985). In contrast, Quaggio et al. (1992) reported slight Mg deficiencies in 'Valencia' trees with leaf Mg concentration below 0.35%, which is within the optimum level reported by Koo et al. (1984) for Florida citrus prior to HLB. Given the devastating impacts HLB has on citrus physiology and growth, cultivar-specific and site-specific critical nutrient tissue concentrations should be developed to better understand the functions and interactions of individual nutrients in HLB-affected plants (Menino, 2012).

Furthermore, leaf Mg guidelines established either before HLB or in locations where HLB has not been reported yet must be updated. For example, the most recent study cited in Table 3 took place in Spain in 2010, a country where HLB is not yet present. In addition, there is no agreement on Mg excess and/or toxicity values, as some are still reported as uncertain. Finally,

Table 3. Leaf magnesium reference concentrations (%) for citrus in five different locations.

Authors	Country	Citrus variety	Deficient (less than)	Low	Normal (optimum)	High	Excess (more than)
Rodriguez et al., 1961	Brazil	Citrus	0.15	0.16–0.29	0.30–0.60	0.70–1.10	1.20 <sup>z</sup>
Embleton et al., 1978	California, USA	Valencia Late orange	0.16	0.16–0.25	0.26–0.60	0.70–1.10	1.20 <sup>z</sup>
Jorgensen et al., 1978	Australia	Citrus	0.16	0.16–0.25	0.25–0.60	0.6–1.20	1.20 <sup>z</sup>
Koo et al., 1984	Florida, USA	Citrus	0.20	0.20–0.29	0.30–0.49	0.50–0.70	0.70
Quiñones et al., 2010	Spain	Citrus	0.15	0.15–0.24	0.24–0.45	0.46–0.90	0.90

<sup>z</sup>Uncertain values.

nutrient guidelines should be revised based on a balanced nutrition approach rather than individual nutrients taken in isolation. Lower Mg:Ca ratios may indicate Mg absorption and translocation to the leaf, and it could be a useful indicator if it correlates well to yields or fruit quality.

### Fruit quality

Citrus fruit quality is evaluated with variables like Brix (i.e., total soluble solids), acidity, the Brix:acidity ratio, kg solids per hectare and kilograms juice per hectare. According to Koo (1988), soil applications of Mg slightly increase juice quality variables such as Brix, Brix:acidity ratio, fruit size and weight, and decrease rind thickness.

Carbohydrates are the most prevalent compound in citrus juice, accounting for about 80 % of total soluble solids (TSS) (Kimball, 1999). Carbohydrates increase with soil applications of N, Mg and Fe and decrease with K (Koo, 1988). Quaggio et al. (1992) found that Mg increases TSS in citrus juice and Koo (1971) found a non-significant increase in TSS with Mg. In contrast, Esteves et al. (2021) found no significant difference in TSS concentration with Mg application (Table 2), although TSS content was higher in the Mg treatment compared to the control, with all treatments above the minimum threshold of 11 established by the USDA for Grade A pasteurized orange juice (Kimball, 1999).

Acids, mainly citric and malic acids, are the second most abundant solids in citrus juice (Kimball, 1999; TETRA PACK, 2004). The Brix:acidity ratio is an indicator of fruit maturity and it measures the balance between the sweet and sour sensation of juice (Kimball, 1999; TETRA PACK, 2004). Since sour and sweet flavors compete for the same receptor sites in the tongue, the ratio between Brix and acidity is more important than the specific amount of each (Kimball, 1999). Previous studies report mixed effects of Mg on juice acidity, as Mg can either increase juice acidity (Moss and Higgins, 1974; Quaggio et al., 1992) or show no effect (Calvert, 1970; Koo, 1971; Koo, 1988; Weir, 1969). As Mg deficiency can decrease acidity and soluble solids (Quiñones et al., 2012), Mg applications can increase the acidity in oranges, possibly through a reduction in Ca uptake (Moss and Higgins, 1974; Quaggio et al., 1992). Vang-Petersen (1980) also found a reduction in Ca uptake due to Mg application in apple trees. However, Weir (1969) found no effect of Mg applications on 'Valencia' fruit juice acidity whereas Koo (1988) reported that Mg increased the Brix:acidity by increasing the Brix content. Esteves et al. (2021) found mixed results: a greater percent acidity and lower Brix:acidity ratio with Mg fertilization relative to the unfertilized control in the first year, compared to a non-significant decrease in juice acidity with Mg fertilization relative to the control in the second year (Table 2). The Brix:acidity ratio was compliant with USDA standards for unsweetened Grade A pasteurized orange juice in Florida for both years, although the acceptable Brix:acidity ratio varies among states (Kimball; 1999), highlighting that the effect of Mg fertilization on this indicator must be evaluated locally.

### Future trends in managing nutrition of HLB-affected citrus

The citrus industry can take multiple paths to address issues related to nutrition and HLB. As the development of tolerant and/or resistant varieties to HLB is underway, plant breeders should develop rootstocks and scions that are resistant and tolerant against HLB and have high nutrient uptake to maximize benefits for improving plant immunity, growth and overall performance. Meanwhile, research that improves our understanding of nutri-

ent placement and delivery mechanisms (foliar, soil application, trunk injection) along with water management practices and plant physiology should be prioritized to improve nutrient use efficiency and environmental quality in the mid- to long-term.

In addition, research should determine the nutritional requirements of emerging citrus varieties under endemic HLB conditions while considering the management of soil health, soil organic matter and soil organisms. The interaction between nutrients in the soil and plant related to the heterogeneity of soils and the responses obtained in the HLB era warrants further investigation not only in Florida, but also in other citrus producing regions.

Finally, despite promising results in terms of vegetative growth and yields, research on Mg fertilization in HLB-affected citrus trees should consider fruit quality variables like Brix:acidity ratio and consumer preference surveys to ensure the quality of juice and fruit produced is acceptable to fruit buyers, processing industries and consumers. Because interactions among nutrients are complex, determining the optimal ratios of Mg among nutrient management programs is key to ensure balanced nutrition in HLB-affected trees.

### Conclusion

Although past research conducted on the effects of Mg application in citrus was mostly focused on using dolomitic lime to raise soil pH, the extent of HLB-affected areas illustrates the need to focus future research on soil – plant – disease interactions, not only with Mg cycling but with the other nutrients as well. This is critical as Mg applications can have positive effects on vegetative growth (e.g., canopy volume) and leaf Mg concentration and either positive or negative effects on juice quality. However, adjusting the supply of a single nutrient may not be optimal, and a holistic grove management that includes a balanced nutrition seems more promising to address HLB. Adequate management of Mg and its interaction with other nutrients may help keep citrus trees in production despite HLB, as a well-designed nutritional program may allow growers to partially overcome the effects of HLB disease on citrus growth and productivity.

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## Split Application of Essential Nutrients Enhances Tree Growth of HLB-affected Citrus Trees on Florida Sandy Soils

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**Additional index words.** huanglongbing, leaf area index, root length density

Studies indicated that greater than 30% of the roots of huanglongbing (HLB)-affected citrus trees are damaged before canopy symptoms appear and that greater than 70% of root loss could be prevalent as citrus deprived of intensive cultural management to reduce abiotic and biotic stresses. The objective of this study was to determine whether split applications of essential nutrients reverse the deterioration of fine root length density (FRLD), tree canopy volume and leaf area index caused by HLB. Statistically significant leaf manganese (Mn) and zinc (Zn) concentrations were detected on the treated trees as compared to the control trees. The results indicated that soil-applied Mn had a positive effect in correcting the nutrient deficiency that might have occurred because of the seasonal crop removal. On the other hand, the inherent bioaccumulation and less mobility of Zn showed that foliar application of Zn could satisfy the annual requirement of Zn by the citrus trees. FRLD and leaf area index (LAI) were significantly affected by the highest micronutrient rate, a manifestation of possible Mn toxicity. These results indicate that citrus trees react positively to the split essential nutrient applications.

Recently, huanglongbing (HLB, or citrus greening) has become the greatest threat to the citrus industry in Florida and worldwide. HLB is a major contributor to citrus yield reduction in Florida (Gottwald et al., 2012; Graham et al., 2020). HLB alters the host plant's physiology, decreases root density by 50%, thus ultimately affecting nutrient absorption, movement, and assimilation (Kadyampakeni et al., 2014; Graham et al., 2013). Poor fibrous density is reflected in weak tree leaf canopy (TCV), deterioration in fruit yield, and poor fruit quality (Morgan et al., 2006; Quiñones et al., 2003). HLB-affected citrus trees with reduced fibrous roots, develop visible symptoms, such as interveinal chlorosis of young leaves, followed by blotchy mottling of older leaves and starch overaccumulation that distorts the grana in the chloroplast (Fan et al., 2013; Hamido et al., 2019). Research has indicated that water uptake by HLB-affected citrus trees is about 18 to 29 % less than by healthy citrus trees (Morgan et al., 2016). Previous studies have reported that trees with lower root density, as occurs in HLB-affected citrus trees, can have lower water uptake and nutrient concentrations (Hamido et al., 2017).

Citrus growers in Florida have been using foliar spraying of micronutrients [manganese (Mn), zinc (Zn), and boron (B)] to supply the required nutrients (Gottwald et al., 2012; citrus huanglongbing (HLB, Morgan et al., 2016). Research results have demonstrated mandarin trees grown in various soil types treated with various fertilizer rates have experienced reduced HLB-induced symptoms by approximately 40% (Pustaka et al., 2008) These findings supported the assumption that HLB-affected trees have limited soil nutrient uptake and eventually nutrient utilization. Hence, foliar-applied nutrients might extend tree life and increased the yield of HLB-affected trees (Morgan et al.,

2016). The rate and method of application of foliar nutrition and their impacts on tree nutrient uptake, accumulation, movement in the soil and the plant are not well understood in HLB-affected citrus trees. Therefore, the objective of this study was to determine whether tree growth, water and nutrient uptake and accumulation in HLB-affected 'Valencia' trees are affected by soil and foliar applied essential nutrients.

### Materials and Methods

The study was conducted at the University of Florida, Southwest Florida Research and Education Center (SWFREC) near Immokalee, FL. The study used 15-year-old sweet orange [*Citrus sinensis* (L.) Osbeck] 'Valencia' trees budded on 'Swingle' (Swingle citrumelo [*C. paradisi* Macf. × *Poncirus trifoliata* (L.) Raf.] rootstocks. The trees were planted on Immokalee fine sand, which is a poorly drained soil in the Flatwoods, containing sandy marine sediments with slopes < 2%. The experiments were set up in a split-split plot design that contained three nitrogen (N) rates (150, 200, and 250 lb/acre/year) as main block and three soil-applied micronutrient rates including an untreated control at all combinations, with four replications. The N fertilizer was fertigated along with the normal irrigation line in a split on a biweekly basis, summing up to 20 times per year from February to November. The sub-plots had received (1×) [Mn (10 lb/acre/year), Zn (10 lb/acre/year), and B (5 lb/acre/year)] treatments randomly within the N rates.

Twenty 4- to 6-month-old leaves were randomly collected across four quadrants (north, south, east, and west) of the canopy of each tree. The leaves were collected from non-fruiting branches located at approximately 2/3 of the height of the canopy. Oven dry samples of 0.5 g dry leaf tissue were weighed and incinerated at 500 °C for 16 h and processed by Inductively Coupled Plasma

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Optical Emission Spectroscopy (ICP-OES) (Spectro Ciros CCD, Fitzburg, MA).

The tree leaf area index (LAI) was estimated using a SunScan canopy device (Dynamax Inc. Huston, TX) during sunny days as per the recommendation of the manufacturer (Kadyampakeni et al., 2014b). Minirhizotron tubes were installed in Mar. 2017 at 0.5 m from either the east or west of the tree trunk (perpendicular to the tree row). Root minirhizotron measurements were conducted on eight trees per treatment (4 micronutrient rates  $\times$  4 replications) using transparent minirhizotron acrylic tubes with an external diameter of 2.75 in and 24 in length. The tubes were inserted at an angle of 45° with the respect to the ground and a perpendicular depth of 18 in.

## Results and Discussion

Leaf Mn concentration was significantly higher when trees received the foliar (1 $\times$ ) coupled with the soil (1 $\times$ ) or foliar (1 $\times$ ) and the soil (2 $\times$ ) doses treated trees than the control trees. Only the foliar-treated trees showed no significant difference as compared with the control trees, yet within the optimum range of nutrient concentration (Fig. 1A). Leaf Mn concentration had

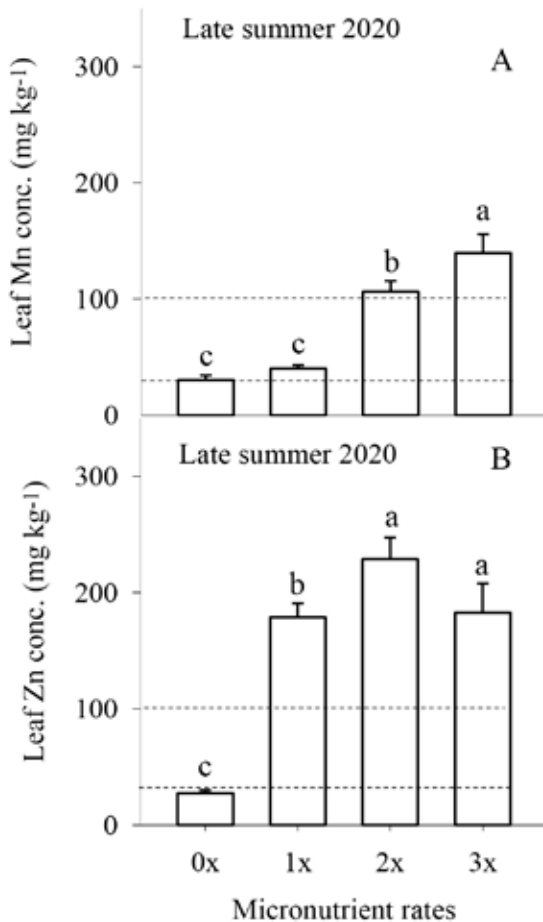


Fig. 1. (A) Leaf manganese (Mn) and (B) zinc (Zn) concentration on sweet orange [*Citrus sinensis* (L.) Osbeck] ‘Valencia’ trees budded on ‘Swingle’ [*Swingle citrumelo* (*Citrus paradisi* Macf.  $\times$  *Poncirus trifoliata* (L.) Raf.) rootstocks in reaction to the control (0 $\times$ ), foliar (1 $\times$ ) (1 $\times$ ), foliar (1 $\times$ ) and ground (1 $\times$ ) (2 $\times$ ), and foliar (1 $\times$ ) and ground (2 $\times$ ) (3 $\times$ ), (1 $\times$  = 10 lb/acre of manganese (Mn) and zinc (Zn) each and 5 lb/acre/year of boron. Error bars indicate the mean values (n = 12 trees)  $\pm$  standard error of the mean. Different letters on the bars indicate significance at  $P < 0.0001$ . Horizontal broken lines across the two panels indicate the optimum range of critical nutrient concentrations of leaf Mn and Zn.

a magnitude of 1.1 $\times$ , 2.2 $\times$ , and 3.2 $\times$  greater for trees under the foliar (1 $\times$ ) only, the foliar (1 $\times$ ) and soil (1 $\times$ ) or foliar (1 $\times$ ) and soil (2 $\times$ ) treatments, respectively. This indicates that the addition 1 $\times$  more dose to the soil was not worthwhile. In addition, the highest leaf Mn concentration of the highest micronutrient rate was an indication of the highest mobility of Mn from the soil to the tree.

All of the treated trees showed excess leaf Zn concentration regardless of the rates and methods of applications (Fig. 1B). Leaf Zn concentration had a magnitude of 2.6 $\times$ , 5.7 $\times$ , and 5.4 $\times$  greater for trees under the foliar (1 $\times$ ) only, the foliar (1 $\times$ ) and soil (1 $\times$ ) or foliar (1 $\times$ ) and soil (2 $\times$ ) treatments, respectively. This indicates that persistent only foliar application can satisfy the annual requirement of Zn to HLB-affected citrus trees. Previous studies also indicated that soil-applied Zn was susceptible to unprecedented soil fixation (Fu et al., 2016) and was less mobile in the trees (Atta et al., 2021b).

There was lower LAI in trees under the highest micronutrient rates, while the other treatments had no significant difference for LAI (Fig. 2). There was 8%, 5%, and -17% of LAI in reaction to the only foliar (1 $\times$ ), the foliar (1 $\times$ ) coupled with the soil (1 $\times$ ) or foliar (1 $\times$ ) and the soil (2 $\times$ ) rate treated trees, respectively. The relative reduction in LAI to the highest micronutrient could be potentially be attributed to Mn toxicity. We also observed the lowest fine root length density (FRLD) when the tree received the highest micronutrient rates.

After the winter season, trees generally started to grow from February onward. Thus, the fine roots simultaneously started to grow accordingly. There was a significant FRLD growth for trees that received foliar or control trees from Feb. to Aug. 2021 (Fig. 3). Since the micronutrients promote above-ground biomass, the FRLD was affected as trees need to balance the above and below-ground growth. However, the highest micronutrient FRLD was significantly lower as compared to the other treatments. This result could be related to the Mn toxicity described earlier. HLB-affected trees react to foliar and ground-applied micronutrient rates such that leaf Mn, Zn concentrations were increased

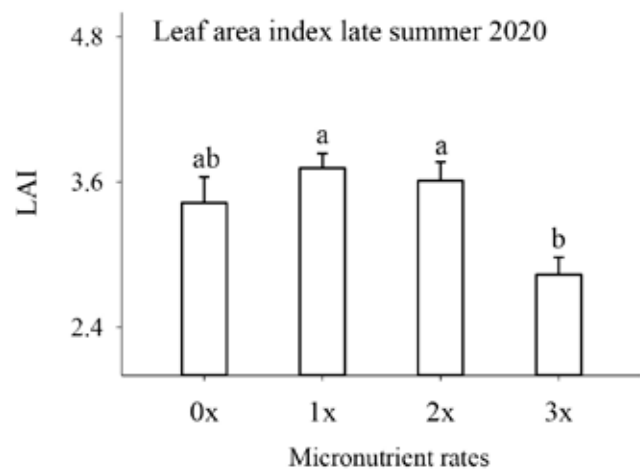


Fig. 2. Leaf area index in sweet orange [*Citrus sinensis* (L.) Osbeck] ‘Valencia’ trees budded on ‘Swingle’ [*Swingle citrumelo* (*Citrus paradisi* Macf.  $\times$  *Poncirus trifoliata* (L.) Raf.) rootstocks in reaction to micronutrient rates: control (0 $\times$ ), foliar (1 $\times$ ) (1 $\times$ ), foliar (1 $\times$ ) and ground (1 $\times$ ) (2 $\times$ ), and foliar (1 $\times$ ) and ground (2 $\times$ ) (3 $\times$ ), (1 $\times$  = 10 lb/acre of manganese and zinc each and 5 lb/acre/year of boron. Error bars indicate the mean values (n = 12 trees)  $\pm$  standard error of the mean. Different letters on the bars indicate significance at  $P < 0.05$ .

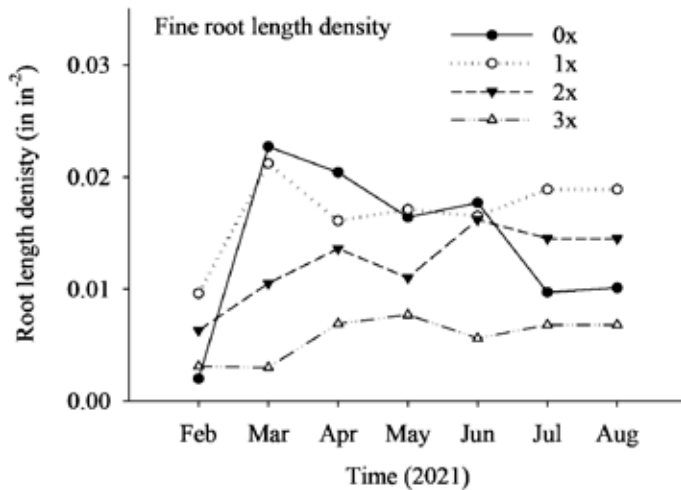


Fig. 3. The effect of micronutrients on fine root length density on sweet orange [*Citrus sinensis* (L.) Osbeck] 'Valencia' trees budded on 'Swingle' (Swingle citrumelo [*Citrus paradisi* Macf. × *Poncirus trifoliata* (L.) Raf.] rootstocks: control (0x), foliar (1x) (1x), foliar (1x) and ground (1x) (2x), and foliar (1x) and ground (2x) (3x), (1x = 10 lb/acre of manganese and zinc each and 5 lb/acre/year of boron on fine root length density during the Spring and Summer 2021 growing seasons.

accordingly. However, micronutrients promoted vegetative growth and compromised FRLD as excess micronutrient was applied to the soil. The highest micronutrient rate (30 lb/acre/year) had a detrimental effect on both above and below-ground tree growth, probably because of Mn toxicity.

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## Improved Irrigation Increases Water Use Efficiency and Tree Growth on Growers' Citrus Groves

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**Additional index words.** branching angle, canopy volume, root length density, soil moisture

The agriculture industry consumes the highest freshwater withdrawals and the Florida citrus industry uses about 30% of irrigated cropland acreage. Availability of water is one of the most significant restrictions on crop production. Therefore, the development of new management approaches to improve irrigation efficiency is an urgent need. The objective of this study was to identify commercial citrus groves, investigate the impact of irrigation rates based on crop water requirements, and to determine the best water use efficiency techniques at selected irrigation rates and tree densities. The study was conducted at a commercial citrus grove near Immokalee, Florida, on nine-year-old sweet orange (*Citrus sinensis*) trees. The experiment consisted of three tree densities (145, 196, and 373 trees per acre) and three irrigation rates: 50%, 78%, or 100% of the reference evapotranspiration (ET<sub>o</sub>). In reaction to the different irrigation rates, soil moisture on the top three soil layers (0–6, 6–12, and 12–18 inches) remained uniformly distributed when the tree received 78% irrigation rate on the highest tree density (373 trees/acre). Meanwhile, the highest tree density which received the lowest irrigation (50%) rate had the greatest root length density (RLD), tree root volume (TRV), and average root branching angle (ABA) indicating that lower irrigation promoted more root growth than the highest irrigation rates. Root branching was significantly higher on trees that received the lowest irrigation volume than the highest which may be an indication for the highest resource utilization efficiency. The moderate irrigation rate and tree densities had the highest RLD, TRV, but similar ABA. The highest tree canopy volume occurred at the lowest tree density, yet no significant variation was detected because of the irrigation rates.

The agriculture industry consumes the highest water volume and accounts for 70% of worldwide freshwater withdrawals (Mbabazi et al. 2017; United Nations, 2015). The Florida citrus industry accounts for the greatest share (30%) of irrigated cropland acreage (The Balmoral Group, 2018). Availability of water is one of the most significant restrictions on crop production (Graham et al. 2013). Due to the increasing demand for drinking and industrial usages, the percentage of agricultural water is predicted to decrease (United Nations, 2015). The future higher crop production requirements to feed an increasing population with decreased water availability represents a significant challenge to researchers in crop production (Kadyampakeni et al., 2014). In citrus, the lack of water at any stage of the growth decreases the yield and fruit quality. In contrast, higher irrigation amounts could result in substantial loss of nutrients and herbicides from the citrus root zone through deep percolation and surface runoff, which can cause polluted water resources in the surrounding environment include lakes, rivers, and groundwater (United Nations, 2015). Therefore, developing better water management is urgently needed. Typical methods for monitoring crop water stress include measurements of soil water content, plant growth, root development, and meteorological variables including evapotranspiration (ET<sub>o</sub>) and precipitation. The objectives of this study were: 1) identify commercial citrus groves with tree densities ranging from 1145–370 trees per acre; 2) investigate the impact of irrigation rates as a function of evapotranspiration (ET) on each tree density level and 3) determine water use and gas exchange rate of tree at selected irrigation rates.

### Materials and Methods

#### Site description and experimental design

The study was conducted at Ranch One Corporation, a commercial citrus producer. The site had sandy soils consisting more than 90% sand, located in Southwestern Florida near Immokalee, Florida (lat. 26.50° N, long. 81.27° W) during 2019–2022. The grove was planted during 2010 with 'Valencia' (*Citrus sinensis*) trees grafted on the Carrizo, US-812, and US-897 citrus rootstock with planting densities of 163, 283, and 373 trees per acre. The experiment was designed as a split-plot design on three blocks of selected densities and irrigation patterns.

The experimental area consists of three-1000-foot-long beds with drainage swales on each side. The three beds were divided into 42 plots of three trees densities and were replicated six times and arranged as randomized complete-block design. Trees spacing were: a) 8 feet between trees and 14.6 ft between rows (373 trees/acre); b) 12 feet between trees and 22.2 ft between rows (145 trees/acre); and c) 6.8 feet between trees and 22.6 feet between rows (196 trees /acre). All trees were planted in Malabar fine sand soil (loamy, siliceous, active, hyperthermic Grossarenic Endoaqualfs).

**TREATMENTS AND DATA COLLECTION.** Trees received irrigation with the micro-jet sprinkler method, with one emitter per tree (lower density) or one emitter per two trees (higher density) and different flow rate to meet the proposed irrigation treatment per tree as following: 8.3 gallons/h, 12.8 gallons/h, and 16.5 gallons/h emitter for 50% ET<sub>o</sub>, 78% ET<sub>o</sub>, and 100% ET<sub>o</sub> emitters, respectively. Irrigation emitters were placed one foot away from each tree trunk at the lower density rate or in the middle between two trees at higher planting rates.

Six soil moisture devices (SDI-12 Drill and Drop Probe; Sentek, Stepney, South Australia) were installed per tree and used for each irrigation rate at the three densities to record the soil moisture

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configuration along with the three soil profiles (Ayankojo et al. 2020). The soil moisture sensors use the capacitance method of estimating volumetric water content and were used to determine irrigation effects on the soil moisture status. Root growth pattern data were collected using minirhizotron tubes installed in the soil where about 2-ft long acrylic tubes were inserted 1 ft from the tree at 45° to the ground. Root images were captured using a CI-600 In-Situ Root Imager camera (CID-Bioscience, Camas, WA), processed, and converted these images into a quantitative variables using Root Snap CI-690 software (version 1.3.2.25, CID-Bioscience, Camas, WA, USA). The tree canopy volume of a tree was determined by measuring the average diameter width of each tree in the east-west and north-south and canopy height:

$$TCV = \frac{4}{3} \times \pi \times r^2 \times \left(\frac{h}{2}\right)$$

where: TCV = Tree canopy volume (ft<sup>3</sup>); r = mean canopy radius (ft<sup>2</sup>); and h = canopy height (ft).

## Results and Discussion

### Soil moisture

**TWO-ROW TREES (145 TREES/ACRE).** The soil moisture extended as far as 18 in and most of which eventually accumulated in the lowest soil layers when the grower's preferred irrigation rate (100%) was applied under the highest irrigation rate (Fig. 1). This also had implications on the root growth of the trees. When trees received the lower irrigation (78%), the soil moisture had uniform distribution across the three soil layers during the entire season.

**TWO ROWS (196 TREES/ACRE).** The soil moisture under the full irrigation had about 200% more water in the lower soil layers (12 and 18 inches) during the spring season (January – mid-April). The distribution of the soil moisture was higher during summer than the spring season making a continuous soil moisture pattern across the three layers in the full irrigation (Fig. 2). The pattern of soil moisture and the distribution across the three layers were uniform when the tree received lower irrigation (78%) as compared with the grower full irrigation (100%). Irrigation water accumulation at the lowest soil profile was considered wastewater beyond the reach of the root zone. The tree orientation in the moderate tree density had less penetration of sunlight and wind gust that enabled the soil moisture to remain moist. This had also an impact on the RLD, TRV, and ABA of the root remains

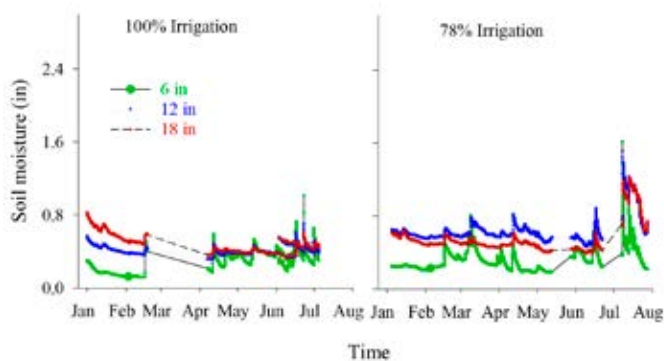


Fig. 1. Soil moisture of three soil layers (0–6, 6–12, and 12–18 in) under two-rows 'Valencia' (*Citrus sinensis*) trees with a single sprinkler per tree and tree density (145 trees /acre) at Ranch One Corporation, near Immokalee, FL during 2021.

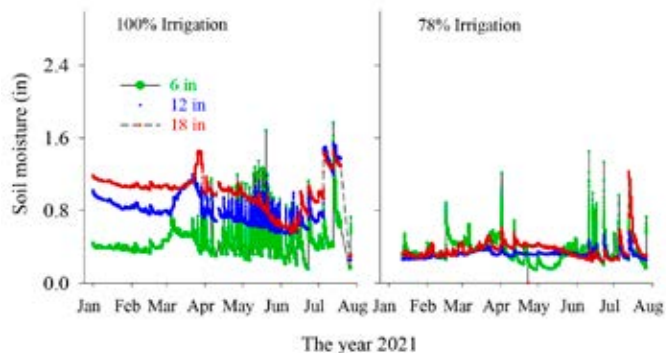


Fig. 2. Soil moisture of three soil layers (0–6, 6–12, and 12–18 in) under a two-row 'Valencia' (*Citrus sinensis*) tree with a single sprinkler per two trees and tree density (196 trees /acre) at Ranch One Corporation during 2021.

the lowest as compared to the other tree densities and irrigation.

**THREE-ROW TREES (373 TREES/ACRE).** The soil moisture on the top three soil layers (0–6, 6–12, and 12–18 inches) remained uniformly distributed when the tree receive 78% of the grower rate (Fig. 3). The higher and lower rates had either over moisture or uneven moisture distribution within the active root zone soil layer (0–18 inches). Higher accumulation of water was observed under the two extreme irrigations. The sharp increase in the soil moisture in all the graphs in July and August was the result of the commencement of the rainy summer season. The highest increase in the soil moisture during this season on the lowest soil depth was because of the increase in the soil water table.

### Root Growth Pattern

The lowest tree density (145 trees /acre) showed the highest RLD, TRV, but similar ABA when trees received the lower irrigation (78%) than the highest irrigation (100%) rate (Fig. 4 and Table 1). Similarly, the moderate tree density (196 trees /acre)

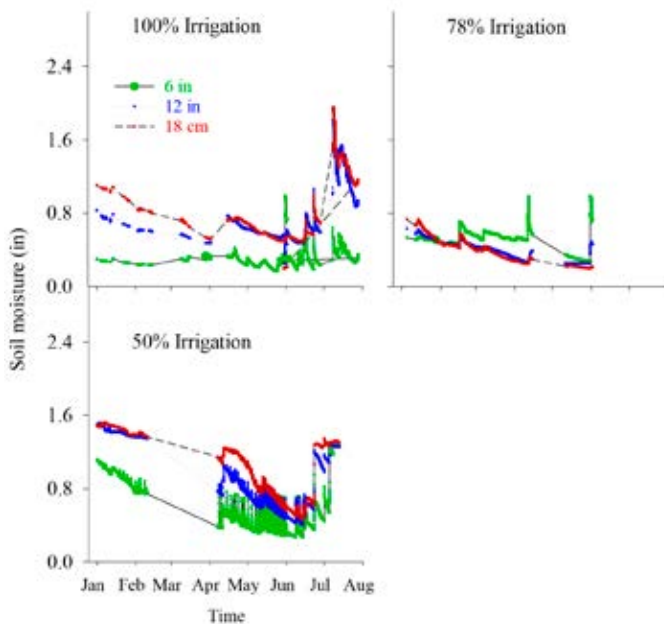


Fig. 3. Soil moisture of three soil layers (0–6, 6–12, and 12–18 in) under three-row 'Valencia' (*Citrus sinensis*) trees with a single sprinkler per tree and tree density (372 trees /acre) at Ranch One Corporation, near Immokalee, Florida during 2021.

Table 1. Effect of plant density and irrigation volume on citrus ‘Valencia’ (*Citrus sinensis*) tree roots: tree root volume (TRV), and average root branching angle (ABA) during 2021 growing season.

Treatments	50% (ET <sub>0</sub> )		78% (ET <sub>0</sub> )		100% (ET <sub>0</sub> )	
	TRV (in <sup>3</sup> )	ABA (°)	TRV (in <sup>3</sup> )	ABA (°)	TRV (in <sup>3</sup> )	ABA (°)
Two rows <sup>z</sup>	— <sup>y</sup>	—	12.5	55.3	4.9	52.4
Two rows <sup>x</sup>	—	—	9.8	53.9	3.8	0
Three rows <sup>w</sup>	3.1	62.2	2.0	34.3	0.78	0

<sup>z</sup>Two-row tree with a single sprinkler per tree and tree density (145 trees /acre).

<sup>y</sup>No data available for 50% ET<sub>0</sub> irrigation for these tree densities.

<sup>x</sup>Two-row trees with a single sprinkler per two trees and tree density (196 trees /acre).

<sup>w</sup>Three-row trees with a single sprinkler per tree and tree density (372 trees /acre).

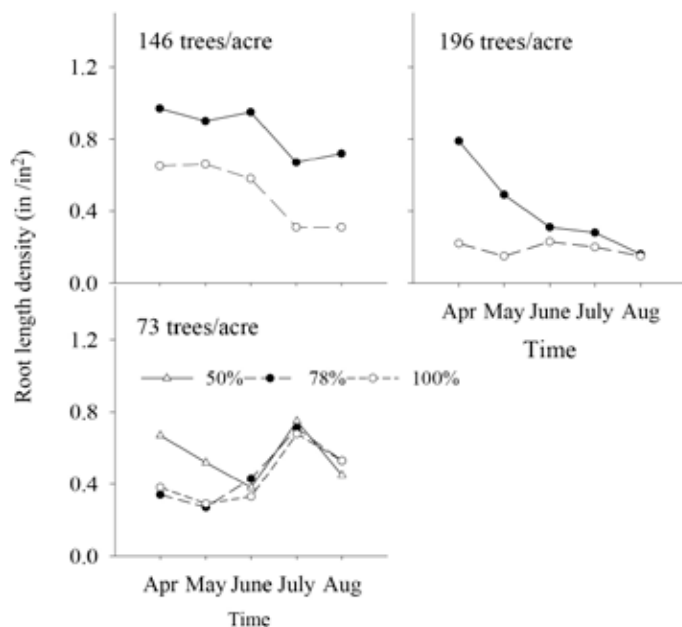


Fig. 4. Root length density under different ‘Valencia’ (*Citrus sinensis*) tree densities at Ranch One Corporation, near Immokalee, FL during 2021.

showed a significant RLD, TRV, and ABA when trees received the lower irrigation (78%) than the highest irrigation. Therefore, under the lowest irrigation, root growth was greater than the highest irrigation that resulted in increased water uptake efficiency. Trees at the highest density (373 trees /acre) and the lowest irrigation (50%) had the greatest RLD, TRV, and ABA indicating that lower irrigation rates promotes root growth. Root branching was significantly higher on trees receiving the lowest irrigation volume than the highest (100%) which is an indication for the highest resource utilization.

### Tree Canopy Volume

The tree canopy volume was significantly higher when the trees had wider spaces to grow than high-density tree arrangements. (Fig. 5)

### Conclusion

The current results indicated that the moderate irrigation rate (78%) and moderate tree density (196 trees /acre) showed the highest water use efficiency and its impact on root developments and tree growth. The pattern of soil moisture across the three soil layers was uniform when the tree received moderate irrigation

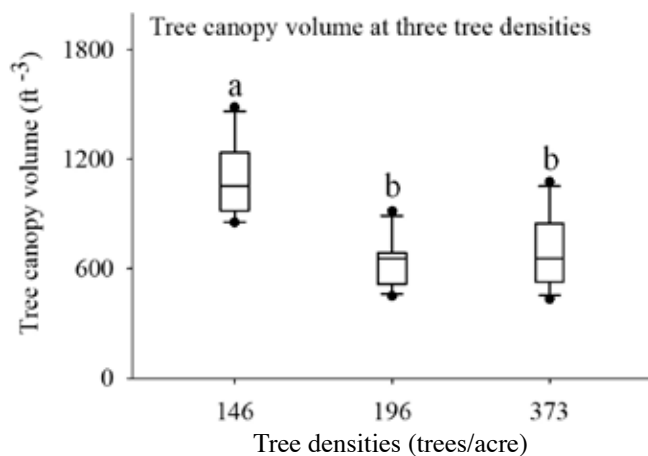


Fig. 5. Tree canopy volume of ‘Valencia’ (*Citrus sinensis*) under three planting densities.

(78%) as compared with the grower’s preferred full irrigation (100%). However, the increase in the soil moisture in all irrigation rates and tree densities in summer (July and August) because of the increase in soil water, suggesting water-saving strategies need to be applied or the entire halt of irrigation during the rainy season. Meanwhile, the highest tree density which received the lowest irrigation (50%) rate had the greatest RLD, TRV, and ABA indicating that lower irrigation promotes more root growth, hence, the highest water use efficiency. Generally, reduced irrigation promoted the highest RLD, TRV, and ABA for increased water uptake and resource optimization.

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—Scientific Note—

## Efficacy and Effects of Trunk Injection for Delivering Imidacloprid and Oxytetracycline to HLB-affected Sweet Orange Trees

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**Additional index words.** huanglongbing

Huanglongbing (HLB), associated with the phloem-limited bacterial pathogen, *Candidatus Liberibacter asiaticus* (CLAs), is vectored by the Asian citrus psyllid, *Diaphorina citri*. The location of the pathogen in the tree vascular tissue limits the effectiveness of foliar-applied therapeutic compounds. Because of the abundance of the psyllid vector throughout Florida, management is challenging and typically relies on insecticides applied as a foliar spray or by soil drenching. Trunk injection is an alternative delivery method which supplies crop protection materials directly into the xylem of a woody species. This technique can optimize the availability of the compound while minimizing drift, runoff, or damage to non-target organisms.

Five-year-old HLB-affected 'Valencia' sweet orange (*Citrus sinensis*) trees were injected with therapeutic compounds in Oct. 2020 and Apr. 2021 using two ChemJet tree injectors on opposite sides of the trunk. Trees received injections of either 2 g of ArborOTC (37% oxytetracycline) dissolved in 40 mL water, 4 mL of Xytect (10% imidacloprid), or 40 mL of water as a control. Trees were monitored for differences in: i) canopy health measured using visual ratings; ii) psyllid mortality by caging laboratory reared adults on individual shoots; iii), bacterial titer in roots, bark, and leaves using RT-qPCR; and iv) fruit quality, fruit drop, and yield at harvest.

Four months after the first injection, trees injected with oxytetracycline had a significantly higher mean Ct-value in both root ( $P < 0.0001$ ) and leaf tissue ( $P = 0.006$ ) compared to the mean Ct-values of trees injected with the water control. From December until February, fruit drop averaged 20% in oxytetracycline

treated trees, compared to 82% in control trees ( $P < 0.0001$ ). This corresponded to an average yield at harvest of 9.4 kg after oxytetracycline injection and 2.5 kg in the water control ( $P = 0.0012$ ). Fruit quality ( $P = 0.003$ ) and peel color ( $P < 0.0001$ ) at harvest were significantly improved for oxytetracycline-injected trees compared to the water control. Tree health was significantly ( $P < 0.0001$ ) improved in oxytetracycline injected trees compared to the controls six months after injection.

Leaf concentrations of imidacloprid two weeks after injection were 271 ppb, which caused a 63% mortality of reared adult psyllids and reduced progeny survival by 80% ( $P = 0.0038$ ) compared with the control. Two months after injection, leaf concentrations of imidacloprid were below 35 ppb, corresponding to 18% psyllid mortality. No significant difference in psyllid mortality was evident five months after injection, when leaf imidacloprid concentrations were below 10 ppb. CLAs titers were not affected by imidacloprid injections.

Neither the oxytetracycline nor the imidacloprid formulation used in this study are currently labeled for injection in bearing citrus trees. Although we detected no oxytetracycline residues in harvestable fruits five months after injection, this will need to be confirmed in additional studies. Moreover, the long-term effects of trunk injection on citrus tree health will need to be determined specifically regarding effects of wounding and of phytotoxicity of the applied compounds. These results suggest trunk injection could be an effective delivery method for existing or novel therapeutics to manage HLB by targeting the insect vector or the pathogen causing the disease.

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—Scientific Note—

## Evaluation of Varied Fertilization Rates on Root Growth and Distribution of HLB-affected ‘Valencia’ Orange Trees

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**Additional index words.** huanglongbing, nutrient availability

*Candidatus Liberibacter asiaticus* (CLAs), which causes Huanglongbing (HLB) in citrus trees, has a great impact on tree root health, fruit development, and juice quality. HLB-affected trees have a fibrous root density loss of about 30 to 80%. CLAs blocks the phloem, disrupting the functionality of phloem in the transportation of sugars from sources to sinks. The leaves act as sources due to production of sugars in the photosynthetic process while roots are the sinks due to a higher demand of sugars for growth and nutrient uptake. Thus, the flow of sugars in phloem tubes of HLB-affected trees transports CLAs to the roots resulting in loss of fibrous roots. Loss of fibrous root loss subsequently interferes with water and nutrient uptake, which negatively affects tree overall performance and productivity. There are no current fertilization guidelines to determine optimal nutrient concentrations in citrus roots that can lead to a better understanding of the relationship between root growth, distribution and HLB-affected trees for improved management strategies. Therefore, a two-year study was conducted to evaluate the effect of varied soil fertilization rates on root growth and distribution of 5- to 8-year-old HLB-affected ‘Valencia’ (*Citrus sinensis*) oranges on ‘Swingle’ citrumelo rootstock on the Florida Ridge and Flatwoods soils. The Ridge soils at Citrus Research and Education Center (CREC) site are excessively drained Entisols formed from eolian deposits and sandy marine deposits. Soils at the southwest Flatwoods site are poorly drained and rapidly permeable soils that are formed in sandy marine sediment underlain by limestone. The Ridge soils have a high density of trees of about 450 trees/acre while the Flatwoods soils of southwest Florida have a lower tree density of about 300 trees/acre.

The experimental design for the orange trees was a randomized complete-block factorial design with an evaluation of macronutrients K and Ca at 220 lb/acre K and 40 lb/acre Ca (1× macronutrients) and 440 lb/acre K and 80 lb/acre Ca (2× macronutrients); and micronutrients (Zn and Fe) at a) 5 lb/acre (1× micronutrients), b) 10 lb/acre (2× micronutrients) and

c) 20 lb/acre (4× of micronutrients) of the current UF/IFAS fertilization guidelines. Root scans were done using minirhizotrons at 0–19.1 cm, 19.1–40.7 cm, 38.2–59.8 cm, and 57.3–78.9 cm soil depths. Root growth and dieback were estimated using the CID-600 root imager. Results obtained from the study showed that root growth and distribution were greater in 0–19.1-cm than 19.1–40.7 cm to 57.3–78.9-cm soil depths. Thus, root growth decreased ( $P < 0.0004$ ) with increasing soil depth due to variation in nutrient availability for tree uptake. Root growth and dieback was also influenced by season. At Flatwoods site, root growth increased from Nov. 2019 till Feb. 2020 (fall/winter season), however sharp decreases in root growth were observed by July 2020 and at the end of winter season. Root dieback was reduced during the fall and spring season. Sharp increases in root dieback were observed throughout Summer 2020. However, root dieback decreased from mid-Fall 2020 until the end of the study. Similarly, at Ridge site, root growth decreased sharply from Nov. 2019 to Jan. 2020. Root growth increased during winter season until toward the end of the spring season (Apr. 2020) when a decrease in root growth was observed. Root dieback increased from Nov. 2019 to Dec. 2019, however during the winter season, root dieback decreased sharply. This was followed by an increase in root dieback once the spring season began and another decrease in root dieback at the beginning of Fall 2020.

In conclusion, nutrient availability affects root distribution and growth in the soil. With increased nutrient availability in the topmost layer, there is more root growth while root growth is decreased with depth. Additionally, increased nutrient availability at occurrence of physiological processes in citrus trees results in root growth flushes. Processes such as fruit formation, growth of shoots, and flower formation affect root growth. When these processes are occurring, nutrient expenditure is mostly on the vegetative growth instead of root growth. Therefore, at higher fertilization rates, nutrient availability was increased, thus promoting root growth and distribution in HLB-affected orange trees.

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—Scientific Note—

## Diagnosis of Nutrient Deficiencies, Pest, and Disease Disorders on Citrus Leaves using Deep Learning Machine Vision

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**Additional index words.** citrus leaf, CNNs, diagnosis, machine vision, symptoms

Late and imprecise diagnosis of crop biotic and abiotic stresses cause significant decreases in productivity and subsequent economic losses as a result of delays in implementing corrective actions. Therefore, supplemental tools are needed to complement the laborious conventional analytical methods employed to identify these stresses. Visual diagnosis of citrus leaf symptoms is challenging in the presence of confounding factors from plant-pathogen-environment interactions. The advances in artificial intelligence (AI) and machine vision have made it possible to develop accurate and inexpensive diagnostic tools to analyze plant properties from digital images. In this study, machine vision in the form of convolutional neural networks (CNNs) was applied to develop image classification models for rapid, non-destructive, and accurate analysis of citrus leaves from digital images captured by a smartphone camera.

The research was conducted at the Citrus Research and Education Center, Soil and Precision Agriculture Laboratory <<https://crec.ifas.ufl.edu/>>. A targeted leaf sampling was carried out to create a database of leaf images with common citrus disorders encountered in HLB-endemic Florida groves: nutrient deficiencies (N, Mg, Zn, Mn, and Fe), disease symptoms (citrus scab, HLB, citrus canker, phytophthora chlorosis, and greasy spot), pest damage (spider mites) and asymptomatic leaves (i.e., healthy leaves). The top and the underside of each leaf were photographed using a Samsung Galaxy S8 smartphone camera. Leaf tissue analysis along with the Diagnosis and Recommendation Integrated System (DRIS) web tool were used to identify the most limiting essential nutrient in samples. A total of 14,400 images divided into 24 classes (including the top and the underside of the leaf) were used for model calibration. Also, 1400 images from an external dataset were used for independent validation. Each class was trained with 600 images of representative samples of citrus cultivars and distinct degrees of symptoms initial/early, moderate/intermediate, and severe/late.

Transfer learning was implemented to calibrate two pre-trained CNN models, EfficientNet-B4 and VGG-16 to develop the citrus leaf diagnosis models (CLD-Models). The models were then fine-tuned on the database of digital images, with an 80%:20% training and validation ratio. Calibration was implemented in a Jupyter Notebook developed by Pérez and Granger in 2018, using the Keras API, developed by François Chollet in 2015, written

in Python 3, running on the TensorFlow framework version 2.4, an open source platform developed by the Google Brain team. A subset of 240 images from the external dataset was used to develop a web survey <<https://s.surveypal.com/YC17pXmhH>>, to assess human classification performance in comparison to the models. The variables used to evaluate model performance were: accuracy, precision, recall, and F<sub>1</sub> scores.

Five CLD-Models were developed: four models based on EfficientNet-B4, and one model based on VGG-16. The EfficientNet-B4 models had a better performance than the VGG-16 model during calibration and testing. The results showed a validation accuracy of 98% for the VGG-16 and 99% for the EfficientNet-B4 models. The CLD-Models achieved an independent validation accuracy ranging from 96 to 100% for most of the classes on the external dataset. The average model performance ranged between 97.99% and 98.26% for the EfficientNet-B4 models compared to 95.90% for the VGG-16 model. The model results were compared to the classification performance of two groups of individuals with distinct levels of expertise diagnosing citrus disorders, the Experts, and the Novices (Fig. 1). The Pearson's Chi-square test showed that the models outperformed human Experts and Novices ( $P < 0.001$ ). These results suggest that the CLD-Models are a reliable tool to supplement field and laboratory assessment of biotic and abiotic stress.

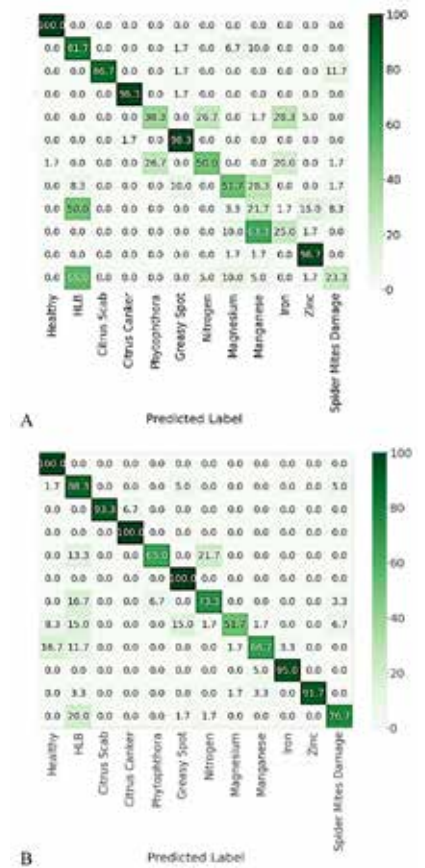


Fig. 1. (A) Confusion matrix with classification results from of group of novice scout and (B) the group experienced professionals.

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—Scientific Note—

## Water Use Assessment for Citrus Trees Affected by Huanglongbing in Florida

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**Additional index words.** evapotranspiration, huanglongbing, sap flow, stem water potential

Proper irrigation scheduling and water management efficiency are keys to achieving higher yields in citrus. As the human population increases, water allocated to citrus production may decrease. Therefore, efficiency of citrus irrigation systems should be improved to better manage irrigation water without compromising yield. The soils in central Florida, like Entisols, and those of south Florida flatwoods like Spodosols and Alfisols are characterized by rapid infiltration of rain and irrigation water, since they constitute > 83% sand, as well as low water and nutrient retention capacities. Thus, when irrigation water, rich in nutrients, is lost by deep percolation due to the low water holding capacity of the soil, it may end up in the groundwater, which may have detrimental consequences on the environment. In order to minimize or prevent the above mentioned scenario from occurring, water supply should meet the plant's water requirement. Citrus trees affected by huanglongbing (HLB) tend to lose more than 40% of their root systems, which may affect the tree's water use at a given time.

A greenhouse experiment was conducted in Oct. 2019 at the Citrus Research and Education Center in Lake Alfred, FL, to assess water use dynamics in 2- to 4-year-old HLB-affected 'Valencia' orange (*Citrus sinensis*) trees grown on 'Kuharske citrange' rootstock (*Citrus sinensis* × *Poncirus trifoliata*).

Four treatments comprising of trees receiving 100% evapotranspiration (ET) and 80% ET, on HLB- and non HLB-affected (NHLB) trees were applied on a randomized complete-block design with five replicates.

We used a drip irrigation system and a timer was used to schedule irrigation events. All trees received equal amount of

fertilizer and soil surfaces were covered with mulch to minimize surface evaporation. Stem water potential, sap flow, root length, root diameter, root area, and root volume were monitored and compared among all treatments.

For root length and diameter, non HLB trees that received 100% and 80% ET generally showed increased root diameter and length as compared to HLB trees that received 100% and 80% ET. However, between HLB-affected trees, no significant differences were observed showing that irrigation rate of HLB-affected trees could be lowered to 80% ET. Similar growth in terms of root area and volume was observed for HLB-affected trees at 100% and 80% ET. There was no significant water stress among HLB-affected trees at 100% and 80% ET ( $P = 0.6681$ ). However, trees showed higher water stress in Spring than in Fall 2021. Comparable water consumption rates were observed between 100% ET and 80% ET for HLB and NHLB trees in Fall 2020.

For Spring 2021, HLB-affected trees irrigated at 100% ET showed higher water consumption than trees receiving 80% ET. In Summer 2021, HLB-affected trees at 80% ET had similar water use as HLB-affected trees at 100% ET. A correlation between water-use and stem water potential for the spring data was fairly weak ( $r = 0.568$  and  $R^2 = 0.32$ ).

In summary, HLB-affected trees that received 100% ET had comparable water use and similar stress level compared to trees that received 80% ET. Higher water use observed for healthy trees was manifested in more rapid root growth. Therefore, irrigating at 80% ET might be appropriate for HLB-affected trees, subject to field validation.

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—Scientific Note—

## ***Candidatus* Liberibacter asiaticus Movement and Carbohydrate Allocation in Different Citrus Scion–Rootstock Combinations during the First Year After Infection**

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**Additional index words.** citrus greening, HLB tolerance, scion/rootstock interaction, starch accumulation

Huanglongbing (HLB) is associated with a phloem-limited bacterium, *Candidatus* *Liberibacter asiaticus* (CLAs), vectored by the Asian citrus psyllid. Studies have shown that HLB causes starch accumulation in aboveground tissues in citrus plants while causing starch depletion in the roots (Etteberria et al., 2009); however, changes in starch distribution following natural infection in the field have not been documented. The objectives of this study were: 1) to determine CLAs distribution in leaves and roots of newly planted citrus trees after natural inoculation in the field; and 2) to determine rootstock effects on CLAs and starch allocation in graft combinations with sweet orange during the first year after infection.

Seven commercially important rootstock cultivars were grafted with ‘Valencia’ orange (*Citrus sinensis*) scion and planted in a southwest Florida citrus orchard in Mar. 2019, where HLB is endemic. Previous studies (Folimonova et al., 2009; Albrecht and Bowman, 2012; Boava et al., 2015) have documented that trifoliolate orange and some hybrids are more HLB-tolerant than cultivars without trifoliolate orange in their parentage. The rootstock cultivars used were ‘US-802’, ‘US-897’, ‘US-942’, ‘Carrizo’, and ‘Swingle’, which are hybrids of citrus and trifoliolate orange (*Poncirus trifoliata*), sour orange (*C. aurantium*), and ‘Cleopatra’ mandarin (*C. reticulata*). The experimental design was a randomized complete-block design with 10 replications. Trees were planted in March 2019 at the Southwest Florida Research and Education Center in Immokalee, Florida. Leaves and fibrous roots were collected in Fall and Winter 2019 and in Spring and Summer 2020 to measure starch concentrations and CLAs titers.

Three months after planting (Summer 2019), 35% and 62% of trees were CLAs-infected based on leaf and root analysis, respectively, whereas 94% and 61%, respectively, were infected by Summer 2020 (15 months after planting) (Fig. 1). Starch concentrations in the leaves were significantly higher than in the roots throughout all seasons and, like the CLAs titers, increased from Spring 2020 to Summer 2020 (Fig. 2). Root starch concentrations and CLAs titers were lowest in spring 2020 and increased in Summer 2020. There was a significant rootstock effect for root starch concentrations in Fall and Winter 2019, but this effect was

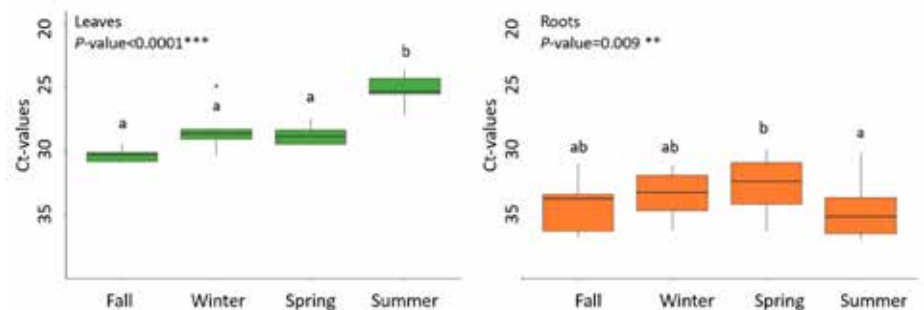


Fig. 1. Ct-values of leaves and roots of *Citrus sinensis* ‘Valencia’ trees grafted on different rootstocks during the first year after CLAs infection.

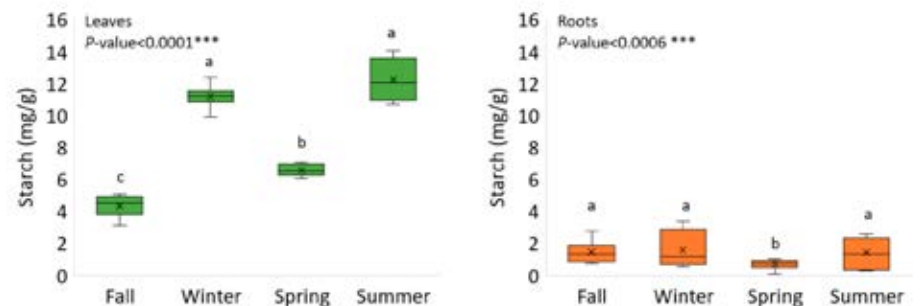


Fig. 2. Starch concentrations of leaves and roots of *Citrus sinensis* ‘Valencia’ trees grafted on different rootstocks during the first year after CLAs infection.

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not consistent and not related to HLB tolerance. There was also no clear indication that starch depletion followed by root decline is one of the early consequences of CLas infection.

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—Scientific Note—

## Evaluation of the Productivity of HLB-affected Citrus Varieties Grafted on US-942 and Swingle Rootstocks

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**Additional index words.** fruit drop, pre-harvest, productivity, rootstock, Valencia, yield

Huanglongbing (HLB) disease and abiotic stresses such as temperature variation, drought, and flooding have been associated to exacerbate citrus preharvest fruit drop in Florida. The preharvest fruit drop can be as high as 50% of expected yield per cropping season. Attempts to control the fruit drop rate have not been successful to date. There is no known citrus variety that is resistant to HLB, but some varietal-rootstock combinations show some tolerance and are preferred by growers. We evaluated the productivity of HLB-affected mature *Citrus sinensis* 'Valencia' trees grafted on US-942 and 'Swingle' rootstocks. The trees were six-years old. A total of 96 trees per variety-rootstock combination were used, and each experimental unit consisted of six trees.

Preharvest fruit drop, nutritional status, internal fruit quality, and yield were determined during the crop season. The productivity status was classified using the following scale: > 90 lbs = most productive; 70–89 lb = moderately high production; 50–69 lb = moderately low production; and < 50 lb = low production. Proc Glimmix (SAS Institute Inc., Cary, NC) was used for statistical analysis of the data. At harvest, 65% of the trees on US-942 rootstock fell within the most productive category. On

the contrary, most of the 'Valencia' trees on 'Swingle' rootstocks fell within the moderately low category. In trees with 'Swingle' rootstock, fruit drop per tree was significantly higher when compared with trees with US-942 rootstock.

Regardless of the rootstock, the most productive individual trees had fruit drop around 10%. In the other productivity categories, fruit drop was greater in trees on 'Swingle' rootstock. This shows that fruit drop rate was related to rootstock type and this may imply that the health and productivity of HLB-affected 'Valencia' trees grafted on 'Swingle' rootstock decline faster when compared with trees on US-942 rootstock. Fruit quality was not affected by productivity status, as both Brix increased and fruit detachment force declined with time regardless of the rootstock, but these parameters were not significantly different between each rootstock-scion combination. In all plots, leaf and soil nutrient concentrations were within or above the optimum ranges. In summary, HLB infected *C. sinensis* 'Valencia' trees grown on US-942 rootstock had higher fruit yield than *C. sinensis* 'Valencia' trees grown on 'Swingle' rootstock and this is likely due to a differential fruit drop behavior that is influenced by rootstock.

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—Scientific Note—

## Fertilizer Inputs Affect Grapefruit Root Health

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**Additional index words.** *Citrus paradisi*, planting density, soil nutrient

Huanglongbing (HLB) is a disease caused by *Candidatus Liberibacter asiaticus* that impacts citrus production areas on a global scale and has decreased citrus yield in Florida by more than 70% over the past decade. Root structure–function relationships remain an understudied component of the tree’s responses to infection and may play a key role in tolerance. An HLB-damaged root system has poor water and nutrient uptake capacity, which leads to nutrient deficiencies and impaired tree performance. Furthermore, current fertilization guidelines were developed prior to HLB. However, these guidelines must be revisited given the widespread prevalence of HLB-affected trees. The aim of this study was to evaluate the effects of different fertilizer forms and rates with different planting densities on HLB-affected citrus root health. The study consisted of four foliar fertilizer treatments, which included 0×, 0.5×, 3×, and 6× from the current University of Florida, Institute of Food and Agriculture (UF/IFAS) recommendation. Plant material used in this study consisted of eight-year-old ‘Ray Ruby’ grapefruit trees (*Citrus × paradisi*) grown on Kuharske citrange (*Citrus*

*sinensis* × *Poncirus trifoliata*) rootstock. The planting densities studied were low (300 trees/ha), medium (440 trees/ha), and high (975 trees/ha). Additionally, two granular fertilizer treatments were used, specifically, two controlled-release fertilizer blends (16–3–20 and 12–3–9).

Trees planted at high density had significantly greater root density and total root length than those planted at low density. Soil samples from citrus fertilized with 3× and 6× foliar treatments had significantly higher zinc and manganese concentrations than those fertilized with 0× foliar treatment. Soil samples from plots fertilized with the standard granular treatment had significantly higher potassium, calcium, boron, and pH than those fertilized with the improved granular treatment.

Rhizosphere samples that were previously collected will be analyzed to determine possibly correlations between changes in the bacterial community composition and fertilizer treatments. Both soil and root nutrient data will also be used to identify potential correlations with rhizosphere bacterial community composition.

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—Scientific Note—

## Effect of HLB on Leaves and its Implication on Nutrient Profile

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**Additional index words.** citrus greening, sweet orange, citrus, fertilizer, starch

Huanglongbing [(HLB); *Candidatus Liberibacter asiaticus*] is a bacterial citrus disease that has resulted in more than 70% decline of Florida sweet orange (*Citrus sinensis*) production. HLB symptoms are quite evident, ranging from blotchy mottled leaves, nutrient deficiencies, starch accumulation and reduced leaf size. HLB affected trees undergo significant root and shoot dieback, which affects nutrient uptake, assimilation, and translocation. Based on newly developing research, it is recommended that citrus growers regularly perform leaf nutrient analysis to assess the nutritional status of the HLB-affected sweet orange trees and hence accordingly tailor their fertilizer program to address the nutritional needs of the trees. However, due to the changes in the morphology and composition of leaves on HLB-affected trees, it is critical to understand if there is any effect on leaf nutrient profile. Therefore, the goals of this study were to assess the effect of HLB on mineral nutrient content in different plant parts, HLB effects on leaf size, leaf weight, and starch accumulation on nutrient accumulation in sweet orange.

HLB-affected leaves were about 20% smaller than healthy leaves. On fresh weight basis, HLB affected leaves were about 20% lighter (Fig. 1) and had 10 times more starch than healthy leaves. Interestingly, when dry weight was measured there was no difference in leaf weight between healthy and HLB affected leaves, and the starch content was 2.5% in HLB-affected trees as compared to 1.5% in healthy trees. During the drying process, healthy trees lost significantly more water as compared to those that were HLB affected, indicating water deficit in HLB-affected leaves. HLB-affected trees are known to be under water deficit; therefore, it is likely that water accumulation is lower in HLB-affected leaves thereby resulting in smaller leaf area.

In regard to leaf nutrient analysis, usually measured on dry weight basis, in HLB affected trees calcium (Ca), sulfur (S), zinc (Zn), manganese (Mn), and iron (Fe) values were lower, but nitrogen (N) was higher in leaves of HLB-affected trees compared to leaves on healthy trees. However, N, potassium (K), B, copper (Cu) and Mn values were lower in roots of HLB affected trees. Most of nutrients were found to be deficient in HLB affected trees as compared to healthy trees. Out of all the nutrients, B and Cu had significant positive relation with starch content in HLB affected trees, but not in healthy trees. When biomass of leaves, stem and roots were taken into consideration separately, no significant difference was seen in total nutrient values between healthy and HLB-affected trees; N along with

Ca, B, and Cu values were found to be higher in stem tissue of healthy trees and Mg, B, and Zn found to be deficient in the roots of HLB-affected trees, respectively. When total nutrients present in a tree were taken into consideration, N, Ca and Mn were significantly lower in HLB-affected trees than in healthy trees. Macronutrients like Ca, Mg, and S along with micronutrients such as B, Zn, Mn, and Cu showed a significant relation for the parameters of plant growth. A significant variation of nutrient content in plant parts was seen when total biomass of trees was taken and most of nutrients were deficient in HLB-affected trees.

Our preliminary results suggest that the leaf nutrient profile is not affected by excessive starch buildup or differences in leaf morphology. The observed deficiencies of most of the nutrients in the leaves of HLB-affected trees is possibly due to higher nutrient requirements.

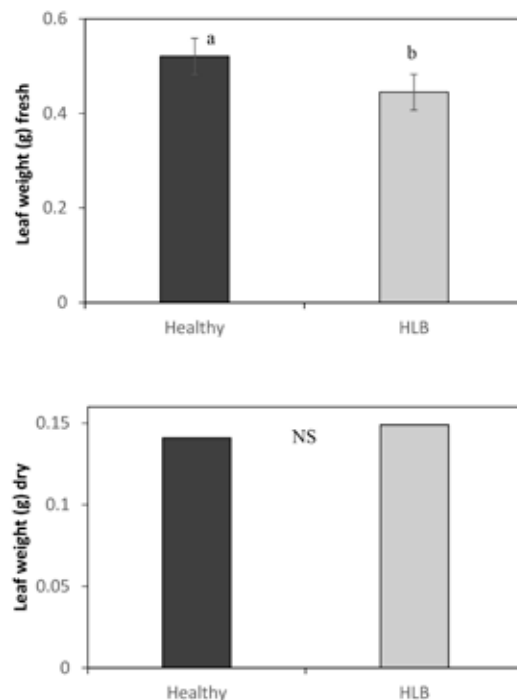


Fig. 1. Average weight of healthy and huanglongbing-affected sweet orange (*Citrus sinensis*) leaves, grown under greenhouse conditions. Different letters indicate significant differences ( $P < 0.05$ ) among treatments. NS = not significantly different.

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—Scientific Note—

## Root Growth and Nutrient Uptake of HLB-affected Grapefruit on Florida Flatwood Soils

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**Additional index words.** citrus greening, *Citrus paradisi*, huanglongbing, Indian River district

Root growth and development is an understudied yet essential component in Florida citrus production. Currently, the greatest challenge in the industry is the bacterial disease, Huanglongbing (HLB; citrus greening). The disease causes a slow decline and eventual death of the trees. Additionally, research has shown that one of the early symptoms of HLB is the reduction of fine root mass and root life span. With no cure for HLB commercially available and nutrient uptake of affected trees compromised due to loss of root biomass, nutrient management guidelines may need to be revised. The objectives of this study were to identify which application methods and rates lead to increased micronutrient uptake in HLB-affected grapefruit (*Citrus ×paradisi*) trees and further explore how increased nutrient rates impact root growth and development.

A large-scale field study consisting of six-year-old HLB-affected 'Ruby Red' (*C ×paradisi*) grapefruit trees grafted on sour orange rootstock was conducted at the University of Florida, Institute of Food and Agricultural Science (UF/IFAS) Indian River Research and Education Center in Fort Pierce, Florida. Eight different treatments consisting of four liquid and four solid fertilizers with 1×, 2×, and 4× the current UF/IFAS recommended rates of boron (B), zinc (Zn), manganese (Mn), and iron (Fe) were applied. The soil, root, and leaf nutrient analyses were conducted every six months during the two-year study. Additionally, root density and titer were collected at the same time as nutrient sampling. Higher rates of B, Zn, and Mn were observed in the soil of plots treated with liquid fertilizers

with 2× and 4× the micronutrient recommendations. Root and leaf micronutrient analysis revealed significant differences in B, Zn, Mn, and Fe among treatments which varied between seasons, but no consistent patterns were observed. No significant differences were observed in root density but differences in root titer were observed.

Our preliminary results showed that increased micronutrient rates increased soil nutrient levels. No consistent patterns have been established for the first two years between increased micronutrient fertilizer rates and root and leaf micronutrient content. It is important to note that the trees used in this study were grown in a high HLB incidence environment since the time they were planted. All trees showed moderate to severe HLB symptoms and were HLB positive before the experiment began. The results may show that rehabilitation of severely HLB-affected trees using overdoses of micronutrients is limited by both tree age and years the tree has been exposed to HLB. Additionally, physiological responses due to increased micronutrient fertilizers may take many years to become evident, longer than the two years that this study was conducted.

Future studies on the use of micronutrients for HLB-affected tree management are needed to reevaluate current nutritional guidelines. These studies should focus on different rates and ground application methods for macro and micronutrients as well as explore the integration of foliar nutritional sprays into nutrient management. These studies should be conducted in the field for long periods of time to account for a lag in tree response.

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—Scientific Note—

## Nutrient Management to Enhance Citrus Fruit Color Break and Quality at Maturity

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**Additional index words.** fertilization, subtropical climate

In Florida's subtropical climate, color break usually occurs naturally during the Fall when day length decreases and temperatures drop. During the color break process, the decline in peel chlorophyll content occurs over a several month period while carotenoid content increases, and is affected by environmental conditions, nutrient availability and phytohormones such as ethylene. Optimized fertilization may be one of the most cost-effective and practical methods for enhancing color break in Florida citrus, especially when warmer temperatures during the Fall inhibit the process. We investigated the relationships between peel color and leaf nutrients of 'Honey' Murcott (*Citrus reticulata*) measured on 8. Dec. 2020 in a screen house located in Lake Alfred, FL. Leaf and peel tissue nutrient concentrations were determined in samples (5 fruit, 25 leaves per tree) from 20 trees, and average peel color was determined for fruit samples by digital image processing according to the CIE L\*a\*b\* color measurement system. Regression methods were used to determine the relationships between peel color in the a\* (green-red) or b\* (blue-yellow) axes, and leaf or peel nutrient concentrations. Loss of green color and increase of red peel color was negatively correlated with leaf nitrogen (N), phosphorus (P), sulphur, and iron concentrations, and positively correlated with leaf magnesium concentrations (Fig. 1). Peel nutrient results mirrored the leaf data, but with weaker correlations. Total soluble solids (Brix) concentrations in the juice were correlated with leaf nutrients in similar fashion to the peel a\* color, but a negative correlation with leaf P was the most significant (Fig. 2).

Pearson's correlation coefficient (r) for **Brix%**

Fruit variables	Leaf nutrients	Peel nutrients
Diameter	N % ** -0.577	N % * -0.445
L*	P % *** -0.789	P % -0.392
b*, red	K % ** -0.61	K % -0.261
b*, yellow	Mg % 0.349	Mg % * 0.475
Juice %	Ca % 0.146	Ca % 0.398
Acid % * 0.488	S % ** -0.658	S % -0.286
	B mg/kg -0.333	B mg/kg * -0.458
	Zn mg/kg 0.035	Zn mg/kg -0.132
	Mn mg/kg -0.263	Mn mg/kg 0.21
	Fe mg/kg* -0.553	Fe mg/kg* -0.446
	Cu mg/kg -0.061	Cu mg/kg -0.14

\*, \*\*, \*\*\* indicate statistical significance at 5%, 1% and 0.1%

Fig. 2. Correlation coefficient (r) for juice Brix with other fruit quality variables, leaf and peel nutrients in *Citrus reticulata*.

The detrimental effects of high leaf N during the fruit maturation phase are illustrated in Fig. 3, where leaf N concentrations in the low to deficient ranges are conducive to the best color break. To enhance early, complete color break and high juice quality, we recommend that annual P fertilization should be completed at post-bloom, and N fertilization should be completed mid- to end-summer, depending on whether the citrus variety is early- or late-maturing.

Pearson's correlation coefficient (r) for peel a\* (green → red color)

Fruit variables	Leaf nutrients	Peel nutrients
Diameter	N % *** -0.723	N % ** -0.665
L*	P % *** -0.762	P % -0.417
b*, yellow	K % ** -0.630	K % -0.343
Brix % *** 0.816	Mg % * 0.498	Mg % 0.342
Juice %	Ca % 0.353	Ca % * 0.534
Acid %	S % *** -0.687	S % * -0.536
Ratio	B mg/kg -0.253	B mg/kg * -0.472
SS /box	Zn mg/kg 0.124	Zn mg/kg -0.021
	Mn mg/kg -0.368	Mn mg/kg -0.050
	Fe mg/kg** -0.607	Fe mg/kg* -0.460
	Cu mg/kg 0.107	Cu mg/kg -0.142

\*, \*\*, \*\*\* indicate statistical significance at 5%, 1% and 0.1%

Fig. 1. Correlation coefficient (r) for peel a\* color with fruit quality, leaf and peel nutrients in *Citrus reticulata*.

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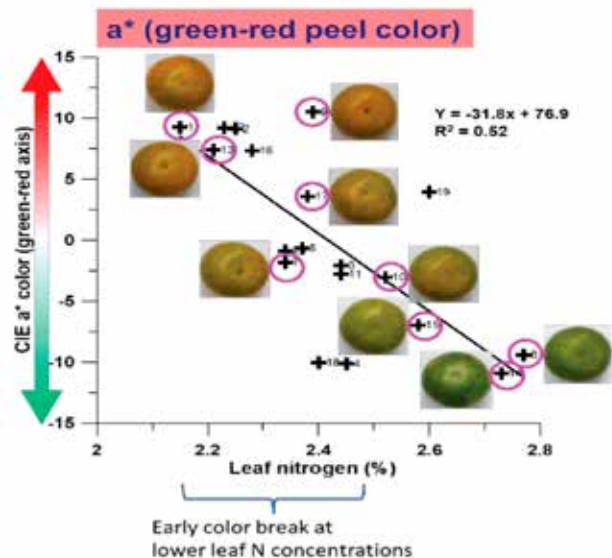


Fig. 3. Scatter plot and linear regression of peel a\* color with leaf N concentrations in *Citrus reticulata*.



—Scientific Note—

## Oak Mulch Applications Improved Soil Characteristics in an HLB-affected Citrus Grove

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**Additional index words.** citrus greening, *Citrus ×sinensis*, huanglongbing, soil amendments

Florida's citrus industry has experienced a massive decline since the arrival of Huanglongbing (HLB) disease. In Florida, the disease is caused by the phloem-limited bacterium *Candidatus Liberibacter asiaticus* (CLAs) and is vectored by the Asian citrus psyllid, *Diaphorina citri*. With nearly all commercial citrus groves affected and no cure on the horizon, growers and researchers have been searching for alternative strategies to manage diseased trees. After reports showed reduced HLB symptoms in citrus trees growing in close proximity to oak trees, many growers began incorporating oak mulch into their production systems. No studies have investigated the impact of oak mulch on HLB-affected citrus trees or oak mulches' impact on soil characteristics.

This study was created to (i) to study the capability of oak mulch to contain and suppress CLAs and (ii) to measure the effect of oak mulch on soil characteristics and nutrient uptake in HLB-affected trees. Twenty-four field grown 'Navel' orange (*Citrus sinensis*) trees were assigned to a complete randomized block design replicated three times. Branches from mature laurel oak trees (*Quercus laurifolia*) were ground into mulch. Three inches (7.6 cm) of oak mulch was applied at the beginning of the experiment and one year after project began. Control trees did not receive mulch.

Soil, root, and leaf nutrient analysis, leaf bacteria titer, and soil moisture levels were measured monthly. Results showed higher levels of phosphorus (P), potassium (K), and magnesium (Mg) in oak mulch treated trees and soils in certain months of the year. No significant differences were observed in leaf bacteria titer and leaf nutrient levels. Differences were observed in soil moisture levels in certain months between the control and mulch treatments. The results of this study show that applying oak mulch may not suppress CLAs but can lead to higher nutrient levels in the soil. Higher soil nutrient levels may increase the productivity of HLB-affected citrus trees and reduce costly fertilizer inputs for growers. Although previous work on leaf mulches showed an effect on CLAs titer, our data was inconsistent with these findings because this work used a wood-based mulch. Future studies should compare the effectiveness of mulches from different sources including different species of hardwood trees. Additionally, an economic analysis on the use of mulch in HLB-affected citrus production may help increase operational profitability.

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—Scientific Note—

## Using Colored Mesh Bags to Induce Early Color Development in Fresh Market Citrus Varieties

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**Additional index words.** fruit color, shade cloth.

Citrus under protective screen (CUPS) growing systems are only successful when the quality of fruit meets the standards to fetch high prices in the market. One aspect of high fruit quality is the color of the fruit peel. Many popular varieties in CUPS systems reach internal maturity standards before the color break is complete. Finding a method for hastening color development so fruit can be harvested as soon as internal fruit maturity is reached is essential. Research has shown that factors such as cool temperatures, nutrient concentrations (nitrogen, phosphorus, and potassium) and light intensity can cause the breakdown of chlorophyll and the development of carotenoids. These experiments aim to take advantage of the latter mentioned factor by using various colored light filtering bags to shade fruit.

At the Citrus Research and Education Center (CREC) CUPS facility, three experiments were done over two seasons (2019–20 and 2020–21). Fruit from three varieties ‘UF914’, ‘Ray Ruby’ and ‘W. Murcott’ were covered with colored knitted shade cloth mesh bags (Fig. 1a and 1b). In the first experiment, fruit from ‘UF914’, a hybrid of grapefruit (*Citrus × paradisi*) and pomelo (*C. maxima*) were covered with black, blue, green, and red mesh bags. In the second and third experiments using ‘Ray Ruby’ grapefruit (*C. × paradisi*) and ‘W. Murcott’ tangerines (*C. reticulata*), pink and white bags were added. Unbagged fruit were also sampled as a control comparison. Fruit was tested for both internal and external quality. External fruit color was analyzed using digital images of the fruit at a fixed white balance. Images were then analyzed using ImageJ software to calculate CIE L\* a\* b\* color coordinates. Internal fruit



Fig. 1. Mesh bags deployed in the research field (a) and mesh shade cloth colors used to make bags (b).

quality was measured using standard brix and acid juice analysis, as well as internal color analyzed using the same methods employed for the external color analysis.

Results (Table 1), of the 2019 ‘UF914’ experiment found that covering fruit with green mesh bags significantly increased the a\* (redness) value. Results from the ‘Ray Ruby’ experiment (harvested Nov. 2020) agree and show fruit within the green bags had significantly higher redness (a\*) values. The ‘W. Murcott’ experiment (harvested Dec. 2020) failed to show any significant differences between colored bags and unbagged controls. While there were no significant differences in the a\* values, all treatments evenly developed orange peel color. We believe this is due to a reduced nitrogen fertilizer program that was put in place in the CUPS research block for the 2020 harvest season. Ongoing research in the 2021–22 season is attempting to test this hypothesis. Across all three variety experiments and all colored bag treatments, there were no significant differences in internal fruit color or juice quality between bagged treatments and unbagged controls.

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Table 1. External color analysis using the CIE color mapping system. L\* represents lightness from 0–100 black to white, a\* represents green-red color from –100 to +100, and b\* represents blue-yellow color from –100 to +100. ‘UF914’ is a hybrid of grapefruit (*Citrus × paradisi*) and pomelo (*C. maxima*). ‘Ray Ruby’ is grapefruit (*C. × paradisi*) and ‘W. Murcott’ is a tangerine (*C. reticulata*)

Bag color	UF914 (2019)			Ray Ruby (2020)			W. Murcott (2020)		
	L* (ns)	a* (P < 0.001)	b* (ns)	L* (P < 0.001)	a* (P < 0.001)	b* (P < 0.001)	L* (ns)	a* (ns)	b* (ns)
Unbagged	48.52	–3.43 a	43.35	49.3 ab	1.8 a	40.9 a	38.9	19.4	41.1
White	--	--	--	51.5 b	2.7 a	43.7 b	39.2	24.5	41.4
Black	47.74	1.34 ab	41.43	47.4 a	9.6 bc	39.9 a	38.9	28.4	42.6
Red	48.64	2.06 b	43.48	48.2 a	7.9 b	41.3 a	39.9	16.3	41.1
Green	47.95	5.07 b	42.25	48.2 a	13.1 c	41.1 a	44.3	18.0	44.5
Blue	48.8	2.16 b	42.47	48.0 a	9.1 bc	40.7 a	42.1	14.7	41.4
Pink	--	--	--	46.6 a	5.3 ab	40.2 a	43.8	21.5	46.7



—Scientific Note—

## Developing Neutral Electrolyzed Water for Pest Management: 2020 Update

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**Additional index words.** fruit quality, greasy spot, melanose

Citrus under protective screen (CUPS) is an approach to producing fresh market citrus by growing trees in an enclosed space covered with fine mesh screen. The screen excludes the Asian citrus psyllid [*Diaphorina citri* (Hemiptera: Liviidae)], the vector of huanglongbing. This enables production of high yields of fruit with better internal and external quality. The two fungal pathogens greasy spot (*Zasmidium citri-griseum*) and melanose (*Diaporthe citri*) are important problems in CUPS.

Neutral electrolyzed water (NEW) is a short-lived surface sterilant that is made on-site by passing an electric current through a salt solution to produce hypochlorous acid. Our goal is to develop NEW for use in CUPS. If successful it provides growers another tool for disease management with a different mode of action than current products. However, high rates of NEW are phytotoxic. The goal is to understand the balance between these two effects.

'Ray Ruby' grapefruit (*Citrus xparadisi*) were sprayed to runoff once per week with NEW water produced using sodium chloride. Plots of three plants were sprayed with 0, 25, 50, or 100 ppm chlorine once per week. Treatments started either 29 July or 2 Sept. and ended 9 Dec. in time for harvest on 14 Dec. 2020. We used a handgun sprayer at 100 psi to treat 3-plant experimental plots with four or five replicates/treatment. Trees were spaced on 1.5 m centers with 3.05 m row centers equaling a density of 2152 trees/hectare (871/acre), and an application rate of 679 L·ha<sup>-1</sup> (72.59 gallons/acre).

This two-factor experimental design was recast by calculating a total dose that was the sum of the applied dose over the number of weeks (19 or 14 weeks): 100 ppm applied for 19 weeks gives a total dose of 1900. All NEW treatments were in addition to a commercial disease management program. Statistical analyses were done in SAS running under SAS Enterprise Guide, and R running under RStudio. Evaluation was made using yield, external fruit quality, internal fruit quality, and tree growth.

There was no significant effect of NEW on fruit diameter, fruit weight, boxes per tree, or percent juice. Fruit quality was assessed photographically for both color and disease. CIE L\*a\*b\* color was used but only the a\* showed a significant difference where a\* declined with increasing total dose ( $P > F = 0.002$ ,  $r^2 = 0.32$  for  $a^* = 13.0 - 0.0028 \times \text{total dose}$ ). However, all fruit had a\* values above zero and were therefore marketable (Fig. 1A). Fruit damage was caused by greasy spot and melanose. The combined damage was reduced by NEW, though the effect was marginally

significant (Fig. 1B). There was also a significant reduction in Brix from 7.75 to 7.25 (not shown). With no significant change in acid, the ratio and BrimA values declined. There was no effect of NEW on internal color. Research continues to better minimize the adverse effects while maintaining efficacy.

NEW continues to show promise as an approach to reducing disease on grapefruit. This should improve the fruit value. However, NEW treated fruits were slightly greener. While still marketable, this may cause problems if other factors also inhibit color break. Further research is needed for this to become a functional tool for pest management in citrus.

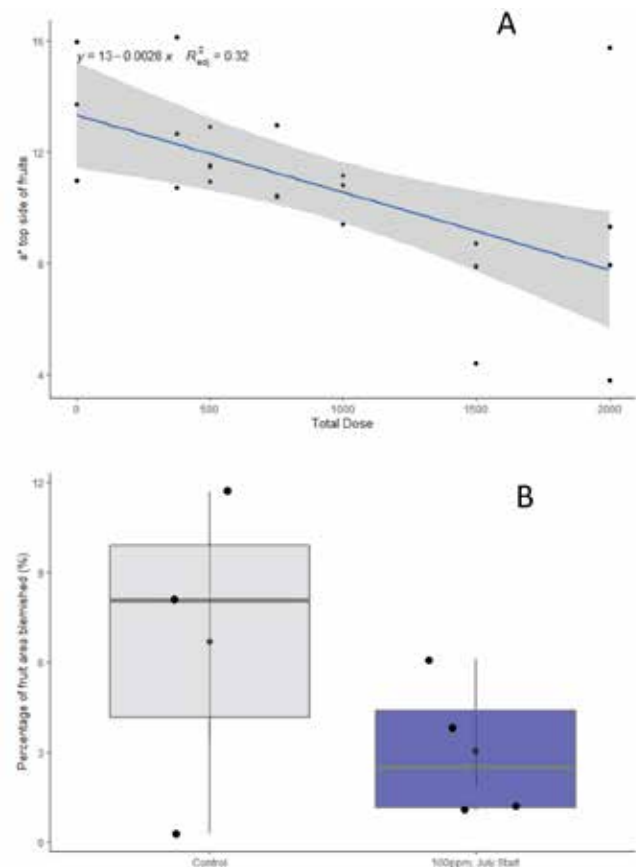


Fig. 1. (A) decline in a\* color scale values on grapefruit (*Citrus xparadisi*) with increasing total dose of neutral electrolyzed water (NEW). (B) The overall disease on fruits was reduced by NEW, though just shy of achieving statistical significance. Dots are the plot average (ten fruits per plot). Only the control and high rates are presented.

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—Scientific Note—

## Pest Population Dynamics in Citrus under Protective Screen Production Enclosures: Experiences in Lake Alfred, Florida

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**Additional index words.** Asian citrus psyllid, Citrus rust mite, mealybugs, spider mites

Citrus Under Protective Screen (CUPS) systems are used for the production of fresh citrus fruit varieties for the commercial market. The objective of CUPS growing systems is the exclusion of the Asian citrus psyllid, the vector of huanglongbing. The 1.33-acre CUPS structure in Lake Alfred, Florida uses 50 mesh screens and was built in 2014. This mesh size excludes larger insects but is less effective against organisms like mites and thrips that may damage leaves or fruit peels. To make the CUPS system profitable, high-quality fruit is imperative. Therefore, scouting is necessary to monitor pest populations and determine the right time for applying a pesticide to protect fruit quality. Weekly scouting involved inspecting two leaves and two fruit per tree with a 10× hand lens. Scouted trees were selected using a grid pattern, with 19 rows and 11 locations per row totaling 209 positions. When young flush was present, the tap-sampling method was used. A white sheet of laminated paper was placed underneath the young flush, which was then gently tapped, displacing thrips and other pests onto the sheet below. The number of pests that appear are

recorded in a Microsoft Excel spreadsheet. Grapher 8 (Golden Software, Golden, CO), was used to create line graphs (Fig. 1), and GS+ (GammaDesign Software, Plainwell, MI), was used for the Kriging Interpolated population heat maps (Fig. 2) which were used to monitor development of hot spots as pest population densities increased.

Over the past two years of scouting, major pests found include spider mites (*Tetranychus urticae*), Citrus rust mites (*Phyllocoptruta oleivora*), thrips, (Thysanoptera, *Frankliniella bispinosa* and *Scirtothrips citri*), leaf miner (*Phyllocnistis citrella*), and mealy bugs (Pseudococcidae). Figure 1 shows spikes in spider mites (Fig. 1a), rust mites (Fig. 1b), and thrips (Fig. 1c) populations. Spider mite population peaks tended to coincide with leaf flushes (Spring and Summer), citrus rust mite population peaks tended to coincide with the maturation of fruit (August–January), and thrips population peaks coincided with the flowering period (February–April).

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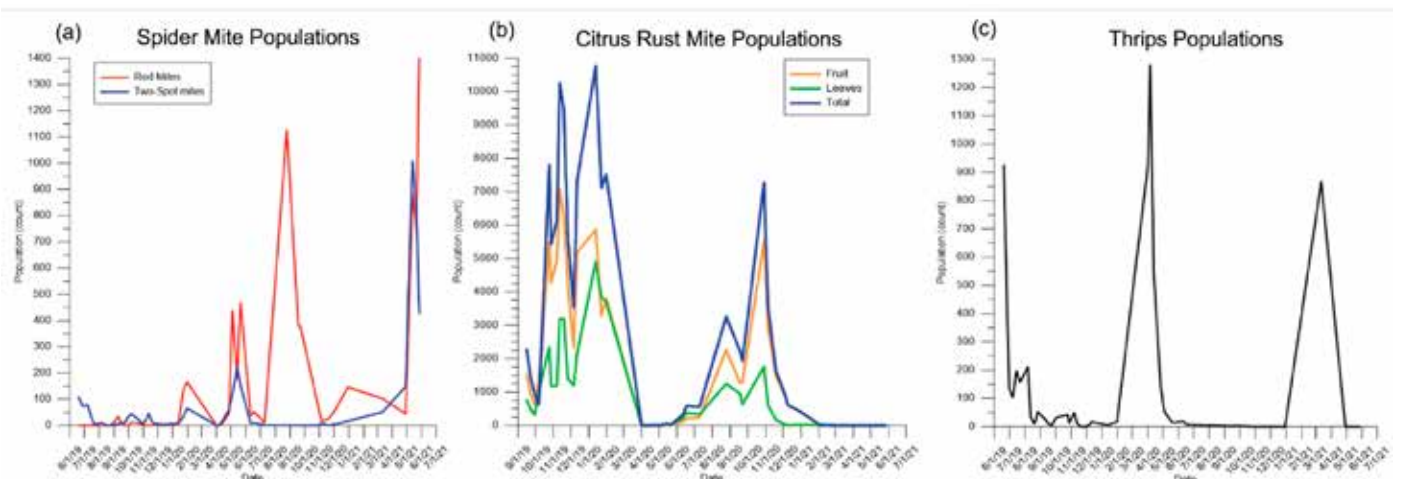


Fig. 1. (a) Spider mite (*Tetranychus urticae*), (b) citrus rust mite, (*Phyllocoptruta oleivora*) and (c) thrips (*Frankliniella bispinosa* and *Scirtothrips citri*) populations during a 2-year period in a citrus under protective screen system in Lake Alfred, FL.

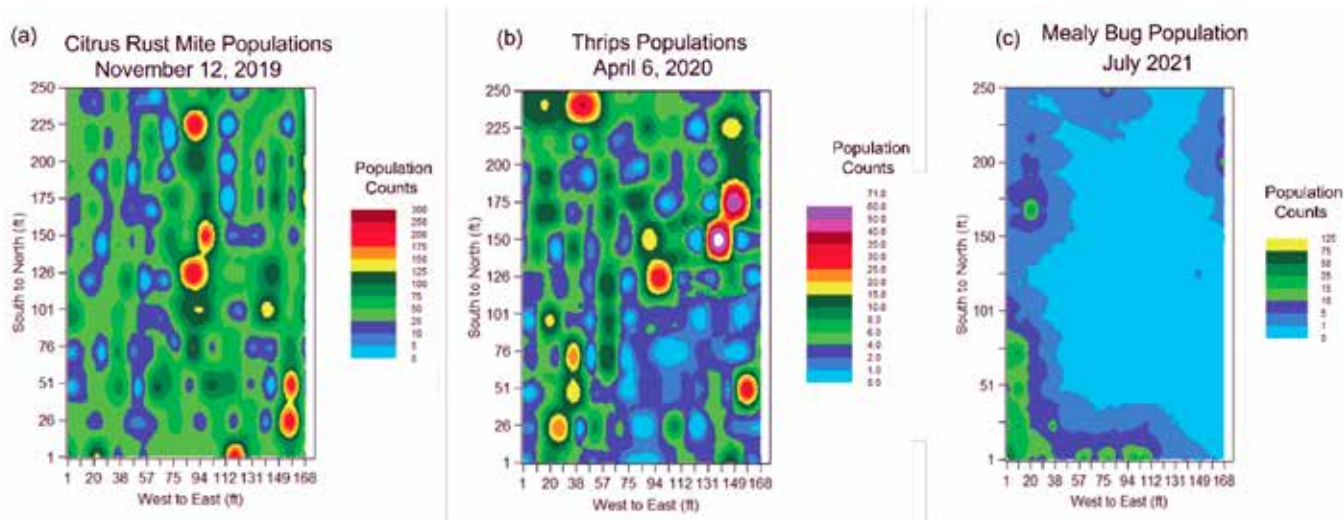


Fig. 2. Heat maps of (a) citrus rust mites (*Phyllocoptura oleivora*), (b) thrips (*Frankliniella bispinosa* and *Scirtothrips citri*), and (c) mealybug populations (Pseudococcidae) in a citrus under protective screen system in Lake Alfred, FL.

Figure 2 shows the population intensities over space, with axis tick marks representing rows (x axis) and scouting locations (y axis). Citrus rust mite population hotspots in Nov. 2019 (Fig. 2a) were found where fruit density was high. Thrips hotspots in Apr. 2020 (Fig. 2b) coincided with the locations of the late flower blooming varieties. Population dynamics of mealybugs in July 2021 (Fig. 2c) showed the highest populations are concentrated nearest personnel and equipment (tractor, sprayer, etc.) entrances with populations radiating outward.

The screen was replaced in Feb. 2020 and psyllids entered and established a population that was detected in Sept. 2020. A rotation of chemical controls was used to remove the psyllids shortly after detection. The minor pests detected were leaf rollers,

snow scale, Florida wax scale, Glover scale, ants, grasshoppers, whiteflies, and fruit flies. As part of the pest control strategy the following biocontrol agents were released: minute pirate bug (*Orius insidiosus*), ladybugs (Coccinellidae, convergent and mealybug destroyers), and predatory mites (*Galendromus occidentalis* and *Amblyseius andersoni*). Of these, only the predatory mites are routinely found during scouting and the minute pirate bugs have been found rarely. The CUPS environment provides an opportunity for a diverse population of pests and beneficial organisms. Furthermore, with scouting, pest populations can be controlled to reduce economic damage and maintain high quality fruit standards.



—Scientific Note—

## Compatibility of Scions on Two New Rootstocks

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**Additional index words.** grafting, graft formation, incompatibility, citrus rootstocks

Rootstocks are an important component of citrus production systems around the world because they can have profound effects on tree size, fruit quality, and tree health (Martínez-Cuenca et al., 2016). Citrus rootstock development is a long process during which selections undergo extensive testing to cull those cultivars that have undesirable traits. Graft incompatibility, defined generally as the inability of the rootstock and scion to form a successful union (Goldschmidt, 2014), is an undesirable trait and can be a major concern with many fruit tree species. Recently, graft incompatibility symptoms have been observed on several commercial citrus cultivars when grafted on two new citrus rootstocks, ‘US-1283’ and ‘US-1284’. In this study, we sought to characterize incompatibility symptoms of three scion cultivars on these rootstocks in comparison to a control rootstock, ‘US-812’, and describe the physiological effects on the trees.

True-to-type seedlings of rootstock cultivars ‘US-1283’, ‘US-1284’ and ‘US-812’, all hybrids of mandarin (*Citrus reticulata*) and trifoliolate orange (*Poncirus trifoliata*) were grafted with scion cultivars ‘Bears’ lemon (*C. limon*), ‘Star Ruby’ grapefruit (*C. paradisi*), and ‘Valencia’ sweet orange (*C. sinensis*). Trees were grown for one year in an environmentally controlled greenhouse and were irrigated by an automated dripper system 3–4 times a week with weekly fertigation. Tree growth and incompatibility symptom development were tracked monthly by measuring trunk diameters, visual rating of graft union swelling and rootstock stem grooving. In addition, hydraulic conductivity was measured through the scion, the rootstock and the graft union tissue. Transverse sections of rootstock stems were sectioned, stained with toluidine blue O, and visualized by light microscopy.

After 52 weeks, all scion combinations with ‘US-1283’ and, to a lesser extent, with ‘US-1284’ developed grooving on the rootstock stem in addition to swelling of the scion directly above the graft union (Fig.1) while combinations with ‘US-812’ developed none. However, no differences in rootstock diameter were observed between the rootstocks for any of the combinations. Except for two trees which died, trees looked healthy. Hydraulic resistivity was highest in the scion combinations with ‘US-1283’ when compared to combinations with ‘US-812’; however, the hydraulic resistivity through the graft union

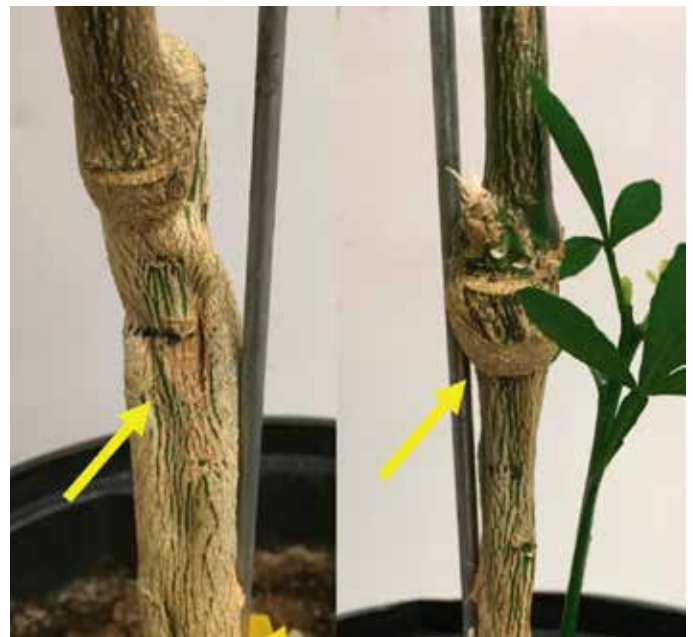


Fig. 1. Rootstock stem grooving and scion swelling on incompatible graft combinations of ‘Bears’ lemon (*C. limon*), and ‘US-1283’ rootstock.

was an order of magnitude higher than that of the rootstock and the scion, regardless of the graft combination. There was a moderate, negative correlation between graft union resistivity and rootstock diameter in combinations with ‘US-1283’, which was not observed in combinations with ‘US-1284’ or with ‘US-812’. Transverse rootstock sections of symptomatic ‘US-1283’ revealed that secondary xylem development was inhibited in the grooved regions (Fig. 2). In addition, necrotic tissue, varying in the degree of severity, was often present in the grooves extending into the wood. These abnormalities were absent in all graft combinations with ‘US-812’.

It has been noted that previous grafted trees of sweet orange on rootstocks of ‘US-1283’ and ‘US-1284’ propagated by stem cuttings did not exhibit grooving or swelling at the union and were superior in field performance to other rootstocks (Bowman and McCollum, 2015). It is unclear why there was a large dif-

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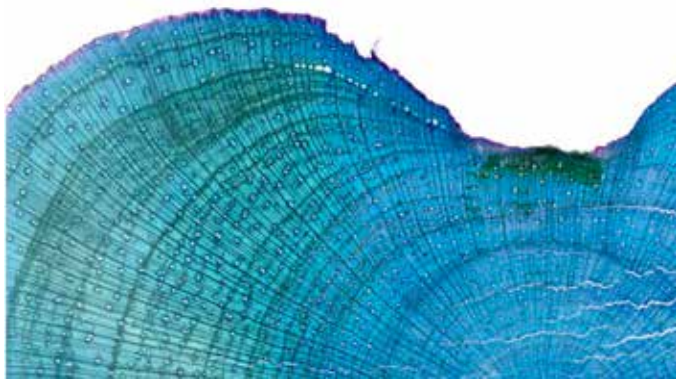


Fig. 2. Transverse light microscopic section of a grooved region on 'US-1283' rootstock grafted with 'Bearss' lemon (*C. limon*). The grooved region shows little secondary xylem growth and necrotic vascular tissue.

ference in compatibility reaction for rootstocks propagated by cuttings and nucellar seedlings. Transcriptomics and metabolomic study are in progress to identify the underlying physiological factors.

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—Scientific Note—

## Horticultural Evaluation of ‘Valencia’ Sweet Orange Grafted onto Pummelo Interstocks and Swingle Rootstocks

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**Additional index words.** canopy density, huanglongbing, interstocks, pummelo

Citrus greening disease [huanglongbing (HLB)] is a devastating disease caused by gram negative, phloem-limited, fastidious bacteria *Candidatus Liberibacter* spp. (Davis et al., 2008). HLB infects almost all the commercial cultivated citrus, causing a major loss of tree vigor, production, fruit development, and quality (Trivedi et al., 2010). An interstock is a graft of a citrus selection which can be used as a “bridge” between the scion and rootstock. Interstocks can increase tree longevity, production and improve fruit quality, regulate tree size (Girardi and Mourão Filho, 2006). Interstocks have been reported to improve abiotic stress tolerance (Aboutalebi and Hasanzadeh, 2014). HLB-tolerant interstocks would allow growers to use the rootstocks they liked prior to HLB becoming a problem (Shokrollah et al., 2011).

In this study, pummelo derived interstock candidates were selected based upon their field performance in which they showed few or no HLB symptoms. The ‘Swingle’ rootstock was cleft grafted with 6-inch interstock sticks obtained from trees in the field. The interstock was subsequently cleft grafted with HLB infected ‘Valencia’ scion. A year following grafting, trees were planted out in the field. There were nine of pummelo selections: UKP-1 (unknown origin), HBJL-1, HBJL-4, 5-1-99-3, and 5-1-99-2-S5 (derived from ‘Hirado Buntan’ pummelo); 5-4-99-3 and 5-4-99-7 (derived from ‘Red Shaddock’ pummelo); 7-2-99-11 (derived from ‘Large Pink’ pummelo); and 8-1-99-1B (derived from ‘Liang Ping Yau’ pummelo). ‘Swingle’ interstock served as the control in this study.

Leaf samples were periodically collected for physiological analysis (total chlorophyll content, starch content, total phenolic compounds content (TPC). The expression of two Pathogenesis Related (PR1 and PR2) genes were investigated from RNA obtained from the ‘Valencia’ scion leaves. Soluble solid content (SSC) was estimated in ‘Valencia’ fruit. CLas titer diagnosis of field trees in 2021 showed a low bacterial titer ranging between

29.74–33.56 in ‘Valencia’ grafted onto the pummelo selections and 32.69 in ‘Valencia’ grafted onto ‘Swingle’. ‘Valencia’ grafted onto HBJL-4 interstock and ‘Swingle’ control recorded the highest foliar starch content (34.50 and 38.22  $\mu\text{g}\cdot\text{mm}^{-2}$ ). 5-1-99-3 and HBJL-4 interstock trees exhibited the highest values of TPC (46.44 and 46.362  $\text{mg}\cdot\text{g}^{-1}$  gallic acid FW). The highest relative expression of PR1 and PR2 genes was recorded in HBJL-1 interstock trees followed by 8-1-99-1B and UKP-1 interstock trees. All interstocks influenced tree growth rate canopy density in the field ( $P$ -value = 0.0085). Trees on the 5-4-99-7 and 8-1-99-1B interstock had higher canopy density compared with the other trees. Additionally, some of the interstock combinations resulted in higher soluble solid content (SSC), which ranged between 5.15–6.15 lb solids/box. Our results indicate that HLB-tolerant interstocks can be used to provide enhanced tolerance to the susceptible scions and improve the soluble solid content in fruits, potentially resulting in increased income.

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—Scientific Note—

## Shading Effects on the Physiology, Growth and Yield of HLB-infected ‘Hamlin’ Sweet Orange Trees

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**Additional index words.** citrus, HLB disease, shade, yield

The citrus juice industry of Florida has been incurring huge economic losses due to HLB disease which is spread by an insect vector (Asian citrus psyllid; *Diaphorina citri* Kuwayama; ACP). To improve the management of infected trees, one of our ongoing field-based strategies deals with optimizing whole-tree light environment throughout the year. An earlier study (Jifon and Syvertsen, 2003) and a survey (Vincent et al., 2021) indicated benefits of shaded environments on citrus photosynthetic performance, but empirical data on shading effects on HLB-infected trees are not available. At the University of Florida, Institute of Food and Agricultural Science Citrus Research and Education Center at Lake Alfred, a field experiment was initiated in Oct. 2018. In a randomized block design, four-year-old, 1–1.5 m tall ‘Hamlin’ (*Citrus sinensis*) trees (naturally infected by *Candidatus Liberibacter asiaticus* (CLas.)) were treated with different levels of shade (0, 30, 50, and 70% shade) using shade structures consisting of black polyethylene nets with different mesh sizes. Our major objective was to investigate whether shade environments positively affect whole-plant physiology, growth, and yield characteristics of citrus trees. We also assessed what percentage of shade is optimum for citrus growth and productivity.

Measurements of tree canopy architecture indicated significant differences in important growth characteristics across treatments. Among the shading levels, 30% shade showed significant positive impacts on tree growth and yield performance. Within two years of treatment onset, the 30% shade grown trees manifested higher magnitude of plant volume, density, biomass, and area compared to the non-shaded cohorts. Yield data from years 2019 and 2020 indicated an increasing trend in fruit quantity in 30%

shaded trees, though longer term data collection is needed to confirm this. Physiological measurements conducted in past two years indicate better photosynthetic efficiency, higher carbon and water fluxes, safer range of leaf operational temperature and more balanced source-sink relationship in the 30% shade grown trees compared to their non-shaded counterparts.

Our study provides evidence at the whole-plant and leaf physiological levels that shade ameliorates the symptoms of HLB disease, resulting in enhanced growth and yield. The agronomic conclusion is that mild to moderate shade treatments may be a valuable tool in dealing with HLB disease which is endemic in several citrus-producing regions. Future work should address the economic cost-benefit of shading commercial citrus groves, as well as identify the optimum shading deployment for HLB-affected trees. Further, different colored shade nets could be tested to examine if they alter light quality and induce differential shading impacts on citrus physiology and yield performance. Future work should also address the degree to which shading impacts ACP vector pressure and populations of CLas. in the plant.

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## **Citrus Nutrition Box Program: Year One Review**

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### **Abstract**

Huanglongbing (HLB) has plagued the Florida citrus industry since 2005 and over 90% of commercial citrus groves are affected by HLB. Since the discovery of HLB, citrus nutrition has been of interest as a tool for improving and maintaining HLB-affected trees. HLB-affected trees have a smaller and weaker root system; therefore roots cannot take up the same amount of nutrients as healthy trees. Research studies have shown fertilizer applications should be done in smaller doses and more frequently, which allows nutrients to be consistently available. This coincides with another research project that showed HLB-affected trees cannot maintain nutrient levels compared to healthy trees. This new concept of collecting leaf samples to get an accurate nutrient analysis and applying fertilizer multiple times a year is a change in practice for citrus growers. To assist in teaching and creating a behavior change, the citrus nutrition box program was created. The box program is both a service project and a scientific demonstration. Through this service, one year of leaf analysis was collected throughout the Florida citrus industry. Early results indicate leaf nutrient levels change on certain varieties in particular seasons. Although this is normal in healthy trees, some varieties seem to struggle more than others in the presence of HLB. This study identified leaf analysis variability throughout the Florida citrus industry. Interesting patterns between soil pH and calcium has been observed. All these observations are critical in understanding the ongoing nutrition trends and formulating new extension strategies for nutrient management for citrus growers.

The abstract was presented at the 2021 FSHS Annual Meeting.

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## **Evaluation of the Susceptibility of Different Citrus Rootstocks to Burrowing Nematodes (*Radopholus similis*)**

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### **Abstract**

Citrus greening or huanglongbing is not the only concern for citrus growers. Underground pests such as plant parasitic nematodes and root weevils are endemic in Florida and of great concern. Rootstock programs produce many new rootstocks that may contribute to mitigating huanglongbing. Evaluating the newly produced rootstocks for their susceptibility to underground pests is extremely important before releasing them to growers. Burrowing nematodes (*Radopholus similis*) are tropical species with a wide range of crop hosts; however, a race known only in Florida is the only burrowing nematode that attacks citrus, causing the disease known as “spreading decline.” We established a strategy to evaluate the citrus rootstocks’ susceptibility to burrowing nematodes. Our methods included challenging the rootstock with the nematode following by morphological, histological and metabolomic studies. Information from this work will certainly assist in making useful recommendation about these rootstocks to growers.

The abstract was presented at the 2021 FSHS Annual Meeting.

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## **Determination of Citrate in Citrus Plants Using Europium Sensitized Fluorescence Assay**

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### **Abstract**

Citrate is one of several intermediates in the tricarboxylic acid cycle (TCA), which is the center for energy production in the plant cell. Citrate is found at high levels in citrus fruits and different tissues. It has been found that citrate plays an important role in phosphate uptake from the soil. Additionally, it is believed that citrate is the main carbon source for *Candidatus Liberibacter asiaticus*, the causal agent of huanglongbing disease. Several different methods have been developed for the detection and quantification of citrate in different sample matrixes. However, most of these methods are time-consuming, expensive, require a cleanup step before analysis, and are not available in most laboratories. We previously established a europium-based method for the detection and quantification of oxytetracycline in citrus tissues after the treatment with this antibiotic. We reported a significant increase in the fluorescence intensity of the europium tetracycline complex after the addition of citrate as sensitizing agent. We now report a fluorescence method for the determination of citrate in citrus plants using europium and oxytetracycline as a sensitizing agent. Our results showed a wide linear range ( $R^2 = 0.99$ ) between the log of citrate concentration (5 ppm to 500 ppm) and fluorescence intensity. We also were able to estimate citrate in juice and phloem and xylem saps in lime, lemon, and sweet oranges. The recovery of citrate from citrus juice and saps ranged from 80 to 108%. Currently, we are optimizing the method for the determination of citrate in citrus leaves and other tissues.

The abstract was presented at the 2021 FSHS Annual Meeting.

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## Nitrogen Management and Recovery Efficiency and Response to Nitrogen Sources in Drip-irrigated Tomato

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**Additional index words.** fertilizer management practices, nutrient management, nutrient use efficiency, *Solanum lycopersicum*, vegetable production

Plant use efficiency of applied N is estimated to be < 50% for most crops, particularly in coastal plains sandy soils, where N losses through leaching and volatilization in warmer climates may be a predominant pathway for N loss to the environment. The use of best management practices (BMPs) such as controlled release fertilizer (CRF) is one potential way to increase plant N uptake and decrease N loss to the environment. The use of CRF allows for one preplant application of N fertilizer that could potentially supply sufficient N for a growing season. Less labor would be associated with application of N fertilizer which could lead to economic benefits as well. This research study evaluates the suitability of CRF as an alternative nitrogen (N) fertilizer source to conventional urea (U) for tomato production. The dissimilar management of controlled-release urea CRU and U can potentially lead to different N use efficiencies (NUE) and losses in a vegetable production system. In addition, the incorporation of cover crops between seasons of vegetable production can be beneficial as certain cover crops function as N catch crops, which hold residual N at the soil surface. A two-year fall tomato-winter rye-spring tomato-sorghum-sudan rotation was conducted to evaluate the management of N sources such as CRU and U. Lower N rates, timing of N fertilizer application, and placement were also evaluated. Data results from the first-year fall tomato season will be presented.

Nitrogen is a common limiting factor for tomato production in the southeastern United States. (Everett, 1976; Locascio et al., 1997; Rhoads et al., 1996), resulting in significant N use. In Florida, the University of Florida, IFAS (UF/IFAS) recommended rate of N fertilization for tomato production is 224 kg·ha<sup>-1</sup> (Mylavarapu et al., 2017a), although commercial growers may apply more than double this amount (400–560 kg·ha<sup>-1</sup>) (Everett, 1976; Rhoads et al., 1996). Excessive application of synthetic N fertilizer eventually leads to leaching of N into groundwater through nitrate (NO<sub>3</sub>-N) pollution (Paltineanu et al., 1980). Degradation of surface water bodies can occur through eutrophication (Carpenter et al., 1998). With increases in the utilization of N fertilizer occurring, it is critical to produce information on crop N fertilizer accumulation to improve production practices that can increase use efficiency and decrease N fertilizer loss.

Use of control release fertilizer (CRF) is a best management practices (BMP) tool that can provide N to the crop gradually throughout the season, reduce nitrogen (N) pollution to the environment, and reduce the costs of labor due to one preplant application compared to multiple application of N. The mechanism of release (polymer coating) of CRFs is highly affected by temperature and water. Manufacturers of CRF typically report their release durations of CRF at 25 °C, however field conditions are not always at 25 °C, especially if growers decide to plant early or late in the season. Therefore, there is a need to investigate the release pattern of CRF to determine if CRF can sustain the release of N for crop N requirement (CNR) of tomatoes.

The CNR is defined as the N concentration in the aboveground dry matter at near maximum yield (Meisinger et al., 2015), where approximately 2.0 to 4.5% N can be contained in dry matter (Mengel and Kirkby, 2001). The CNR by crops is predominantly

determined by physiological need (Mengel, 1983). To study the impact of CRF on CNR, sampling whole plants multiple times throughout the growing season was done. This allowed monitoring N uptake in crop. This study helped determine the effect of CRF on N uptake in various tomato tissues (fruit, petioles, leaf blades, stems, and roots). This study used controlled-release urea (CRU) and conventional urea (U) as N sources. Two CRU fertilizers with different release durations of 60 (CRU-60) and 75 (CRU-75) days were to determine the optimum N release rate for the CNR of tomatoes. This study will be conducted twice for two years. Both years will have a crop sequence of fall tomato–winter rye, spring tomato–sorghum–sudan. Results from this study are from the first fall tomato season of the tomato–cover crop rotation.

### Materials and Methods

A field experiment was conducted at the University of Florida, Plant Science Research and Education Unit in Citra, FL during the Fall 2019 season. On 12 Aug. 2019, the study area was rototilled, and six false beds were made. Controlled release fertilizer was applied to the false beds and rototilled in, then the beds (0.3 m high on 1.5 m centers) were made. Each row is approximately 50 m long. Pic-Clor 60 umigant (TriEst Ag. Group Inc, Greenville, NC) was applied at a rate of 336 kg·ha<sup>-1</sup> to prevent nematode infestation and suppress weeds. During fumigant application, drip tape (12-inch emitter spacing) was laid, and beds were covered with black plastic mulch. In preparation for transplanting, holes were punched into the plastic about 0.46 m apart.

A randomized complete block design (r = 3) was established, where 10 N treatments were randomly assigned to plots that were 9.1 m long and 0.91 m wide with 0.91 m buffers in between each plot. In addition to the control (0 kg·ha<sup>-1</sup>) treatment, soluble urea (U) and two CRU fertilizers 1) a 60-day (CRU-60) and 2) 75-day (CRU-75) release duration, were applied at three N rates (140,

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168, and 224 kg·ha<sup>-1</sup>). A treatment name such as 140 U means soluble urea applied at the 140 kg ha<sup>-1</sup> N rate. CRUs were applied at the time of bed formation, whereas U treatments were applied one week after planting. Soil P (196 mg·kg<sup>-1</sup>), Ca (431 mg·kg<sup>-1</sup>), and pH (6.5) levels were sufficient, so application of P, Ca, and lime was not done. Potassium (K) as potassium sulphate and magnesium (Mg) as magnesium sulphate were applied at 252 kg·ha<sup>-1</sup> and 38 kg·ha<sup>-1</sup> respectively in weekly doses until the conclusion of the study.

Three weeks after fumigation, tomato (var. HM 1823) seedlings were transplanted on 5 Sept. 2019, for the fall season. Fifteen seedlings were planted per treatment. One random plant was collected for sampling every two weeks and three plants were collected at harvest. Irrigation was used after transplanting. Seven days after transplanting (DAT), U treatments were put out through the drip and then were applied in equal weekly doses over the growing period.

Plant samples were analyzed at the (UF/IFAS) Analytical Services Laboratories in Gainesville using standard procedures (Mylavarapu et al., 2017b). Whole plant samples were dried at 65 °C for a week and were subsequently separated into fruit, leaf, stem, and root components. Dry weights of each plant component were taken and then samples were ground and analyzed for total N (TN) and total carbon (C; TC). These measurements were used to calculate the C:N ratio. Dry weights of each plant component were used to calculate dry matter weight of the whole plant. The weight was then multiplied by whole plant TN to calculate the N accumulation (kg·ha<sup>-1</sup>) in the whole plant.

The agronomic calculation of apparent N recovery (APR) is defined as the difference in N uptake (kg·ha<sup>-1</sup> N) between plots receiving fertilizer and unfertilized plots and is in proportion to the amount of N fertilizer applied. Nitrogen use efficiency (NUE) is defined as N uptake by plants divided by the total amount of N fertilizer applied. The APR and NUE were calculated using the following equations:

$$APR (\%) = \left( \frac{N_f - N_{uf}}{N_a} \right) \times 100 \quad [1]$$

where  $N_f$  and  $N_{uf}$  were total N uptake in fertilized and unfertilized plots (kg·ha<sup>-1</sup> N) and  $N_a$  is the amount of N fertilizer applied (kg·ha<sup>-1</sup> N); and

$$NUE (\%) = \frac{N_{uptake\ in\ plant}}{N_a} \times 100 \quad [2]$$

where  $N_a$  is the amount of N fertilizer applied (kg·ha<sup>-1</sup> N).

Data were analyzed using statistical software (JMP 14.1; SAS Institute, Cary, NC). A one-way analysis of variance, with the N rate as a fixed factor and the block as a random effect was used to evaluate treatment effects on mean fruit yield, whole plant dry matter, and whole plant N accumulation using Dunnett's test at  $\alpha = 0.05$ . Treatment effects on NUE and APR were evaluated using Tukey-Kramer honestly significant difference (HSD) post-hoc test at  $\alpha = 0.05$ . A second order polynomial regression analysis ( $y = a + bx + cx^2$ ) was used for fruit yield, whole plant dry matter, and whole plant N accumulation to determine the N rate at which yield is maximized.

## Results and Discussion

No fruit yield, whole plant dry matter, and N accumulation results for CRU-60 are shown as the regression analysis was nonsignificant.

A regression analysis and derivative of the quadratic whole plant N accumulation kg·ha<sup>-1</sup> data showed that whole plant N accumulations were maximized at 207 kg·ha<sup>-1</sup> and 217 kg·ha<sup>-1</sup> for CRU-75 and soluble urea, respectively (Fig. 1).

Highest efficiencies and recoveries were found at lower N rates (140 and 168 kg·ha<sup>-1</sup>) from both the CRF and U treatments, resulting in an NUE of 61.6% and APR of 44.8% (Table 1). Nitrogen use efficiency decreased significantly with increasing rates of N fertilizer in Fall 2019, which is a typical result in many N fertilizer rate studies (Anderson et al., 1999; Hochmuth and Cordasco, 2000; Hochmuth and Hanlon, 2014; Zotarelli et al., 2009a, 2009b).

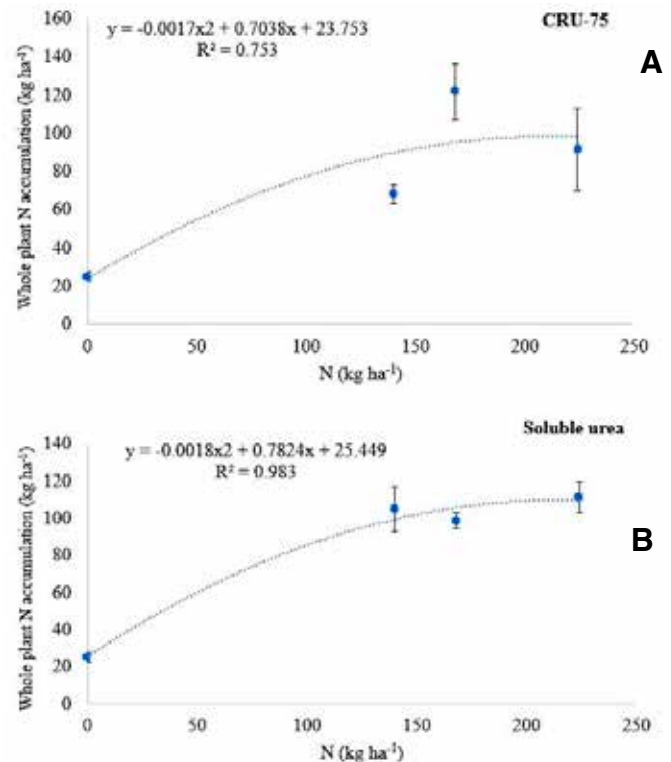


Fig. 1. Regressions of nitrogen (N) fertilizer rate to whole plant N accumulation in Fall 2019 season in Northern Florida. (A) Regression of controlled release urea at 75-day duration (CRU-75). (B) Soluble urea N source.

Table 1. Effect of N fertilizer treatments on the nitrogen use efficiency (NUE) and apparent recovery of N (APR) values.

Treatment	NUE (%)	APR (%)
140 CRU-60	59.2 ± 1.1 a <sup>z</sup>	40.1 ± 0.7 ab
140-CRU-75	45.2 ± 1.1 c	26.1 ± 0.7 cd
140 U	66.6 ± 0.6 a	47.4 ± 2.3 a
168 CRU-60	35.7 ± 3.4 cd	22.0 ± 2.5 cd
168 CRU-75	64.2 ± 2.6 a	50.5 ± 3.5 a
168 U	56.5 ± 1.3 ab	41.4 ± 3.6 ab
224 CRU-60	43.1 ± 0.9 c	31.8 ± 0.8 bcd
224 CRU-75	31.4 ± 2.0 d	19.4 ± 0.9 d
224 U	46.3 ± 2.3 bc	34.3 ± 1.2 bc
P value	< 0.0001	< 0.0001

<sup>z</sup>Values followed by the same letter within a column indicate not statistically significantly different ( $P > 0.05$ ) within the nine N fertilizer treatments according to Holm-Tukey adjust. N rates = 140, 168, and 224 kg·ha<sup>-1</sup>.

CRU-60 = Controlled release urea with a release duration of 60 days.  
CRU-75 = Controlled release urea with a release duration of 75 days.  
U = Soluble urea.



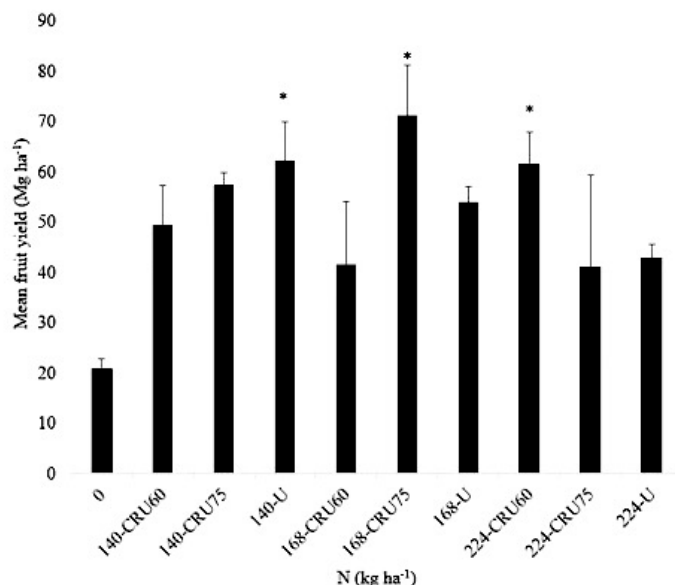


Fig. 2. Effect of nitrogen (N) fertilizer rate on mean fruit yield in Fall 2019 season in northern Florida. Means followed by a \* were significantly different from the control (0 lb/acre N) according to Dunnett's test at ( $P < 0.05$ ).

Treatments 140 U, 168 CRU-75, and 224 CRU-60 resulted in the highest yield compared to the control (Fig. 2). A regression analysis and derivative of the quadratic fruit yield data showed that yields were maximized at 131 kg ha<sup>-1</sup> and 135 kg ha<sup>-1</sup> for CRU-75 and U, respectively (Fig. 3). Additionally, whole plant dry matter was maximized at 136 kg ha<sup>-1</sup> and 132 kg ha<sup>-1</sup> for CRU-75 and soluble urea, respectively (Fig. 4). Various N fertilizer rate studies conducted on sandy soils have concluded that no appreciable tomato yield increase occurred with N fertilizer rates

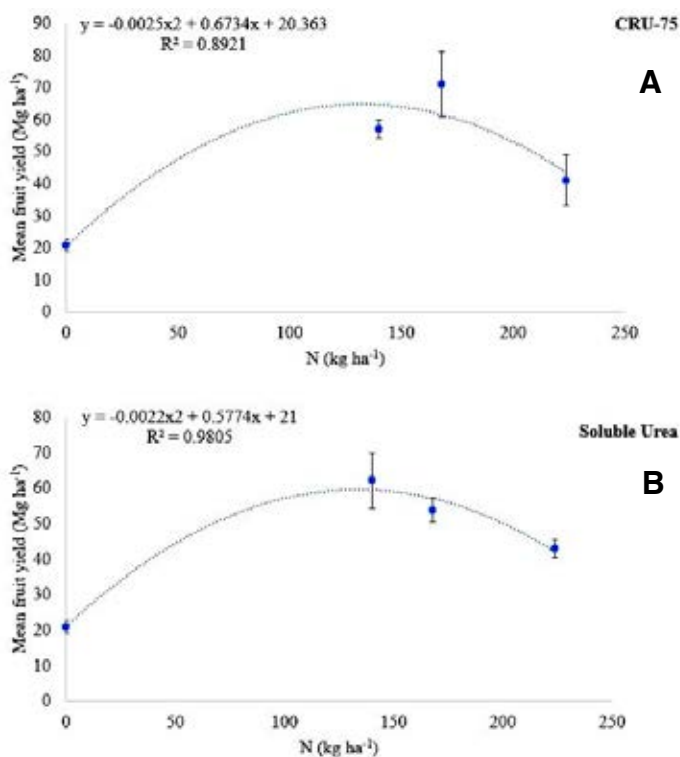


Fig. 3. Regression of nitrogen (N) fertilizer rate to fruit yield in Fall 2019 season in northern Florida. (A) Regression of controlled release urea at 75-day duration (CRU-75). (B) shows the soluble urea N source.

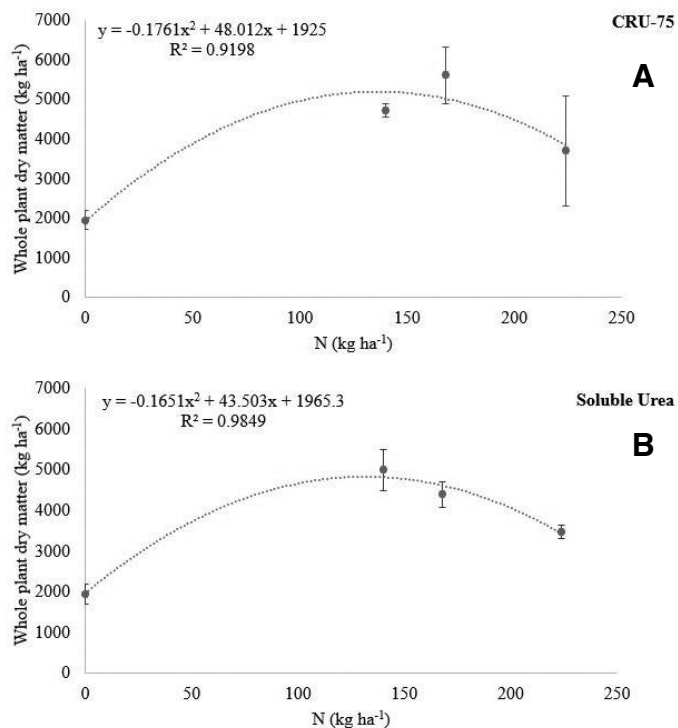


Fig. 4. Regression of nitrogen (N) fertilizer rate to whole plant dry matter in Fall 2019 season in northern Florida. (A) Regression of controlled release urea at 75-day duration (CRU-75). (B) shows the soluble urea N source.

above the recommended N rate of 224 kg·ha<sup>-1</sup> (Anderson et al., 1999; Hochmuth and Cordasco, 2000; Hochmuth and Hanlon, 2014; Jalpa et al., 2020; Jalpa et al., 2021; Zotarelli et al., 2009a, 2009b). Our results showed that no appreciable increase in tomato yield was obtained with higher N rates and that the recommended N fertilizer amount 224 kg·ha<sup>-1</sup> seems to be adequate for tomato production.

## Conclusions

Fruit yield, whole plant dry matter, and N accumulations were maximized at N rates below the recommended N rate for tomato production, showing that the current UF/IFAS recommendation of 224 kg ha<sup>-1</sup> seems to be adequate. Out of the two durations of CRFs tested, CRU-75 seems to be a suitable N source for producing tomatoes that is comparable to the conventional N source. Future steps for this project will be to assess leaching, cover crop benefits, and conduct a cost-benefit analysis for the two-year tomato-cover crop rotation.

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## Biological Interactions Between Root-knot Nematode (*Meloidogyne incognita*) and Pepper (*Capsicum annuum*)

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Root-knot nematodes (RKNs; *Meloidogyne* spp.) are soilborne plant parasites that severely decrease productivity of susceptible pepper (*Capsicum annuum*) varieties. Genetic resistance to RKNs serves as a nonchemical alternative to nematode management strategies. Although previous studies of pepper resistance genes to RKNs demonstrate a hypersensitive reaction-based mechanism, an advanced inbred line of pepper ('UFRJ107(6)A3', named 'Ruby') completely inhibits penetration to *M. incognita*. To gain insight into the mechanism of resistance in 'Ruby', responses of *M. incognita* infective juveniles to root tissues and root extracts from pepper test lines were evaluated in vitro using Pluronic F-127 gel. First, infective second-stage juveniles (J2) were able to penetrate root tips of *C. annuum* cv. Jimmy Nardello Italian (JNI, a susceptible cultivar), and 'Ruby' when 3-week-old seedlings were used. The ability of J2 to penetrate root tips of 'Ruby' suggests that resistance in 'Ruby' could be associated with plant growth developmental stage. Second, hydrophilic compounds from root extracts of both 'JNI' and 'Ruby' showed a negative effect on chemoreception by J2s. The results of the current study can be used to better understand the multiple components of RKN resistance unique to 'Ruby'.

Pepper (*Capsicum annuum*) is a vegetable crop with high economic impact and nutritional value. Root-knot nematodes (RKN; *Meloidogyne* spp.) are the most widely distributed plant-parasitic nematode that severely decreases productivity of susceptible crops including peppers. Damage caused by nematodes in agricultural crops results in an estimated annual loss of 80–157 billion USD globally (Coyne et al., 2018). The primary method to manage RKNs in pepper production has been broad-spectrum soil fumigants. Genetic resistance to RKNs serves as a nonchemical alternative to nematode management strategies involving fumigants.

Currently in pepper, there are 10 reported genes controlling resistance to RKN with varying species specificity (Djian-Caporalino et al., 1999; Hendy, et al., 1985; Wang and Bosland, 2006). Two resistance genes, *Me3* and *Me7*, inherent in the lines HDA149 and 'Criollo de Morellos' (CM334), respectively, are effective against *M. incognita*, *M. arenaria*, *M. javanica*, and *M. hapla*. The resistance mechanisms of *Me3* and *Me7* have been reported to act through a hypersensitive response (HR) with some differences. In HDA149, RKN root penetration induces an immediate HR in the root epidermis while a delayed HR localized to root vasculature is observed in CM334 after penetration. Furthermore, resistance in CM334 is uniquely triggered after a feeding site has been established (i.e., giant cells have formed) (Bleve-Zacheo et al., 1998; Pegard et al., 2005). In contrast to these HR-based mechanisms of resistance, 6-week-old seedlings of an advanced inbred pepper cultivar, UFRJ107(6)A3 ('Ruby'), showed complete inhibition of *M. incognita* penetration based on the results of root staining 7 d after inoculation (Maquilan et al., 2020). Thus, the mechanism and genes involved in resistance unique to 'Ruby' are unknown.

As one of many avenues of enquiry, we aimed to test whether plant root extracts made in specific solvents likely containing chemical compounds from roots in high enough concentrations could influence nematode behavior. Others have successfully used such methods. For example, infective J2 of *M. incognita* and *Heterodera glycines* were highly repelled and attracted, respectively, to extracts of three different plant species, including pepper, in an in vitro assay (C. Wang et al., 2018). An understanding of the role of various plant root extracts on nematode behavior has implications for differentiating resistance mechanisms and may allow future identification of metabolites that function as components of RKN resistance.

To gain an understanding of the mechanisms involved during the early interactions between *M. incognita* and the pepper genotypes tested, an in vitro, Pluronic F-127 gel-based assay was conducted. Pluronic F-127 gel, a copolymer that forms a gel at room temperature, allows for free movement of J2s, is transparent, and allows for diffusion of chemical gradients. The objectives of the current study were to: 1) compare attraction or repulsion of *M. incognita* to the roots of 'Ruby' and a susceptible cultivar [Jimmy Nardello Italian (JNI)] and 2) evaluate the response of *M. incognita* to root extracts of 'Ruby' and 'JNI' in a choice experiment.

### Materials and Methods

**INOCULUM PREPARATION.** *Meloidogyne incognita* race 3 originated from cotton grown in an agricultural field in South Georgia. An isolate was derived by making a single egg mass isolate and then grown on tomato in a greenhouse in the Entomology and Nematology Department, University of Florida at 27 ± 5 °C at 75% relative humidity (RH). Host plant maintenance and egg

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extractions were conducted as described in (Maquilan et al., 2020) for the preparation of freshly hatched J2 suspensions. Freshly hatched J2s were collected on a sieve with 500  $\mu\text{m}$  openings, then transferred to a 50-mL Falcon tube with 20 mL of water. The J2-Pluronic F-127 suspension was prepared through an adapted protocol as described by (Williamson and Čepulytė, 2017). Pluronic F-127 23% (wt/vol) (PF-127; Sigma-Aldrich, USA) was first prepared in 10 mM Tris- morpholinoethanesulfonic acid (MES) buffer. The solution was stirred with a magnetic stir bar at 4 °C overnight until dissolved, then kept in a refrigerator at 4 °C. Freshly hatched J2 suspended in 1–2 mL  $\text{H}_2\text{O}$  were added to the PF-127, then stirred for 10 min. at 4 °C with a magnetic stir bar. Solutions were kept on ice before adding to the wells.

**PEPPER GENOTYPES.** Seeds of *M. incognita* ‘Jimmy Nardello Italian’ (‘JNI’) were purchased from Baker Creek Heirloom Seeds (Mansfield, MO). ‘JNI’ is an heirloom cultivar that is highly susceptible to *M. incognita* race 3. Advanced inbred line UFRJ107(6)A3 (‘Ruby’) is a specialty pepper that shows high resistance to *M. incognita*, *M. arenaria*, and *M. javanica* based on RKN reproduction and penetration measurements (Maquilan et al., 2020). An  $F_1$  derived from a cross between ‘JNI’ and ‘Ruby’ was also evaluated for root attraction assays.

**PREPARATION OF ROOT EXTRACT.** Root extracts were prepared through an adapted protocol as described by C. Wang et al., (2018). Entire root systems of 4-week-old seedlings were rinsed and cut into pieces. Roots were ground and frozen in a mortar and pestle with liquid nitrogen into a fine powder. Approximately 0.042 g of root material was transferred to 1.5-mL microcentrifuge tubes, in which 250  $\mu\text{L}$  of extraction solvents were added. The two extraction solvents used in this study were distilled water ( $\text{H}_2\text{O}$ ) and methanol (MeOH) 80% (v/v). Extracts were centrifuged (20 min., 13,000  $\times g$ ) and supernatant volumes were transferred to a separate 1.5-mL microcentrifuge tube.

**ROOT ATTRACTION AND PENETRATION ASSAY.** Root systems from seedlings of each pepper genotype were removed from vermiculite after 3 weeks and rinsed with sterile water. Terminal 5-mm of root tips were excised individually and placed in a cell of a 12-well tissue culture plate (Nunc™ Non-Treated Multidishes, Thermo Scientific, Waltham, MA) at room temperature. One mL of PF-127 23% (wt/v) and 10 mM Tris-MES (pH 8) containing 100 J2 was delivered to each well. The media was allowed to solidify over 10 min before recording any observations. Plates were covered in aluminum foil and kept at room temperature. The total number of J2s in contact with the root were then counted under a stereomicroscope equipped with an overhead illuminator at 2 h, 4 h, 6 h, 19 h, and 24 h. At the end of the root attraction assay, the root tips were immediately removed from the well, stained with fuchsin-acetic acid (Byrd et al., 1983), and the number of J2 were counted. Six replicate assays were used for each data point.

**ROOT EXTRACTS CHOICE TEST.** For the root extracts choice test, each of the test and control solutions were suspended in PF-127 23%. The test controls used in the root extracts choice test were salicylic acid (50 ng/mL) (SA; attractant), acetic acid 1% (v/v in  $\text{H}_2\text{O}$ ) (HOAc; repellent),  $\text{H}_2\text{O}$ , and MeOH 80% (v/v in  $\text{H}_2\text{O}$ ). For each well of a 12 well tissue culture plate, approximately 330 freshly hatched J2 suspended in 1 mL of PF-127 23% was added. After the gel solidified at room temperature, 3.5  $\mu\text{L}$  of test solution and 3.5  $\mu\text{L}$  water or other control as indicated were injected into the gel at marked points 5.5 mm apart. Plates were covered in aluminum foil and kept at room temperature. The number of

J2 within a 5 mm circle centered on the injection site of the test solution or control was counted under a stereomicroscope after 6 h and 24 h. Three replicate assays were used for each data point.

**STATISTICAL ANALYSIS.** A completely randomized design was used during all experiments. Quantitative data were used to calculate means and the standard error. The statistical significance of the difference in data collected from all experiments was tested using Student’s *t* test ( $P \leq 0.05$ ) with the RStudio program (RStudio, Boston, MA).

## Results and Discussion

Root tips of ‘Ruby’, ‘JNI’, and the  $F_1$  hybrid were studied to evaluate any differences in attraction by J2 between the genotypes (Fig. 1A). The number of J2 in contact with root tips of ‘Ruby’ was significantly lower compared to root tips of ‘JNI’ at 6 h, while the opposite trend was seen at 24 h (Fig. 1B). In addition, the number of J2 in root tip in contact with the  $F_1$  hybrid resembled that of ‘Ruby’. The decrease in number of J2 in contact with root tips of ‘JNI’ suggested that J2 were unable to penetrate roots of ‘Ruby’ but able to penetrate roots of ‘JNI’. To confirm this, the root tips were then removed from the wells and subjected to fuchsin-acetic acid staining to determine the number of J2 inside the root. The J2 were able to penetrate the root tips of both ‘JNI’ and ‘Ruby’ (Fig. 1C). Furthermore, the difference in mean number of J2 inside the roots of both genotypes was insignificant.

The results from this experiment contrasts with previous findings that J2 of *M. incognita* were unable to penetrate roots of ‘Ruby’ (Maquilan et al., 2020). In comparing variables between the previous and current experiment, two that differed are the medium used and seedling age. It is possible that the PF-127 23% media exogenously induced sensitivity to penetration in ‘Ruby’. The media used for ‘Ruby’ seedlings may play a role in the components of resistance unique to ‘Ruby’. In regard to seedling age, the previous and current study evaluated roots of Ruby seedlings at 8 weeks and 3 weeks, respectively. The results of both experiments imply that different developmental plant-growth stages are associated with penetration inhibition in ‘Ruby’. For example, resistance that is developed at later stages of plant-growth is referred to as adult plant resistance. In contrast to highly specific HR-based resistance, adult plant-growth resistance confers broad-spectrum resistance and is quantitative in nature (Johnson, 1984). Further studies are needed to evaluate differences between penetration ability in ‘Ruby’ at different plant-growth developmental stages.

Evaluating root extract activity on J2 behavior serves to test chemical components of resistance. Thus, root extracts of ‘Ruby’ and ‘JNI’ were compared to evaluate any effects on J2 behavior at 6 h and 24 h. Salicylic acid (SA) served as an attractant control (Wuyts et al., 2006). Although acetic acid 1% (v/v) acted as a repellent control in a preliminary experiment, the same effect was not observed in the root extracts choice test (data not shown) possibly due to diffusion of the solution into the gel to below optimal concentrations after placement. Both ‘Ruby’ and ‘JNI’ root extracts obtained through ddH<sub>2</sub>O extraction demonstrated significantly reduced attractance compared to  $\text{H}_2\text{O}$  (Table 1). Furthermore, the reduced attractance was observed at 24 h, suggesting that the compounds in both roots are stable in solution. In contrast, both ‘Ruby’ and ‘JNI’ root extracts obtained through MeOH 80% (v/v in ddH<sub>2</sub>O) extraction did not show any reduced attractance except for ‘Ruby’ at 6 h (Table 1). In summary, roots

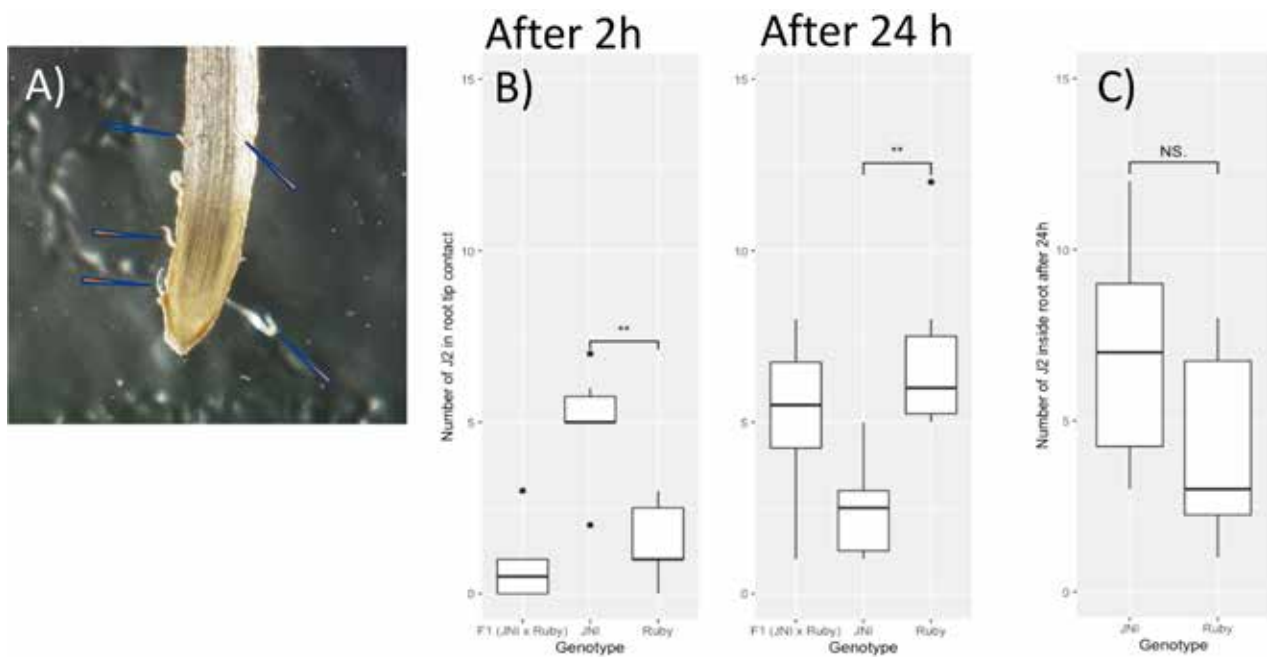


Fig. 1. **A**) Infective-stage juveniles of *Meloidogyne incognita* race 3 on a root tip of 'Jimmy Nardello Italian' ('JNI') at 2 h; triangular blocks point to individual J2s; magnification = 24.0x. **B**) Mean number of J2 in contact with root tips of 'Ruby', 'JNI', and the F<sub>1</sub> hybrid at 6 h and 24 h. Statistical significance was determined by mean separation by Student's *t* test at  $P \leq 0.05$  (\*\* significant at  $P \leq 0.01$ ). **C**) Mean number of J2 inside roots of 'Ruby' and 'JNI' after staining with fuchsin-acetic acid at 24 h.

Table 1. Preferred choice of J2 between two choices presented in a pluronic gel matrix. Comparisons were made between 'Ruby' and 'Jimmy Nardello Italian' (JNI) root extracts obtained through water and MeOH 80% (v/v), as well as control solutions, at 6 h and 24 h.

Choice A	Choice B	Observation time (h)	Mean number of J2s in A + SE	Mean number of J2s in B + SE	Preferred choice	Statistical significance <sup>z</sup>
SA	H <sub>2</sub> O	6	19 ± 0.882	9 ± 1.528	A	***
JNI (H <sub>2</sub> O)	H <sub>2</sub> O	6	2 ± 0.333	14 ± 3	B	****
Ruby (H <sub>2</sub> O)	H <sub>2</sub> O	6	5 ± 0.3333	13 ± 2.333	B	**
JNI (MeOH)	MeOH	6	5 ± 2.3333	14 ± 3.606	neither	ns
Ruby (MeOH)	MeOH	6	5 ± 2.081	9 ± 1.155	B	*
JNI (H <sub>2</sub> O)	Ruby (H <sub>2</sub> O)	6	8 ± 1.333	3 ± 0.333	A	**
JNI (MeOH)	Ruby (MeOH)	6	6 ± 0.577	5 ± 0.882	neither	ns
SA	H <sub>2</sub> O	24	14 ± 2.404	9 ± 1.856	A	NS
JNI (H <sub>2</sub> O)	H <sub>2</sub> O	24	6 ± 1.856	10 ± 1.202	B	*
Ruby (H <sub>2</sub> O)	H <sub>2</sub> O	24	5 ± 1.202	10 ± 0.882	B	*
JNI (MeOH)	MeOH	24	11 ± 2.186	15 ± 2.403	neither	ns
Ruby (MeOH)	MeOH	24	6 ± 1.528	7 ± 0.333	neither	ns
JNI (H <sub>2</sub> O)	Ruby (H <sub>2</sub> O)	24	10 ± 0.667	9 ± 1.453	neither	ns
JNI (MeOH)	Ruby (MeOH)	24	8 ± 0.882	9 ± 1.202	neither	ns

<sup>z</sup>Statistical significance was tested between mean comparisons between A and B using Student's *t* test.

\*\*\*\* =  $P \leq 0.0001$ , \*\*\* =  $P \leq 0.001$ , \*\* =  $P \leq 0.01$ , \*  $P \leq 0.05$ , ns = nonsignificant.

of both 'JNI' and 'Ruby' contain hydrophilic compounds that negatively affect chemoreception of *M. incognita* J2.

Previous reports indicate the effects of plant metabolites on J2 behavior under different systems (Sikder and Vestergård, 2020). However, there are few studies involving pepper specifically (Bleve-Zacheo et al., 1998; Kihika et al., 2017; Pegard et al., 2005). Chlorogenic acid was identified as the primary chemical constituent of hypersensitive response sites in CM334 (Pegard

et al., 2005). Nonetheless, the results in our root extracts choice test were similar to a previous study, in which extracts of a susceptible pepper cultivar did not attract *M. incognita* (C. Wang et al., 2018). Further work is required to evaluate the root extract chemical profile for identifying the primary metabolite(s) causing this repellent effect. A potential outcome that would be of great benefit is to identify genes in pepper responsible for biosynthesis of such metabolites.

## Conclusions

In the current study, we evaluated the interactions between J2 of *M. incognita* race 3 and root extracts, as well as terminal root tips of resistant and susceptible pepper varieties. With the exception of *Me3* and *Me7*, RKN resistance genes in pepper have typically been characterized based on galling index, egg mass index, and reproduction factor, while the biological mechanisms of such genes generally remain poorly understood (Changkwian et al., 2019; Djian-Caporalino et al., 1999; Hajihassani et al., 2019; Hendy et al., 1985; Thies and Fery, 2000). Our aim was to test whether the RKN-resistant ‘Ruby’ demonstrated unique mechanisms independent of HR-based resistance as characterized in other RKN-resistance genes (e.g. *Me3* and *Me7*). Our results can be used in further studies to investigate the multiple components of RKN resistance unique to ‘Ruby’.

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## Collaborative, Rapid-Response Research and Extension Efforts for Management of an Invasive Thrips Species, *Megalurothrips usitatus* (Bagnall)

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Florida snapbean production ranks first in the United States, contributing to more than one-third of the national production. In January 2020, *Megalurothrips usitatus* (Bagnall) (Thysanoptera: Thripidae), Asian bean thrips, was first detected in Homestead, FL by Glades Crop Care, Inc. and identified by FDACS as a new pest to the continental U.S. Impacted snapbean growers in Miami-Dade County reported 30% losses (estimated at \$1,620 per acre), which could total more than \$33 million with similar losses across southern Florida. As a pest of other legumes, *M. usitatus* has the potential to negatively impact growers across the southeastern U.S. A rapid-response extension program led by University of Florida/IFAS (UF/IFAS) faculty and extension agents was developed in partnership with commercial crop consultants and growers. Meetings were organized to train growers, scouts, and extension agents, and to foster information sharing across farms and production areas. A regional scouting effort was initiated that covered 25 farms in Palm Beach, Hendry, and Collier Counties to monitor population level dynamics, detect potential alternative hosts, and identify effective grower management practices during the 2020-2021 season. Lab and field pesticide efficacy trials began. A website was developed to provide timely insights from research efforts along with weekly updates of *M. usitatus* population levels throughout the scouted region. This presentation will highlight knowledge gained from the first year of the rapid-response effort. Discussion will include the successes and challenges faced, expansion and improvements to be made for the upcoming season, and takeaways for future collaborative, rapid-response efforts.

Florida ranks #1 in the U.S. for snapbean production, producing nearly half of total national production (Zhang et al., 2017). Florida has about 28,000 acres of snapbeans, with about 7500 acres in Miami-Dade County, 6670 in Palm Beach County, and 7270 acres in Southwest Florida (Hendry, Glades, Collier, and Lee Counties) (USDA-NASS, 2017). Average Florida snapbean yields are approximately 180 thirty-pound bushels/acre (Zhang et al., 2017). At \$30 per bushel, growers can gross about \$5,400 per acre. Production costs are \$4,000 to \$4,700 per acre (Olson et al., 2011), with a net return of \$700 to \$1,400 per acre.

In Jan. 2020, significant damage was reported in a young snapbean field near Homestead, FL, in Miami-Dade County. Field scouts from Glades Crop Care, Inc. noted large black thrips feeding on the young plants. Samples were sent to UF/IFAS entomologist and thrips specialist, Joseph E. Funderburk and Florida Department of Agriculture and Consumer Services Division of Plant Industry (FDACS-DPI) taxonomist Felipe Soto-Adames. Based on its morphology, the new pest was identified as *Megalurothrips usitatus*, Asian bean thrips (ABT). This was later confirmed by DNA molecular analyses. This was the first record in the continental U.S. A PEST ALERT describing this pest was published by FDACS-DPI (Soto-Adames, 2020).

Female ABT are large (nearly 2 mm long), black thrips with two white bands on their wings. Antennae are black except for a white, 3rd antennal segment. Light-colored forelegs are also characteristic of *Megalurothrips*. Males are smaller than females (1 mm long), slender, and are lighter in color. Male color patterns are the same as females', but less distinct. Young larvae are pale yellow like other thrips species, but later instar larvae are orange to red in color (Soto-Adames 2020).

During the 2019–2020 production season, snapbean growers in Miami-Dade County reported losses of 30%. This amounts to a reduction of \$1,620 per acre, resulting in net losses. By the end of the 2019–2020 growing season, ABT were also confirmed in Palm Beach and Collier Counties. The potential negative impact of the ABT is great. If 30% losses were realized across South Florida snapbean production, this could amount to \$35.3 million dollars of losses to Florida growers.

### Response

The University of Florida, Institute of of Food and Agricultural Sciences (UF/IFAS) Extension initiated a collaborative effort between growers, scouts, state and private taxonomists, university researchers, and university extension specialists. The goal was to provide timely information to assist stakeholders in identifying ABT, understanding population dynamics, and implementing effective management practices.

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During the 2020–2021 growing season, a survey effort began to document ABT population dynamics in Palm Beach, Martin, Hendry, Glades, and Collier Counties (subsequently referred to as the “survey region”). Up to 57 locations on 12 farms were monitored weekly by an UF/IFAS extension agent. Information was compiled along with weekly data from four professional scouts. Weekly updates were distributed to a listserv of 1560 subscribers that conveyed population levels throughout the scouting area. UF/IFAS research faculty began pesticide efficacy trials, both in the lab and in the field. The information gathered from the survey and trials were posted on the ABT website <<https://mailchi.mp/da4e89b0186f/south-florida-growers-meeting-asian-bean-thrips>>. Weekly reports were often re-distributed as Specialty Crop Industry <<https://specialtycropindustry.com>> news articles.

## Results

Survey results during the 2020–2021 growing season indicated ABT were first identified in the survey region at the end of Oct. 2020 and became widespread by early Dec. 2020. Population levels spiked in Feb. 2021 and losses were reported in some locations. With a year of surveying and a better understanding of pest thresholds and population dynamics, data are now available for year-by-year comparison in subsequent seasons. Insecticide applications are not needed during the first cropping cycle (September through December) with the exception of Homestead. This scouting effort has enabled producers to eliminate 1–2 preventative insecticide sprays during the first crop cycle, potentially saving up to \$1.70 million (\$60/acre × 2 sprays × 14,200 acres in scouting region).

Through weekly scouting, preliminary management recommendations have also been developed. Weed hosts and pseudo-hosts of ABT were identified. Wild cowpea (*Vigna luteola*), phasey bean (*Macroptilium lathyroides*), and multiple crotalaria species have all been reported as having ABT adults and larvae on blooms. Wild cowpea appears to be a primary alternative host which has been reported from ditch banks on most snapbean farms. Locations where ABT populations reached crop damaging levels all had large areas of wild cowpea nearby. The reproductive ability of ABT has not yet been evaluated on these weeds.

On 9 Mar. 2021, a population gradient of ABT was identified on a 500-acre planting of snapbeans in Hendry County. Data were collected to correlate ABT counts per bloom to percent damage on pods caused by thrips feeding. Scouting at bloom stage at six locations across the planting was done on two different dates (9 Mar. and 12 Mar.). Ten blooms were sampled at each location for each sample date. Harvest occurred on 26 Mar. 2021, with

10 plants were sampled; all beans over 3.5 inches were counted and graded. Grading consisted of marketable, unmarketable due to thrips, and unmarketable due to other reasons. Beans were considered marketable if thrips injury was found only on the bloom tip of a pod and overall damage was < 0.25 inches long. It was included in the other category if the damage was 0.25–0.5” long. If the damage was ≥ 0.5”, the bean pod was considered unmarketable. Commercial harvest occurred on 26 Mar. 2021, although yield data are not available. Results are summarized in Table 1. Examples of ABT damage on pods can be seen in Fig. 1.

Damage increased as the ABT population increased. At high populations, total yield was reduced, confirming reports of flower abortion from growers and scouts in Homestead. No damage was seen on bean pods with populations of 0.4 ABT adult per bloom; and low damage identified at 1.4 ABT adult per bloom may be tolerable. If pod damage is identified using stricter standards than are described above, slightly more damage would be included in the “other” category. More data are needed to better correlate ABT populations and damage at lower population levels to begin developing an economic threshold. Edge effects were also apparent and should be considered when developing thresholds.

Laboratory insecticide efficacy trials were done using eleven pesticides with ten different active ingredients. Adult ABT were collected from 13 different locations and evaluated separately. For each population, 10 ABT were placed in a petri dish and fed bean seedlings that had been treated with the maximum labeled rate of the respective insecticide. Survival was recorded after 72 hours. Methomyl (Lannate®), spinetoram (Radiant®), acetamiprid (Assail), and cyantraniliprole (Exirel®) were the most effective against adults under laboratory conditions. Abamectin (Agri-Mek) was effective, although was only included in a few bioassays. Pyrethroids were not effective and are not recommended for management of ABT. There was greater variation in the larval study, with spirotetramat (Movento®) and novaluron (Rimon) causing the highest mortality. These results are consistent with anecdotal reports from scout and grower field experience.

A field trial was conducted in Palm Beach County to evaluate insecticide efficacy. It was initiated at early bloom stage, when ADT adult numbers were still very low. Four insecticides with five active ingredients were evaluated along with a control. Treatments were applied twice, 7 days apart. Adult numbers in insecticide treated plots were not different from non-treated plots, perhaps due to the very low initial population numbers. Trends indicated that pyrethroids had a negative effect, with higher average ABT detected after treatment than in the control. For larvae control, there was a trend toward lower numbers in spinetoram- and

Table 1. Scouting and damage data from Asian bean thrips across various population levels.

Location	Asian bean thrips adult/bloom (no.)	Yield (g)				Total (g)	Loss (%) <sup>2</sup>	Grower harvested	Other notes
		Marketable pods (g)	Non-marketable pods (g)						
			Due to thrips feeding	Other reasons					
SE	0.43	610	0	4.5	614.5	0.00%	yes	slightly younger planting	
NE	1.4	689	58	230	977	5.90%	yes		
NC	2.2	740	175.5	103.5	1019	17.20%	yes <sup>3</sup>		
NW	4.4	453	327	205	985	33.20%	no		
SC	7.15	135	132.5	110.5	378	35.10%	no	slightly younger planting	
SW	12.55	86	330.5	136	552.5	59.80%	no		

<sup>2</sup>Based on non-marketable pod weight due thrips feeding.

<sup>3</sup>Skipped 40 ft edge effect.





Fig. 1. Asian bean thrips feeding damage to snapbean pods (Photo: Craig Frey).

methomyl-treated plots, but total populations were quite low and variability was high.

### Discussion

There were both opportunities and challenges in developing a collaborative scouting network. Extension agents have vehicles and county operating budgets, and are themselves labor to enable fast responses to new pest and disease situations. Scout and grower collaborations are essential because they greatly expand the area being monitored and substantially increase the amount of information an extension agent can provide to the industry. It is critical for county extension agents to partner with UF/IFAS research specialists to conduct trials to identify potential management strategies. The grower-extension-researcher collaboration fosters greater dialogue regarding the questions that need to be answered and help ensure research is applicable to the different growing systems around the state.

A collaboration is challenging as well. Research is not free and the time lag for writing and obtaining grants is often too slow for the agricultural community, which needs immediate answers to solve urgent problems. Extension agents have the potential to assist by engaging in practical research. Effective researchers are able to leverage similar research projects and industry support to provide timely management recommendations. Additionally,

scout and grower partners are busy with full time jobs and regular contributions require additional work. Sometimes it is difficult to track down data to keep information up to date. In order to increase the efficacy of the work, it is important to combine scouting and research efforts with grower data, and this is often a challenge.

### Conclusion

While grower trust takes time to develop, stakeholder involvement multiplies extension efforts and ensures grower cooperation in research initiatives. Season-long surveying efforts enable year-by-year comparisons that reduce unneeded pesticide applications, increasing grower savings. Research efforts closely associated with extension efforts have provided clear recommendations to manage new pests. They also identify specific active ingredients that can make a situation worse. Extension and research faculty relationships have been critical for the success of this rapid-response initiative, and will be important for the success of similar initiatives in the future.

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## Evaluation of the Potential of Biocontrol Agent *Catolaccus hunteri*, a Hymenopteran Parasitoid in Reducing Pepper Weevil (*Anthonomus eugenii*) Population in Greenhouse Conditions

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**Additional index words.** fallen flowers, fruits and buds, greenhouse, insect cage, larvae, parasitoid, pepper plant, pepper weevil

Pepper *Capsicum annum* L. is an essential crop in the United States. The key pest of pepper is the pepper weevil (PW), *Anthonomus eugenii* Cano (Coleoptera: Curculionidae). It is distributed in tropical and sub-tropical parts of the United States. Adult females oviposit on flowers, buds, and fruits. Larvae cause significant damage by feeding inside the fruit, thus rendering the produce unmarketable. Farmers use insecticides to kill the adults, they are ineffective on the hidden immature stages that cause significant damage. There is a need to develop a sustainable approach that can reduce the damage inflicted by PW larvae. Developing a sustainable approach includes the use of biocontrol as a component for integrated management program. *Catolaccus hunteri*, has been found as a potential biocontrol agent of pepper weevil. However, the potential control offered by the parasitoid needs to be evaluated. In this study we evaluated the potential of *Catolaccus hunteri* to control pepper weevil. An experiment was performed in cages in a glasshouse with three pots of jalapeño plants and a pair of pepper weevil adults in each cage. Treatment includes the release of three levels of *C. hunteri* adult densities 10, 20, 40 and a control without *C. hunteri*. The effectiveness of *C. hunteri* adults in suppressing pepper weevil was evaluated by counting the total number of infested flowers, fruits and buds on each plant at weekly intervals for twelve weeks. Infestation was confirmed by dissecting each plant part and observing them under a binocular microscope at 20×. The results indicated that the infestation rate was significantly lower in the treatment where 40 *C. hunteri* were released at weekly interval as compared to the control. Future studies are needed to incorporate *C. hunteri* as a component to develop sustainable management system for pepper weevil.

*Catolaccus hunteri* Crawford is an ecto-parasitoid of coleopteran larva, particularly of Bruchidae and Curculionidae. It is the most common parasitoid of pepper weevil (PW), *Anthonomus eugenii* Cano, in the United States and Mexico (Seal et al., 2002). The first record of *C. hunteri* as a parasitoid of PW was in Baja California Sur, Mexico, in 1995 (Aguilar and Servin, 2000). Records indicate that *C. hunteri* was also found in Puerto Rico as a parasitoid of PW. Females of *C. hunteri* can lay  $466.35 \pm 28.39$  eggs, out of which only 18.95% reach the adult stage (Rodríguez-Leyva et al., 2000). A couple of studies have demonstrated the potential of *C. hunteri* for management of PW. About 33.4% of *C. hunteri* was recovered from PW-infested nightshade berries. Schuster (2007) showed that the release of *C. hunteri* on bell pepper results in lower infestation than in pepper with no parasitoid. However, *C. hunteri* was not recovered from fruit larger than 2.5 cm (Riley & Schuster, 1992). Releasing adults in the off-season on nightshade and following with a second release in the main pepper season helps suppress larvae. Gómez-Domínguez et al. (2012) found that *C. hunteri* oviposits mainly on third instar larvae of PW, which makes some authors think it is ineffective. Monitor-

ing this parasitoid in the field can be done using the same yellow sticky traps as are used for monitoring PW (Schuster, 2012). In Mexico, 13 species of parasitoid were reported to attack PW. The main species is the ecto-parasitoid, *C. hunteri*, on the third larval stage of PW. The larva develops on the host surface, and after two weeks, the adult emerges (Vásquez et al., 2005; Chabaane et al., 2021). *C. hunteri* feeds on all larval stages of PW but only parasitizes the third instar larva. The first and second larval instars of PW have been found to be more vulnerable to the attack of *C. hunteri* as they are nearer the pericarp. As the larva develops, it moves toward the placenta and seeds. The ovipositor length of *C. hunteri* is  $1.91 \pm 0.71$  cm, and it does not reach the third instar if it is deeper. Mortality caused by the parasitoid is 39% (Riley et al., 1992 and Rodríguez-Leyva et al., 2007). *C. hunteri* feeds on the first and second instar larvae of pepper weevil and parasitizes the third instar (Murillo-Hernández et al., 2019). In a survey of the abundance of this PW parasitoid in Mexico, *C. hunteri* was found at every site (Rodríguez-Leyva et al., 2007). *Triaspis eugenii* Wharton and Lopez-Martinez (Hymenoptera-Braconidae) and *C. hunteri* Crawford (Hymenoptera-Pteromalidae) have been evaluated against PW infestations. *T. eugenii* attacks the egg stage of pepper weevil but fails to establish in the field. *C. hunteri* is native to Florida, where it is the species found most often attacking PW (Rodríguez-Leyva, 2007; Adesso et al., 2014). Seal et al. (2002) found that the optimum temperature for rearing *C. hunteri* is 25 °C. The greatest number of progeny was found when the

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parasitoid was reared on *C. maculatus*. In the present study, we determined the potential of *C. hunteri* to suppress pepper weevil.

## Materials and Methods

**STUDY LOCATION.** Studies were conducted in a greenhouse located at Tropical Research and Education Center (TREC) in Homestead, FL.

**GREENHOUSE PREPARATION.** The roof of a 10.4 × 18.3 m<sup>2</sup> greenhouse was covered with a black, UV-resistant cloth netting which reduced the amount of sunlight coming into the greenhouse.

The plants used in the experiment were Jalapeño pepper (*C. annuum* var. PS 11435810). Styrofoam trays were used to plant the seeds of the pepper. Promix® potting medium, (Premier Tech, Quebec, Canada) was used to fill the trays. In each cell, two seeds were placed by making a shallow hole with the fingertip which was covered with soil. The trays were watered every day at 10 am to enhance germination and prevent desiccation. Three weeks after germination, the seedlings were fertilized with 20:20:20 (N: P: K) Peters water-soluble fertilizer (Scotts Co., Marysville, OH) at the rate of 1.198 kg/m<sup>3</sup> gallons of water. Each seedling was drenched with 30 mL of 20:20:20 solution. Six weeks after sowing, the seedlings were transplanted. The six-week-old seedlings were transplanted into 3.78-L plastic pots. The same potting was used. Plants were manually irrigated everyday with 0.059 L of tap water. All transplants were fertilized with 20:20:20 liquid fertilizer once each week. Granular fertilizer 6:12:12 (N: P: K) 896.6 kg/ha (Diamond-R Fertilizer, Fort Pierce, Fla.) was applied every two weeks. To protect the plants from fungal disease, chlorothalonil (1725 mL/ha, Bravo Weather Stik® (Syngenta crop protection LLC, Greensboro, NC) was applied at weekly intervals. The experiment was started forty five days after transplanting.

**PLOT DESIGN AND DATA COLLECTION.** Black plastic greenhouse benches (2.4m long × 0.91m wide × 60cm high) were used. The experimental design was a randomized complete-block design with four treatments and three replications per treatment. Each cage was of 45.72 cm × 45.72 cm × 91.44 cm size (Bioquip Products Inc., Compton, CA) and contained three jalapeño plants. The cages were placed on the bench in a row, spaced 1.83 m within the row and 0.91 m between rows on an adjacent bench.

The four treatments used in the present study were based on varying the number of *C. hunteri* released each time. The treatments included releasing of 10, 20, or 40 adults of *C. hunteri* (72 hrs old) in each cage plus a control without any adult release. In each cage, a pair (♂ × ♀) of pepper weevil (48-h-old) was released after placing the plants inside the cage. Adults of *C. hunteri* were released at weekly intervals in each cage according to the treatments listed above. Each cage was provided with a vial (30 drums) of 10% sugar solution as alternate nutrition source. The sugar solution was replaced in the cage every third day. Pepper plants were replaced by a new set (n = 3) of non-infested plants

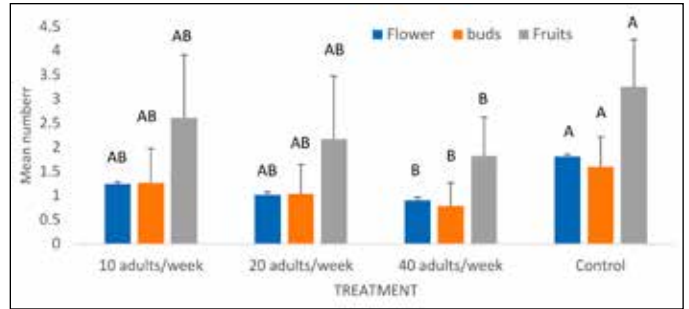


Fig. 1. Mean  $\pm$ SE number of abscised flowers, fruits, and buds of pepper due to infestation of pepper weevil in different treatment plots in a greenhouse. Means within the same color followed by the different uppercase letter are significantly different at  $P \leq 0.05$  according to Tukey's HSD test.

having flowers, buds, and young fruit in each cage three times at monthly intervals. Two pairs (♂ × ♀) of PW adults (48-h-old) were kept constant in each cage and released again if one died.

Treatments were evaluated by collecting all abscised flowers, fruit, and buds from each treatment at weekly intervals for 12 weeks. Samples of abscised flowers, fruits and buds from each treatment plot were placed in a Ziplock bag and were taken to the laboratory to check for the signs of parasitization by *C. hunteri*. Abscised flowers and buds were counted, cut open, and observed under a binocular microscope. The number of PW eggs and larvae, and parasitoid eggs and larvae were recorded. Any presence of PW and plus the parasitoid was considered as parasitization. When discarding old plants, all flowers, buds, and fruit were collected and checked for *C. hunteri* parasitisation.

**STATISTICAL ANALYSIS.** Data were analyzed using SAS statistical software (SAS institute, Cary, NC, USA). Square-root transformation was used to normalize the data before analysis. Non-transformed means are in the tables. Transformed data were further analysed using mix model ANOVA using the mixed effects including treatment, sampling date and their interactions (PROC GLIMMIX model, SAS institute Inc. Cary, NC). Means were separated using Tukey's HSD (Honestly Significant Difference) test in SAS.

## Results

A significantly lower number of abscised flowers ( $F = 3.57$ ,  $df = 3,6$ ,  $P = 0.0152$ ), buds ( $F = 3.04$ ,  $df = 3,6$ ,  $P = 0.03$ ) and fruit ( $F = 2.77$ ,  $df = 3,6$ ,  $P = 0.043$ ) were observed in the treatment where 40 adults of *C. hunteri* were released weekly compared with the control (Fig.1). The highest parasitisation was recorded in the treatment with 40 adults released per week (Table 1).

**MARKETABLE FRUIT NUMBER AND WEIGHT.** There was no significant difference among treatments for the number of marketable fruit (Treatment;  $F = 2.36$ ,  $df = 3,6$ ,  $P = 0.17$ ) or fruit weight

Table 1. The number of parasitoids (*Catolaccus hunteri*) in pepper fruit infested by pepper weevil collected in greenhouse on different sampling dates at TREC, Homestead, FL, in Jan. 2021.

Treatment	Sampling dates								
	15 Jan.	22 Jan.	19 Feb.	26 Feb.	12 Mar.	19 Mar.	26 Mar.	23 Apr.	30 Apr.
Control	0	0	0	0	0	0	0	0	0
10 adults/week	0	0	0	0	0	1	0	0	0
20 adults/week	0	0	0	1	1	1	0	0	0
40 adults/week	0	0	2	0	2	0	1	0	0

Table 2. The effect of the number adult *C. hunteri* parasitoid released weekly on marketable pepper fruit number and weight in a greenhouse at TREC, Homestead, FL, in Jan. 2021.

Treatment	Marketable fruit count (Mean ± SE) <sup>z</sup>	Marketable fruit wt. (Mean ± SE)
10 adults/week	16.3 ± 0.3 a	0.3 ± 0.0 a
20 adults/week	17.3 ± 1.3 a	0.4 ± 0.1a
40 adults/week	17.4 ± 0.3 a	0.4 ± 0.0 a
Control	13.0 ± 3.0 a	0.3 ± 0.2 a

<sup>z</sup>Means within the same column followed by the different letter are significantly different at  $P \leq 0.05$  according to Tukey's HSD test.

(Treatment;  $F = 0.81$ ,  $df = 3,6$ ,  $P = 0.53$ ). However, the number and weight of marketable fruit were the highest in the treatment with 40 adults released per week and the lowest in the control (Table 2).

### Discussion

In the greenhouse study, there were significant differences in the number of abscised flowers, buds, and fruit among treatments. The treatment where 40 *C. hunteri*/week were released had the lowest number of abscised flowers, fruit, and buds. This result is supported by Schuster (2007), who also observed less PW infestation when parasitoids were released on bell pepper compared to the control with no parasitoid released. However, in the present study very few *C. hunteri* larvae were observed in the infested fruit. No adult emergence of *C. hunteri* was observed in the parasitized fruit due to the fruit rotting and the death of larvae after cutting infested fruit open in the laboratory. It was reported that *C. hunteri* fed on the first and second instar larvae of PW and parasitized the third instar (Murillo-Hernández et al., 2019). Feeding by the *C. hunteri* female on first and second instar larva of PW may be the reason for fewer infested fruit. In the present study, parasitisation by the female was observed when *C. hunteri* females were provided with third instar larvae in a Petri dish in the laboratory. Lower infestation of PW in pepper indicates that *C. hunteri* can be incorporated into a PW management program to decrease the damage by larvae and help reduce the PW population. There is good potential for using *C. hunteri* in a greenhouse, but more research is needed for large scale field applications. The appropriate time to start releasing the parasitoid is when the plant is in the flowering stage.

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## Harvest Yields for Brussels Sprouts Cultivar Trials in Northeast Florida

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**Additional index words.** anthocyanins, brussels sprouts, marketable

A brussels sprout cultivar trial was conducted in Northeast Florida at the University of Florida/IFAS (UF/IFAS) Hastings Agriculture Extension Center (HAEC) in Fall 2020. Brussels sprouts are considered a specialty crop in the United States with a reported 9445 harvested acres in 2017, according to the USDA–NASS Census of Agriculture. Brussels sprouts are becoming a trendy treat in upscale restaurants. Over 95% of the harvested acreage goes to fresh market. Currently, 85% of the acreage is grown in California, but Northeast Florida may have a competitive window in the fall. Cultivar trials were planted for two consecutive seasons and marketable yields were measured according to USDA grading standards. Three green varieties ('Marte', 'Speedia', and 'Divino') were planted on 13 November 2019 in Year 1 (Y1) and on 5 Oct. 2020 in Year 2 (Y2). Transplants were planted on 40-inch rows with 16-inch interrow spacing in a randomized complete-plot design with four replications. Average marketable harvest yields (lb/acre) for all four replications in Y1 and Y2 were 4248 and 5275 lb/acre for 'Marte', 3625 and 4670 lb/acre for 'Speedia', and 2694 and 3101 for 'Divino'. The primary differences in production year include days to maturity (i.e., 127–131 in Y1 and 120 in Y2) and in-season pruning in Y1 (i.e. the tops were removed within the first 60 days of planting to allow for fuller sprout production). Three additional red varieties were added to the trial in Y2, namely 'Redarling', 'Rubine', and 'Red Ball'. The only red cultivar that produced any measurable marketable yield was 'Redarling' (2651 lb/acre), but the maturity date was later (135 days after transplanting) and the average yield was significantly lower than the three green varieties. Nutritional benefits in the form of anthocyanin concentrations were detected in 'Redarling' at 13 g/100 g (fresh weight), while none were detected in 'Divino'.

Given the current state of supply chain hindrances and increasing transportation costs, Florida growers and regulators must preserve agricultural land use for food production and economic security. Florida must continue to strive to produce high-value commodities and establish local markets to keep a competitive edge with other high productivity states. In the most recent Census of Agriculture, Florida ranked 5th in vegetable harvested acres after California, Idaho, Washington, and Wisconsin (USDA, 2017a). California is clearly the top producer of brussels sprouts with over 85% of the harvested acreage. A majority (96%) of the harvested acreage is sold on the fresh market (USDA, 2017b).

The Tri-County Agriculture Area (TCAA) in Northeast Florida consists of St. John's, Putnam, and Flagler Counties. The primary cash crops are potatoes and cabbage. Brussels sprouts are a valuable commodity that could be incorporated as 5-acre plots alongside relatively large acreage (> 100 acres) cabbage fields to diversify crop schemes and boost economic viability as a secondary cash crop. Research is currently underway to determine the most productive varieties of brussels sprouts that can be grown in the sandy soils of the TCAA. Specific objectives were to: 1) identify days to maturity for each cultivar grown in a specific season and climate; 2) measure marketable harvest yields for each cultivar; and 3) to determine economic feasibility of brussels sprout production in Northeast Florida.

### Materials and Methods

Field experiments were conducted using three consistent green varieties, namely 'Marte', 'Speedia', and 'Divino' for two consecutive growing seasons at the Hastings Agriculture Extension Center. Additional varieties including 'Dagan' (planted only in Year 1) and 'Red Ball', 'Rubino', and 'Redarling' (planted only in Year 2) were evaluated. Transplants were planted in raised beds using a mechanical transplanter on 40-inch centers with 16-inch in-row spacing, resulting in a planting density of 9803 plants per acre (A). Four replications of each cultivar were planted in a randomized complete block design; each subplot consisted of four 20-ft rows. The brussels sprouts were planted in seepage irrigation fields on 13 Nov. 2019 during Year 1 and 5 Oct. 2020 during Year 2. The nutrient scheme consisted of preplant and two side dressings totaling N–P–K (lb/A) of 200–100–200. Specifics associated with soil preparation, irrigation and management of pest, weeds and diseases followed grower standard practices. A major production variable that differed from Year 1 to Year 2 was topping the sprouts (physically removing the top of the plant to promote sprout growth) approximately 60 days after transplanting in Year 1.

The harvest date was determined for each cultivar when the majority of the sprouts on the stalk had reached marketable size. At harvest, the inner two rows in each subplot were cut and destemmed by hand and graded according to USDA Standard Grades (USDA, 2016). Marketable sprouts, (U.S. No. 1 and No. 2), were

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well-colored, firm, and not withered with a minimum diameter of 1 inch and a maximum length of 2.75 inches (USDA, 2016). Results were statistically analyzed within each year using least squares means differences followed by mean separation using Tukey's honestly significant difference (HSD) test at  $P < 0.05$ .

Postharvest evaluation of anthocyanin concentrations was conducted on two varieties of brussels sprouts as part of the Year 2 trial. Anthocyanins are natural phytochemicals, more specifically flavonoids, with a reddish purple pigment that act as antioxidants and offer health benefits. Seven individual sprouts were removed from each stalk and three sections were cut from a single red cabbage head for comparison purposes then blended ('Redarling' sprouts;  $n = 6$  samples, 'Divino' sprouts;  $n = 4$  samples, and 'Rio Grande' red cabbage;  $n = 3$ ). Ground tissue (0.3 g) was weighed into three individual extraction tubes and from each tube three replicate subsamples were measured. The total number of measurements read were 54 for 'Redarling', 36 for 'Divino', and 9 for 'Rio Grande'. Total anthocyanins were extracted and measured according to Lee et al. (2005).

### Results and Discussion

Maturity dates ranged from 127 to 131 days after transplanting (DAT) in Year 1 and 120 to 135 DAT in Year 2 (Table 1).

Marketable harvest yields for each cultivar in pounds per acre (lb/acre) are shown in Fig. 1 (Year 1) and Fig. 2 (Year 2). The graphical depiction of harvest yields incorporates eight replications since each of the two inner rows in each of the four replicated plots were harvested, graded and weighed separately. During Year 1, the yields from 'Divino' were statistically different and significantly lower than 'Dagan', 'Marte', and 'Speedia'

Table 1. Length of growing season for each cultivar by year.

Trial Year	Cultivar	Maturity (days after transplanting)
Year 1	Dagan	131
Year 1	Marte	127
Year 1	Speedia	127
Year 1	Divino	131
Year 2	Marte	120
Year 2	Speedia	120
Year 2	Divino	120
Year 2	Redarling	135

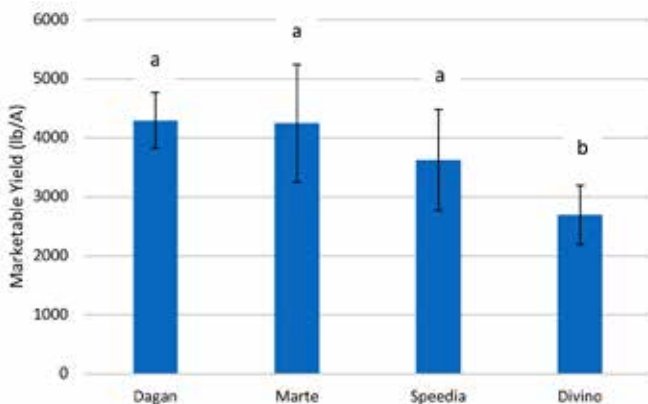


Fig. 1. Marketable Harvest Yields for Brussels Sprout Varieties Grown in Year 1 (bar graph represents averages of eight replications; error bars represent standard deviation; letters on top represent significant differences according to the Tukey test at the 0.05 confidence level)

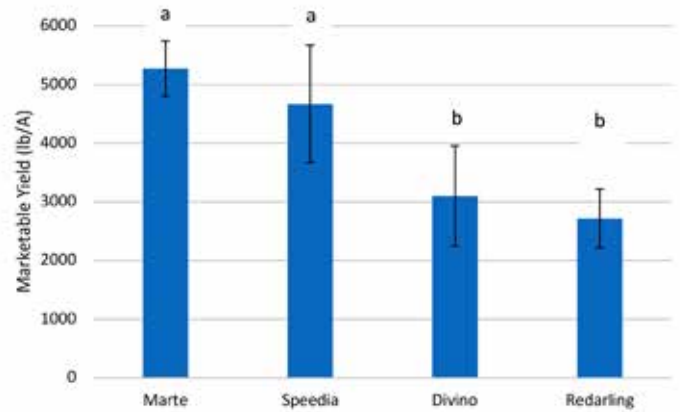


Fig. 2. Marketable Harvest Yields for Brussels Sprout Varieties Grown in Year 2 (bar graph represents averages of eight replications; error bars represent standard deviation; letters on top represent significant differences according to the Tukey test at the 0.05 confidence level)

(Fig. 1). During Year 2, yields from both 'Divino' and 'Redarling' were statistically different and significantly lower than 'Marte' and 'Speedia' (Fig. 2). No significant differences were observed within the same cultivar when comparing Year 1 and Year 2.

Year 2 of the trial involved three red cultivars, namely 'Rubine', 'Red Ball', and 'Redarling', that are unique in color and flavor. They have a reddish-purple color. In the case of 'Redarling', that color penetrates through the entire sprout (Fig. 3). 'Rubine' was bred over 30 years ago by C.N. Vreeken by crossing red cabbage with brussels sprouts, but it is not known which particular



Fig. 3. Cross-section view of Redarling brussels sprout still on the stalk, immediately following harvest.

cultivars were used as parents (Ockendon, 1977). The only red cultivar that produced marketable results was ‘Redarling’. The anthocyanin results are included in Table 2, along with concentrations detected in other fruits and vegetables for comparison purposes (Kalt, 2020). Although anthocyanins are clearly present in ‘Redarling’ sprouts, the concentrations are relatively low compared to red cabbage.

A brief discussion on economics is warranted since price point would be the major driving force for Florida growers to adopt this alternative crop as a secondary cash crop. Since California is the

Table 2. Anthocyanin concentrations of different fruits and vegetables (Kalt, 2020).

Fruit or Vegetable	Anthocyanins (mg/100g fresh)
Blackberry	245
Blueberry (highbush)	387
Blueberry (lowbush)	487
Raspberry	92
Red Plum	20
Apple (red peel)	12
Redarling Sprouts	13 ± 3 <sup>z</sup>
Rio Grande Cabbage	60 ± 11 <sup>z</sup>
Divino Sprouts	0 <sup>z</sup>

<sup>z</sup>Evaluated as part of this research trial.

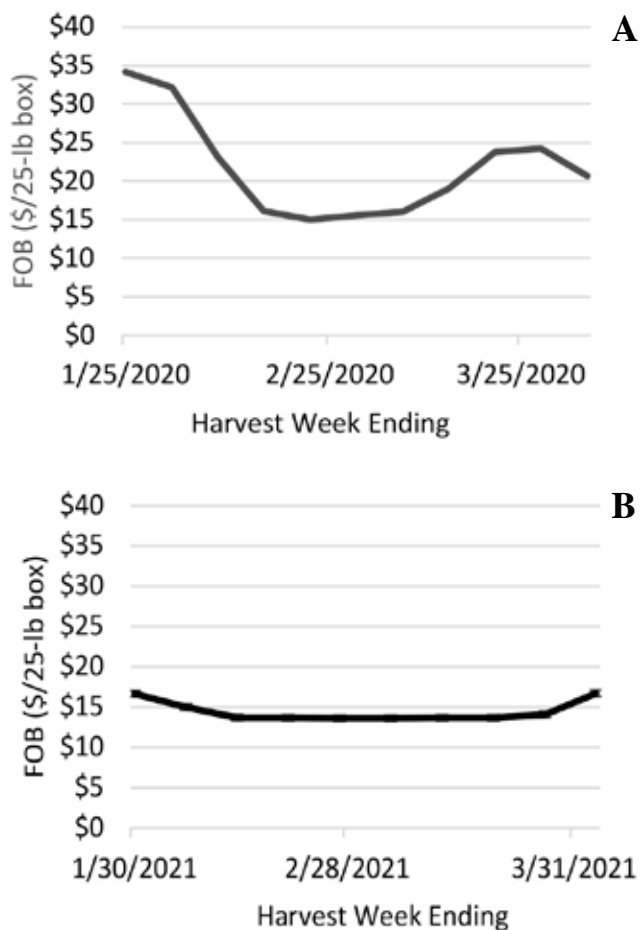


Fig. 4. Free on Board Shipping Point Prices from California for the harvest season in Year 1 (A) and Year 2 (B). Custom reports were generated by the USDA Agriculture Marketing Service <<https://www.ams.usda.gov/market-news/custom-reports>>.

major producer, the free on board shipping point (FOB) reference value was based on prices from California at the time of harvest. Figure 4 shows the FOBs generated by the USDA Agricultural Marketing Service for both production years. Marketable harvest yield and specific harvest dates for ‘Marte’ were used to estimate the crop value to the farmer for each year. The key harvest week was 21 Mar. 2020 for Year 1 when FOB prices were \$24/box and 2 Feb. 21 for Year 2 when FOB prices were \$15/box. Each box weighs 25 lb, so the value for ‘Marte’ is estimated to be \$4078/acre in Year 1 and \$3165/acre in Year 2. Although a thorough economic analysis was not part of the trial, these estimated earnings would not cover all the input costs associated with production, harvest and shipment of the produce to wholesale markets.

## Conclusion

‘Marte’ and ‘Speedia’ had consistently higher yields than ‘Divino’ in both years, and were fairly early maturing varieties (127 DAT in Year 1; 120 DAT in Year 2). Although there were some major production differences (later planting date and topping the sprouts in Year 1), no significant differences in yields were observed in Year 1 compared to Year 2. ‘Redarling’ was the only red cultivar that produced marketable yields; however, they were significantly lower than the green varieties. ‘Redarling’ had anthocyanin concentrations of 13 mg/100 g fresh, which is comparable to those measured in a red apple or a red plum. Although a thorough economic analysis was not completed as part of this trial, a rough estimation based on real-time shipping price points from California indicated that higher production yields would be necessary to generate profits from this alternative crop in Northeast Florida.

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## Pandemic Tilts Local Agriculture and Communities to Behavior Changes

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**Additional index words.** marketing changes, pandemic results, wholesale to retail

Typical agricultural distribution systems and venues temporarily ceased during the COVID-19 pandemic. Commodities were turned under in fields, dumped, or left behind for wildlife to pilfer while food banks ran out of produce. Residents did not know where to get fresh produce and wholesale producers lacked the ability to instantly shift their business model and distribution methods to meet local needs. Advisory board networks helped connect components of the food system: wholesale producers; retail outlets; and consumers to locations where produce was available. I connected volunteer gleaners with wholesale producers to gather produce for distribution to food banks around Manatee County. The Bradenton Downtown Farmer's Market started a Community Supported Agriculture (CSA) venue to accommodate wholesale producers with a retail venue. The county Geographic Information System (GIS) team and I created an interactive map of local agriculture commodities such as vegetables, fruit and vegetable crops, aquaculture, beef and dairy products, and nursery plants. The volunteer group organized over 60 gleaning events and harvested over 72,000 pounds of produce for food insecure residents. Based on the farmer's market model, two producers created CSA markets, in addition to their restaurant venue. As a result of the pandemic, four wholesale producers expanded distribution to local retail venues. The GIS map included over 100 Manatee County agriculture producers, searchable by location and commodity for markets, nurseries, aquaculture, beef and dairy, fruits, and vegetables. Key players in the food system who initially connected during the pandemic lockdown were able to provide beneficial opportunities for everyone. The GIS map has increased awareness of local agricultural production in Manatee County. Some wholesale producers adopted alternative marketing venues to rebound from pandemic deficits and prepare for future market changes. Gleaning events continue to provide local fruits and vegetables to food insecure residents, allowing them to enjoy healthy, accessible produce. Socially, many new connections and relationships have been fostered between the farming and residential communities.

Throughout Florida during the COVID-19 pandemic, a group of University of Florida, Institute of Food and Agricultural Sciences (UF/IFAS) Extension faculty and researchers interviewed small and large scale, commercial fruit and vegetable producers, farmers' market managers, community garden representatives, restaurants, and other parts of the food system. The pandemic had both negative and positive impacts on the agricultural and the residential communities. This paper discusses the local impacts and behavior changes in Manatee County.

Understanding food insecurity is relevant to the need for this project. In 2020, 10.9% or 13.8 million people in the United States faced food insecurity (USDA-ERS, 2020); In 2020, Florida's insecurity level was 10.9% or 2.38 million people (FDACS, 2021) while Manatee County's food insecurity in 2019 was 11.5% or 44,200 people (Feeding America, 2019). Food insecurity increased during the pandemic. Local volunteers worked with local producers to glean crop crops. The volunteers gleaned, transported crops that could not be sold through the "normal" distribution chain to local food banks. Food insecure residents were able to eat fresh, local fruits and vegetables.

Manatee County has roughly 59,541 acres of commercial fruit and vegetable production (USDA-NASS, 2017), producing seven of the top vegetable crops: tomatoes, cucumbers, peppers,

potatoes, green beans, sweet potatoes, and cabbage. At 14,000 acres, tomatoes are the primary crop (USDA-NASS, 2017). Most wholesale vegetables leave the county.

When the pandemic lockdown happened in Mar. 2020, the county was in full swing with fruit and vegetable production and harvesting. The shut down—from distribution points to restaurants, food service to grocery stores—meant commercial producers could not move their crops. A typical scene was of fruit and vegetables piled up with no place to go. The crops were left to rot or for wildlife to pilfer. A local dairy had to dump thousands of gallons of milk when the school district and local food services closed. The growers who sold their produce wholesale were not prepared for closures of this magnitude or to make the shift to selling small quantities on a retail basis.

Grocery stores went from full shelves to bare shelves within a two-month period. Farmer's markets shut down and food banks had no fresh produce. Residents contacted the local extension office seeking solutions.

### Materials and Methods

At the onset of the pandemic lockdown, producers were called weekly to understand and discuss the impacts of the closures. There were virtual advisory board meetings and other meetings with people associated with other parts of the food system. Three goals emerged: producers needed alternative venues to sell crops, volunteer organizations needed to be trained and connected to producers to glean produce that would go to waste otherwise, and

I would like to thank the wholesale producers involved in this project for their willingness to modify their business models during the COVID-19 pandemic to accommodate the community.

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Manatee County residents needed to become aware of locally available produce and other agricultural products.

I discussed the Community Supported Agriculture (CSA) model with the Bradenton Farmer's Market (BFM). This model had previously been used by a network of small farm producers in Manatee County. [Note: A CSA is an alternative marketing style of selling fresh produce where the consumer usually pays up front to help support the farm(s) financially through a subscription or other payment (USDA-NAL, 2021)]. A market volunteer created an online CSA model to sell local crops online through the BFM. Some growers opened produce stands at their farms for direct to consumer sales while others created their own CSAs.

A local non-profit organization trained volunteers on gleaning. The non-profit and growers who were willing to donate excess produce to local food banks were put in touch with each other. Once the gleaners finished, they would haul produce directly to the food banks. Residents needing food received fresh, local produce that had not formerly been available.

Personal interviews with residents showed that many residents did not know about all the produce grown in Manatee County. The author worked with the county's GIS team to create an online map identifying the locations of farms with available items. Extension agents created several social media campaigns to increase community awareness on produce available locally and its nutritional value.

### **Results and Discussion**

Fourteen growers sold their crops at alternative locations: two via a CSA; four to the Bradenton Farmer's Market via the CSA model; two had U-pick options; and six opened produce stands selling directly to consumers. Although the wholesale model for sales is back to normal, six of the 14 have continued their retail sales. The two producers with CSAs feel it is more successful than their traditional sales to restaurants.

The non-profit organized over 60 gleaning events in nine months, harvesting over 72,000 pounds of fruits and vegetables. During and after the lockdown, food insecurity heightened and the influx of fresh, local produce provided some relief to food insecure families. I continue to connect growers with the non-profit organization when they have surpluses. Currently the non-profit has expanded their gleaning efforts to assist homeowners with

over productive, backyard fruit trees. They will pick up the excess fruit and take it to the food banks.

The GIS map increased the community's awareness of local agricultural products. I was able to generate a list of farms, locations, crops, operating hours, and whether they were open to the public for retail sales. The interactive map <[www.tinyurl.com/manateecountyproducers](http://www.tinyurl.com/manateecountyproducers)> has a tutorial for users. It allows the user to search for local produce near where they live, by crop type (e.g. vegetables vs. fruits), or by business style (e.g. farm stand, U-pick, restaurant, wholesale, or retail, etc.) Within four months of launching the map, over 3000 visits lasting  $\geq 3$  minutes were recorded.

### **Conclusion**

Key food system players worked together during and post pandemic which helped all involved. Wholesale growers adopted alternative business models, are on the rebound, and are better equipped for the next major market change. Volunteers gleaned, and food insecure residents reaped the benefits of locally grown, fresh produce. The GIS map has escalated awareness of local agricultural products.

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## Severity of Tomato Chlorotic Spot Virus with Field Geolocations

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**Additional index words.** chlorotic spot virus, geolocation, small unmanned aerial vehicle, tomato

Tomato chlorotic spot virus (TCSV) is a major fatal diseases affecting tomato yield and quality in south Florida. This is especially true for cultivars like ‘Sanibel’ and ‘Florida 47’ that lack the gene *Sw-5*, which confers resistance to TCSV. Knowing a field’s geolocation (exact positioning) including the surrounding environment could play an important role in understanding disease incidence and severity. This paper describes the distribution of TCSV in a tomato field and its severity in relation to the other crops in the surrounding area based on ground surveys and spatial mapping conducted using a small unmanned aerial vehicle (sUAV). The tomato cultivars were ‘Sanibel’ (susceptible) and ‘Red Bounty’ (resistant) to TCSV. The tomato field was adjacent to a 20 acre 25+ year-old Bismark palm field nursery with a severe weed infestation. The results showed a clear patten of disease distribution and severity which was influenced by the grove. It is unknown whether the Bismark palm is a host of TCSV, but the weeds could be. Wind may help spread the disease by carrying thrips, the TCSV vector. Understanding whether surrounding crops that may be reservoirs of viruliferous thrips may help growers select field locations, which are relatively thrips-free. Information on TCSV resistant cultivars may help growers reduce disease pressure by planting TCSV nonhost crops or resistant cultivars.

Outbreaks of tomato (*Lycopersicon esculentum*) chlorotic spot virus (TCSV) (family *Bunyaviridae*, genus *Tospovirus*) in south Florida have caused significant yield losses. An outbreak in the growing season of 2014–2015 caused tomato yield losses as high 40% (Zhang, et al., 2019). TCSV was first reported in south Florida in 2012 (Londoño et al., 2012). It has become a major plant pathogen threatening the tomato industry in Miami-Dade County, FL (Poudel et al., 2019; Zhang et al., 2015). This disease is especially damaging in susceptible cultivars such as ‘Sanibel’ and ‘Florida 47’ (Zhang, et al., 2019).

In peri urban agricultural areas such as Miami-Dade County, vegetable fields are often small and in close proximity or adjacent to other crops such as tropical fruits, nurseries and other vegetables. Pests on one crop can affect other crops. Windy and gusty conditions can play an important role in moving insects and diseases from one field/grove/nursery to another. As described by Liu et al., 2020, the movement of thrips from TCSV reservoirs to tomato fields may be a passive event due to air currents. Since growers of different types of crops (vegetables vs. nursery vs. tropical fruit) may not know each other, there is little communication between/among them which makes pest control challenging. In a recent survey, Poudel et al (2019) observed a gradient of TCSV in a commercial tomato field, in which TCSV incidence was the greatest at the southern edge of the field and decreased as one moved away from the edge. The southern edge of this tomato field was bordered by ornamental nurseries and a row of mature palms.

To understand epidemics such as TCSV, the impact of precise geographical locations of tomato fields, and the influence of other crops, ground surveys and a small unmanned aerial vehicle (sUAV) were used at various locations around a 20-acre 25+ year-old palm nursery filled with weeds.

### Materials and Methods

To investigate the influence of a Bismark palm nursery, (*Bismarkia nobilis* Hildebr. & H. Wendl.) on the incidence of tomato chlorotic spot virus (TCSV), a ground survey of different tomato fields around the nursery was conducted over a couple of years. (Note: tomatoes are planted in the fall and harvested in the spring, hence there is a 3–5 month span which includes part of two calendar years.) The locations of these fields were: a) Field 1, west side of and across the street from the nursery in 2015–16; b) Field 2, cattycorner to the southwest in 2018–19; c) Field 3, north side and directly adjacent also in 2018–19; and d) Field 4, east and northeast in 2018-19 (Fig. 1). Fields 1–3 were planted to ‘Sanibel’ (TCSV vulnerable cultivar) and Field 4 was planted to ‘Red Bounty’ (TCSV resistant cultivar).

A spatial survey with a small Unmanned Aerial Vehicle (sUAV) was done (Wang, 2020) on tomato fields 2–4 (b–d) in 2018–19. The sUAV is a DJI Phantom 4 Pro with a high definition (HD) camera and a double 4 K multispectral sensor preinstalled (Wang, 2019) specifically designed for agriculture.



Fig. 1. Geographical distribution of tomato fields around a Bismark palm field nursery.

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## Results and Discussion

An overview of tomato Field 1 (Fig. 2, left) showed that some tomato plants in this field on west of the palm tree grove (see the top of the picture) were infected by TCSV during the fruiting stage. The initial symptom of the disease was necrotic lesions and chlorotic spots on the top of tomato plants. As the disease progressed, it caused terminal stem and leaf death, wilting, bronzing, necrosis and deformation of the leaves. Infected tomato fruits had necrotic ringspots (Fig. 2, right), and were unmarketable.

The tomato plants in the southwest corner of Field 2 were strong and healthy (Fig. 3, left) because they were away from the nursery (Fig. 3, left, top right). However, the area at northeast corner of the same tomato field closer to, but across the street from the palm nursery, was so severely infected that 60% of plants died or were rouged (Fig. 3, right).

Wang et al. (2020) described a spatial image with ‘Management Zones’ derived from sUAV data which showed a clear pattern of disease incidence. The TCSV infection started from the northeast corner of Field 3 and gradually spread to the south and southeast.

Tomato plants at the northside of the palm field nursery (Fig. 4) in Field 3 had a severe TCSV infection because the field was adjacent to the palm field nursery. Growers rouged infected plants at beginning of the infection to reduce the spread of the disease. This resulted in the removal of many plants.

The areas surveyed with the sUAV (Fig. 5, left) showed that as many as 82% of tomato plants from the area (NE corner) with the heaviest infection had died or were rouged (Fig. 5, right).

‘Red Bounty’ tomato plants to the east and northeast of the palm field nursery were healthy and uniform (Fig. 6). A closer observation showed that a couple of these plants were infected due to a breakthrough, but the yield and quality were barely affected by TCSV.

The results described above indicate that ‘Sanibel’ was vulnerable to TCSV at all the locations around the palm field nursery. ‘Red Bounty’, a resistant cultivar, was able to tolerate or resist TCSV even though the tomato field was adjacent to the palm field nursery. ‘Red Bounty’ is resistance to tomato yellow leaf curl virus (TYLCV) and fortunately, can tolerate TCSV as well



Fig. 2. Field 1: Overview of the tomato field (left); severe tomato chlorotic spot virus infection (right).



Fig. 3. Field 2: Healthy area (SW corner, left) vs. tomato chlorotic spot virus infected area (NE corner, right) of the field.



Fig. 4. Field 3: Rouging infected plants to reduce infection.



Fig. 5: A spot survey with sUAV (left) showed as many as 82% plants died (right) at the NE corner of Field 3.



Fig. 6. Field 4: Healthy ‘Red Bounty’ (tomato chlorotic spot virus resistant cultivar) plants at east(left) and northeast (right) of the palm field nursery.

(Polston et al., 2015; Kang et al., 2005; Soler et al., 2003). It has great potential to be one of cultivars that replace the susceptible cultivar ‘Sanibel’. Using ultraviolet (UV) reflective plastic mulch, may also help reduce TCSV infection (Funderburk et al., 2011; Liu et al., 2020) to some extent, but the best solution would be to use TCSV resistant cultivars carrying the *Sw-5* gene.

The effect of ornamental plants, including palms, on the incidence of TCSV infection in tomato plants was also noted in the other report. In the field adjacent to an ornamental plant nursery with a row of palms along the field edge, up to 56% of tomato plants had symptoms of TCSV, but the incidence gradually decreased to zero in the other side of the same field (Poudel et al., 2019). To date, there has been no any evidence that palms are a host of TCSV. There was a complex mixture of weeds under the palms and these may have contributed to the TCSV infection. Purslane (*Portulaca oleracea*), one of the most common weeds in the subtropics, is a known TCSV host (Raid et al., 2017). TCSV is a relatively new virus, so as time goes, more plants may eventually become hosts.

## Conclusion

The severity of tomato chlorotic spot virus was closely related to the geolocation of tomato fields around a palm field nursery. The tomato plants could be infected at all directions from the nursery. The closer to the nursery, the greater the number of plants that were infected. Thrips, especially western flower thrips (*Frankliniella occidentalis*) and common blossom thrips (*F. schultzei*) are main

vectors which transfer the virus from plant to plant (Webster et al., 2015). Wind plays an important role in moving these thrips in all different directions, causing TCSV infections in the surrounding tomato fields. To reduce the rate of TCSV infection, the geolocation of a specific tomato field should be a serious consideration when a palm field nursery or a similar type of crop which full of weeds is nearby. Selecting TCSV resistant cultivars, such as 'Red Bounty', is another way to avoid TCSV infection.

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## Incidence and Distribution of Tomato Yellow Leaf Curl Virus (TYLCV) and the Potential of TYLCV-resistant Cultivars to Manage TYLCV and Other Tomato Diseases in South Florida

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**Additional index words.** Tomato yellow leaf curl virus, disease resistance, genetic resistance, epidemiology

Tomato yellow leaf curl virus (TYLCV), transmitted by whitefly (*Bemisia tabaci*), has been one of the major limiting factors in tomato production in Florida since its introduction in 1990s. Current control of TYLCV largely relies on heavy sprays for whiteflies. Planting resistant cultivars is the best way to manage TYLCV and reduces grower expenses for pesticides. Tomato cultivars commonly planted in south Florida are resistant to the devastating tomato chlorotic spot tospovirus (TCSV) but are susceptible to TYLCV. In early March 2021, a high level of TYLCV was observed in two tomato fields with cultivar 'Southern Ripe'. The average incidence was 30% and 34% on the south side of Fields 1 and 2, respectively, but 11% on the north side of Field 2. Two field trials were conducted in Homestead, Fla. to evaluate the performance of tomato cultivars resistant to both TCSV and TYLCV. In the first trial, planted December 2020, the incidence of TYLCV in cultivar 'Red Bounty' was 29.2%, while no TYLCV was observed in cultivar 'Varsity'. No TCSV was observed on either cultivar. Total fruit yield was 46,575 and 47,475 kg/ha for 'Red Bounty' and 'Varsity', respectively. Yield of extra-large and large fruit was 40,163 and 45,900 kg/ha for 'Red Bounty' and 'Varsity', respectively. In the second trial, planted February 2021, no TCSV or TYLCV was observed on any of the six cultivars, including 'Varsity', 'Packout', '895FS', 'Vanessa', 'Shanty', and 'Katya'. Fruit yield was 27,900, 24,188, 30,713, 24,863, 26,550, and 24,975 kg/ha, respectively. Severity of bacterial spot was 7.5%, 2.7%, 5.0%, 11.7%, 12.5%, and 11.7%, respectively, at the end of the season with a rotation program of Actigard and ManKocide. This study provided south Florida growers with information about tomato cultivars with yield potential comparable to currently used cultivars and reduced disease management costs.

Florida is the leading producer of fresh market tomatoes in the United States (USDA-NASS, 2016). In Florida, tomatoes are grown almost year-round except the hot summer in south Florida and cold winter in central and north Florida. Besides the limitation of the environmental factors, diseases, including viral diseases are a major production constraint for tomato growers.

Tomato yellow leaf curl virus (TYLCV) was first found in a Florida tomato field in 1997 (Polston et al., 1999). It has become a chronic problem affecting tomato production in the entire state of Florida. TYLCV is vectored by the silverleaf whitefly (*Bemisia tabaci*). Infection of tomato plants is through feeding by a whitefly nymph or adult. Typical symptoms of TYLCV begin to show up around two weeks after infection. Infection early on can lead to severe stunting and no fruit production; infection during late stage does not have much effect on fruit yield. The first six weeks are considered the most critical period for protecting tomato plants from TYLCV infection (Smith and Nagle, 2014). Though planting TYLCV-resistant cultivars is the best and most economic strategy, these resistant cultivars have not been widely planted because of their unfavorable horticultural characters. Currently, management of TYLCV is mainly through controlling the whitefly populations by applying pesticides. In south Florida,

tomato planting usually stops in early January mainly due to the difficulties in controlling whitefly populations even with heavy pesticide use. Tomato production could be extended for at least one month if TYLCV were under control, for example, by using resistant cultivars.

Tomato spotted wilt virus (TSWV) has been affecting tomato production in the northern parts of Florida since early 2000 (Adkins et al., 2006; Momol et al., 2004). Tomato chlorotic spot tospovirus (TCSV), a virus closely related to TSWV, has recently emerged and is a devastating disease in tomato, mainly in south Florida (Londoño et al., 2012; Zhang et al., 2015). TCSV is transmitted by thrips; early infection can kill tomato plants. Because management of TCSV by controlling thrips has been unsuccessful, planting TCSV-resistant cultivars is the most effective way for growers to manage TCSV (Liu et al., 2020; Pappu et al., 2009; Zhang et al., 2019). Tomato cultivars, such as 'Sanibel' and 'FL 47', which used to be widely planted in south Florida but are susceptible to TCSV, have been replaced by TCSV-resistant cultivars, including 'Southern Ripe' and 'Red Bounty'. However, these TCSV-resistant tomato cultivars are highly susceptible to TYLCV. Thus, TYLCV has once again become the major constraint to tomato production in south Florida.

Planting tomato cultivars resistant to both TCSV and TYLCV is the most effective and economical approach in south Florida. 'Red Snapper', a cultivar resistant to both viral diseases, has been planted on a small scale in recent years. Other tomato cultivars

We would like to thank Yuanyuan Wang for help in field survey and trials.

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have not been evaluated for their resistance to both viruses and yield potential in south Florida. In this study, a recent epidemic of TYLCV was found in growers' fields. The performance of tomato cultivars resistant to both viruses were evaluated in the field conditions for their disease resistance and yield potential. Cultivars with dual resistance would provide growers with more options for disease management.

### Materials and Methods

**INCIDENCE AND DISTRIBUTION OF TYLCV IN GROWERS' TOMATO FIELD.** In early Mar. 2021, severe infections of TYLCV were found in two growers' fields in the Homestead area, Miami-Dade County, FL. The incidence and severity of TYLCV were determined. In the first field, two cultivars were planted on white-on-black plastic mulch, including a TYLCV-susceptible cultivar 'Southern Ripe' and a TYLCV-resistant cultivar 'Red Snapper'. Sampling was done on the south side of the field where infection was heavy. The number of plants with typical symptoms of TYLCV infection in the first 100 plants in a row, starting from the south end, was recorded as one sample. A total of four samples were recorded by inspecting every third row. In the second tomato field, 'Southern Ripe' (susceptible) was planted; metalized plastic mulch was used on the first 12 rows on the west side and the rest of the field had black-on-white plastic mulch. Sampling was conducted at various locations due to uneven disease incidence in the field, including the northwest corner, middle location at north side, southwest corner, and middle of the south side. In addition, incidence of TYLCV infection was recorded separately in plants on metalized and black plastic mulches in northwest and southwest corner locations. Differences in disease incidence between tomato cultivars, between locations of the field, and between plastic mulches were analyzed using Student's *t*-test in SAS (SAS version 9.4, Cary, NC).

**PERFORMANCE OF TOMATO CULTIVARS AGAINST VIRAL AND BACTERIAL DISEASES AND THEIR FRUIT YIELD.** The performance of six tomato cultivars that are resistant to both TCSV and TYLCV was evaluated in two field trials in Homestead, FL for disease resistance under field conditions in south Florida against the two viral diseases plus bacterial spot and for fruit yield. These six cultivars included 'Varsity' and 'Packout' from Syngenta, 895FS from Florida Seeds, and 'Katya', 'Shanty', and 'Vanessa' from Hazera Seeds Inc. 'Varsity' and 'Packout' are globe-shaped tomatoes, and other four cultivars are all Romas. In the first field trial, two cultivars 'Red Bounty' and 'Varsity' were transplanted 26 Dec. 2020. Plots consisted of a single 12-ft bed; the buffer zone between plots was 2.5 ft. There were 8 plants per plot. There were three replicates of each cultivar; cultivars were completely randomized. Fertilization was applied according to the recommendations of Vegetable Production Handbook of Florida (\*\*\*) please either cite this reference in the Literature Cited section or delete the reference(\*\*). Plants were sprayed for common diseases (except bacterial spot) and insects in the area. Both cultivars were inoculated around one month after transplanting with a bacterial suspension of *Xanthomonas perforans* ( $1 \times 10^8$  CFU/mL). Disease severity was rated when symptoms were fully developed. Incidence of TCSV and TYLCV were recorded weekly after the first plant showing typical symptoms. Fruit were harvested three times and graded by size. Differences between the two cultivars were analyzed using Student's *t*-test for incidence of TCSV and TYLCV, severity of bacterial spot, yield of extra-large and large sized fruit, and total fruit.

In the second field trial, six tomato cultivars were transplanted on 26 Feb. 2021. Slow-release fertilizer was applied at the base of each plant after transplanting, and 20–20–20 (one teaspoon per gallon of water) liquid fertilizer was applied weekly as a foliar spray. Common diseases and insects were managed through weekly sprays of chemicals recommended in the Vegetable Production Handbook of Florida. Bacterial spot was managed with a rotation of ManKocide and Actigard. Tomato fruit were harvested every 10 days for a total of three times. Disease incidence for TCSV and TYLCV and severity of bacterial spot were recorded after first fruit harvest. Disease severity of bacterial spot and total fruit yield were analyzed by cultivar.

### Results and Discussion

**INCIDENCE AND DISTRIBUTION OF TYLCV IN GROWERS' FIELDS.** In tomato Field 1, there was significantly less TYLCV infection on 'Red Snapper' (1.3%) than that on 'Southern Ripe' (30.0%) (Fig. 1). In tomato Field 2 where 'Southern ripe' was planted, there was significantly less TYLCV incidence on the north side of the field (12.5%) than on the south side of the field (34.5%) (Fig. 1). At the northwest corner of the second field, TYLCV incidence was 8.5% and 19.0% in plants on metalized plastic mulch and black-on-white plastic mulch, respectively (Table 1). TYLCV incidence was 7.5% in plants on black-on-white plastic mulch at the middle location on the north side. At the southwest corner of the second field, TYLCV incidence was 41.0% and

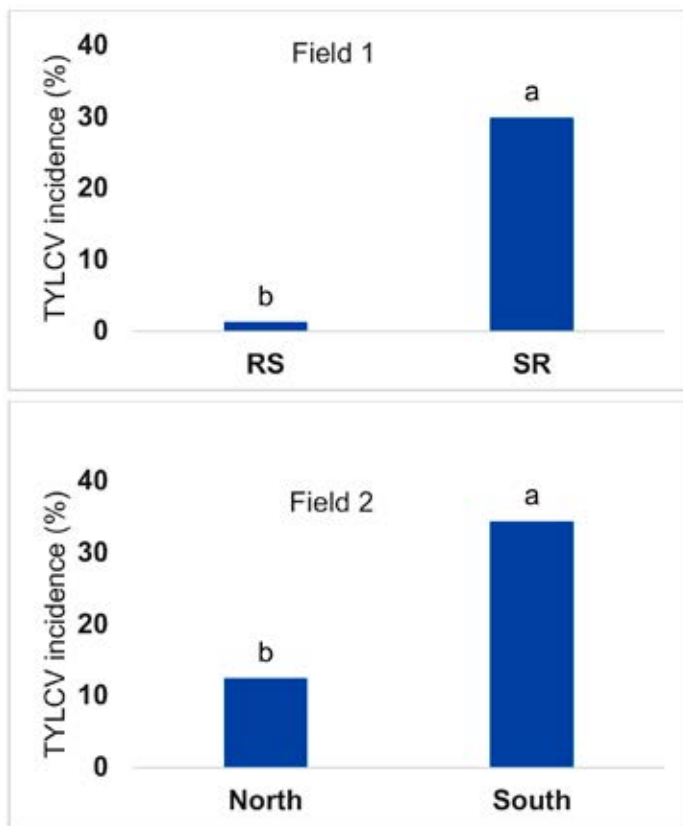


Fig. 1. Incidence of tomato yellow leaf curl virus (TYLCV) in two tomato growers' fields. In Field 1 (top), TYLCV incidence was sampled at the south side of the field for 'Red Snapper' (RS) and 'Southern Ripe' (SR). In Field 2 (bottom), TYLCV incidence in 'Southern Ripe' was sampled at both south and north sides of the field. In each figure, bars labeled with different letters were significantly different at  $P = 0.05$ .

31.5% in plants on metalized plastic mulch and black-on-white plastic mulch, respectively (Table 1). TYLCV incidence was 31.0% in plants on black-on-white plastic mulch at the middle location on the north side. ‘Red Snapper’ tomato has resistance to both TCSV and TYLCV so it was no surprise that very few plants were infected with TYLCV. In both fields, the average TYLCV incidence reached 30% in the susceptible cultivar ‘Southern Ripe’. Fruit production was greatly affected as tomato plants were mildly stunted. In Field 2, there was significantly more TYLCV on south side of the field than the north side suggesting that the prevailing wind direction may play a role in the dispersal of the whitefly vector. This has also been observed with the thrips vector of TCSV in this area (Liu et al., 2020). Metalized plastic mulch has been shown effective in reducing TYLCV incidence during the early growth stages in tomato by affecting the behavior of whitefly (Smith et al., 2007). This was seen at the northwest corner of the field in our study. However, the higher incidence of TYLCV in tomato plants on metalized

plastic mulch compared to black-on-white plastic mulch suggests that the vector population was too high for the metalized plastic mulch to repel the whiteflies effectively which precluded reducing TYLCV incidence. In addition, such disease patterns indicated that the whiteflies may be more likely to be found on the south side of the field rather than on the north (Fig. 1).

**PERFORMANCE OF TOMATO CULTIVARS AGAINST VIRAL AND BACTERIAL DISEASES AND FRUIT YIELD.** In the first field trial, TCSV was not observed on either ‘Red Bounty’ or ‘Varsity’ throughout the trial. However, 29.2% of the plants of ‘Red Bounty’ showed symptoms of TYLCV at the end of the trial, while none of the plants of ‘Varsity’ were infected (Table 2). Both ‘Red Bounty’ and ‘Varsity’ were susceptible to bacterial spot, but ‘Red bounty’ had significantly lower disease severity than ‘Varsity’. There were no significant differences between the two cultivars for total fruit or large and extra-large sized fruit. ‘Varsity’ produced relatively more fruit compared to the major local cultivar ‘Red Bounty’. In the second field trial, neither TCSV nor TYLCV was observed on any plants of the six cultivars tested (Table 3). With natural infection by *X. perforans*, disease severity of bacterial spot was 7.5%, 2.7%, 5.0%, 11.7%, 12.5%, and 11.7% on cultivars ‘Varsity’, ‘Packout’, ‘895FS’, ‘Shanty’, ‘Vanessa’, and ‘Katya’, respectively (Table 3). Total fruit yield (kg/ha) was 27,900, 24,188, 30,713, 24,863, 26,550, and 24,975 for cultivars ‘Varsity’, ‘Packout’, ‘895FS’, ‘Shanty’, ‘Vanessa’, and ‘Katya’, respectively (Table 3). These six cultivars had excellent disease resistance to TCSV and TYLCV under field conditions in south Florida, even though the tomato seedlings were planted quite late by area standards and with high whitefly populations during the growing period. All six cultivars had good fruit yield potential during this initial field trial, especially cultivar ‘895FS’. Further field evaluation need to be conducted on a relatively larger scale to confirm fruit yield potential and other horticultural characters as well.

Table 1. Incidence of tomato yellow leaf curl virus (TYLCV) in tomato ‘Southern Ripe’ at different locations of the field and on different plastic mulches

Location	Plastic mulch	
	Metalized	Black on white
Northwest	8.5 ± 0.5 <sup>z</sup>	19.0 ± 2.0
North middle	–	7.5 ± 0.5
Southwest	41.0 ± 1.0	31.5 ± 1.5
South middle	–	31.0 ± 2.0

<sup>z</sup>Average TYLCV incidence of two samples, followed by standard error of the mean.

Table 2. Disease incidence, severity, and fruit yield of two tomato cultivars in the first field trial.

Action	Cultivar	
	Red Bounty	Varsity
Incidence of TCSV (%) <sup>z</sup>	0	0
Incidence of TYLCV (%) <sup>z</sup>	29.2	0
Severity of bacterial spot (%) <sup>z,y</sup>	34.6 b	50.4 a
Yield of extra-large and large fruit (kg/ha) <sup>z,x</sup>	40163 a	45900 a
Total fruit yield (kg/ha) <sup>z,x</sup>	46575 a	47475 a

<sup>z</sup>Means of three replicates. Values followed by the same letter were not significantly different at  $P = 0.05$  according to the Fisher’s protected least significant difference value.

<sup>y</sup>Plants were artificially inoculated with *Xanthomonas perforans* ( $1 \times 10^8$  CFU/mL) around one month after transplanting.

<sup>x</sup>Yield was the total of three harvests.

TCSV = tomato chlorotic spot tospovirus; TYLCV = tomato yellow leaf curl virus

Table 3. Disease incidence, severity, and fruit yield of six tomato cultivars in the second field trial.

Performance of six tomato cultivars for disease resistance and fruit yield <sup>z</sup>				
Cultivar	Incidence of TCSV (%)	Incidence of TYLCV (%)	Severity of bacterial spot (%)	Fruit yield (kg/ha)
Varsity	0	0	7.5	27,900
Packout	0	0	2.7	24,188
895FS	0	0	5.0	30,713
Shanty	0	0	11.7	24,863
Vanessa	0	0	12.5	26,550
Katya	0	0	11.7	24,975

<sup>z</sup>Values were the average of three replicates for each cultivar.

TCSV = tomato chlorotic spot tospovirus; TYLCV = tomato yellow leaf curl virus

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## –Scientific Note–

# Determination of Potato Nitrogen Status Using a Handheld SPAD Device

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**Additional index words.** nitrogen management, petiole sap testing, potato production

The potential for handheld chlorophyll leaf-greenness meters (e.g. SPAD) to estimate crop nutrient status has been studied for more than 30 years (Bullock and Anderson, 1998). Traditionally growers and agronomists have relied on plant tissue analysis for mid-crop assessments of plant nutrients such as nitrogen (N). SPAD meters can indirectly assess plant N by estimating leaf greenness and have the benefit of being fast and easy to use. SPAD measurements have been demonstrated to correlate well with rates of N application and leaf N content, but models need to be specific to crops and cultivars to be predictive (Xiong et al., 2015).

The goal of this study was to correlate SPAD measurements with fertilization rates in commercial chipping potatoes (*Solanum tuberosum*, CV. 'Atlantic'). The plants were grown under conditions as close to local production methods as possible. Plant petiole sap nitrate and SPAD readings were measured four times: at 33 days after planting (DAP), 46 DAP, 60 DAP, and 75 DAP. We measured SPAD readings with a Konica Minolta 502 Plus SPAD meter (Konica Minolta, Tokyo, Japan) and took the average of 30 SPAD readings in each plot. To measure petiole sap nitrate, we sampled 30 leaves per plot, cut equal sized pieces from the petioles and juiced them to collect a pooled petiole sap sample. The fresh sap was immediately measured with a Horiba nitrate-N cardy meter. We used JMP to model both linear and quadratic responses of SPAD and sap nitrate N to N fertilization rate.

Results from this year of data showed that both petiole sap nitrate and SPAD readings correlated well with total N rate. In modelling SPAD response, linear models provided the best fit as determined by Akaike Information Criterion (AIC) score on all but the third sampling, where the quadratic model provided the best fit. Treatment effects from the last three sampling dates were all significant, with the last two having very high coefficients of determination:  $R^2 = 0.865$  on 6 Apr. 2021 and  $0.824$  on 21 Apr. 2021. The coefficient of determination from the second sampling date was lower, with  $R^2 = 0.522$ , but still significant.

Respective equations for the 2nd, 3rd, and 4th sampling SPAD response to yield were:

$$\text{SPAD} = 43.41 + 0.0203 \cdot x,$$

$$\text{SPAD} = 43.59 + 0.0657 \cdot x - 0.0001 \cdot x^2$$

and

$$\text{SPAD} = 34.22 + 0.231 \cdot x.$$

Quadratic models provided best results in all cases when modelling sap nitrate N. N application rate produced significant differences in sap N at all sampling dates, with  $R^2$  of 0.667, 0.701, 0.712, and 0.881 at the 1st, 2nd, 3rd, and 4th samplings respectively. Equations for sap nitrate N by fertilization rate were:

$$y = 507.8 + 3.873 \cdot x - 0.0081 \cdot x^2$$

$$y = 558.5 + 6.641 \cdot x - 0.0126 \cdot x^2,$$

$$y = 182 + 6.176 \cdot x - 0.0062 \cdot x^2,$$

$$y = 47.8 + 0.5015 \cdot x - 0.0056 \cdot x^2$$

for the 1st, 2nd, 3rd, and 4th samplings, respectively.

This result is promising for the use of SPAD measurements for fertilizer management of potato. More replications are needed to confirm these data. If future data are promising, there may be an opportunity to establish SPAD-based thresholds for N fertilizer and develop more nuanced models with respect to SPAD readings and nitrogen availability. Florida potato growers could benefit significantly from a rapid and simple tool to monitor plant nitrogen status and schedule fertilizer applications.

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—Scientific Note—

## Rate and Timing of Phosphorus Application for Cabbage in Northeast Florida

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**Additional index words.** *Brassica oleraceae* var. *capitata*, liquid fertilization, phosphorus-pentoxide, supplementary fertilization

Northeast Florida growers have reported unfavorable marketable cabbage yields despite applying rates of phosphorus (P) recommended by the University of Florida, Institute of Food and Agricultural Sciences (UF/IFAS). The objective of this study was to evaluate the response of marketable cabbage yield, biomass accumulation, and P-uptake to four P-fertilizers rates (0, 56, 112 and 168 kg·ha<sup>-1</sup> P<sub>2</sub>O<sub>5</sub>) applied pre-plant (P<sub>pp</sub>) in combination with supplemental liquid P fertilizer (28 kg·ha<sup>-1</sup> P<sub>2</sub>O<sub>5</sub>) applied at pre-cupping (Sd<sub>1</sub>) and cupping (Sd<sub>2</sub>) growth stages for a total of twelve treatments. Field trials were conducted in 2018 and 2019 in Hastings, FL. In late October 2018, cabbage (cv. Bravo) seedlings were transplanted in the field, while in 2019, seedlings were transplanted in late September. Soil samples measuring soil available P (Mehlich-3) were collected before treatment application and after the cupping stage. At harvest, whole plant samples were collected for dry biomass accumulation and P uptake. Cabbage heads that weighted ≥ 0.9 kg were considered marketable.

The initial soil available P (Mehlich-3) for the 2018 season averaged 44 mg·kg<sup>-1</sup> and 56 mg·kg<sup>-1</sup> in 2019. There was no effect for the interaction among P<sub>pp</sub>, Sd<sub>1</sub>, and Sd<sub>2</sub> on cabbage biomass accumulation and P uptake. Biomass accumulation and P uptake were significantly affected by P<sub>pp</sub> application rates in the 2018 and 2019 seasons (Table 1). In both years, cabbage dry biomass accumulation for the 0 kg·ha<sup>-1</sup> P<sub>2</sub>O<sub>5</sub> at P<sub>pp</sub> were significantly lower than the accumulated biomass at 56, 112 and 168 kg·ha<sup>-1</sup> P<sub>2</sub>O<sub>5</sub>. There were no significant differences among P treatments > 0 kg·ha<sup>-1</sup>. There was an increase of 0.9 Mg·ha<sup>-1</sup> dry biomass when P<sub>pp</sub> fertilizer was applied. In 2018 and 2019, plant P uptake tended to increase with increasing P<sub>pp</sub> rates. In 2018, there were no significant differences in P uptake for 0 and 56 kg·ha<sup>-1</sup> P<sub>2</sub>O<sub>5</sub> (13.9 and 16.2 kg·ha<sup>-1</sup> of P, respectively), but these were significantly lower than P uptake reached for 112 and 168 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> (19.2 and 21.4 kg·ha<sup>-1</sup> P<sub>2</sub>O<sub>5</sub>, respectively). In

Table 1. Main effect of rate and timing of phosphorous (P-fertilizer application on cabbage biomass accumulation and plant P uptake cultivated in Hastings, FL in 2018 and 2019.

P-fertilizer rate (P <sub>2</sub> O <sub>5</sub> )	Biomass accumulation (Mg·ha <sup>-1</sup> )		P uptake (kg·ha <sup>-1</sup> )	
	2018	2019	2018	2019
P pre-plant (kg·ha <sup>-1</sup> )				
0	3.77 b <sup>z</sup>	3.76 b	13.93 b	9.79 c
56	4.46 a	4.53 a	16.27 b	13.90 b
112	4.80 a	4.80 a	19.27 a	15.66 ab
168	4.79 a	4.76 a	21.14 a	17.15 a
P side-dress (kg·ha <sup>-1</sup> )				
0	4.35	4.50	16.63	13.13
28 Pre-cupping	4.61	4.61	18.02	14.47
28 Cupping	4.40	4.27	18.30	14.78

<sup>z</sup>Mean values within a column followed by same letters are not significantly different at  $P < 0.05$  according to Tukey test. Absence of a letter denotes means are nonsignificant.

2019, P uptake with 0 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> at P<sub>pp</sub> was 9.7 kg·ha<sup>-1</sup> P<sub>2</sub>O<sub>5</sub> and was significantly lower than the 13.3 kg·ha<sup>-1</sup> P for 56 kg·ha<sup>-1</sup> P<sub>2</sub>O<sub>5</sub>. There was no significant response to biomass accumulation and P uptake with the additional liquid fertilizer.

The interaction among P<sub>pp</sub>, Sd<sub>1</sub>, and Sd<sub>2</sub> was insignificant for marketable yield cabbage. The main effect of P<sub>pp</sub> in the 2018 and 2019 seasons on marketable yield was significant (Fig. 1). Marketable yields were relatively higher in 2019, compared to the 2018 season. This can be attributed to different transplanting dates among seasons. Daily air temperatures were warmer on average for cabbage planted in 2019 compared to those for the late October planting. Marketable yield in both seasons increased with increasing rates of P<sub>pp</sub>. In 2018, the marketable yield obtained with no P<sub>pp</sub> applied was 15.9 Mg·ha<sup>-1</sup> and was significantly lower than the other P<sub>pp</sub> rates. 168 kg·ha<sup>-1</sup> P<sub>2</sub>O<sub>5</sub> resulted in a significantly higher marketable yield than 56 kg·ha<sup>-1</sup> P<sub>2</sub>O<sub>5</sub> but

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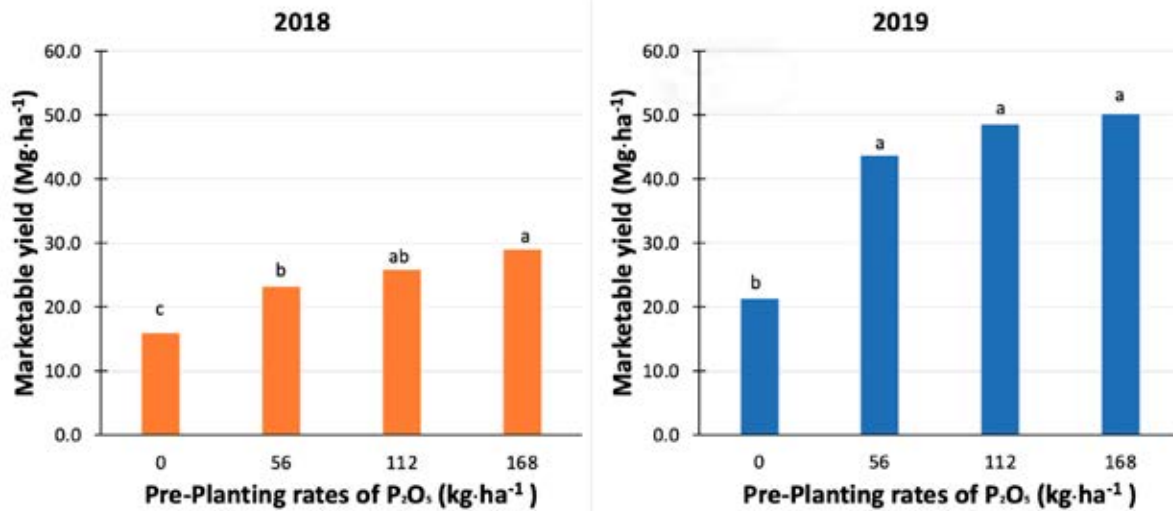


Fig. 1. Main effect of pre-planting ( $P_{pp}$ ) application rates on cabbage marketable yield cultivated in Hastings, FL in 2018 and 2019.

not significantly different than 112 kg·ha<sup>-1</sup> P<sub>2</sub>O<sub>5</sub>. A similar trend of yield response occurred during 2019, but with no significant differences among marketable yield with the application rates of 56, 112, and 168 kg·ha<sup>-1</sup> P<sub>2</sub>O<sub>5</sub>. However, they were only different to marketable yield when no P was added to  $P_{pp}$ . In-season liquid fertilizer application effect to cabbage marketability was not significant in both seasons.

The current UF/IFAS recommendation for P-fertilizer application for soils with 44 and 56 mg·kg<sup>-1</sup> (Mehlich-3) P content are 112 and 0 kg·ha<sup>-1</sup> P<sub>2</sub>O<sub>5</sub>, respectively. Therefore, marketable yield increase should have been expected during the 2018

season with added P-fertilizer, while no response should have been expected to additional P application for the 2019 season. However, cabbage biomass accumulation, P uptake, and marketable yield responded to P-fertilizer rates at  $P_{pp}$  in soils with medium and high P availability. These results suggest that the current recommendations for P-fertilization for cabbage may need further adjustments based on field experiments. Multi-year P-fertilizer calibration studies carried out in agricultural land with low, medium, and high P content are needed. Pre-plant P fertilizer treatments must include at least 6 rates of application to conduct regression analysis.



## —Scientific Note—

# Assessing In-season Soil and Potato Plant Nitrogen Status using Integrated Approaches

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**Additional index words.** leaf chlorophyll content, normalized difference vegetation index, *Solanum tuberosum*

A potato crop requires considerable amounts of nitrogen (N). Proper timing of application can increase its nitrogen use efficiency (NUE) by potato roots. Early applications of N-fertilizer at pre-planting weeks before potato planting have been shown to make a very limited contribution to plant nutrients due to the risk of N leaching. The ideal window for N-fertilizer application is between planting and tuber initiation (0–50 days after planting). Thus, the assessment of soil and plant N status early in the crop season can provide growers with reliable information on the necessity of supplemental N-fertilization. The assessment of soil N availability throughout the season can be achieved using traditional soil test lab (STL) or soil nitrate quick test (SNQT), while plant N status can be determined by traditional plant N content (PNC), non-destructive measurement of leaf chlorophyll concentrations using the chlorophyll-meter (SPAD), and a normalized difference vegetation index (NDVI) sensor.

The study objective was to determine optimum N fertilizer rates and application timing using in-season measurements of soil and plant N to maximize yield and plant growth under seepage and subirrigation with drain tile (SDT) systems. The study was conducted in two commercial farms located in Hastings, FL. Three N-fertilizer rates (200, 224, and 260 lb/ac) were tested under seepage and drain-tile irrigation in two commercial farms in spring 2020. Leaf greenness and NDVI were measured throughout the potato season. Soil samples were collected and analyzed for nitrate (NO<sub>3</sub><sup>-</sup>) and ammonium (NH<sub>4</sub><sup>+</sup>) content in the soil at 0–15 cm soil depth and submitted to the soil testing lab. A subsample was analyzed using the soil nitrate quick test. Data analysis was done using R software (R Core Team, 2020). Pearson correlation was used to determine the relationship between

tuber yield and in-season measurements taken for soil and plant N status. The spectral properties recorded for leaf chlorophyll and NDVI measurements ranged from 42.4 to 57.9 and 0.60–0.93, respectively. Neither leaf chlorophyll nor NDVI were well correlated with plant N content and total yield. The Normalized Difference Vegetation Index was significantly correlated with soil mineral N determined using the soil test lab ( $r = 0.41$ ,  $P = 0.007$ ) and soil nitrate quick test ( $r = 0.334$ ,  $P = 0.331$ ). Soil mineral N determined using the soil nitrate quick test was well correlated with the soil test lab ( $r = 0.84$ ). Total yield ranged from 298–312 and 304–323 cwt/ac for Farms 1 and 2, respectively. There were no significant differences for tuber yield response between N-fertilizer rates within each irrigation system. Plant N content correlated with the soil test lab ( $r = 0.395$ ,  $P = 0.01$ ) and the soil nitrate quick test ( $r = 0.377$ ,  $P = 0.014$ ). Neither leaf chlorophyll SPAD nor NDVI results could be used successfully to identify the early needs for in-season N-fertilizer application with the N-fertilizer level applied in this study. In contrast, due to the high correlation with the soil test lab, the soil nitrate quick test seemed to be the most reliable tool to help growers with the in-season decision for N-fertilizer application. A second season of data collection is needed to confirm the reliability of early in-season strategies to assess soil and plant N to make N-fertilizer recommendations for potatoes.

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—Scientific Note—

## Pre-planting and Supplementary In-season Liquid Phosphorus Fertilization for Potato in Florida

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**Additional index words.** phosphoric acid, phosphorus-pentoxide, *Solanum tuberosum*

Potato tuber yield responds to soils with high P content and have responded to phosphorus (P) fertilizer application, despite applications not being recommended. The objectives of this field experiment were to evaluate the effect of four P-fertilizer rates (0, 56, 112, and 168 kg·ha<sup>-1</sup> P<sub>2</sub>O<sub>5</sub>) applied pre-plant (P<sub>pp</sub>), combined with in-season liquid P-fertilizer applications of 0 and 28 kg·ha<sup>-1</sup> P<sub>2</sub>O<sub>5</sub> (phosphoric acid) applied at emergence (P<sub>eme</sub>) and at tuber initiation (P<sub>ini</sub>). The combination of P rates and application timing resulted in a total of sixteen P-fertilizer application treatments. Chipping potato ‘Atlantic’ was planted in January 2019 and 2020 in Hastings, FL. Soil samples were collected at 0–15, 15–30, and 30–45 cm soil depth before treatment applications and after tuber initiation for soil available P (Mehlich-3) and pH. Petiole samples were collected on a weekly basis from 35 to 75 days after planting for P concentration assessment. Before plant senescence, aboveground and tuber tissues were collected for total dry biomass accumulation and total P uptake. Total and marketable yield (tubers diameter >4.8 cm) were measured.

Initial soil available P was 128–151 and 137–160 mg·kg<sup>-1</sup> in 2019 and 2020, respectively. In both years, there were no significant interactions among P<sub>pp</sub>, P<sub>eme</sub>, and P<sub>ini</sub> for dry biomass accumulation, P uptake, total and marketable yield. Dry biomass accumulation and P uptake responded to P<sub>pp</sub> in both seasons. In 2019, dry biomass accumulated with 168 kg·ha<sup>-1</sup> P<sub>2</sub>O<sub>5</sub> was 11.2 Mg·ha<sup>-1</sup>, which was 1.1 Mg·ha<sup>-1</sup> greater than with 56 kg·ha<sup>-1</sup> P<sub>2</sub>O<sub>5</sub> (Table 1). In 2020, the biomass accumulated (9.10 Mg·ha<sup>-1</sup>) with zero P<sub>pp</sub> was significantly lower than the 11.38 Mg·ha<sup>-1</sup> accumulated with 112 kg·ha<sup>-1</sup> P<sub>2</sub>O<sub>5</sub>. For both seasons, the total plant P uptake ranged from 18.86 to 22.84 kg·ha<sup>-1</sup> P. In 2019, 168 kg·ha<sup>-1</sup> P<sub>2</sub>O<sub>5</sub> resulted in a uptake of 3.5 kg·ha<sup>-1</sup> greater than potatoes fertilized with 56 kg·ha<sup>-1</sup> P<sub>2</sub>O<sub>5</sub>. In 2020, there were no differences in P uptake among P<sub>pp</sub> rates of 56, 112 and 168 kg·ha<sup>-1</sup> P<sub>2</sub>O<sub>5</sub> with zero applied P-fertilizer, resulting in lower plant P uptake. Supplemental liquid P-fertilizer only affected dry biomass accumulation in 2019. With 28 kg·ha<sup>-1</sup> P<sub>2</sub>O<sub>5</sub> at P<sub>ini</sub>, the accumulated biomass was 10.9 Mg·ha<sup>-1</sup>, which was 540 kg·ha<sup>-1</sup> greater than the dry biomass accumulated when no liquid fertilizer at that stage.

Table 1. Effect of pre-planting (P<sub>pp</sub>), emergence (P<sub>eme</sub>), and tuber initiation (P<sub>ini</sub>) P-fertilization dry biomass and plant phosphorus (P) uptake of potato cultivated in Hastings, FL in 2019 and 2020.

P-fertilizer rate	Total biomass (Mg·ha <sup>-1</sup> )		Total uptake (kg·ha <sup>-1</sup> P)	
	2019	2020	2019	2020
<b>P<sub>pp</sub> (kg·ha<sup>-1</sup> P<sub>2</sub>O<sub>5</sub>)</b>				
0	10.53 ab <sup>y</sup>	9.1 b	20.14 ab	16.59 b
56	10.13 b	10.18 ab	19.35 b	18.86 a
112	10.66 ab	11.38 a	20.49 ab	20.85 a
168	11.23 a	9.84 ab	22.84 a	19.14 a
<b>P<sub>eme</sub> (kg·ha<sup>-1</sup> P<sub>2</sub>O<sub>5</sub>)</b>				
0	10.89 a	10.11 ns	20.95 ns	18.67 ns
28	10.39 b	10.14 ns	20.45 ns	19.05 ns
<b>P<sub>ini</sub> (kg·ha<sup>-1</sup> P<sub>2</sub>O<sub>5</sub>)</b>				
0	10.36 b	9.92 ns	19.71 ns	18.01 ns
28	10.91 a	10.33 ns	21.68 ns	19.71 ns

<sup>y</sup>Mean values followed by different lowercase letters denote significant differences at *P* < 0.05 according to Tukey test. Absence of letters denotes means are nonsignificant.

Tuber total and marketable yield responded similarly to P<sub>pp</sub> in both seasons. Overall yield in 2019 was higher in 2020 and may be due to a relatively drier season in 2020. Total yield responses to P<sub>pp</sub> are in Fig. 1. In 2019, total yield (41 Mg·ha<sup>-1</sup>) with 56 kg·ha<sup>-1</sup> P<sub>2</sub>O<sub>5</sub> was significantly lower than the 45.3 Mg·ha<sup>-1</sup> with 168 kg·ha<sup>-1</sup> P<sub>2</sub>O<sub>5</sub>. However, there were no significant differences between total yield obtained when applying 0, 112, and 168 kg·ha<sup>-1</sup> P<sub>2</sub>O<sub>5</sub>. In 2020, total yield with zero P<sub>pp</sub> was 33.3 Mg·ha<sup>-1</sup> which increased significantly by 4.1, 5.2, and 5.1 Mg·ha<sup>-1</sup> with P<sub>pp</sub> rates of 56, 112, and 168 kg·ha<sup>-1</sup> P<sub>2</sub>O<sub>5</sub>, with no significant differences with P<sub>pp</sub> rates. Total and marketable yield only responded to supplementary P fertilization at P<sub>pp</sub> in 2019 (Table 2). Total yield and marketable yield were significantly higher with 28 kg·ha<sup>-1</sup> P<sub>2</sub>O<sub>5</sub> at P<sub>ini</sub> when compared to a P<sub>pp</sub> rate of zero P.

Based on current University of Florida/IFAS interpretations of soil P content, for soils with a P content between 137–160 mg·kg<sup>-1</sup> (Mehlich-3), no (0) kg·ha<sup>-1</sup> P<sub>2</sub>O<sub>5</sub> is recommended. This means that no response to added P fertilizer should be expected.

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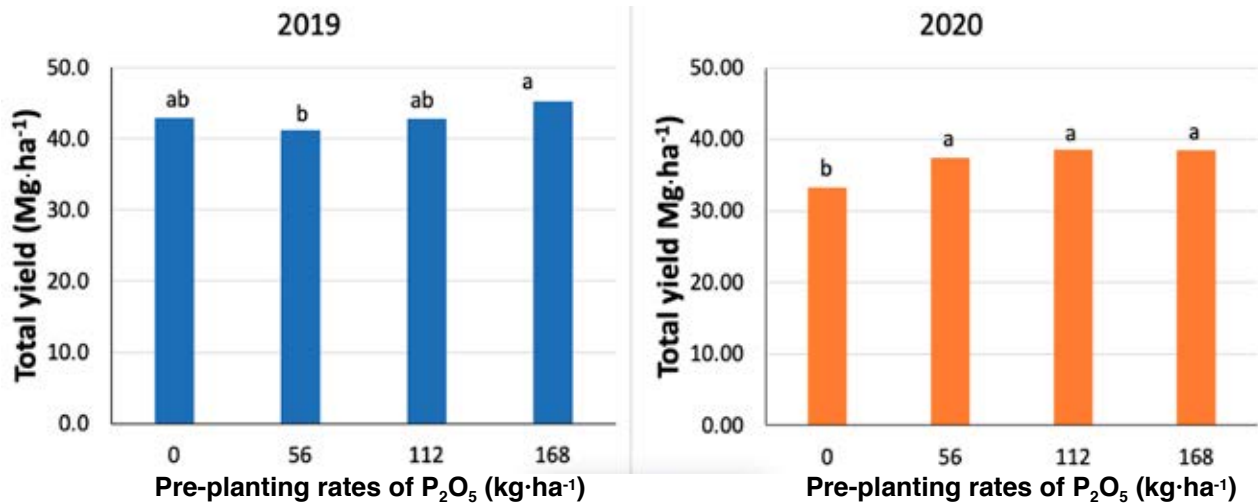


Fig. 1. Main effect of pre-planting ( $P_{pp}$ ) application rates on potato yield cultivated in Hastings, FL in 2019 and 2020. Mean values followed by different lowercase letters denote significant differences at  $P < 0.05$  according to Tukey test.

Table 2. Effect of liquid P-fertilization applied at emergence ( $P_{emc}$ ) and tuber initiation ( $P_{ini}$ ) on total and marketable yield of potato cv. Atlantic cultivated in Hastings, FL in 2019 and 2020.

P-fertilizer rate	Total yield (Mg·ha <sup>-1</sup> )		Marketable yield (Mg·ha <sup>-1</sup> )	
	2019	2020	2019	2020
$P_{emc}$ (kg·ha <sup>-1</sup> P <sub>2</sub> O <sub>5</sub> )				
0	43.7	36.5	38.3	30.7
28	42.4	37.4	37.4	31.6
$P_{ini}$ (kg·ha <sup>-1</sup> P <sub>2</sub> O <sub>5</sub> )				
0	42.0 b <sup>y</sup>	37.8	36.9 b	31.9
28	44.2 a	36.1	38.8 a	30.4

<sup>y</sup>Mean values followed by different lowercase letters denote significant differences at  $P < 0.05$  according to Tukey test. Absence of letters denotes means are nonsignificant.

The results from this study indicate that there has been an increase in both total and marketable tuber yield due to added P fertilizer applied at  $P_{pp}$ . Further investigation, based on field evaluation of tuber yield responses across the state and for multiple soils is required to improve interpretation of Mehlich-3 test result. The in-season liquid application at  $P_{ini}$  affected tuber yield, dry biomass accumulation, and P uptake in only one season. Therefore, it is likely that P availability was improved near the tuber initiation growth stage. Further verification of in-season liquid P application is required before becoming a recommended practice.



—Scientific Note—

## Nitrogen Management for Greenhouse Production of Luffa, A High Value Asian Vegetable

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**Additional index words.** *Luffa acutangula* (L.) Roxb, luffa yield, nitrogen management

Luffa (*Luffa acutangula*) is an Asian vegetable in the Cucurbitaceae family known for its nutritional and medicinal value. It is suitable for cultivation in tropical and subtropical regions including Florida (Xie et al., 2019). An experiment was designed to find the optimal nitrogen levels, in the form of calcium nitrate, for greenhouse production of luffa. Key factors for growing luffa were identified, including susceptibility to pests.

### Findings

Over a 20-week period, optimal fertilizer rates for pot-grown luffa were tested under greenhouse conditions. Luffa seeds were sown in transplant trays on 8 Mar. 2021 and watered daily. Twenty-four (24) luffa 21-day old seedlings, cultivar ‘Jiao Gua’ (Tainong Seeds, Inc.), were planted in 4-gallon containers filled with 3.5 gallons of Promix Mycorrhizae with one plant per pot. There were four nitrogen (N) rates: T1, T2, T3, and T4 – 0, 72.6, 108.9 and 145.2 lb/acre (0, 13, 20, and 27 g/plant) N as  $\text{Ca}(\text{NO}_3)_2$ , respectively. There were six replications. All plants received 122 lb/acre  $\text{P}_2\text{O}_5$  as triple superphosphate (TSP), and 120 lb/acre  $\text{K}_2\text{O}$  as potassium sulfate. The fertilizers were split applied once a week for 8 weeks from 21 Apr. thru 18 Jun. 2021. Plants were irrigated manually to field capacity daily with 2 L a day until flowering and 4 L a day after flowering. T1 and T4 received a total of 168 L/plant while T2 and T3 received a total of 276 L/plant.

Phosphorous and potassium applications were the same for all treatments, with each plant receiving a total 43.3 grams of triple superphosphate and 50 grams of potassium sulfate. Nitrogen, in the form of calcium nitrate, was treatment specific. The N treatment groups: T1, T2, T3, and T4 received 0, 13, 20, and 27 g of N per plant per season. The zero nitrogen treatment (T1) produced no fruit. T2 and T3 produced numerous fruit. The highest nitrogen treatment (T4) had tissue necrosis and wilting leaves consistent with symptoms of salt injury which may have been caused by build-up which has been reported previously in many different vegetable crops. T2 produced 17 total fruit, for an average of 2.83 fruit/plant, and a total fresh weight of 4023 g. T3 produced 18 total fruit, for an average of 3 fruit/plant, and a total fresh weight of 5168 g. During this experiment, plants were affected by spider mites and aphids and which were managed with Agrimek and

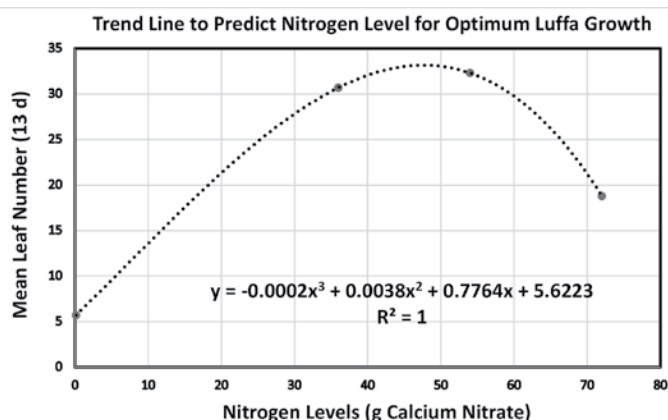


Fig. 1. Trend line to predict nitrogen level for optimum luffa growth in a greenhouse.

neem oil. We also found that the number of leaves on the luffa plant fitted a polynomial third order equation with an optimal level of 20 grams of nitrogen per plant under the conditions of this experiment (Fig. 1).

### Suggestions for Future Direction

Using the results of this study as a guide, future experiments are needed to determine the optimal levels of nutrients especially nitrogen. It will also be useful to optimize additional nutritional programs and pest management options for greenhouse production of this vegetable.

An analysis between nitrogen concentration, the amount supplied, and the number of leaves on the luffa plant fitted a polynomial third order equation with an optimal level of 20 g of nitrogen per plant for this experiment.

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—Scientific Note—

## Effects of Irrigation Rate on Evapotranspiration Rates of Three Sweet Corn Cultivars

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**Additional index words.** irrigation, evapotranspiration, sweet corn, South Florida

Florida is one of the top producers of sweet corn in the United States. The crop is grown in soils that have low water holding capacity and highly permeability. Irrigation management is crucial as the state heavily depends on shallow aquifers for both drinking water and irrigation for crop production. Developing optimal irrigation management practices is critical for optimizing sweet corn yield and improving water conservation and water quality. Water stress responses of sweet corn vary between cultivars and geographic locations. Weather variability also plays a significant role in a plant's response to water stress. This study was conducted to investigate changes in crop evapotranspiration (ET<sub>c</sub>) rates of three sweet corn cultivars ('1170', '8021', and 'Battalion') under three irrigation rates targeted to replenish 50, 75, and 100% of the soil water deficit. The consumptive water use results presented in this study are a first approximation to establish the water requirements for these three cultivars and provide insights for developing irrigation strategies that conserve water without significantly affecting crop yield.

### Materials and Methods

This study was conducted at the Tropical Research and Education Center (TREC) University of Florida, IFAS, Homestead, FL. The experiment was conducted in an open field using 3.79-L plastic containers filled with Krome gravelly loam soil. A 3 × 3 factorial experimental design was used with three irrigation rates, three cultivars, and four replications. This resulted in a total of 180 plants (3 cultivars × 3 irrigation levels × 4 replications × 5 plants per replication). Irrigation was supplied with an automated drip irrigation system with an average emitter delivery rate of 127 mL/

min and distribution uniformity of 90%. Irrigation treatments were targeted to replenish 50, 75, and 100% of the soil management allowable depletion (MAD). Fertilization included 75, 65, and 125 kg/ha N, P, and K. Daily ET<sub>c</sub> rates were measured from 36 pots by monitoring daily weight loss of each replication (pot, soil, and plant) with a digital scale. Reference evapotranspiration (ET<sub>o</sub>) was calculated using the FAO-Penman-Monteith equation based on weather and crop-specific information. For each cultivar, crop coefficient (K<sub>c</sub>) values were then calculated, for different crop growth stages, as a ratio of measured ET<sub>c</sub> and calculated ET<sub>o</sub>. Sub-hourly weather data was retrieved from a weather station of the University of Florida, IFAS Florida Automated Weather Network (FAWN) located approximately one mile from the study site <<https://fawn.ifas.ufl.edu/>>.

### Results and Discussion

Total irrigation applied under the 50, 75, and 100% treatments were 116, 162, and 216 mm, respectively. Fully irrigated plants had the highest ET<sub>c</sub>, while plants that received 50% irrigation had the lowest ET<sub>c</sub>. Consumptive water use for the 100% treatment was 387 mm, followed by 75% with 333 mm and 50% with 287 mm. Cultivar 1170 had slightly higher ET<sub>c</sub> compared to '8021' and 'Battalion'. On average, a 25% reduction in irrigation resulted in a 10% reduction in ET<sub>c</sub>. During most of the sweet corn growth stages, K<sub>c</sub> values were greater than 1 for the 75 and 100% IRRs regardless of cultivar. Peak K<sub>c</sub> values reached as high as 1.5 during the vegetative growth stage for the three cultivars. Fresh ear weight was not significantly affected by irrigation rate or cultivar. Ear weight was slightly higher, but not significantly, in the 75% irrigation treatments compared to the 100% and 50% treatments. Among the three cultivars evaluated in this study, fresh biomass weight was lower for '8021' compared to '1170' or 'Battalion' for the same irrigation level.

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—Scientific Note—

## Pedotransfer Function to Estimate Soil Penetration Resistance of the Vegetable Production Areas in Northeast Florida

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**Additional index words.** sandy soils; subirrigation; seepage irrigation; soil bulk density

Northeast Florida (NE) is responsible for 35% of the spring potato production in the United States. The development of tools to assist growers assess soil physical qualities for vegetable production is needed. Penetration resistance (PR) has been used to quantify soil physical conditions related to soil management practices. Potato root development might be limited by PR >1.5 MPa (PR<sub>critical</sub>). PR can be measured in-situ or in a laboratory using penetrometer devices readily available on the market. Correlations between PR and soil water content ( $\theta$ ) and others soil properties (e.g., soil bulk density (Bd) and soil organic matter (SOM) and texture) still need to be considered. Thus, individual PR measurements might not be sufficient to assess soil physics conditions for root growth. Alternatively, soil pedotransfer functions (PTF) have been used as a practical tool to estimate PR as a function of  $\theta$  and other soil physical properties. This study aimed to develop a PTF to evaluate PR considering  $\theta$ , Bd, SOM, and particle-size distribution < 250  $\mu\text{m}$  (PSD<sub>fine</sub>) variation across subirrigated commercial potato fields. The area of interest was located in the vegetable production areas of St. Johns, Flagler, and Putnam counties, NE. Five of 20 representative areas were selected, with PSD<sub>fine</sub> ranging from 526 (lower) to 937 (upper)  $\text{g}\cdot\text{kg}^{-1}$ . Sixty-six undisturbed soil samples were taken from at 0–0.40 m soil depth in each area from which PR,  $\theta$ , Bd, SOM, and PSD<sub>fine</sub> were determined. The soil penetration resistance curve (SPRC) was estimated using a multilinear regression approach. The fitted SPRC (Eq.1) explained 91% of the PR variation at the 0–0.20m soil depth. PR was negatively influenced by  $\theta$ , and positively by Bd, SOM and PSD<sub>fine</sub>.

$$\text{PR} = e^{(-4.9575 + 0.0676 \cdot \text{SOM} + 0.0009 \cdot \text{PSD}_{\text{fine}}) \cdot \theta^{(-0.311)} \cdot \text{Bd}^{(8.9189)}}; \quad n=292; F=531.5; P < 0.0001 \quad [\text{Eq.1}]$$

The PR was estimated using Eq.1 for lower and upper PSD<sub>fine</sub>, considering a Bd range of 1.18–1.58  $\text{g}\cdot\text{cm}^{-3}$ , SOM of 6.00  $\text{g}\cdot\text{kg}^{-1}$ , and  $\theta$  at field capacity ( $\theta_{\text{FC}}$ ) of 0.12 and 0.15  $\text{cm}^3\cdot\text{cm}^{-3}$  for lower and upper PSD<sub>fine</sub>, respectively. The average PR  $\pm$  standard deviation was 0.80  $\pm$  0.51 and 1.08  $\pm$  0.70 MPa for lower (Fig. 1A) and upper (Fig. 1B) PSD<sub>fine</sub>, respectively. There was no PR<sub>critical</sub> below a Bd of 1.48 and 1.45  $\text{g}\cdot\text{cm}^{-3}$  for lower and upper

PSD<sub>fine</sub>, respectively, regardless of  $\theta$  conditions. However,  $\theta$  lower than  $\theta_{\text{FC}}$  for soils with a Bd higher than 1.48 and 1.45  $\text{g}\cdot\text{cm}^{-3}$ , for lower and upper PSD<sub>fine</sub>, respectively, can result in PR<sub>critical</sub>, thus limiting potato root growth (Fig. 1A and B). The PTF allowed us to estimate PR using  $\theta$  and soil physical properties routinely measured in NE. SPRC should be integrated in irrigation recommendation models as an indicator of the soil physics quality for potato root development.

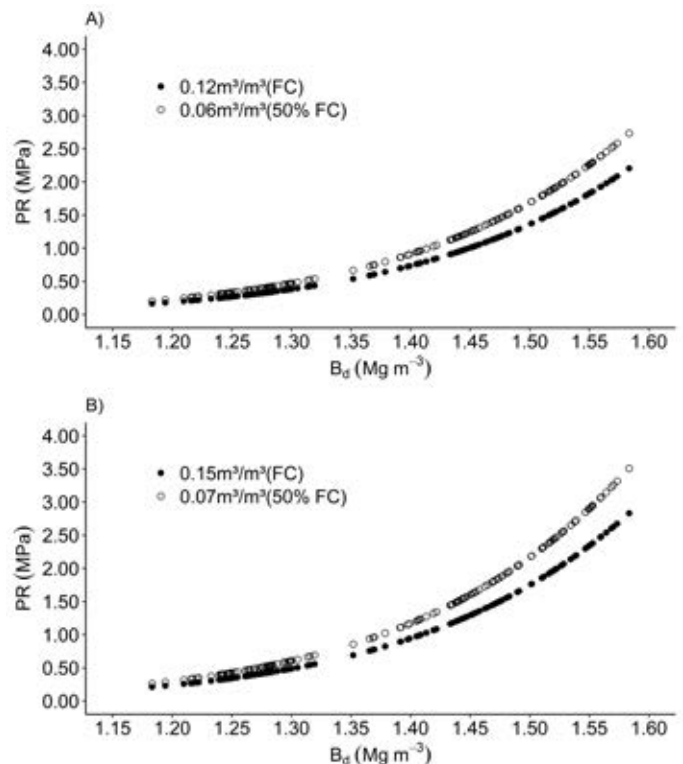


Fig. 1. Soil penetration resistance curve (SPRC) estimated to soils with particle-size distribution (PSD<sub>fine</sub> < 250  $\mu\text{m}$ ); A) 526  $\text{g}\cdot\text{kg}^{-1}$  (lower), and B) 937  $\text{g}\cdot\text{kg}^{-1}$  (upper), with soil organic matter content of 6.00  $\text{g}\cdot\text{kg}^{-1}$ , and soil water content at the field capacity (solid circles) and 50% below of the field capacity (open circles) at the 0–0.20 m soil depth layer.

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—Scientific Note—

## Planting Hole Application of Controlled-release Fertilizer Can Reduce Nitrogen Use without Yield Loss in Strawberry Grown in Sandy Soil in a Subtropical Climate

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**Additional index words.** *Fragaria ×ananassa*, polymer-coated fertilizer, slow-release fertilizer; soil fertility

High temperatures and poor water retention in sandy soils in subtropical climates can lead to quick losses of water-soluble nitrogen (N) fertilizer to the environment before it can be taken up by plant roots (Shukla et al., 2010). This is especially true for bare-root strawberry (*Fragaria ×ananassa* Duch.) transplants during establishment, because their fine roots are desiccated at the time of transplanting and can only absorb small amounts of water. Florida growers often apply high concentrations of soluble N (41–47 kg·ha<sup>-1</sup>) during establishment to maximize N absorbed by the plant (Agehara, 2021). Controlled-release fertilizer (CRF) can be comparable to soluble N fertilizer in supplying the N necessary for vegetable cropping systems, while using less fertilizer due to fewer environmental losses (Incrocci et al., 2020; Wilson et al., 2010). The objective of this experiment was to examine the efficacy of planting hole application of CRF as a new fertilization strategy to replace conventional fertigation for bare-root strawberry transplants during establishment.

'Brilliance' strawberry was grown on sandy soil (97% sand) in West Central Florida during the 2020–21 winter production season. There were 11 treatments arranged in a complete block design: four N rates (0, 11.8, 23.5, and 47.0 kg·ha<sup>-1</sup>, respectively) by fertigation with urea ammonium nitrate (UAN) over 3 weeks during establishment and seven N rates (0, 2.4, 4.7, 11.8, 23.5, 35.3, and 47.0 kg·ha<sup>-1</sup>, respectively) using planting hole application of polymer-coated urea (PCU) at planting. Data were analyzed using analysis of variance followed by the Tukey-Kramer test for multiple comparison. For PCU treatments, curve fitting was used to characterize the dose responses as a dependent variable to N rates. Five models, including linear, quadratic, exponential plateau, exponential decay, and sigmoidal models, were fit to each data set, and the best-fit model was selected based on the smallest corrected Akaike information criterion.

Model fitting analysis revealed positive dose-responses to PCU rate in many growth and yield variables. Both canopy projected area during establishment and shoot (leaves + stems) N uptake at the end of the season showed linear increases. Monthly marketable yields recorded from November through February also showed

linear increases, except for December. Total season marketable yield increased linearly by up to 32%. Interestingly, the proportion of misshapen fruit showed a linear reduction.

Some PCU treatments had better results compared to the UAN treatment at 47 kg·ha<sup>-1</sup> N (the common practice for strawberry production in Florida). During establishment, canopy projected area was 40% greater for the PCU treatment at 11.8 kg·ha<sup>-1</sup> N. November marketable yields for the PCU treatments at 11.8 and 47 kg·ha<sup>-1</sup> N were 108% and 106% greater, respectively. February marketable yield for the PCU treatment at 47 kg·ha<sup>-1</sup> N was 24% greater.

These results suggest that small amounts of CRF in planting holes can achieve high early yields while maintaining total yields and fruit quality and saving up to 35 kg·ha<sup>-1</sup> N. This new fertilization method can replace conventional fertigation during establishment in the winter strawberry production system.

This study will be repeated in the 2021–22 strawberry winter production season, with an emphasis on spatial and temporal variations in soil electrical conductivity in the rhizosphere.

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—Scientific Note—

## Population Dynamics of Pepper Weevil, *Anthonomus eugenii* Cano (Coleoptera: Curculionidae) in Jalapeño Pepper under Field Conditions in South Florida

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**Additional index words.** jalapeño, marking pheromone, pepper weevil

Pepper, *Capsicum annuum* L. is in Solanaceae. It is one of five most-cultivated species in tropical and subtropical regions. There are different types and varieties of pepper which are cultivated worldwide, including sweet and hot types. Characteristics like pungency, flavor, and aroma make them important ingredients in many people's daily diet. Jalapeño pepper is in the hot pepper group. It is one of the common hot peppers for cooking.

Pests and diseases are constraints to pepper production. Pepper weevil (PW), *Anthonomus eugenii* Cano (Coleoptera: Curculionidae), an economically important pepper pest, originated in Mexico. It was first reported in Florida in 1935. The female lays eggs in fruits, flowers, and buds. The life cycle is 20–30 days; there are 3–5 complete generations per year depending on temperature, host, and availability of food.

Different insects in various orders deposit marking pheromones after oviposition, mostly under conditions with limited resources for growth and survival (Addesso et al. 2021). Laboratory studies show that PW uses these marking pheromone to signal conspecifics and prevent additional oviposition. This reduces competition for available resources among larvae, as well as increasing host-finding efficiency. It is important to evaluate the significance of marking pheromones under field conditions. The objective of this study was to validate the idea that PW conspecifics use the same strategy under field conditions as they do under laboratory conditions. We determined PW density in infested jalapeño fruits, fruit size preference for oviposition and did a correlation between fruit length and PW density.

### Materials and Methods

One hundred and fifty infested jalapeño peppers were collected randomly from an infested field for different growing seasons, Fall 2019, Spring 2020, Summer 2020, Fall 2020, and Spring 2021 to evaluate PW density. They were dissected in the lab where the number of PW per fruit were counted and recorded by fruit size. We measured the fruit length (cm) to see if there was a correlation between PW density and fruit length. We used the following range of fruit sizes: small ( $\leq 1.5$  cm); medium (1.5–3 cm); large (3–5 cm) and extra-large ( $> 5$  cm).

### Results and Discussion

The result shows that there is no real correlation between fruit size and PW preference (density/fruit) under field conditions. Small fruits  $\leq 1.5$  cm generally had only 1 PW/fruit. This could be a way to minimize competition when resources are limited and fruit too small to feed more than one PW. More than 70% of larger fruits had more than two larvae, at least in the fall seasons. In the field, all fruit sizes were infested (Fig. 1a). Lower infestations were recorded in small fruits  $\leq 1.5$  cm. Most ( $> 80\%$ ) medium, large, and extra-large fruit had higher rates of PW across the seasons, except in the summer (Fig. 2).

### Conclusion and Future Directions

Pepper fruit need to be protected from PW damage at all stages up to harvest. PW does not discriminate among different fruit sizes, but per fruit density is lower in small fruit. There have been cases of fruit with over 3 larvae (Fig. 1b), but their survival to the adult stages is uncertain. It is important to develop effective strategies to delay the infestation and establishment of



Fig. 1. (A) Different sizes of infested jalapeño pepper fruit. (B) Infested jalapeño fruit with larval and pupal life stages.

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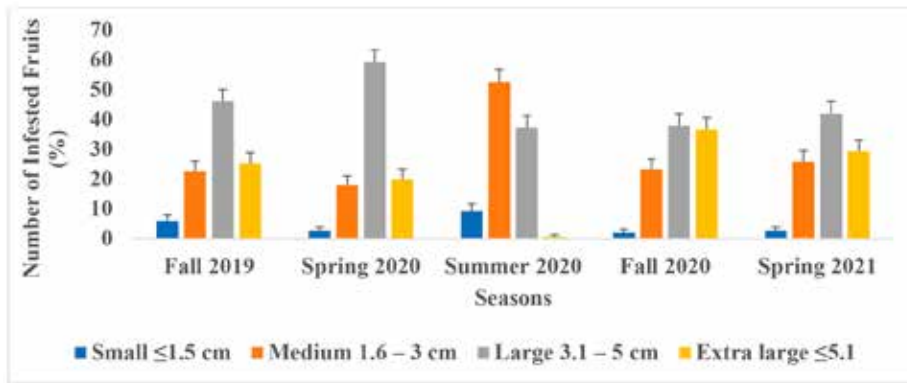


Fig. 2. Percentage of pepper weevil infested fruits by fruit size.

PW in fields because, once they become established, it is difficult to manage them. Having a better understanding of the nature of the PW host marking pheromone under field conditions could be a useful IPM tool. It will require a lot of work to isolate and identify the active components in female frass and oviposition plugs in PW.

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—Scientific Note—

## Lettuce Downy Mildew Differential Cultivars Are Resistant to Other Important Diseases

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**Additional index words.** Breeding, common disease resistance, *Lactuca sativa*

Lettuce (*Lactuca sativa* L.) is planted on 10,000 acres in the Everglades Agricultural Area (EAA), generating over 80 million dollars revenue. The crop is susceptible to diseases due to the region's unique environmental conditions. Lettuce downy mildew (LDM) is caused by the oomycete *Bremia lactucae*, that infects the leaves causing chlorotic yellow spots that eventually coalesce and turn necrotic (Michelmore et al., 2009). *Bremia lactucae* is genetically diverse with 10 races identified in the western United States and 15 races in Europe (van Treuren et al., 2011). Race structure of *B. lactucae* is established utilizing differential cultivars that have known resistant and susceptible reactions. Another disease problem for EAA lettuce growers is bacterial leaf spot (BLS) caused by *Xanthomonas hortorum* pv. *vitians* (*Xhv*). The pathogen produces water-soaked lesions delimited by veins that progress to tissue collapse, becoming dry with a papery appearance. A newly discovered disease in Florida is fusarium wilt of lettuce (FWL) caused by *Fusarium oxysporum* forma *specialis* (f.sp.) *lactucae* (*Fol*). *Fol* is a soil-borne fungus that produces taproot discoloration, usually pink to reddish-brown. Lettuce infested with *Fol* exhibits stunting, chlorosis, and wilting until plant death due to water and nutrient transport blockage. Host resistance is the preferred method to fight these diseases but breeding for resistance is time consuming. Considering that plant breeders must develop multi-disease resistant cultivars, the process could become exponential if only one disease is considered at a time. Previous studies indicate that resistant gene candidates (RGCs) to many diseases are grouped in clades in the lettuce genome. Four RGCs families were found to provide resistance to at least 16 different diseases (Christopoulou et al., 2016). Therefore, a lettuce accession could be resistant to several diseases. This study aimed to identify common resistance lettuce accessions to BLS and FWL in an LDM differential cultivar set that already possesses race-specific resistance to *B. lactucae*.

Four independent greenhouse experiments were conducted to test 17 differentials and controls (depending on the pathogen) with inoculations of isolates L7, L44, and Sc8B of *Xhv* and #51 of *Fol*. Each experiment was planted in a randomized complete-block design with three replicates and was conducted twice. Trays of 30-day-old lettuce seedlings were inoculated with a hand sprayer containing a suspension of  $10^6$ -mL<sup>-1</sup> CFU of each *Xhv* isolate. Plants were maintained in a growth chamber for 48 hours

and then were transferred to a greenhouse. After 10 days, foliar damage was assessed using a rating scale of 0 (no symptoms) to 5 (> 75% of total foliage infected). FWL trials were conducted using the same cultivars. Plant roots were submerged in a *Fol* suspension of  $1 \times 10^6$  spores/mL and transplanted into 4-inch pots. Plants were evaluated for foliar damage weekly and root discoloration was evaluated after 30 days using the rating scale described above. Statistical analyses were computed using SAS statistical software (ver. 9.4; SAS Institute Inc., Cary, NC) and analysis of variance was performed following a nonparametric statistic to obtain relative marginal effects (RME) and their confidence intervals.

Significant differences were found among the tested accessions ( $P < 0.001$ ) for foliar damage. No differences were detected between experiments ( $P > 0.05$ ) when inoculated with isolates L7, L44, and Sc8B. 'Muraies' and 'Silvinas' were as resistant as the controls PI 358001-1 and PI 667690 when tested with all *Xhv* isolates. Fifteen cultivars showed similar disease severity as the susceptible control 'Okeechobee'. Likewise, significant differences were found between the tested accessions ( $P < 0.001$ ) for foliar (FDS) and root (RDS) disease severity in the experiment with *Fol*. 'Balesta', 'Colorado', and 'Silvinas' had low FDS and RDS similar to the resistant controls PI 667690 and 60182. Fourteen cultivars were as susceptible as the control 'Chosen' for both parameters evaluated. The aforementioned differentials are likewise resistant to *B. lactucae* races 7, 8, and 9. Therefore, it was possible to identify a lettuce accession resistant to several diseases, creating the possibility of improving lettuce resistance to LDM, FWL, and BLS at the same time.

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—Scientific Note—

## Influence of Nutrient Management Practices on Crop Productivity and Quality in High Tunnel Organic Production of Leafy Greens

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**Additional index words.** cover crop, compost, lettuce, organic fertilizer, pac choi, spinach

The interest in using high tunnels for organic vegetable (OV) production is growing in Florida along with increasing demand for local organic vegetables. Leafy greens (LG) are among the most common high-value crops in high tunnel systems (Hochmuth and Toro, 2014). Integrated nutrient management in organic high tunnel vegetable production is crucial for optimizing crop productivity and enhancing soil health (Bi et al., 2021). However, the information regarding optimal nutrient management for organic LG in high tunnels is still limited. The objective of this study was to examine the impacts of soil and fertility management practices, including cover crops, compost, and organic fertilizer (OF), on yield and quality attributes of LG to provide research-based information on integrated nutrient management for high tunnel OV systems under Florida conditions.

This three-year study was conducted during 2018–20 in a multi-bay high tunnel on certified organic land at the University of Florida, Institute of Food and Agricultural Sciences (UF/IFAS) Plant Science Research and Education Unit in Citra, FL. A split-split plot design with three replications was used each year. Cowpea (*Vigna unguiculata* ‘Iron Clay’) cover crop and weedy fallow plots were established before the LG season as the whole plots, while OF application (preplant granular OF vs. weekly injection of liquid OF) was the subplot factor. Different types of composts (yard waste-based compost, cow manure-based compost, vermicompost, and no compost control) were included in the sub-subplots. After terminating the cowpea cover crop, pac choi (*Brassica rapa* subsp. *chinensis* ‘Mei Qing Choi’) was transplanted in Sept. or Oct. and harvested after about five weeks. The subsequent crop included direct-seeded spinach (*Spinacia oleracea* ‘Corvair’) or lettuce (*Lactuca sativa* ‘Outredgeous’), followed by tomatoes each year prior to cowpea planting. All nutrient management treatments were implemented during the pac choi season. Each treatment plot remained in the same location across different seasons. Plant fresh biomass was collected weekly until the final harvest during pac choi production, while lettuce and spinach were measured only at harvest. The dynamics of fresh biomass and nitrogen (N) accumulation in pac choi were evaluated. Vegetable phytochemical contents were assessed in 2019 and 2020, including ascorbic acid, total phenolic content, and total antioxidant capacity. Data were analyzed in the GLIM-

MIX procedure using SAS (SAS Institute, Cary, NC) with Fisher’s least significant difference test ( $P \leq 0.05$ ).

There were no consistent interactions found among the cover crop, organic fertilizer, and compost factors for fresh biomass and vegetable phytochemical content. Results showed that the cowpea cover crop had little influence on crop yield performance. Further research is needed to evaluate the cover crop contribution to vegetable crop N management under different levels of N fertilization. In 2018 and 2019, granular fertilizer led to significantly higher fresh biomass of pac choi two weeks after transplanting, while liquid fertilizer resulted in significant increases of fresh biomass three weeks after transplanting in all seasons. A similar trend was observed for N accumulation of pac choi. Weekly application of liquid fertilizer might have better matched crop nutrient demand to help improve crop yield. However, the magnitude of yield increase resulting from liquid fertilizer generally declined from 2018 to 2020, suggesting a possible legacy effect of organic granular fertilizer on soil fertility. The long-term impact and cost effectiveness of different types of organic fertilizers on nutrient supply deserve further examination. Compared with no compost plots, yard waste compost significantly increased marketable pac choi yield in all seasons, and spinach yield in 2018. Yard waste compost also led to enhanced fresh biomass and tended to increase N accumulation in pac choi three weeks after transplanting. Granular fertilizer led to a significant increase in ascorbic acid content of pac choi in 2019 and 2020, and tended to increase total phenolic content of lettuce in 2019 relative to liquid fertilizer. Overall, compost application did not affect the phytochemical content of pac choi, but a reduction of ascorbic acid content and total antioxidant capacity in lettuce was observed in some compost treatments in 2019. More systematic research is needed to optimize integrated nutrient management practices for enhancing nutrient availability and use efficiency, soil health, and crop yield and quality in high tunnel OV production systems.

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—Scientific Note—

## Soil Pedotransfer Function to Estimate Available Water of Northeast Florida Sandy Soils

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**Additional index words.** seepage; soil physics; subirrigation

Northeast Florida (NE) is an important vegetable production area with approximately 15,000 ha irrigated by the subirrigation method (e.g., seepage, drainage-tile). Development of irrigation tools to assist growers increase water conservation are needed. Most subirrigated areas in NE are sandy soils characterized by a high proportion of larger pores which drain easily and have low organic matter content (SOM), leading to low water holding capacity. To improve water use efficiency, the recommended irrigation models need to take into account soil physical and hydrological variation to reduce water application and nutrient loss and enhance crop production. The soil water available (AW) to plants can be estimated using a soil water retention curve (SWR). However, the determination of SWR involves intensive soil sampling and mastery of advanced laboratory analysis techniques, which is time consuming. Alternatively, soil pedotransfer functions (PTF) can indirectly estimate AW faster and at lower cost than direct determination of the SWR. This study aimed to develop a PTF to estimate AW considering soil bulk density (Bd), SOM, and particle-size distribution < 250 μm (PSD<sub>fine</sub>) variation across the vegetable production areas in NE. The areas of interest included agricultural areas under vegetable production in St. Johns, Flagler, and Putnam counties in Florida. Five out of a possible 20 representative areas were selected, with a PSD<sub>fine</sub> ranging from 526 (lower) to 937 (upper) g·kg<sup>-1</sup>. Sixty-six undisturbed soil samples were taken at 0–0.40 m soil depth in each area in which SWR parameters had been determined. The influence of Bd, SOM, and PSD<sub>fine</sub> on the SWR model was evaluated using a multilinear regression approach. AW was calculated by the difference between soil water content (θ) at field capacity (soil water potential, ψ = -60 hPa) and permanent wilting point (ψ = -15,000 hPa) obtained using SWR model. The SWR model [Eq.1] explained 74% of the θ variation and was negatively influenced by Ψ, and positively by Bd, SOM and PSD<sub>fine</sub>.

$$\theta = e^{(-3.1663 + 1.2235 * Bd + 0.0642 * SOM + 0.0006 * PSD_{fine}) * \Psi^{-0.3550}}; \quad (P < 0.0001, F = 224.3, r^2 = 0.74, n = 330) \quad [Eq.1]$$

The AW was estimated for lower and upper PSD<sub>fine</sub>, considering Bd range from 1.18 to 1.70 g·cm<sup>-3</sup> and SOM average of 6.00 g·kg<sup>-1</sup>, for the 0–0.40 m soil depth layer. The average AW ± standard deviations were similar at 0.11 ± 0.02 and 0.14 ± 0.02 cm<sup>3</sup>·cm<sup>-3</sup> between lower (Fig. 1A) and upper (Fig. 1B) PSD<sub>fine</sub>,

respectively. There was a significant increase in AW with the increase of SOM (e.g., increasing SOM by 50% resulted in additional 0.03 cm<sup>3</sup>·cm<sup>-3</sup> of AW, corresponding to 27% of the AW relatively to the lower PSD<sub>fine</sub> soils). Increasing AW range can reduce irrigation needs, which means saving groundwater and energy costs associated with pump irrigation. The PTF allowed for estimating AW using soil physical properties and SOM routinely measured in NE. Additional physical hydrological indicators, such as soil least limit water range, will be determined for these sandy soils. These indicators should be used for the development of recommended irrigation models for specific soils, irrigation methods and vegetable crops.

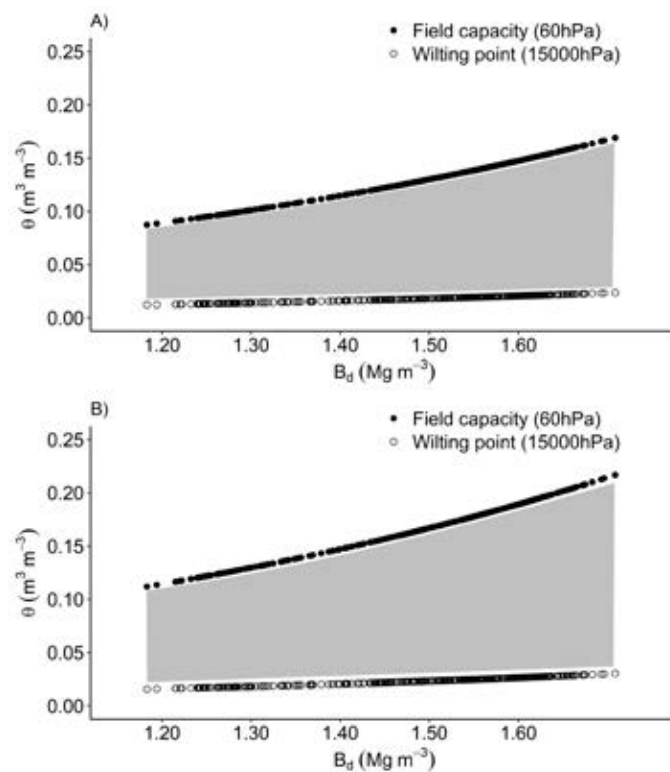


Fig. 1. Soil available water (AW) estimated to soils with particle-size distribution (PSD<sub>fine</sub> < 250 μm); A) 526 g·kg<sup>-1</sup> (lower) and B) 937 g·kg<sup>-1</sup> (upper) with soil organic matter content of 6.00 g·kg<sup>-1</sup> at the 0–0.40 m soil depth layer. The gray areas correspond to the AW.

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—Scientific Note—

## Evaluating Sunn Hemp Biomass as a Carbon Source for Anaerobic Soil Disinfestation in Organic Strawberry Production

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**Additional index words.** ASD, biological soil disinfestation, cover crop, fruit yield, soil redox potential, strawberry cultivar

Anaerobic soil disinfestation (ASD) is an effective non-chemical alternative to manage soilborne pests and pathogens in a wide range of environmental conditions. ASD is initiated by incorporating a carbon source, application of water to fill soil pore spaces, and covering the soil with a gas impermeable film. Under anaerobic soil conditions, facultative and obligate anaerobic organisms utilize organic carbon resulting in reductive soil environments (Di Gioia et al., 2017; Shennan et al., 2018). While ASD has shown potential as a preplant strategy for managing soilborne pests and diseases, further research is warranted to optimize the ASD application technique, particularly in terms of carbon source used to stimulate soil microbial activity. To date, molasses as a byproduct of the Florida sugarcane industry has been used as a carbon source. The potential of alternative carbon sources, including cover crops, should be investigated to address grower concerns related to the cost of implementing ASD. The objective of this pilot study was to assess the effectiveness of sunn hemp (*Crotalaria juncea*) biomass as a carbon source for ASD in organic strawberry (*Fragaria × ananassa*) production.

A field trial was conducted from Oct. 2018 to May 2019 on certified organic land at the University of Florida Plant Science Research and Education Unit (Citra, FL.) using a split plot design with four replications. Whole plots of soil treatment consisted of ASD with molasses (ASD+M), ASD with sunn hemp (ASD+SH), and non-ASD fallow control (Control). Two strawberry cultivars were included in the subplots: 'Florida Brilliance' and Sweet Sensation® 'Florida127'. The ASD treatment lasted 21 days, during which cumulative soil redox potential and soil pH were monitored continuously. Strawberry plants were transplanted into raised beds mulched with totally impermeable film on 17 Oct. 2018 in double rows with 40 plants in each experimental unit. Fruit was harvested twice a week from Dec. 2018 to May 2019. Marketable and non-marketable fruit number and weight were recorded. Overall, there was a low incidence of soilborne diseases.

The ASD+SH and ASD+M soil treatments achieved similar levels of cumulative soil redox potential which were higher than Control ( $P = 0.06$ ) at the end of the 21-day treatment. The main effects of soil treatment and strawberry cultivar on marketable yield were evident, but there were no significant two-way interactions. Whole-season marketable fruit yield increased significantly by 26% and 16% under ASD+SH compared with ASD+M and

Control, respectively. The differences in whole-season marketable fruit yield were mainly attributed to significant increases in marketable fruit yield during Jan., Feb., and Mar. 2019. The effect of strawberry cultivar on marketable fruit yield was inconsistent between Dec. 2018 and May 2019. During the early season harvest in Jan. and the peak harvest period in Mar., 'Florida Brilliance' produced significantly higher marketable fruit yield by 40% and 21% compared with 'Florida127', respectively. However, during the Apr. late season harvest, marketable fruit yield of 'Florida127' was 52% greater than 'Florida Brilliance'. By the end of the production season, marketable fruit yield did not differ between the two cultivars, but the number of fruit was significantly higher in 'Florida Brilliance' compared with 'Florida127'. The observed differences in marketable fruit yield between ASD+SH and ASD+M may be related to the contribution of sunn hemp residue. The low C:N ratio of sunn hemp leaves (13:1) may have resulted in rapid N mineralization during the early season, while sunn hemp stems (C:N 29:1) may have contributed to a longer-term nutrient pool and increased water holding capacity compared with ASD+M and Control plots. The marketable fruit yield differences between the two strawberry cultivars may be related to cultivar-specific traits and are in line with previous findings by Whitaker et al. (2019). The impacts of sunn hemp on soil redox potential and marketable fruit yield suggest the need for further evaluations of the potential of sunn hemp biomass as a carbon source for ASD application in organic strawberry production in Florida. Future research should optimize the seeding rate, growing period, and termination method of sunn hemp as a carbon source for ASD application, while taking into account the contribution of organic matter and mineral nutrients when determining organic strawberry fertility management.

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—Scientific Note—

## Insecticide Lethal and Sublethal Effects on Corn Silk Flies (Diptera: Ulidiidae) following Topical Exposure

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**Additional index words.** sweet corn, bioassay, pyrethroid, spinetoram, corn silk flies

Florida ranks second nationally in terms of sweet corn (*Zea mays* L.) production for the fresh market, being responsible for 29% of the sales in the United States (USDA–NASS, 2019). Sweet corn ranks fourth among the most valuable vegetable, berry, and melon crops in Florida representing an annual production value of 150 million dollars. Thirty-nine thousand acres of sweet corn are grown in Florida with 74% of this acreage concentrated in Palm Beach County in the Everglades Agricultural Area (USDA NASS 2017). Locally known as corn silk flies, *Euxesta stigmatias*, *Euxesta eluta*, and *Chaetopsis massyla*, are three species of picture-winged flies (Diptera: Ulidiidae) that have become the most damaging pests of sweet corn in southern Florida. Corn silk fly adults lay eggs on corn silks, and larvae injure silks and kernels. Due to the high damage potential of corn silk flies and the low tolerance for ear injury using current fresh market standards, these insects are responsible for the rejection of sweet corn loads every year.

Corn silk fly management relies on frequent applications of pyrethroids targeting adults. Because this management strategy relies on a single class of insecticides sharing the same mode of action, the possibility of insecticide resistance has become a concern, emphasizing the need for pyrethroid alternatives. Laboratory experiments were conducted on *E. eluta* to determine the effects of 20 registered and non-registered insecticides with potential activity against corn silk flies. Six pyrethroids, four neonicotinoids, four insect growth regulators, two diamides, one spinosyn, one avermectin, and one oxadiazine were evaluated.

Topical assays were conducted using a Generation III Research Spray booth to deliver commercial formulations of insecticides at high field rates to *E. eluta* adults. A first study determined *E. eluta* adult mortality 24 hours after exposure. A follow-up study was conducted on insecticides associated with less than 15% mortality to determine female *E. eluta* fertility. Twenty-four hours after insecticide exposure, females were allowed to oviposit for 3 days on artificial diet, and the number of larvae was counted

10 days after the end of the oviposition period. The cumulative female-days during 3 days of oviposition and the resulting number of larvae per female per day were determined

The first study evaluating mortality following topical exposure showed that all six pyrethroids were associated with the highest mortality (86 to 100%) whereas spinetoram exposure resulted in intermediate mortality (50%). The remaining 13 insecticides were associated with  $\leq 13\%$  mortality. In the second study, among the 13 insecticides causing minimal mortality, abamectin and dinotefuran were associated with 47% and 36% reductions, respectively, in cumulative female-days. However, only abamectin negatively impacted female fertility, causing a 61% reduction in the number of *E. eluta* larvae per female per day.

Observations of *E. eluta* adult mortality after topical insecticide exposure suggest that pyrethroids remain the only viable option for foliar applications targeting adults, emphasizing the need for new insecticides. Spinetoram was associated with intermediate mortality, suggesting that foliar applications might suppress corn silk fly infestations. This insecticide caused high mortality following ingestion exposure in additional experiments. Thus, spinetoram might be an alternative to pyrethroids if delivered with baits promoting insecticide ingestion. A delayed effect on mortality was observed for both abamectin and dinotefuran, but only abamectin impacted fertility. This suggests that in the field, abamectin could aid by killing flies over time and decreasing their ability to cause larval infestations. Insecticide resistance remains a concern and should be addressed by looking into additional insecticides concentrations and types of exposure.

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—Scientific Note—

## Screening Broad-spectrum Herbicides to Identify Lettuce Lines with Tolerance

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**Additional index words.** herbicide tolerance, lettuce injury, postemergence herbicides, preemergence herbicides

Production of leafy vegetables such as lettuce (*Lactuca sativa* L.) on organic soils in the Everglades Agricultural Area in south Florida is hindered by lack of effective weed management programs, particularly chemical control due to limited number of herbicides available for weed control. Lettuce is very sensitive to weed competition from early in the season because of its relatively slow growth, low stature, and short growing season. Weeds including common lambsquarters (*Chenopodium album* L.), common purslane (*Portulaca oleracea* L.), *Amaranthus* spp. and nutsedges (*Cyperus* spp.) are the most problematic in lettuce. Season-long competition from these weeds can result in up to 52% lettuce yield reduction (Santos et al., 2004a, 2004b). There are limited herbicide options for use in leafy vegetables compared to major row crops (Lati et al., 2015). Preemergence herbicides registered for selective broadleaf and sedge weed control in lettuce are not efficacious on organic soils (Kanissery et al., 2019). Imazethapyr is the only selective broadleaf herbicide for postemergence weed control in lettuce in Florida. Since discovery and development of new herbicides for small acreage crops such as leafy vegetables is limited, utility of existing herbicides with broad-spectrum weed control to develop varieties through enhanced non-transgenic genetic resistance using chemical mutagenesis and conventional breeding is important.

A study was conducted to screen broad-spectrum postemergence and preemergence herbicides on 189 lettuce lines at the Everglades Research and Education Center (EREC) in Belle Glade, FL. The lines included 98 University of Florida (UF) breeding lines, 5 UF historic cultivars 13 commercial cultivars, 3 UF mapping populations, 20 parents of mapping populations, 8 USDA–Pullman lines, and 42 wild types. A total of 13 post-emergence herbicides (6 modes of action) were used to screen the 189 lettuce lines in the greenhouse. An untreated control was included for comparison. The herbicides were applied 30 days after emergence. Plants were visually rated as dead or alive at 7, 14, and 28 days after treatment. The experiment was conducted twice. For the preemergence herbicide screening, a total of 3 herbicides (2 modes of action) were used to screen

the 189 lettuce lines in EREC fields. Preemergence herbicides were applied immediately after planting and incorporated into the soil using overhead irrigation. The experiment was repeated twice. Germination was recorded at 21 days after planting. For the preemergence screening, 9 UF breeding lines, 1 commercial cultivar, 1 parent of mapping population, and 1 wildtype showed tolerance to pronamide, metribuzin, and hexazinone. All 189 lettuce lines showed tolerance to postemergence flumetsulam, imazamox, imazapic, and rimsulfuron. Eight UF breeding lines, 1 commercial cultivar, 1 UF historic cultivar, 1 USDA–Pullman lines, and 1 wildtype showed tolerance to either postemergence linuron, saflufenacil, or topremezone. The 24 lines that showed tolerance to preemergence or postemergence will be further evaluated using replicated greenhouse and field experiments. Any of the aforementioned lines in which herbicide tolerance is confirmed will be subjected to chemical mutagenesis to help isolate herbicide resistant mutants. These lines will be used in the lettuce breeding program in the future to develop cultivars with herbicide tolerance and enable efficacious weed control in this crop.

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—Scientific Note—

## Soil Management Strategies to Reduce Fusarium Wilt of Lettuce in the Organic Soils of the Everglades Agricultural Area

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**Additional index words.** Fusarium wilt, disease management, histosols, lettuce

Lettuce (*Lactuca sativa* L.) cultivation worldwide is threatened by the soil-borne fungus *Fusarium oxysporum* f. sp. *lactucae* (*Fol*) which causes Fusarium wilt. *Fol* colonizes taproot vascular tissue and develops a root rot that deforms or kills plants. In 2017, *Fol* race 1 was confirmed in an isolated field of the primary lettuce producing region of the Everglades Agricultural Area (EAA) in Florida (Murray et al., 2020). Once established, Fusarium wilt is difficult to manage because chemical, cultural, and genetic interventions do not provide complete control. The soils in the EAA have high organic matter (> 80%) and require specific management such as maintaining soil pH, applying phosphorus (P) fertilizers and flooding fallow fields to reduce soil loss and biotic stresses. Potentially, these strategies could reduce *Fol* in different soil types; however, this information is lacking for histosol systems like the EAA. As a result, the goal of this research was to identify cultural practices that could further reduce disease symptoms.

Two experiments were conducted at the Everglades Research and Education Center (EREC) in Mar.–Apr. 2021 to test the effect of soil pH amendments and P fertilizer on Fusarium wilt. An isolated, disease-free field was quarantined to build 18 self-contained microplots (1.2 m<sup>2</sup> plastic beds). *Fol* was established in the microplots by incorporating *Fol*-infested oat seeds and repeatedly planting susceptible ‘Chosen’. Wooden boards were then inserted to subdivide the microplots into four subplots, for a total of 72 (0.61 m<sup>2</sup>). Both experiments were conducted as a randomized complete-block design with 12 replications utilizing a factorial arrangement. The first factor consisted of six soil treatments: low pH (5.6); high pH (7.5); recommended P rate (75 kg·ha<sup>-1</sup>, Rec. P); half-recommended P (Low P); double-recommended P (High P); and untreated control (No Trt). The second factor was two different cultivars: susceptible (S) Chosen and partially resistant (PR) Floricrisp 1265 in Expt. 1, and Floricrisp 1265 and UF breeding line 60182 (PR) in Expt. 2. Five lettuce seeds per cultivar were planted in separate rows for each subplot and monitored daily until moderate foliar symptoms appeared.

Disease severity was rated by dissecting the taproot longitudinally and scoring the vascular tissue for root discoloration severity (RDS) on a 0 to 5 scale, where 0 = no discoloration and 5 = dead plant (Fang et al., 2012). Root discoloration incidence (RDI) was calculated as the proportion of plants displaying RDS ≥ 1 and

only results for RDI are presented. A non-parametric analysis of ranked means was used to test differences among treatments (SAS Institute Version 9.4., Cary, NC). A third experiment (Experiment 3) was initiated in a greenhouse in Mar. 2021 to test three flooding durations for reducing the concentration of *Fol*. Soil was obtained from the field microplots and homogenized to fill 12 buckets with 4.5 kg of soil. Buckets were flooded for either 7, 21, or 30 days. The concentration of *Fol* colony forming units (CFU/g soil) was measured on a soil sample per bucket before and after flooding by performing serial dilution plating on Komada’s Semi-Selective Medium (Randall et al., 2020). A *t*-test was conducted to compare the reduction in *Fol* inoculum within each treatment.

Significant differences ( $P < 0.0001$ ) in RDI were found among soil treatments and cultivars in Expts. 1 and 2. In both experiments, Low P and Rec. P significantly reduced RDI compared to Low pH. In Expt. 2, they also reduced RDI relative to No Trt. The decrease in RDI was greater for ‘Floricrisp 1265’ (PR) than ‘Chosen’ (S) in Expt. 1, and greater than 60182 (PR) in Expt. 2. These results suggest that applying P fertilizers at or below the recommended rate in infested soils may help reduce disease symptoms for PR cultivars. Flooding *Fol* infested soil in Expt. 3 resulted in an insignificant increase of 25.7% after 7 days, but significant reductions of 22.2% and 27.0% *Fol* CFUs were observed for 21- and 30-day flooding, respectively. Despite the observed reduction of *Fol* under 21- and 30-days flooding, over 1200 CFU/g soil were present. In the EAA, growers flood fallow fields for 40 days as a recommended practice, therefore, longer flooding durations need to be investigated to reduce *Fol* concentration to below a safe threshold.

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—Scientific Note—

## Abundance and Diversity of Corn Silk Flies (Diptera: Ulidiidae) in Sweet Corn and Surrounding Habitats in the Everglades Agricultural Area and Homestead Regions

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**Additional index words.** picture-winged flies, pest management, landscape ecology

Sweet corn (*Zea mays* L.) is the most valuable specialty crop in Florida after strawberries, tomatoes, and bell peppers. The total value of sweet corn production in Florida approaches \$150 million annually. Approximately 20% of US sweet corn for the fresh market is produced in Florida, with much of the acreage in the southern region of the state. A complex of picture-winged flies (Diptera: Ulidiidae), locally referred to as corn silk flies (CSF), threatens the production of sweet corn in Florida. CSF include *Euxesta stigmatias*, *Euxesta eluta*, and *Chaetopsis massyla*. These pests are managed using frequent insecticide applications targeting adults. However, CSF are abundant throughout the year, and their larvae and adults have been documented from non-sweet corn plants including sugarcane, cabbage, radish, pepper, tomato, papaya, banana, orange, amaranth, cattail, and johnsongrass. Thus, we hypothesized that habitats surrounding sweet corn fields might provide habitats for CSF population build up. The goal of this study was to determine the abundance and diversity of CSF in sweet corn and surrounding habitats.

A survey was conducted in the Everglades Agricultural Area (EAA) during Fall 2020, Winter 2021, and Spring 2021. For each season, eight different sites on commercial farms were sampled. During the winter, four additional sites were sampled in the Homestead region (HR). Each site included three habitats, with sweet corn fields, non-crop habitats, and sugarcane fields (EAA) or palm tree nurseries (HR). The non-crop habitats were composed of Brazilian pepper, cabbage palm, lantana, ragweed parthenium, ragweed, and numerous grasses. Sampling in each

habitat was conducted using five multi-lure traps baited with ammonium acetate and 1,4 dimethoxybenzene. Trapping was initiated in all habitats at a site when sweet corn was between tassel push and early silking. CSF adults were collected weekly for 2–4 weeks. CSF specimens were identified to species and data were analyzed using analysis of variance.

The three CSF species occurred from fall to spring in all sampled habitats of the EAA. In the fall, there was a trend for higher numbers of CSF adults in non-crop habitats than in sugarcane and sweet corn fields ( $P < 0.1$ ). In the winter, differences in the numbers of CSF adults captured in the three habitats in the EAA were not detected ( $P > 0.05$ ). However, in the HR, the numbers of CSF adults in non-crop habitats were greater than in palm tree nurseries ( $P < 0.05$ ), with intermediate numbers observed in sweet corn fields. In the spring, CSF adults were captured in significantly higher numbers in non-crop habitats and sugarcane fields than in sweet corn fields ( $P < 0.05$ ). CSF were generally captured in higher numbers in non-crop habitats than in sweet corn fields. Hence, avoiding weedy fields and borders that might act as refuges for CSF might improve management. CSF were also present in sugarcane fields surrounding sweet corn fields. These results emphasize the need for frequent insecticide applications to protect sweet corn fields from CSF immigrating from surrounding habitats. Further work is needed to better understand the relationship between CSF abundance and landscape composition.

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—Scientific Note—

## Expansion of Ethnic Vegetable Production in Florida

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**Additional index words.** new income opportunities, summer vegetables, vegetable amaranth

There are currently over 40 ethnic vegetable crops including 4 root, 10 leafy, 2 flower, and 26 fruiting vegetables being grown in the state of Florida. Most of them are originally from Asia and are known as Asian vegetables. They may appear unusual when first encountered, but can diversify Florida's vegetable production systems and provide foods with health and medicinal properties. The rapidly growing interest in Asian vegetables is partial because Florida's Asian population increased by nearly 50% between 2005 and 2015, solidifying it as the fastest growing ethnic group in the state. The purposes of this paper are to provide an overview of various novel Asian vegetable crops gaining popularity among Florida growers and to describe production of vegetable amaranth as a summer crop.

Growing ethnic vegetables is attractive to Florida farmers for a variety of reasons. Florida's climate provides ideal growing conditions for many Asian vegetables, in some cases reducing the amount of applied fertilizer necessary and extending the growing season into the typically unproductive late summer months. Ethnic vegetables also provide new income opportunities for producers and are often more profitable than traditional vegetables. However, farmers may be disinclined from including ethnic vegetables in their crop calendars due to lack of growing information available. The cultivation guide for one such crop, which is published in the University of Florida IFAS Electronic Data Information Source (EDIS), is vegetable amaranth. Vegetable amaranth (*Amaranthus tricolor* L.) is a visually attractive and nutritious vegetable that is typically grown as a leafy green.

Amaranth can be categorized into four groups based on use: vegetable, grain, ornamental, and weed. Vegetable amaranth is native to tropical Asia and includes 17 species that are cosmopolitan. It is popular in parts of the Caribbean where it is known as callaloo. Seeds will germinate about one week after planting within the optimal temperature of 70 °F to 75 °F. As a warm season annual native to the tropics, amaranth prefers full

sun. Leafy amaranth grows best when temperatures are between 70 °F and 85 °F, but can tolerate temperatures from 50 to 110 °F, making it particularly well-suited for Florida's summers. Amaranth grows best with a soil pH between 5.5–7.0 (the soil pH range of the Hastings, FL potato growing region). Fertilizer recommendations for vegetable amaranth are currently not standardized, but IFAS suggests fertilizer recommendations for leaf lettuce as a close approximation. Vegetable amaranth requires 150 lb/A of N, and 100 lb/A of both P<sub>2</sub>O<sub>5</sub> and K<sub>2</sub>O at low to medium M-3 soil indexes. It is susceptible to damage from stem- and leaf-chewing insects, as well as several common fungal diseases such as fusarium wilt and white rust. Using proper spacing is an effective deterrent of these diseases, and insect pests can be removed by hand for production in small gardens or spraying appropriate pesticide and/or broadcasting traditional wood ash for commercial production. Vegetable amaranth is ready for harvest approximately 30 days after planting and can be harvested by pulling from the soil for a once over harvest. For successive harvests, growers can remove some leaves for the first harvest, allow plants to regrow, and harvest again in two weeks later. The foliage wilts rapidly, so a short postharvest cooling of the leaves at 40 to 50 °F and 75% relative humidity is recommended. Vegetable amaranth's high yields and adaptability to hot climates increases its appeal to Florida farmers seeking to expand their production into the late summer months.

Vegetable amaranth is rich in mineral nutrients and vitamins, contributing to its popularity with Western consumers. In the future, distribution of cooking and preparation instructions for Asian vegetables to grocery stores, markets, and buyers will increase awareness—and thus desirability—of the wide variety of Asian vegetable crops that can be grown in Florida. More information is available to growers who are interested in cultivating Asian vegetables online at <<https://edis.ifas.ufl.edu/publication/CV301>> and <<https://edis.ifas.ufl.edu/publication/HS1407>>.

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—Scientific Note—

## Exploring Pumpkin Varieties for Northeast Florida Production

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The Tri-County Agricultural Area (TCAA) within Northeast Florida is primarily composed of potatoes, cabbage, and sod, with agricultural acreage dwindling due to development, and tight profit margins from traditional crops. Small and large-scale farmers need alternative crops to sustain their livelihoods, since the increasing costs of materials and stricter regulations has limited the profitability of traditional crops. Specialty crops with high value and lower costs of production could help keep farmers in business, while creating a food systems effort alongside local chefs. Currently, pumpkins (*Cucurbita spp.*) are not a major part of Florida’s commercial agricultural landscape, despite the plethora of fruits and vegetables produced throughout the state. Northern states (e.g., Illinois and Pennsylvania) are better suited for cucurbit production due to reduced environmental stressors compared to those observed in Florida (e.g., high precipitation events, disease and insect pressure, and poorly drained soils). The goal of this demonstration was to plant six cucurbit varieties in the summer (mid to late July) for Halloween or Thanksgiving markets.

### Materials and Methods

A variety demonstration was planted at the UF/IFAS Hastings Agriculture and Education Center–Cowpen Branch Road Research Facility in Hastings, St. Johns County, FL. The half-acre trial was planted on Holopaw fine sand on July 2020. There were

no blocks or replicates. This was simply a demonstration of six varieties (ranging from miniature to large in size and cookability).

The field was prepped with a fumigation treatment 15 days prior to planting. One variety was planted per single, 420-ft-long row. Pretreated seeds were purchased from Seedway, apart from ‘Seminole’ which was sourced from Southern Exposure Seed Exchange. The varieties selected included two ornamentals (‘Baby Pam’ and ‘Jill-Be-Little’) and four edible varieties (‘Jarrahdale’, ‘La Estrella’, ‘Marina di Chioggia’, and ‘Seminole’). Each variety was direct seeded (triple-drilled) every 32 inches in-row on reflective plastic mulch (80-inch row centers) with double-line drip irrigation. The plot received a 4–8–4 CRF pre-plant, followed by six weeks of fertigation with 8–0–8 for a total seasonal application of 160 lb N, 50 lb P<sub>2</sub>O<sub>5</sub> and 160 lb K<sub>2</sub>O per acre. Seedlings were later thinned by hand to a single plant every 32 inches in-row. Insects and diseases were monitored throughout the season. A rotation of one insecticide and one fungicide was used every other week.

### Results and Conclusions

Harvest began on 1 Oct. 2020 for the smaller varieties, such as ‘Jill-Be-Little’ and ‘Seminole.’ Every marketable and matured pumpkin fruit was harvested within 300’ of the 420’ long row. Varieties were weighed in bulk, and up to 10 individual fruit weights, along with total fruit counts by variety were done (Fig. 1). Unfortunately, the field had an irrigation leak on 13 Oct. 2020 that caused the field to be flooded with standing water for approximately five days. The larger varieties, ‘Jarrahdale’, ‘La

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Variety	Yield by fruit count	Yield by weight (lb)	Yield per acre (lb)	Average weight per fruit (lb)	Weight ranges (lb)
	per 300-ft long row	per 300-ft long row			
La Estrella	296	1310	11,266	4.43	1.05 - 6.06
Seminole	565	975	8,385	1.73	0.69 - 3.94
Jarrahdale	178	1497	12,874	8.41	6.06 - 11.63
Marina Di Chioggia	115	817	7,026	7.10	4.31 - 12.44
Baby Pam	202	237	2,038	1.17	1.06 - 1.68
Jill-Be-Little	669	230	1,978	0.34	0.18 - 0.56

Fig. 1. Comparisons of pumpkins for northeast Florida.

Estrella' and 'Marina di Chioggia,' were unable to fully mature prior to retrieving them from the flooded field.

Pie-type pumpkins are marketed as small or medium fruits in 24-inch bins, or 1 1/9-bushel cartons. Although the Florida's heirloom 'Seminole' was the only untreated seed, it still had impressive fruit production with 565 fruits in 300 feet of row length, averaging 5.6 fruits per plant. 'Jill-Be-Little' exceeded that volume, but the miniature types also produce more fruits per vine. Even without reaching full maturity, 'Jarrahdale' produced the greatest weight of fruits at 1497 lb within the plot, and proved to be the most highly desired by culinary judges based on color, flavor, and ornamental uses. Both 'Marina di Chioggia' and 'Jill-Be-Little' exceeded the expectations of fruit weights by the seed company, even though these two were of the least interest by the surveyed farmers and public.

### **Suggestions for Future Studies**

While pumpkin production is currently limited in Northeast Florida, with the expansion of agritourism and desire for locally produced pumpkins, there is great opportunity to explore pumpkin as a feasible alternative crop for the region. Since the demonstration, two small farmers within the TCAA have since incorporated pumpkins into their rotation. Local chefs are especially excited about edible and heirloom types, which will be the focus of the 2021 variety trial. Although the integrated pest management program was not intended to be a major focal point of the demonstration, with proper cultural practices we can grow pumpkins with few pesticide inputs in the near future.



—Scientific Note—

## Lettuce Cultivated in Hydroponics Responds to Less Phosphorus Inputs

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**Additional index words.** breeding, hydroponic production, lettuce yield, phosphorus use efficiency

Lettuce (*Lactuca sativa* L.) is the most widely consumed leafy vegetable worldwide. In recent years, hydroponics lettuce production has increased in Florida. Despite the lower pest, disease and weed pressure, and the reduction of water and nutrient requirements, the increasing prices of fertilizers, including phosphorus (P), present a major hurdle for hydroponic lettuce production. Identifying lettuce genotypes capable of producing high yields with low P inputs may help reduce fertilizer use, production costs, and excess P in wastewater. The objective of this study was to identify lettuce genotypes that use P more efficiently in a hydroponic system.

Two greenhouse experiments were conducted at the University of Florida/IFAS Everglades Research and Education Center in Belle Glade, FL. In each experiment, 12 lettuce genotypes: romaine (6); crisphead (5); and bibb (1) types were grown in two nutrient film technique (NFT) systems. Each system contained a modified Howard Resh solution plus one P treatment: either low P (3.1 ppm) or high P (31 ppm). Phosphorus was supplied in the form of phosphoric acid (H<sub>3</sub>PO<sub>4</sub>) with all other macro and micronutrients kept constant. Solutions were replaced every 14 days to avoid nutrient imbalance. Electrical conductivity and pH of solutions were adjusted to ranges of 1.4 to 1.8 mS/cm and 6.0 and 6.5, respectively. The genotypes were replicated three times in each NFT system, with each replicate consisting of a single plant. Seedlings were started in rockwool cubes and transplanted into the NFT 11 days after sowing. Plants were harvested at horticultural maturity to measure fresh weight [FW (g)] of shoots and roots and tipburn incidence. After, shoot and root tissues were oven-dried at 65 °C for 7 days to obtain dry weight [DW (g)], then the root-shoot DW ratio was calculated. Tissue total-P concentration [TTP (mg·g<sup>-1</sup>)] was measured in Experiment 1 only, following a hydrochloric acid extraction and analysis using an inductively coupled plasma optical emission spectrometer (ICP-OES). Phosphorus uptake efficiency [PU<sub>p</sub>E (mg P mg<sup>-1</sup> L<sup>-1</sup> P applied)] and P utilization efficiency [PU<sub>t</sub>E (g·mg<sup>-1</sup> P DW)] were calculated for each genotype according to the formulae:

$$PU_{pE} = \frac{TTP \cdot DW}{\text{Phosphorus applied (mg} \cdot \text{L}^{-1})}$$

$$PU_{tE} = \frac{DW}{DW \cdot TTP}$$

Data were analyzed using SAS® software, Version 9.4 (SAS Institute Inc., Cary, NC, USA).

Yields of ‘Little Gem’, 60183, ‘Valmaine’, ‘Green Lightning’, and BG19-0539 were statistically similar ( $P > 0.05$ ) for FW with low and high P. The root-shoot DW ratio of most genotypes, except for ‘Little Gem’ and ‘Honcho II’, increased at low P vs. high P, suggesting that lettuce responds to P-limited conditions by modifying its root morphology, as previously noted in lettuce transplants under a floatation irrigation system (Soundy et al., 2001). At low P, ‘Manatee’ and ‘Valmaine’ had the highest tissue P content and P uptake (PU<sub>p</sub>E) among all genotypes. In contrast, ‘Okeechobee’ and ‘Sun Devil’ had the lowest tissue P content and the highest P utilization (PU<sub>t</sub>E) at low P. Lettuce accessions may use P differently due to differences in P-starvation responses associated with plant morphology and physiology (Fageria et al., 2017). Tipburn incidence was significantly higher ( $P = 0.0253$ ) at low P than at high P. No tipburn was detected in ‘Green Lightning’, ‘H1078’, and ‘Sun Devil’, whereas ‘Little Gem’ had the highest tipburn incidence in both P treatments. Other genotypes had only minor symptoms at low and/or high P. Tipburn incidence in hydroponic lettuce was found to be influenced by cultivar and availability of nutrients, such as calcium (Leskovar et al., 2016). Perhaps the lack of adequate P has a negative influence in tipburn development. Results from this research indicate the presence of genetic variation for FW, tipburn, PU<sub>p</sub>E, and PU<sub>t</sub>E among hydroponically grown lettuce genotypes. Preliminary results from this research can contribute to breeding programs that aim to develop P (or any other nutrient) efficient lettuce cultivars for hydroponic production. Further investigations are needed to determine the specific mechanisms of PU<sub>p</sub>E and PU<sub>t</sub>E in hydroponic lettuce.

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—Scientific Note—

## Toward Improvement of Lettuce for Vitamin C in Protected Agriculture

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**Additional index words.** controlled environments, hydroponics, nutrition

Lettuce (*Lactuca sativa*) is an important crop for protected agriculture including hydroponics, aquaponics and vertical towers. This sector is becoming an increasingly contributor to the leafy vegetable industry. In Florida, 386 acres of protected agriculture structures are planted with vegetables, including 25 acres of lettuce. Lettuce is popularly believed to have no nutritional value, but vitamins and minerals are present in reasonable amounts. The concentration of compounds beneficial to humans in lettuce is not as high as in some other leafy vegetables, including kale and spinach, but lettuce is among the top-10 vegetables in the United States. This makes it an ideal candidate to breed for greater nutritional compounds. Vitamins in lettuce vary by type, with romaine lettuce typically containing a higher concentration of several vitamins (C and pro-vitamin A), especially compared to the less nutritious crisphead lettuce (Still, 2007). Vitamin C (ascorbic acid) is crucial for preventing the debilitating scurvy disease and has antioxidant properties that are beneficial to human health (Aćamović-Djoković et al., 2011). It is recommended to help human immune systems fight respiratory illnesses caused by bacteria and viruses. In lettuce, vitamin C is present in small amounts and the content can be type and cultivar dependent. For breeders to increase nutritional content of lettuce, sources of breeding material are needed. The objectives of this research were to identify hydroponically grown lettuce with high vitamin C content and to understand the horticultural performance of lettuce planted during three planting seasons.

Three experiments were conducted in a greenhouse using a Nutrient Film Technique hydroponic system at the North Florida Research and Education Center—Suwanee Valley in Live Oak, FL. A randomized complete-block design with three replicates was used in each experiment with five plants per rep. Thirty commercial lettuce cultivars and breeding lines of four different types (romaine, Boston, butter/bibb, and leaf). Breeding lines

are elite lines from the University of Florida, Institute of Food and Agricultural Sciences (UF/IFAS) lettuce breeding program. Planting dates were 9 Sept. 2020 (summer/fall), 18 Nov. 2020 (winter), and 22 Jan. 2021 (winter/spring). Vitamin C analysis was conducted using one composite sample for each accession, consisting of the edible portion of 2–4 heads per replicate. Additional sample protection protocols followed Simonne et al. (2002). Horticultural traits were evaluated for five plants at harvest including head weight (HW), marketability, head height and width and core length. Lettuce disorders were registered (tipburn, chlorosis, and bolting) to understand if these accessions were affected by the planting season.

Significant differences ( $P < 0.0001$ ) were found among accessions, experiments, and the interaction accession  $\times$  experiment for vitamin C content. Overall, romaine lettuce had the highest vitamin C content (mg/100 g of fresh tissue) followed by leaf, Boston, and butterhead/bibb, respectively. As previously noted, romaine lettuce seems to be a consistently good source of vitamin C (Simonne et al., 2002; Still, 2007). Accessions with the highest vitamin C content were found in the breeding lines and the commercial cultivar groups. Vitamin C content higher in the middle season planting and harvesting, denoting an important genotype  $\times$  environment ( $G \times E$ ) interaction consistent with previous observations that nutritional compounds are highly influenced by the growing environment (Mou, 2009). Additional significant differences ( $P < 0.05$ ) were found for horticultural characteristics (head weight, width and length, core length) and disorders (tipburn, bolting, chlorosis) among the accessions tested within each type as well. Interactions between accessions  $\times$  experiment were significant for all these traits denoting a  $G \times E$  interaction that should be considered in the breeding process. The differences detected among accessions indicates the availability of genetic variation among the tested material for improving vitamin C content of lettuce. Romaine and leaf lettuce should be considered as sources of improvement for vitamin C since they tend to have a higher content, but breeders must take into consideration the  $G \times E$  controlling the trait. Accessions with an acceptable HW and less tipburn and bolting were identified during this study. The identified germplasm can be used as

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sources for breeding cultivars for hydroponic production with an increased vitamin C content.

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—Scientific Note—

## Quantitative Trait Loci Identification and Marker Development for Papaya Ringspot Virus Resistance in Squash

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Papaya Ringspot Virus (PRSV) is an aphid transmitted pathogenic plant virus threatening squash production worldwide. Viruses being obligate intracellular parasites, chemical and cultural practices for their control are not fully effective. Using a genetic source of resistance is the most effective way to minimize crop loss due to virus. Current study identified quantitative trait loci (QTL) and molecular markers associated with PRSV resistance using whole genome sequencing based on bulked segregant analysis technique. An  $F_2$  population derived from a cross between ‘Nigerian Local’ (PRSV resistant) and ‘Waltham Butternut’ (susceptible) were mechanically inoculated with PRSV and disease ratings were recorded. At 28 days after inoculation 30% of the  $F_2$  individuals were found to be resistant. Two DNA bulks, each from 10 resistant and 10 susceptible  $F_2$  individuals were selected for whole genome resequencing and subsequent QTL mapping. QTL mapping using *QTLseqr* identified one major QTL significantly associated with PRSV resistance on chromosome 9 ( $P < 0.05$ ) which was designated as *QtlPRSV-C09*. Thirteen kompetitive allele specific PCR (KASP) markers were developed within the QTL region and two KASP markers were found to be tightly linked with resistance. Overall, QTL and associated markers identified in the study will facilitate accelerated breeding of stable PRSV resistance in squash through marker assisted selection.

Papaya Ringspot Virus (PRSV) is one of the major constraints in all of the squash growing regions worldwide. However, genomic regions associated with PRSV resistance remain unknown in squash making virus resistance breeding tedious and time-consuming with rigorous phenotypic assays.

### Materials and Methods

Mapping  $F_2$  population was developed by crossing ‘Nigerian Local’ (PRSV resistant) × ‘Waltham Butternut’ (susceptible).  $F_2$  plants were mechanically inoculated with PRSV and screened phenotypically. The 10 most resistant and the 10 most susceptible individuals were selected. DNA from the 10 most resistant and the 10 most susceptible individuals were pooled together to prepare a resistant and a susceptible bulk,

respectively. Resistant and susceptible parents, and resistant and susceptible bulks were sent for whole genome re-sequencing. Sequencing results were used for variant calling and quantitative trait loci (QTL) analysis.

### Results

QTL associated with PRSV resistance were identified on Chromosome 9 that extended from 785,532 bp to 5,093,314 bp and harbored 12,245 SNPs. Thirteen kompetitive allele specific PCR (KASP) markers were developed from the region out of which two were found to be significantly associated with resistance. The markers can be used for accelerated selection of PRSV resistance individuals.

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—Scientific Note—

## Performance of Selective Lettuce Varieties and Evaluation of Bolting in a Top Vent High Tunnel and Open Field Conditions in North Florida

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**Additional index words.** high tunnel, organic production, leaf lettuce

In the South, it is common for organic lettuce producers to suspend lettuce production during the summer months due to an increase in hot temperatures that can trigger bolting. This study evaluated the plant performance of eleven organically grown leafy lettuce in a top vented high tunnel structure and in open field conditions

Four plant growth parameters were used to visually assess the crop weekly. These were head size, plant vigor, and plant health, along with the incidence of bolting. The study was conducted in spring 2021 at the Florida A&M University Research and Extension Center in Quincy, FL. Five types of leaf lettuce (followed by their corresponding varieties) were selected for the evaluation based on the seed supplier's (Johnny's Selected Seeds) heat and bolting attributes. These were green leaf ('Green Star', 'Star Fighter', and 'Oakleaf Pansise'); red leaf ('Vulcan'); romaine ('Coastal Star', 'Monte Carlo', and 'Jericho'); butter head ('Adriana' and 'Buttercrunch'); summer crisp ('Nevada' and 'Magenta'). Each variety was planted in a nine-plant subplot arrangement in a three-way replicated block. Temperature and relative humidity inside the high tunnel and in the open field was recorded using a HOBO USB micro station data logger and analyzed using SAS ver. 9.4. (Cary, NC).

### Findings

Lettuce performance during the cool spring months was varied. Ambient air temperatures were similar inside the high tunnel and in the open field. Regarding head size, the greatest percentage of heads ranked in the average category with 60.8% in the open

field and 57.3% in the high tunnel. Plant vigor ratings were also greatest in the moderate category, with 59.7% in the open field and 54.5% in the high tunnel. Healthier plants were observed in the high tunnel, where 86.3% were in the very healthy category. Conversely, only 51.6% of the plants in the open field were in the very healthy category (Table 1).

Early bolting varieties (< 100 days after seeding) were 'Vulcan' in the open field and 'Vulcan', 'Green Star', and 'Star Fighter' in the high tunnel. Late bolting varieties were 'Nevada', 'Magenta', and 'Pansise' at 120 days in the open field and high tunnel (Fig. 1). Fresh market weight was greatest for 'Magenta' (143.9 g) in the open field and 'Star Fighter' (82.5 g) in the high tunnel.

Further evaluations are being conducted to determine leaf lettuce performance in a high tunnel vs. open field and to evaluate summer, fall, and winter variations.

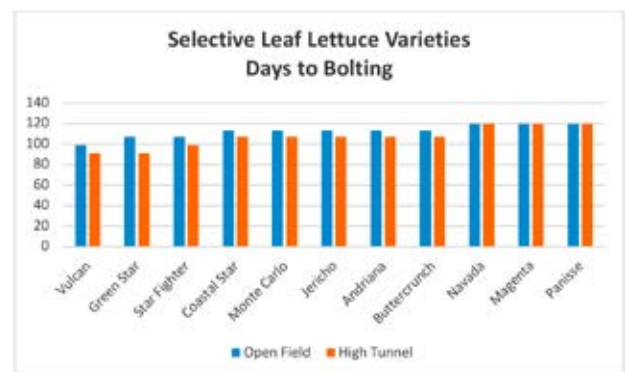


Fig. 1. Days to plant bolting for eleven selective leaf lettuce varieties.

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Table 1. Overall leaf lettuce performance percentages in open field vs. high tunnel by size, vigor, and health visual assessment.

Treat.	Size	Percent	Vigor	Percent	Health	Percent
Open field	Small	15.7	Low	15.1	Not healthy	1.2
	Average	60.8	Moderate	59.7	Acceptable	47.3
	Large	23.5	High	25.2	Very healthy	51.6
High tunnel	Small	2.8	Low	3.7	Not healthy	0.5
	Average	57.3	Moderate	54.5	Acceptable	13.2
	Large	39.9	High	41.8	Very healthy	86.3



## **Discovery and Characterization of Genes Conferring Resistance to Charcoal Rot Caused by *Macrophomina phaseolina* in Cultivated Strawberry**

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### **Abstract**

In the United States, Florida and California produce the vast majority of marketed strawberry (*Fragaria × ananassa*). However, during recent years, both areas have faced increasing threats from disease proliferation. Since the 2005 phase-out of methyl bromide fumigation, *Macrophomina phaseolina* has become a soil-borne pathogen of particular concern for strawberry growers nationwide. *Macrophomina phaseolina* spreads rapidly by soil contamination, causing charcoal rot of the plant crown leading to plant collapse. Current chemical controls for charcoal rot have limitations, making resistant plant cultivars the preferred management strategy. Moderately resistant cultivars exist, and previous work has investigated the genetic architecture of resistance in University of Florida, Institute of Food and Agricultural Sciences (UF/IFAS) strawberry germplasm. Three quantitative trait loci (QTLs) associated with resistance to *M. phaseolina* were discovered on chromosomes 2-2, 4-3, and 4-4, and named *FaRMp1*, *FaRMp2*, and *FaRMp3*, respectively. In this study, we sought to more precisely understand the architecture of resistance by comparative investigation of the QTLs *FaRMp1* and *FaRMp2* using high-quality chromosome-scale octoploid strawberry genomes and molecular markers. Based on these data, high-throughput high-resolution melting (HRM) markers were developed for implementation in marker-assisted seedling selection and breeding for charcoal rot resistance. We conducted further comparative genome sequence analysis and RNA sequencing to identify candidate genes mediating resistance to *M. phaseolina*. This information will provide the practical tools and understanding necessary to enhance genetic resistance to charcoal rot in cultivated strawberry.

The abstract was presented at the 2021 FSHS Annual Meeting.

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## Fine Mapping and Candidate Gene Discovery for the Resistance to Angular Leaf Spot in Cultivated Octoploid Strawberry (*Fragaria ×ananassa* Duch.)

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### Abstract

Strawberry is one of the economically important fruit crops worldwide. Angular leaf spot (ALS) caused by *Xanthomonas fragariae* is the only bacterial disease in strawberry and is problematic in Florida strawberry production. Developing ALS-resistant cultivars is important as all commercial varieties are susceptible to ALS. In the previous study, a major quantitative trait locus (QTL) conferring resistance to ALS, *FaRXfl*, was identified, but the genomic region and genetic mechanisms for ALS resistance have not been characterized. We recently identified candidate genes for *FaRXfl* by fine mapping and transcriptome analysis. For defining the *FaRXfl* locus, seven newly developed molecular markers were used to genotype three populations (n = 663). The *FaRXfl* region was delimited to a 330-kb interval on chromosome 6-2 in the 'Camarosa' reference genome. To identify candidate genes for *FaRXfl*, RNA sequencing was performed with five resistant or susceptible genotypes. We identified one candidate gene that is located within the genomic region of *FaRXfl*. This information will facilitate developing functional DNA markers and elucidating the genetic mechanism of *FaRXfl* controlling resistance to ALS in cultivated octoploid strawberry.

The abstract was presented at the 2021 FSHS Annual Meeting.

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## **Impact of Deep Fumigation on Root-knot Nematodes (*Meloidogyne* spp.), Reniform Nematode (*Rotylenchulus reniformis*), and Nutsedge (*Cyperus* spp.) in Plasticulture Production in Florida**

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### **Abstract**

Fumigation with methyl bromide (MBr) was the primary choice to manage plant-parasitic nematodes and weeds in many horticultural crops in the United States for decades. Since the ban of MBr due to its deleterious effects to the ozone layer has come into effect, producers have transitioned to the use of alternative fumigants. Unfortunately, these alternatives have limited movement in the soil and can provide reduced effectiveness on pests and diseases compared to MBr. The deep application of such fumigants could improve nematode management in but the management potential on other pests is unclear. The objective of this research was to compare the efficacy of three fumigants drip applied at a shallow (2.5 cm) and or deep (38.1 cm) depth for the control of weeds and nematodes in a plasticulture vegetable production system. Double and single cropping field trials were conducted in Quincy, FL, at the North Florida Research and Education Center in 2019 and 2020. Results from the current study have shown that shallow fumigation with dimethyl disulfide (DMDS) (375 L/ha), 1,3-Dichloropropene (1,3-D) (169 L/ha), and metam sodium (MNa) (703 L/ha) is more effective on nutsedge compared to a deep application of these chemicals in both single and double crop systems. However, deep fumigation of 1,3-D (169 L/ha), MNa (703 L/ha), and DMDS (375 L/ha) can be more effective than shallow fumigation to manage reniform nematode in both single and double crop plasticulture systems. No significant effects were observed on root-knot nematode populations in either a single or double crop system. These data illustrate the complexity of soil-borne pest management and the necessity of a systems approach in the post methyl bromide era.

The abstract was presented at the 2021 FSHS Annual Meeting.

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## **Watermelon Planting Decisions with Multiple Risks: A Simulation Analysis**

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### **Abstract**

Watermelon growers choose planting dates every year considering multiple risks. North Florida growers report that timing their harvest to coincide with the best market window is their primary consideration. Earlier harvests typically find more favorable markets, but earlier planting has a higher risk of freeze damage. Research also indicates that risk of Fusarium wilt (a soilborne disease) is higher during cooler weather, adding to the risk of planting earlier. Thus, growers need to balance market risk (e.g., getting a low price) and production risk (e.g., lower harvest or higher cost due to freezing temperatures or disease) in selecting a planting date. The objective of our analysis is to examine the effect of planting date on the distribution of economic returns, considering these multiple risks. We estimate parameters of the probability distributions for key risk factors, based on input from watermelon growers, published price data, historical freeze data, experiment station trials, and the experience of specialists. The distribution of economic returns is then simulated for three planting windows using Simetar<sup>®</sup>, an add-in for Excel<sup>®</sup>. Results demonstrate planting date risk-return tradeoffs, validate the growers' drive to plant early despite higher production risks, and identify thresholds at which delayed planting could be a favorable risk-management strategy.

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## **Comparative Transcriptomes Analysis Reveals Candidate Genes Associated with Powdery Mildew Resistance in Cultivated Strawberry**

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### **Abstract**

Powdery mildew (PM) caused by *Podosphaera aphanis* is one of the major fungal diseases in cultivated strawberry. The development of cultivars resistant to the PM pathogen is a long-term goal of the University of Florida strawberry breeding program. However, the genetics underlying the resistance trait are largely unknown. This study aimed to discover genes associated with PM resistance using transcriptome profiling of selected UF strawberry accessions with varying responses to PM infection. A total of 16 RNA-seq libraries were generated from two resistant ('13.55-195' and '14.34-33') and two susceptible ('Sensation®Florida127' and '12.55-220') accessions at 0 and 24 hours post infection (hpi). RNA-seq analysis showed that the number of differentially expressed genes (DEGs) were significantly higher in susceptible genotypes than resistant genotypes at 24 hpi. In total, 6679 and 7420 DEGs were detected in '13.55-195' and '14.34-33' while 8965 and 8358 DEGs were detected in 'Sensation®Florida127' and '12.55-220', respectively. Among all the DEGs, 31 genes are specifically expressed in both resistant genotypes. These include MYB transcription factor, ABC transporter, hypersensitive-induced response, and photosynthesis-associated genes that could be essential for pathogen-triggered immunity. We also detected 48 DEGs specifically expressed in both susceptible genotypes and identified few candidate susceptibility genes that include *Mildew Resistance Locus O (MLO)* gene members known to play key role during PM infection. Selected candidate genes from RNA-seq were validated using qRT-PCR. Overall, our study provides a valuable information to elucidate the defense mechanism of strawberry during *P. aphanis* infection.

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## **Field Evaluation of CRISPR-driven Jointless Pedicel Fresh-market Tomatoes**

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### **Abstract.**

Clustered regularly interspaced short palindromic repeats (CRISPR)-driven traits are commonly introduced into tomato (*Solanum lycopersicum*) in less than a year. Although CRISPR technology is highly suitable for rapid incorporation of new traits into plants with superior breeding backgrounds, the usefulness of these traits must be demonstrated prior to large-scale distribution of any new variety possessing them. The jointless pedicel trait is crucial for stem-free harvest of tomato fruits and is therefore an essential requirement for developing low-labor tomato varieties. Here, we aimed to evaluate the effect of CRISPR-driven jointless pedicel trait on the yield and horticultural traits of fresh-market tomatoes. During three consecutive seasons, we conducted field trials using three related genotypes of two elite fresh-market tomatoes, namely a jointed pedicel tomato, a CRISPR-driven jointless pedicel mutant of the jointed pedicel tomato, and a conventionally-bred jointless pedicel near isogenic line (NIL) to the jointed pedicel tomato. Field evaluations confirmed that the total and medium-, large-, or extra-large-size fruit yields of the CRISPR-driven mutants were not statistically different for those corresponding to their backgrounds and that the fruit yield stratified by fruit size might be varied under different growth conditions. We did not observe any negative correlation between the genotype and the fruit/abscission joint detachment force for any of the genotypes under study. Field evaluation of the jointless pedicel-correlated traits could be effectively conducted by combining objective fruit-quality measurement with high-performance computing to accelerate analysis and decision making.

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## **Screening for Biotic and Abiotic Stress Resistant Somatic Clones From Cultivated Strawberry**

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### **Abstract**

Variation in somatic clones occur naturally through the process of shoot regeneration from callus using tissue culture. This variation can be heritable by means of genetic or epigenetic changes and provides an alternative approach to conventional breeding or transgenic methods to introduce desirable important agronomic traits to the crops. Here, we report that our University of Florida, Institute of Food and Agricultural Sciences strawberry breeding program developed a protocol for shoot regeneration from callus induced either from runner segment or in vitro grown leaf tissue of octoploid strawberry as an explant. Preliminary field evaluation showed a considerable amount of phenotypic variation among the regenerants. We then selected for increased *Phytophthora* crown rot resistance by inoculating somatic clones of Sweet Sensation® 'Florida 127' and 'Florida Radiance'. Furthermore, we screened clones from 'Florida Radiance' and 'Florida Beauty' for heat resistance. Generally, 10% of the population survived the biotic or abiotic stress. Our findings suggest that the method of somatic variation has a great potential to improve elite strawberry varieties for disease resistance and fruit quality. In this presentation, we will discuss the procedure and the results in detail.

The abstract was presented at the 2021 FSHS Annual Meeting.

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## **A Simple Marker-assay for Selecting Hull-less Seed Trait in Pumpkin**

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### **Abstract**

Pumpkin (*Cucurbita pepo*) seeds are nutritious and valued as a source of protein (35% w/w), vegetable oil (50%), and healthy unsaturated fatty acids (80%). Hull-less pumpkin seeds are preferred by the processing and snacking industry because they do not require de-hulling prior to use. Other important traits for hull-less pumpkin include seed yield, seed size, and most recently, flesh quality for dual-purpose use. A single recessive gene, designated *n*, controls the hull-less seed trait in pumpkin. Phenotypic selection for the hull-less seed trait in segregating populations is resource intensive because a large number of individuals must be evaluated at fruit-maturity to increase the likelihood of identifying those homozygous at the *n* loci. Marker-assisted selection could facilitate detection of the hull-less seed trait in pumpkin, but simple high throughput marker-assays are not currently available. In the current study, QTL-seq bulk segregant analysis was applied to a segregating F<sub>2</sub> population derived from a cross between 'Kakai' (hull-less) × 'Table Gold Acorn' (hulled). A single QTL peak (*QTLh\_C12*) significantly ( $P < 0.05$ ) associated with the hull-less seed trait in pumpkin was detected on chromosome 12. Twenty single nucleotide polymorphism (SNP) markers within the QTL's confidence interval were converted into KASP assays and tested for association with the hull-less seed trait. One SNP marker (*Chr12-3*) was significantly associated with the trait, and successfully differentiated the hull-less from the hulled individuals in the F<sub>2</sub> population. This marker will facilitate marker-assisted selection for the hull-less seed trait in pumpkin.

The abstract was presented at the 2021 FSHS Annual Meeting.

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## Ripening Inhibition and Quality of Selected Tropical Fruits in Relation to 1-MCP Controlled Release Technology from Hazel Technologies

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**Additional index words:** 1-methylcyclopropene, shelf life

**Abstract:** The use of in-package 1-MCP technology shows potential to extend distribution and broaden consumer availability of some tropical fruits, as well as reduce food waste by extending the shelf life of these ethylene-sensitive commodities. In this study, the Hazel Technologies 1-MCP slow-release sachet was evaluated for its effectiveness in extending the shelf life of six tropical fruits. These were mango (*Mangifera indica*), atemoya (*Annona × atemoya*), passionfruit (*Passiflora edulis*), Guatemalan-West Indian hybrid avocado (*Persea americana*), guava (climacteric and nonclimacteric types) (*Psidium guajava*), and Maridol type papaya (*Carica papaya*). The fruits were harvested from the University of Florida/IFAS (UF/IFAS) Tropical Research and Education Center in Homestead, FL and transported to the Postharvest Lab at UF/IFAS in Gainesville. Initial evaluations of ripeness and quality were performed before placing the fruit samples in the appropriate storage containers and conditions. Ripening parameters were monitored for control fruit and those treated with 1-MCP during storage until a significant percentage of the fruit were judged either to be overripe or unmarketable or when sufficient time had passed to accommodate any conceivable distribution marketing chain duration. Fruit quality was periodically assessed during storage. Based on the results of these experiments, the shelf life of these tropical fruits can be extended from 5 days up to 3 weeks with 1-MCP. Hazel Technology 1-MCP slow-release sachets resulted in slower ripening for 1-MCP-treated fruit compared to the control fruit, especially during the first week of storage. Irregular and non-uniform ripening was seen in some fruit, but the majority ripened normally if they did not become unmarketable due to decay.

1-Methylcyclopropene is a potent gaseous ethylene inhibitor that binds irreversibly to the ethylene receptors in plant tissues (Blankenship and Dole, 2003). Climacteric fruits serve as the predominant target for the investigation of 1-MCP, and the responses of these fruits confirm that the antagonist operates in opposition to ethylene (Huber, 2008). Ethylene management plays a pivotal role in maintaining postharvest life and the quality of horticultural commodities (Mahajan et al., 2014). For highly perishable and fast ripening crops like tropical fruits, ethylene management is key to slowing ripening and gaining flexibility during shipping. The use of in-package 1-MCP technology shows potential to extend distribution and broaden consumer availability of some tropical fruits, as well as reduce food waste by extending the shelf life of these ethylene-sensitive commodities (Hazel Technologies, 2021). In this study, the Hazel Technologies 1-MCP slow-release sachet was evaluated for its effectiveness in extending the shelf life of six tropical fruits. These fruits were mango (*Mangifera indica*), atemoya (*Annona × atemoya*), passionfruit (*Passiflora edulis*), Guatemalan-West Indian hybrid avocado (*Persea americana*), guava (climacteric and nonclimacteric types) (*Psidium guajava*), and Maridol type papaya (*Carica papaya*).

### Materials and Methods

The fruits used in this study were harvested at different ripeness stages (chosen as appropriate for each fruit type) and transported from the University of Florida Institute of Food and Agricultural Sciences (UF/IFAS) Tropical Research & Education Center in Homestead, FL to the Postharvest Laboratory at UF/IFAS in Gainesville. Initial evaluations of ripeness stage and quality were performed. The fruit samples were stored in controlled temperature and humidity storage rooms at the appropriate temperature for each type to avoid chilling injury (Gross et al., 2016), with 85 to 95% relative humidity (RH) maintained unless otherwise noted. In most cases, storage temperature was around 10–15 °C. The fruit in the air control and 1-MCP treatments were kept in separate rooms for the duration of storage. After different storage periods, the fruit were transferred to 20 °C for ripening. The fruit were maintained in their shipping cartons (corrugated, waxed fiberboard with no lid) if available.

Ripening parameters were monitored for control fruit and those treated with 1-MCP during storage until a significant percentage (typically > 25%) of the fruit were judged to be either overripe or unmarketable or when sufficient time had passed to accommodate any conceivable distribution marketing chain duration. Fruit quality was periodically assessed during storage (approximately 1 time per week) by evaluating or measuring appearance, weight changes, firmness, uniformity of external/internal color, soluble solids content (SSC), and total titratable

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Table 1. Effectiveness of Hazel Technology 1-MCP slow-release sachets in extending shelf life of several tropical fruits.

Fruit type	Effective in extending shelf life? (Yes/No)	Shelf life extension with 1-MCP (days or weeks)	Needs more research? (Yes/No)
Atemoya	No	n/a <sup>2</sup>	Yes
Avocado	Yes	2–3 weeks	No
Guava (climacteric)	Yes	1–1.5 weeks	No
Guava (nonclimacteric)	No	n/a	No
Mango	Yes	2 weeks	No
Papaya	Yes	1–1.5 weeks	No
Passionfruit	Yes	5 days	Yes

<sup>2</sup>n/a = not applicable.

acidity (TTA). For visual evaluations and documentation, pictures and notes were taken on visual changes.

### Results and Discussion

For mangos, it was determined in preliminary research that one sachet was effective for 4.5 kg or one standard carton of mangos stored at 12 °C. This is a standard that was carried over to the other experiments. The 1-MCP resulted in less green to yellow color changes, less softening, and lower TTA than the control. Anthracnose decay affected the mangos in all treatments and the decay progressed with ripening. Nonuniform ripening was observed. The Hazel 1-MCP sachets were somewhat effective in extending the shelf life of ‘Tommy Atkins’ and ‘Keitt’ mangos by about 2 weeks.

For atemoya, due to their high sensitivity to chilling injury, a ripening temperature of 20°C was selected for this treatment. The treatments for this experiment consisted of 0, 1, or 3 sachets. After 4 days at 20 °C, both the control and 1-MCP-treated fruit showed surface browning and decay, which made them unmarketable. This change revealed that a lower temperature should have been used for the 1-MCP application to mimic shipping conditions. Whether 1 or 3 sachets were more effective could not be concluded. This experiment may be repeated with a larger quantity of fruit and an updated protocol for more accurate results.

For passionfruit, 0, 1, or 3 sachets were used on groups of 10 ‘Ruby Red’ fruit. The sachets showed potential for extending the shelf life. However, similar to the atemoya, this result is based on a small number of fruit and needs to be re-tested on more fruit with an improved experimental protocol. After 6 days of storage at 20 °C, the fruits were evaluated and each treatment (control, 1 sachet, and 3 sachets) developed approximately 20% decay (gram-negative grey mucoid bacteria). Pending availability, this fruit may be tested again.

For avocado, the 1-MCP sachets showed a significant impact in extending the shelf life of multiple varieties of avocado by approximately 2–3 weeks at 7 °C. ‘Choquette’, ‘Arue’, ‘Donnie’, and ‘Booth 8’ avocados were studied during the 2020 and 2021 seasons. From 2–4 sachets were used on each treatment group based on weight. After 14 days of storage, there was a noticeable difference between the control and 1-MCP-treated avocados. The control fruit developed browning and softening while the 1-MCP-treated fruit stayed green with little softening. By day 20, the control fruit were 80% brown and the 1-MCP-treated fruit were still green, though they were starting to soften. From this research, we can say that 1-MCP treatment of avocados can extend their shelf life by 12–20 days depending on the variety and the treated fruit still complete ripening in a normal manner.

For guava, climacteric ‘Para’ guavas stored at 7 °C were studied. The 1-MCP sachets (2 sachets per 24 fruit) were effective in extending the shelf life of these fruit by about 1–1.5 weeks, but were not effective in suppressing decay from Phoma leaf spot and gram-negative white mucoid bacteria. ‘Thai’ and ‘Watermelon’ nonclimacteric guava were also studied but the 1-MCP sachets had minimal effect in extending the shelf life of those fruit because ethylene is not involved in the ripening process. Minimal changes were observed through all methods of fruit quality analysis. After 2 weeks, *Pestalotia* spp. fungus that causes bitter rot was observed in >25% of the fruit.

For papaya, ‘Tainung’ Maridol type papayas were harvested at a range of ripeness stages from mature green to 3/4 color. Prochloraz and thiabendazole (TBZ) fungicides were applied according to label instructions to attempt to prevent anthracnose and other decays. The 1-MCP treatment was 3 sachets per 11–14 fruit (about 14 kg). The papayas were evaluated periodically during storage for 2 weeks at 12 °C. The 1-week evaluation showed 25% decay in 1-MCP-treated fruit while the control had 50% decay. The 2-week evaluation had 41% decay in the 1-MCP-treated papayas compared to 53% decay of the control fruit. Phoma leaf spot and gram-negative white mucoid bacteria were detected. Due to the incidence of decay, it was concluded that Hazel’s 1-MCP sachets were somewhat effective in extending the shelf life by 4–10 days, but without effectively controlling decay.

### Conclusions

Overall, Hazel Technology 1-MCP slow-release sachets resulted in slower ripening for treated fruit compared to the control fruit, especially during the first week of storage (Table 1). Irregular and non-uniform ripening was seen in some fruit, but the majority ripened normally if they did not become unmarketable due to decay. No significant differences were measured in SSC, pH, or TTA at the fully ripe stage for any of the fruits tested. Overall, the results confirmed that 1-MCP-treated fruit were eventually able to resume and complete normal ripening. Decay was a significant factor in rendering many fruits unsuitable for further measurement. The 1-MCP sachets had a small effect in inhibiting decay. Anthracnose, Phoma leaf spot, and gram-negative white mucoid bacteria were the most common decay pathogens.

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## Total Phenolics as an Indicator of Quality in Florida-grown Tea

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**Additional index words.** *Camellia sinensis*, phytochemistry, specialty crops, tea, total phenolics

Tea (*Camellia sinensis*) is emerging as a potential specialty crop for Florida, but quality evaluations of Florida-grown tea have not yet been reported. Phytochemicals contributing to tea quality include phenolics, such as catechins, tannins, and flavonoids, which contribute astringency and bitterness. We harvested two varieties of Florida-grown tea and processed them into green tea, then evaluated each variety of made-tea for total phenolics (TP): 'Fairhope', sourced from Alabama and 'Georgian' from Mississippi State University. Both have been growing in North-central Florida since 2016. TP levels were comparable to commercially-sourced green tea in both methanolic extracts and hot water tea infusions, measured as milligrams of catechin equivalent (CE) per gram dry weight of made-tea. In methanolic extracts, 'Fairhope' had  $99.67 \pm 5.77$  mg·g<sup>-1</sup> CE and  $91.90 \pm 5.54$  mg·g<sup>-1</sup> CE in July 2020 and May 2021 harvests, respectively. 'Georgian' extracts had  $106.27 \pm 5.96$  mg·g<sup>-1</sup> CE and  $124.86 \pm 6.46$  mg·g<sup>-1</sup> CE in July 2020 and May 2021 respectively. A commercially-sourced green tea showed TP levels of  $81.58 \pm 5.22$  mg·g<sup>-1</sup> CE. There were no significant differences ( $P = 0.05$ ) between spring and summer harvests, or between the Florida-grown teas and the commercially-sourced tea, except for the May harvest of 'Georgian,' which had higher TP than both 'Fairhope' and the commercial standard. Hot water infusions of made-tea from the 2020 harvests of 'Fairhope' and 'Georgian' and the commercial control also did not differ significantly, with  $25.95 \pm 2.94$  mg·g<sup>-1</sup> CE,  $30.55 \pm 3.19$  mg·g<sup>-1</sup> CE and  $24.19 \pm 2.86$  mg·g<sup>-1</sup> CE, respectively. This work indicates that Florida-grown tea has phenolics levels of suitable quality for processing and marketing as green tea.

Tea (*Camellia sinensis*) is an important beverage worldwide, both culturally and for health-promoting qualities (Khalesi et al., 2014). A variety of compounds present in tea leaves contribute to the quality of prepared tea (Kottawa-Arachchi et al., 2019). One important class of compounds found in tea is phenolics; these include catechins, tannins, and flavonoids. Catechins are the dominant fraction of this class; the total dry weight of tea leaves can comprise up to 30% catechins (Han et al., 2016). Phenolics in tea leaves have been reported to change with processing method and season of harvest, contributing to changes in flavor and quality (Fang et al., 2017). This can have varying effects on quality of made-tea. Phenolics primarily contribute to the astringency, bitterness, and color aspects of tea quality (Zhang et al., 2020).

Tea is emerging as a specialty crop in the United States. Tea thrives in the subtropics, preferring sandy, acidic soils and humid conditions. Tea is of special interest for Florida growers, since it thrives in conditions similar to those preferred by citrus and blueberry and could be a potential economic buffer crop. Tea has marketing potential both as a finished horticultural product (made-tea) and as a nursery crop intended for home growers (Mavrakis, 2016). Establishment trials have identified several accessions of tea that perform well in North Florida field conditions, and some accessions that, despite showing lower vigor, may have high quality traits. The quality of teas made from mature ( $\geq 5$ -year-old) tea plants grown in field conditions has not yet been assessed. Given the large contribution of phenolics to tea quality, an assessment was made of the total phenolics (TP) of green tea made from two accessions growing in North Florida field conditions. One of these accessions, 'Fairhope', has shown good performance in the field

regarding survivorship and vigor, and produces a mid-quality green tea. The other accession, 'Georgian', has proved to be less vigorous than 'Fairhope,' but produces a green tea with pleasant taste and aroma. Green tea made from these two accessions was compared to Maeda-en Sencha, a sencha-style green tea produced in Japan and commercially available in the US.

The objectives of this study were to determine the total levels of phenolics present in each accession and whether they differ between seasons or by accession, and compare the TP levels to a commercial standard to assess the quality of Florida-grown teas.

### Materials and Methods

Tea plants were grown at the Plant Science Research and Education Unit in Citra, FL (29°24'27.6"N; 82°08'27.7"W). One-year-old plants were established in Spring 2016. Two accessions were used in this study: Fairhope, sourced from Fairhope Tea Plantation in Fairhope, AL, and Georgian, sourced from Mississippi State University.

The apical meristem and first two leaves of the two different tea accessions were harvested in July of 2020 and May of 2021. Harvested leaves were processed into green tea. This procedure includes withering (5 h at 20 °C.), steaming (5 min), rolling (10–20 min), and drying (2 h at 77 °C) (Fig. 1). Processed tea leaves were stored at 4 °C until analysis.

Three biological replicates were used for every sample in both methanol and hot water extractions.

**Methanol Extraction.** Dry processed tea leaves (50 mg) were frozen in liquid nitrogen, homogenized in 6 mL of 95% methanol for 30 s, inverted 3x, and incubated for 16 h at 22 °C. Samples were centrifuged at 200 g for 10 min in an IEC Clinical Centri-

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Fig. 1. Processing harvested tea leaves into green tea. Leaves were plucked at two-and-a-bud stage, then withered in paper bags for about 5h. The leaves were then steamed for 5 min, after which they were rolled in a stand mixer for 15 min. The processed leaves were dried at 77 °C until a stable weight was reached.

fuge (IEC, Irvine, CA) and the supernatant was collected (Yao et al., 2004).

Extraction efficiency was evaluated by three sequential extractions of two samples of Maeda-en Sencha and one sample of 'Fairhope.' Dry processed tea leaves (50 mg) were extracted according to the method described above. The tissue was re-extracted in a fresh 6 mL aliquot of 95% methanol for 2 h at 22°C, and this step was then repeated. First, second, and third extracts were then reacted in the Folin-Ciocalteu assay to determine TP levels in each successive extraction.

**Hot Water Extraction.** Dry processed tea leaves (500 mg) were placed in glass tubes with 29.5 mL of water and held at 80 °C in a hot water bath for 1 min. The tea leaves were removed and the extract was used in the Folin-Ciocalteu TP Assay.

**Folin-Ciocalteu TP Assay.** Extracts were reacted in a Folin-Ciocalteu assay modified after Ainsworth and Gillespie (2007). Diluted extract samples (10 µL of extract and 90 µL 95% MeOH) were placed into glass test tubes. Freshly prepared Folin-Ciocalteu reagent (10%, 200 µL) was added. Samples were vortexed and 800 µL of 700 mM sodium carbonate (Na<sub>2</sub>CO<sub>3</sub>) were added. The reaction was incubated at 22 °C for 2 h and the absorbance at 765 nm was read using a DU730 UV visible spectrophotometer (Beckman Coulter, Brea, CA). The standard used was ± catechin (C<sub>15</sub>H<sub>14</sub>O<sub>6</sub>). Total phenolics levels are reported as catechin equivalents (CE).

## Results and Discussion

**Methanol Extraction.** Total phenolics levels for green tea made from Florida-grown tea plants were found to be comparable to commercial standard green tea (Fig. 2). 'Fairhope' had 99.67 ± 5.77 mg·g<sup>-1</sup> CE in leaves from the July 2020 harvest and 91.90 ± 5.54 mg·g<sup>-1</sup> CE in leaves from May 2021. 'Georgian' extracts had 106.27 ± 5.96 mg·g<sup>-1</sup> CE in July 2020 and 124.86 ± 6.46 mg·g<sup>-1</sup> CE in May 2021. Maeda-en Sencha, the commercially-sourced green tea, showed TP levels of 81.58 ± 5.22 mg·g<sup>-1</sup> CE. There was no difference between Maeda-en Sencha and the July 2020 harvests of 'Fairhope' and 'Georgian,' or the May 2021 harvest of 'Fairhope.'

There was no significant seasonal change in TP within accessions. Among accessions, the May 2021 harvest of 'Georgian,' showed TP levels significantly higher than the May 2021 harvest of 'Fairhope' ( $P < 0.01$ ) and Maeda-en Sencha ( $P < 0.001$ ). The

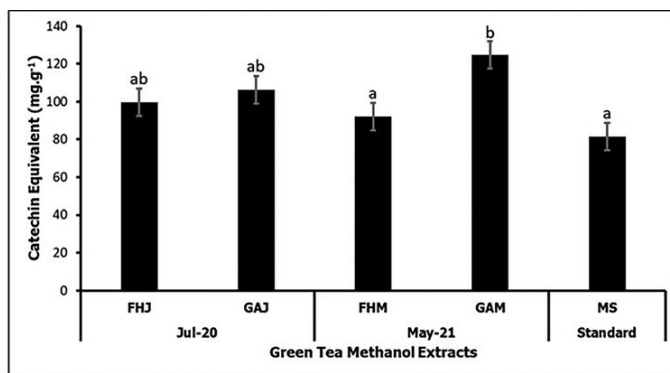


Fig. 2. Total phenolics (TP) of methanol extractions of green tea, expressed as catechin equivalents (mg·g<sup>-1</sup>). FHJ = 'Fairhope' July 2020. FHM = 'Fairhope' May 2021. GAJ = 'Georgian' July 2020. GAM = 'Georgian' May 2021. MS = Maeda-en Sencha.

higher levels of TP seen in the May 2021 harvest of 'Georgian' could be explained by the presence of lesions on the leaves of most 'Georgian' plants that season. A separate assay of only lesioned leaves collected in June 2021 showed TP levels higher than all samples except for the May 2021 'Georgian' harvest (data not shown).

The extraction efficiency for the methanolic extractions was > 97% recovery of TP in the first of three sequential extractions tested (data not shown).

**Hot Water Extraction.** The July 2020 harvests of 'Fairhope' and 'Georgian' used for the hot water extractions showed TP levels comparable to Maeda-en Sencha, with 'Fairhope' at 25.95 ± 2.94 mg·g<sup>-1</sup> CE, 'Georgian' at 30.55 ± 3.19 mg·g<sup>-1</sup> CE and Maeda-en Sencha at 24.19 ± 2.86 mg·g<sup>-1</sup> CE (Fig. 3). These data indicate that total phenolics of Florida-grown tea extracted by hot water, as would be done to make tea for drinking, are of a suitable level to meet commercial quality standards. The hot water extracts showed much lower levels of TP in comparison with the methanolic extracts, with 26% of methanol-extracted TP present in 'Fairhope,' 29% in 'Georgian,' and 30% in Maeda-en Sencha. The hot water extracts were only incubated for 1 min, while the methanolic extractions were incubated for 16 h. However, the temperature of incubation was much higher for the hot water extraction (80 °C) compared to the methanolic extraction (22 °C). Previous studies have found that methanol results in higher recovery of phenolics from plant matter, compared to other solvents including hot water, ethanol, and chloroform (Yao

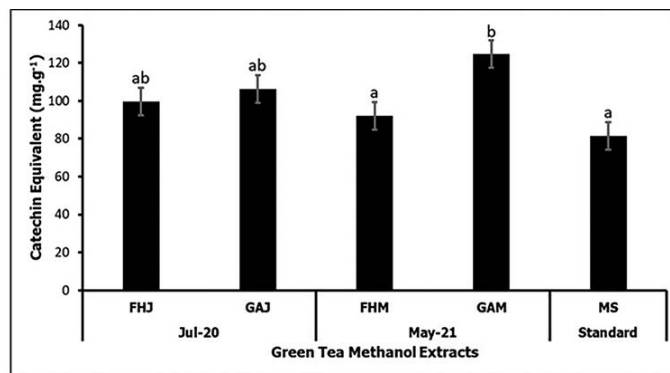


Fig. 3. Total phenolics (TP) of green tea extracted in hot water (80 °C) expressed as catechin equivalents (mg·g<sup>-1</sup>). FHT = 'Fairhope' tea. GAT = 'Georgian' tea. MST = Madea-en Sencha tea.

et al., 2004). This result can serve to inform an estimate of what proportion of the total plant phenolics are being consumed when drinking green tea.

These results indicate that the TP levels found in Florida-grown green teas are comparable to those found in commercially available green tea. Further analyses will investigate the profile of phenolics present in each accession by mass spectrometry, as well as other quality factors including L-theanine and caffeine.

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## Preservation of Postharvest Quality of Fresh Bamboo Shoots via Suppression of Lignification, Browning, and Decay: A Review

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**Additional index words.** 1-methylcyclopropene, *Bambusa*, hydrocooling, melatonin, modified atmosphere packaging, *Phyllostachys*, *Zizania*

Bamboo shoot, the peeled, growing meristematic tissue of several edible species of bamboo, is a desirable food crop because, once cooked, it is high in protein and vitamins, low in fat, and has a hedonically pleasurable crisp texture. The potential exists for bamboo shoot to become a lucrative crop for Florida growers given typical returns of \$3.50 per pound wholesale. When striving to preserve shelf life of fresh bamboo shoot, growers and packers face issues such as lignification (toughening of tender edible tissue to a woody, inedible state), yellowing, moisture loss, and chilling injury. This review discusses the three main categories of techniques available to packers to address these concerns: environmental management, applied compounds, and packaging. Environmental management techniques range from the very simple, beginning with field temperatures at harvest and transport time, heat treatment, thorough cooling techniques like hydrocooling and forced-air cooling, proper storage conditions, to the more complex such as ultraviolet light, hypobaric storage, and hydrostatic pressure, to sophisticated techniques unlikely to be applied in the packinghouse, such as gamma radiation. Applied compounds such as 1-methylcyclopropene (1-MCP), brassinolide, melatonin, oxalic acid, salicylic acid, and chlorine dioxide in combination with chitosan have also been reported to have beneficial effects in suppressing lignification and otherwise preserving edible quality of bamboo shoot. Packaging films focus on the composition of the film overwrap, use of perforations, modified atmosphere packaging, and packing density. Further, practical studies are suggested to determine the appropriate storage temperature and to compare these techniques individually and in combination to determine which are the most effective and the most realistic under typical packinghouse conditions.

Bamboo refers to a group of nearly 1500 species of plants in the grass family (*Poaceae*) that have firm stems and grow to heights ranging from 4 to 30 meters. The peeled, actively growing shoot tip, known as meristematic tissue, is referred to as the 'bamboo shoot', a somewhat crunchy vegetable that must be processed for consumption by pickling, drying, or other methods. The leading producer and exporter of bamboo shoot, and subsequently bamboo shoot research, is China. Current production values are difficult to find, but in 1999, China alone consumed over 1.3 million tons of bamboo shoot from 4 million hectares of production. India was another key production center with nearly 3 million ha (Collins and Keilar, 2005).

Bamboo shoot has potential to become more desirable to the consumer due to its nutritional composition—it is low in fat but high in proteins and vitamins (Nongdam and Tikendra, 2014). A fresh bamboo shoot of the highest consumable quality will be crunchy/crisp in texture yet firm rather than woody, with a sweet flavor. Freshly processed bamboo shoots are reported to have better flavor than those that are canned. Ideal pulp tissue color is cream-colored to pale yellow; however, over time the fresh shoot darkens to a deeper yellow or browning as one sign of senescence.

Not all bamboo species produce edible shoot tips; some are simply too woody to consume. Genera usually cultivated for consumption and discussed in this review include *Bambusa*, *Phyllostachys*, and *Zizania*. It is important to note that the edible species contain cyanogenic glucosides that break down to toxic

hydrogen cyanide—harmful to an adult when as little as 0.002 ounce (57 mg) is consumed raw. These compounds are readily inactivated by boiling for 30 min.

Sheaths (bracts) surround and protect the edible shoot. The entire shoot is harvested by sawing at approximately 30–45 cm in length (Fig. 1), then transported in bulk to the packing facility. For fresh market the sheaths are cleaned (Figs. 2–4). There are several postharvest challenges that bamboo shoot farmers and packer/shippers face. Lignification is the toughening of the shoot tissue that renders it inedible. Lignin is quickly synthesized in living plant tissues during room temperature storage by the enzymes phenylalanine ammonia lyase (PAL), cinnamyl alcohol dehydrogenase (CAD), and peroxidase (POD). This process also occurs with asparagus, another actively growing shoot tip. Other postharvest challenges include discoloration of the sheath, proliferation of decay-causing microorganisms, moisture loss, and yellowing or browning at the cut surface. The edible shoots are also susceptible to chilling injury which is characterized by lignification and water soaking.

Many solutions have been identified to address these concerns and extend the quality of fresh bamboo shoots. The following sections review pertinent literature that describes tests in which intact bamboo shoots (sheath and shoot tips, as in Fig. 2) were stored under various conditions while the quality changes refer to the edible shoot portion. They are grouped here under environmental management, applied compounds, and packaging. Proper cooling is the most important factor in maintaining postharvest quality; other treatments, while considered secondary, can further extend shelf life beyond that obtained by cooling alone.

The authors would like to express their appreciation to Kevin Barley at OnlyMoso.

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Fig 1. Bamboo shoot in process of harvest.



Fig. 3. Edible portion of the bamboo shoot.



Fig. 4. Edible bamboo shoot, sliced open.



Fig. 2. Freshly harvested bamboo shoots after cleaning/brushing in the packinghouse. (Credit: Kevin Barley, OnlyMoso).

### Environmental Management

*Temperature management.* Refrigeration is a key factor in preserving postharvest quality of fresh bamboo shoots. Temperatures ranging from 1, 2, and 5 °C have been investigated compared with warmer alternatives and in each case, the warmer alternative resulted in quicker loss of quality. Room-temperature storage at 20 to 25 °C without packaging resulted in a 25% loss in the product's fresh weight due to transpiration through the cut end of this rapidly expanding tissue, limiting usable shelf life to a single day (Kleinhenz et al., 2000). Higher storage temperatures also favor growth of decay microbes. Chen et al. (1989) observed that bamboo shoots stored at 30 °C (ambient temperature in their region) for 5 d contained twice the crude fiber content of shoots stored at 5 °C. Their work found that lower temperature storage suppressed activity of the enzymes PAL and POD, thus delaying lignification. Chang and Yen (2008) also verified that shoots remained marketable after 6 d at 5 °C whereas those stored at 25 °C were no longer marketable.

Other researchers reported that bamboo shoots stored for 10 d at 2 °C were 10 N softer, contained higher amounts of total soluble sugars, had lower cellulose and lignin contents, and demonstrated lower PAL activity than shoots stored at 20 °C (Luo et al., 2008). However, during long-term storage (30 d) shoots stored at 2 °C

continued to toughen, indicating that the lignification was only delayed by the cold temperature, but not prevented. This delay then subsequent increase in lignification was reflected in increased lignin and cellulose contents and PAL activity and a corresponding decrease in sugars. After this long storage period (30 d), chilling injury was implicated in the lignification of tissue due to the visual observation of watersoaking. Shoots stored at 1 °C developed only minor yellowing, while those stored at 8 °C developed dark, soft patches after 6 d of storage (Kleinhenz et al., 2000).

**Cooling methods.** Rapid cooling (“precooling”) has long been employed to maximize postharvest life (shelf life) of fresh produce by reducing the pulp temperature shortly after harvest. Techniques like hydrocooling (showering with or immersing in near-freezing water) and forced-air cooling (using high-velocity fans to draw refrigerated air through freshly-harvested product) are the most commonly employed methods for a wide variety of fruit and vegetable commodities.

During cooling, the field heat (also called sensible heat) from the product is transferred to the cooling medium (air, water, crushed ice). Effective cooling depends on careful management of three factors: time, temperature and contact. To maximize cooling, 1) the product must remain in the cooler for sufficient time to remove heat, 2) the cooling medium must be maintained at constant temperature throughout the cooling cycle, and 3) the cooling medium must have continuous, intimate contact with the surfaces of the crop. If the crop is packed in containers with insufficient vent or drain openings or if the containers are incorrectly stacked on pallets so that the vent holes do not align, the flow of the cooling medium can be severely restricted, significantly extending cooling times (Sargent et al., 2008).

Effective cooling significantly benefits the shipping life of the product, and a widely accepted definition of commercial cooling for perishable crops is: “The rapid removal of at least 7/8 of the field heat from the crop by a compatible cooling method”. The time required to remove 7/8 of the field heat is called the “7/8 Cooling Time”, and is the most effective and efficient time (Fig. 5). Removal of the remaining 1/8 of the field heat occurs during subsequent refrigerated storage and handling with minimal effect on crop quality. Although the cooling curve in Fig. 5 was

based on room cooling, the intermediate time/temperature relationships (i.e., 1/2 Cooling; 3/4 Cooling) can be extrapolated to other cooling methods.

Kleinhenz and Midmore (2002) stated that hydrocooling reduces pulp temperature more rapidly than air cooling, and the rate at which a shoot cools is affected by the weight of the shoot, with smaller shoots cooling more rapidly. They reported that 2.5 h of hydrocooling was necessary to cool 1 kg of bamboo shoots from 30 °C to 1 °C using ice water, compared with 10 h of room cooling (air temperature not specified). It is critical that an approved sanitizer be added to the hydrocooling water and maintained during cooling to avoid accumulation of decay pathogens in the water during cooling (Sargent et al., 2008). Chang and Yen (2008) found that hydrocooling reduced internal temperature of the bamboo shoot more rapidly than forced-air cooling. They also noted that soluble solids content was higher in forced-air cooled bamboo shoots. Soluble solids content (SSC), or degrees Brix as a measure of sweetness, is around 5–7 °Brix for bamboo shoot depending on storage temperature; the longer a shoot was hydrocooled, the lower the SSC. These authors ultimately recommended that a two-step combination be employed for maximum cooling benefit with minimum loss of nutritional value. First, hydrocooling water was maintained at 5 °C, cooling the pulp of freshly harvested bamboo shoots to below 10 °C after 20 min. Second, the shoots were then transferred to forced-air cooling for a second 20-min cycle in 5 °C air. Nutritional composition, therefore, was maximized, and the cooling air dried the cut surfaces and sheath tissues, thus reducing the potential for decay to develop during subsequent handling and shipping. However, actual cooling times and storage temperatures for this cooling procedure need to be verified under Florida conditions, particularly regarding effects on shoot quality and shelf life. Forced-air cooling may benefit nutritional composition, and drying the cut surface and sheath tissues may reduce the potential for decay to develop during subsequent handling and shipping.

**Heat treatment.** Elevated temperatures can slow or stop the activity of the enzymes involved in lignification. Bamboo shoots pretreated for 5 h at 45 °C/90% relative humidity (RH) had lower amounts of lignin and cellulose during storage for 12 d at 20 °C/90% RH compared to unheated control tissue held constantly at 20 °C (Luo et al., 2012). Under these conditions, lignin was initially just below 0.50% and increased to nearly 0.75% in unheated control shoots but to less than 0.65% in heated shoots. Cellulose, which was initially just above 0.8%, was approximately 1.05% in control shoots and approximately 0.95% in treated shoots. PAL and POD enzyme activity was also higher in control tissue than in heat treated tissue.

**Ultraviolet (UV) light.** Ultraviolet light, at wavelengths of 100–400 nanometers (nm), is invisible to the human eye. The UV spectrum is divided into UV-A (400–315 nm), UV-B (314–280 nm), and UV-C (100–279 nm). All three types of UV light have been documented as potentially beneficial to preserving the shelf life of a wide range of commodities, either by inactivating pathogens by exposing them to sufficient energy to disrupt the nucleic acids or by stimulating the tissue to form beneficial secondary metabolites that can protect it from pathogen attack. In this case, UV-C (254 nm) has been observed to retard toughening in bamboo shoots (Bo et al., 2019) by inducing the formation of antioxidants that remove reactive oxygen species (ROS) and hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) that stimulate lignification. Bamboo shoots pretreated with a 4.24-kJ/m<sup>2</sup> (kiloJoules

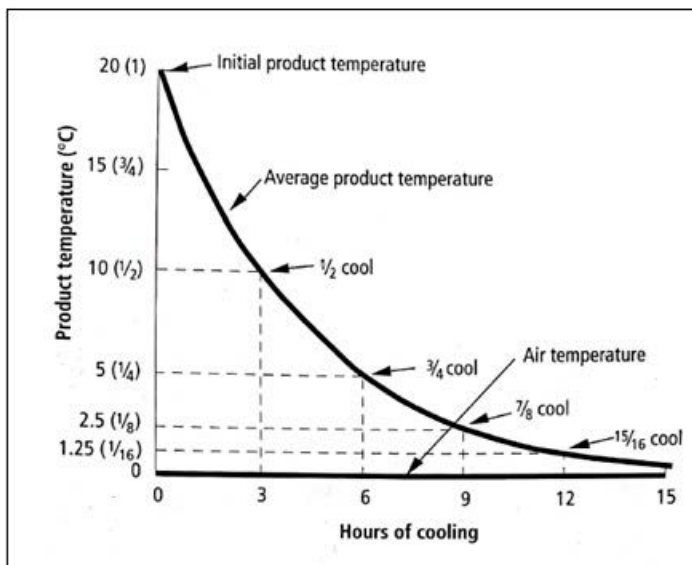


Fig. 5. Ideal cooling curve showing intermediate cooling times. Source: Thompson et al., 2008.

per square meter) dose of UV-C light (254 nm) for 8 min, then stored for 8 d at 10 °C were more tender, a “cutting force” that was 25 Newtons (N) lower and a higher total phenolic content than untreated controls.

**Gamma radiation** Gamma rays are a high-energy radiation created by the degradation of the atomic nucleus. They have the shortest wavelength, shorter than X-rays or UV light, and are measured in “Gray” (Gy) units, defined as the absorption of one Joule of radiation energy per kg of matter. Bamboo shoots pretreated with 0.5 kGy of gamma radiation (40 min, room temperature) then stored for 28 d at 2 °C/90% RH were more tender (20% increase in firmness vs. a 30% increase), had a 7-d longer shelf life, and had 12% higher phenolic content as compared to untreated controls (Zeng et al., 2015). These authors also found that the gamma radiation suppressed PAL and POD enzymatic activity, and that overall decay was 71% lower than control shoots throughout the storage period. Fresh, peeled bamboo shoots packaged in vacuum-sealed polyethylene bags in cartons were pretreated with 0.3 kGy of gamma radiation; after 45 d at 4 °C these shoots had approximately half as much browning and lignin and cellulose contents as compared to the non-irradiated controls (Wang et al., 2019). As with the previous report, PAL and POD activities were reduced in irradiated tissue.

**Hydrostatic pressure.** Hydrostatic pressure is defined as the force per unit of area exerted by a fluid, and is measured in Pascals, as is air pressure. High hydrostatic pressure can interfere with the functionality of the enzymes PAL and POD which regulate the lignification of shoot tissue. Bamboo shoots pretreated in polyethylene bags for 10 min in a high-pressure chamber (600 MegaPascals, MPa) and stored at 4 °C for 7 d exhibited 25% lower lignin content, cellulose content, and overall firmness when compared to untreated controls (Miao et al., 2011). The authors noted that flavor, color, and nutritional content were not investigated, and so further research is necessary to determine if overall quality is preserved by this treatment.

**Hypobaric storage.** The standard pressure of the Earth’s atmosphere is 101 kilopascals (kPa). Storage in low-pressure or ‘hypobaric’ atmosphere at a pressure of 50 kPa was effective at maintaining edible quality of bamboo shoots after 35 d at 2 °C/90 to 95% RH. Treated shoots were more tender (20 N softer), exhibited less browning, and contained less cellulose and lignin compared with controls stored in standard atmospheric pressure (Chen et al., 2013). Initial lignin content of 0.48 g/100 g (fresh weight basis, FW) increased to 1.06 g/100 g FW in control tissue but only to 0.62 g/100 gFW in treated tissue.

## Applied Compounds

**1-MCP.** 1-methylcyclopropene (1-MCP) is a gaseous compound that inhibits ethylene by out-competing it for binding with its receptor molecule. There are several commercial products, such as SmartFresh™ (AgroFresh, Philadelphia, PA) and Hazel 100™ sachet technology (Hazel Technologies, Inc., Chicago IL). Blocking ethylene action slows senescent processes that make crops unmarketable. It has been validated in the literature to delay ripening of many fruit and vegetable commodities, including fresh bamboo shoots. 1-MCP was effective at slowing lignification in bamboo shoots at either room temperature (20 °C) or at 2 °C. At 2 °C toughening was delayed, a benefit also observed in asparagus (Liu and Jiang, 2006). Bamboo shoots treated with 1-MCP at 20 °C also received a higher subjective visual rating than control tissue by a sensory panel.

Bamboo shoots pretreated with 1  $\mu\text{L}\cdot\text{L}^{-1}$  1-MCP for 8 h at 20 °C and stored for 12 d at 20 °C/90% RH had 50% less lignin content when compared to untreated controls (Luo et al., 2007). Lignin content of control shoots nearly doubled, while treated shoots only exhibited a 50% increase. Cellulose content of control shoots did not increase as much as lignin but still increased more than in treated shoots. Control shoots also exhibited twice as much activity of the enzymes PAL, POD, and CAD as treated shoots, which explains the higher percentage of tissue toughening. In a later study, these authors reported that bamboo shoots pretreated as above and stored for 40 d at 2 °C/90% RH developed 1/3 less chilling injury (as indicated by water soaking) and were an average of 5 N softer than untreated controls throughout storage (Luo et al., 2008).

Bamboo shoots were pretreated with 0.5  $\mu\text{L}\cdot\text{L}^{-1}$  1-MCP (powder form converted to gaseous phase by contact with water) for 20 h at 20 °C and stored for 9 d at 20 °C/90 to 95% RH; they were rated twice as visually appealing as untreated controls on days 3, 6, and 9 (Song et al., 2011). Lignin synthesis was suppressed in treated shoots as well as activities of PAL and POD. The reduction in enzymatic activity was found to be the 1-MCP-induced suppression of a specific gene called *ZcExp*.

In addition to extending shelf life of the shoots, 1-MCP may also retain sheath color longer, as reported in other crops such as leafy greens and broccoli.

**Melatonin.** Melatonin is an antioxidant compound found in both plants and animals. In plants it often serves as protection against environmental stresses. The cut bases of whole bamboo shoots were immersed for 1 min in 1.0 mM solution of melatonin; after storage for 12 d at 4 °C/80 to 85% RH they were softer, less yellow, and appeared brighter compared with untreated controls (Li et al., 2019). The melatonin inhibited the enzymes that trigger lignification.

**Brassinolide.** Brassinolide is a plant hormone and a steroid. Bamboo shoots pretreated with a 10-min aqueous dip of 0.5  $\mu\text{M}$  brassinolide and stored for 42 d at 1 °C/95% RH had only 1/3 of the incidence of visually-evaluated chilling injury (browning or water soaking) compared with controls dipped in distilled water (Fig. 6a) (Liu et al., 2016). Electrolyte leakage is another method that quantifies tissue membrane breakdown in response to chilling injury; it was also lower in treated tissue (39.7%) compared with 49.0% in control tissue (Fig. 6b).

**Oxalic acid.** Oxalic acid is an organic acid found in many plants. It has historically been used in many applications such as cleaning and as a mordant (fixative) for dyes and is now being investigated for its many benefits on fruit and vegetable shelf life (Zheng & Brecht, 2018). Bamboo shoots pretreated with a 10-min dip in 10 mmol/L oxalic acid solution and stored for 20 d at 6  $\pm$  1 °C/85 to 90% RH exhibited less browning and were approximately 1/3 (5 N) softer compared with controls dipped in water (Zheng et al. 2019). Disease incidence was also halved by oxalic acid treatment. After 12 d, lignin content rose from an initial content below 0.4% to approximately 1.3% in control tissue, but this increase was suppressed to only 0.8% in treated tissue.

**Salicylic acid.** Salicylic acid, a plant hormone most familiar to humans as the principal ingredient in aspirin, forms an important component of plant defense against pathogens. Bamboo shoots pretreated with a 15-min dip in 1.0 mM salicylic acid and stored for 50 d at 1 °C had half as many incidences of chilling injury, half as much browning, and 10% lower incidence of disease compared with controls dipped in distilled water (Luo et al., 2012). This

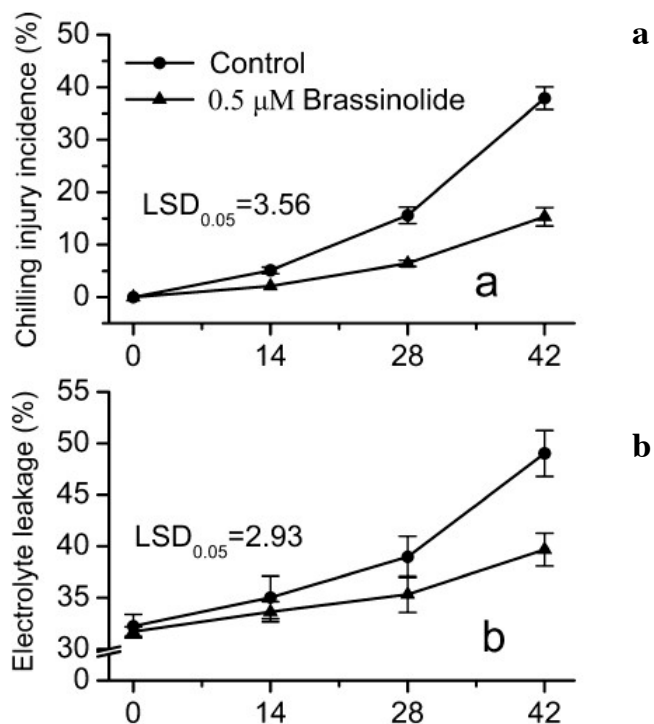


Fig. 6. Effect of brassinolide treatment on chilling injury incidence (a) and electrolyte leakage (b) of bamboo shoots during storage at 1°C. Values are the means  $\pm$  standard deviation (SD) (n=3) (Liu et al., 2016).

preservation of quality is attributed to the suppression of PAL, PPO, and POD synthesis, retarding quality degradation.

**Chlorine dioxide + chitosan.** Chlorine dioxide ( $\text{ClO}_2$ ) is a sanitizer approved to disinfect food and food contact surfaces in either aqueous or gaseous forms. Chitosan is a linear polysaccharide derived from crustacean shells, useful for creating films that act as a semipermeable barrier. Combined aqueous chlorine dioxide and chitosan coating were found to be more effective at reducing microbial counts and delaying lignification and browning than when treated separately. Bamboo shoots pretreated with a 3-min dip in 28 mg/L  $\text{ClO}_2$  followed by a 3-min dip in 1.5% chitosan solution and stored for 6 d at 4°C/60-70% RH had lower microbial count, less toughening, and delayed browning and lignification compared with controls; shoots dipped in either treatment alone still benefited, but the benefits were greater when the two treatments were used in combination (Yang et al., 2011).

**Other compounds.** Other applied compounds have been identified in the literature as having a positive effect on storage quality but are not approved for contact with food in the United States (Yang et al., 2010, Yang et al., 2015, Li et al., 2019, U.S. Food and Drug Administration, 2018).

### Packaging

Many of the environmental and chemical studies cited above also employed film overwraps or bags to reduce moisture loss from fresh shoots and protect exposed cut tissue from pathogens. Polyethylene (PE) and polyvinyl chloride (PVC) are both effective packaging materials.

**Perforated LDPE film.** Storage of fresh bamboo shoots in low-density polyethylene (LDPE) film at 20 to 25 °C extended the shelf life to 6 d and reduced weight loss to about 2%, which is considered acceptable when <5% (Kleinhenz et al. 2000). Weight

loss of 5% water content renders the shoot visually unacceptable, as the cut end dries out and develops cracks. However, storing fresh shoots in macro-perforated (8.94% vent area) LDPE bags at 1 °C for 30 d resulted in a retention of over 90% of the initial fresh weight; those held at 8 °C still maintained over 80% of initial fresh weight. In contrast, when stored at 11 °C, fresh weight was less than 80% while the shoots stored at 25 °C had less than 20% of their initial fresh weight. Therefore, the conclusion from the above experiment was that shoots stored in macro-perforated, LDPE bags at 1°C did not reach the 5% weight loss threshold until day 17 of storage, in contrast to the threshold being reached from 10 d to as little as 1 d at the progressively warmer storage temperatures.

The authors also reported that properly sized and spaced perforations retain sufficient internal RH to reduce moisture loss while minimizing condensation of free moisture which promotes decay. Multiple types of plastic packaging were compared for shoots stored in macro-perforated LDPE bag, micro-perforated LDPE bag, unperforated LDPE bag, unperforated LDPE film, heat-sealed PVC film, and a control in an open fiberboard carton for 28 d at 1 °C. The micro-perforated LDPE (35 μm thick, 0.01% area perforated) retained the highest fresh weight and had one of the lowest condensation indices. In contrast, although the unperforated LDPE bag lost less water, it had more than twice as much condensation.

In another report Kleinhenz and Midmore (2002) recommended combining the advantages of temperature control (hydrocooling) with packaging using the appropriate material and perforation size (Table 1). They reported that weight loss was 5% for hydrocooled shoots stored at 1 °C in microperforated LDPE bags (35μm thick, 0.01% perforation) after 28 d, that additionally suppressed discoloration and fungal growth. Recommended as a good second-choice option was the non-perforated 10.5 μm LDPE film, which also resulted in a 28+ day shelf life at 1°C. These authors also recommended that the packaging fit the shoots snugly so that the bag is in “intimate contact” with the commodity to minimize pulp temperature fluctuations observed when there was more air space within the bag.

**Modified Atmosphere Packaging (MAP).** Properly designed MAP films create a desirable atmosphere around the refrigerated crop that further inhibits respiration while suppressing growth of pathogens. MAP involves use of a selectively permeable, plastic membrane calculated to allow accumulation of the respiring crop’s carbon dioxide ( $\text{CO}_2$ ) as it consumes oxygen ( $\text{O}_2$ ) so that the commodity establishes a beneficial low- $\text{O}_2$ , high- $\text{CO}_2$  environment. Under these conditions the rate of respiration is reduced, decreasing enzymatic action such as PPO and POD, therefore delaying programmed cell death (PCD). PCD is the biological mechanism by which cells naturally reach the end of their life cycle through regulated processes, rather than due to exposure to an external event. MAP is used to prolong the shelf life of many fruits and vegetables—for example, it is the innovation that allows a high-respiring crop like fresh-cut broccoli to stay fresh on supermarket shelves; otherwise, use of impermeable films would allow  $\text{O}_2$  to be completely consumed, resulting in anaerobic conditions that would quickly render the crop unmarketable. Precautions must also be taken to minimize accumulation of free moisture in the sealed bag or container.

MAP’s potential benefit to bamboo shoots was explored by Shen et al. (2006). Peeled shoots were either sealed in 0.04 mm thick LDPE bags to create a modified atmosphere (2%  $\text{O}_2$ /5%  $\text{CO}_2$ ) or left in ambient air (controls) during storage at 10 °C. After

Table 1. Influence of packaging material and storage temperature on shelf life (days) of bamboo (*B. oldhamii*) shoots (data collated from all experiments). (Kleinhenz and Midmore, 2002).

Packaging material	Storage temperature					
	1 °C	2 °C	8 °C	11 °C	20 °C	25 °C
Control (open storage)	7	6	— <sup>z</sup>	—	1.5	—
Macro-perforated LDPE <sup>y</sup> bag	17	—	6–10	5	—	1
Micro-perforated LDPE bag	28	—	—	—	—	—
LDPE film	28	20	—	—	6	—
LDPE bag	21	—	6–10	—	—	—
Heat-sealed PVC <sup>x</sup> film	14	—	—	—	—	—

<sup>z</sup>Not assessed.

<sup>y</sup>LDPE = low-density polyethylene.

<sup>x</sup>PVC = polyvinyl chloride.

10 d of storage, POD and PPO activity in the MAP treatment was halved compared with controls; the browning index for the former was 1 (trace browning; 0 = no browning) compared with a rating of 5 (“extremely severe”) for the latter.

In Song et al. (2013), shoots were minimally processed (outer leaf sheaths removed, and a 5-cm slice of tissue removed from cut end) and placed in either open or sealed 0.05-mm thick LDPE bags with an O<sub>2</sub> transmission rate of  $1.2 \times 10^{-14}$  M/m<sup>2</sup>/s/Pa and a CO<sub>2</sub> transmission rate of  $10.8 \times 10^{-14}$  M m<sup>2</sup>/s/Pa before storage at 2 °C/90% for 60 d or 20 °C/90% RH for 9 d. Sensory quality was subjectively rated on a scale of 1 to 6 with 6 as the highest quality. At 20 °C the sensory quality was unaffected by MAP and declined from 5 to 1 by day 9, but at 2 °C, MAP slowed the decline in sensory quality over 60 d and halved the accumulation of lignin. This second study is important because it indicated that initial flushing with premixed O<sub>2</sub>/CO<sub>2</sub> gas was unnecessary in that the sealed shoots established an effective modified atmosphere.

**Packing density.** Chang and Yen (2008) reported that bamboo shoots sealed in a 0.06 mm polyethylene (PE) bag packed too tightly (8/bag) produced anaerobic conditions and subsequent development of off-odors during storage at 1 °C. However, bamboo shoots packed 5 per bag had a higher SSC and produced a beneficial modified atmosphere necessary to keep the product fresh during 16 d of marine transport from Taiwan to Japan.

### Future Areas for Research

Extensive studies have been performed, exploring environmental conditions, chemical treatments, and packaging methods to extend the quality of fresh bamboo shoots for the fresh market. Temperature management has repeatedly been demonstrated to be the most critical and readily applicable method to extend post-harvest quality and shelf life of fresh bamboo shoots. However, it was also noted that the ideal lowest safe temperature has not been precisely identified; this may actually vary by bamboo cultivar. Storage in air for 10 d at 2 °C resulted in higher quality product than at 20 °C, but after 30 d chilling injury was apparent at the former temperature. Therefore, straightforward storage tests are necessary to compare shoot quality during storage at 1, 2, and 4 °C, with those stored at 10 °C.

The studies highlighted in this review have largely tested single variables or variations of the same treatment and compared with untreated controls. For example, the hypobaric atmosphere study evaluated standard atmosphere against 75, 50, and 25 kPa of pressure. Comparing combined treatments with two or more of the above cited methods with untreated controls would provide very useful information for the farmer, packer, and shipper. Therefore, it is suggested to determine the most effective of the

above treatments and then evaluate these simultaneously. One potential scenario would be to treat bamboo shoots with 1-MCP, melatonin, hydrocooling, heat treatment, or UV-C exposure then store at the same temperature and evaluate shoot quality over time, always comparing with the current handling method as the control. Treatment with gamma radiation, hydrostatic or hypobaric (low) pressure may not be the most cost-effective or feasible options.

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—Scientific Note—

## Evaluation of Thyme Oil Vapor for Control of Postharvest Gray Mold on Blueberry

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**Additional index words.** *Botrytis cinerea*, *Vaccinium* sp.

Gray mold caused by *Botrytis cinerea* is a major postharvest disease of blueberry (*Vaccinium* spp.). It is a necrotrophic fungus that favors moist, humid, and warm environmental conditions between 65 to 75 °F (18.3 to 23.9 °C). It has fluffy mycelium and gray-tan spore masses on brown stalks (Fig. 1). The increasing need to control pathogens in organic fruits promotes the search for safe and effective antimicrobials from natural sources. Essential oils (EOs) are natural, complex, volatile aromatic, hydrophobic, oily liquids synthesized in aromatic plants as secondary metabolites (Bassolé and Juliani 2012). They have broad-spectrum anti-bacterial, anti-fungal, and anti-viral functions. Thymol is the most abundant compound; the major active component of thyme oil (TO). The objectives of this in vivo study were to determine the effects of TO on postharvest gray mold control and to explore the side effects of TO postharvest application.

Inoculated and uninoculated southern highbush blueberries cv. Suziblue were exposed to TO vapor at 0 mL, 0.05 mL, 0.1 mL, 0.25 mL, and 0.5 mL per 500 mL mason jar with 25 berries per jar and four replications per treatment. *B. cinerea* was isolated from infected blueberry fruit and maintained on an acidified potato dextrose agar (APDA) at 71.6 °F (22 °C). Spore suspensions of *B. cinerea* were prepared by wiping the surface of a culture dish, and spore concentration adjusted to  $1 \times 10^5$  CFU/mL with sterile DI water using a hemocytometer. Each fruit was sprayed with 20  $\mu$ L of spore suspension using an airbrush for inoculation. Blueberries were incubated for 24 h at room temperature to establish the pathogen then TO was added to jars and the fruit incubated for 24 h at room temperature. All blueberry fruit were stored in jars in a 41 °F (5 °C) and 95% relative humidity (RH) cold room for 7 d and then moved to clamshells at room temperature (75 °F/24 °C and 50% RH) for another 3 d. The number of decayed fruit was recorded daily after moving to room temperature. Fruit color and firmness were measured at the end of the experiments. The TO vapor effectively inhibited the growth of gray mold, but also reduced fruit firmness and caused significant color change



Fig. 1. Gray mold (*Botrytis cinerea*) on blueberry (*Vaccinium* spp.) under the dissecting microscope.

(a\*, b\*, and C\* values) expressed as a darker, more purplish hue. The highest TO concentration (0.5 mL per 500 mL mason jar) completely eliminated decay development for at least 3 d at room temperature in inoculated and uninoculated groups. Thyme oil vapor may potentially provide a new and effective strategy for controlling postharvest gray mold disease in blueberry fruit.

Further research in sensory evaluation is needed to determine consumer acceptability due to the strong influence of TO on organoleptic properties.

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—Scientific Note—

## Validating Agricultural Water Treatment for Food Safety on Farms

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**Additional index words.** agricultural water, chlorine, *E. coli*, irrigation, peracetic acid, salmonella, water treatment

Agricultural water is identified as one of the major routes for microbial contamination of fresh produce (Harris et al., 2012). Continuing outbreaks of foodborne disease associated with fresh produce in the U.S. (CDC, 2019) have resulted in market (Arizona LGMA, 2020) and regulatory pressures (FDA, 2020) that are driving growers toward treating agricultural surface water that contacts the harvestable portion of the crop. Very little information exists for growers to validate water treatment systems on their farms. The objective of this study was to validate in the field the effectiveness of agricultural water treatment methods.

Eight agricultural ponds in West Central Florida were sampled 3 times between February 2021 and June 2021. Surface water was treated with injection systems [diaphragm, aqueous chlorine (NaOCl) or peristaltic, peroxyacetic acid (PAA)] to achieve concentrations of 2–4 ppm free residual chlorine and 5–10 ppm PAA. Contact time was 31 s. Water samples were collected at 0, 1, 20, 40, and 60 min [neutralized with 0.12% w/v sodium thiosulphate (NaOCl) or sodium metabisulphite (PAA)] and evaluated for physicochemical attributes. Populations of generic *E. coli* and total coliforms were enumerated using IDEXX Quanti-Trays.

Average values for oxidation-reduction potential, pH, electrical conductivity, and chemical oxygen demand ranged from 38.50–524.55 mV, 4.26–10.70 (pH), 59.40–1116  $\mu$ S/cm, and 7.50–804.50 ppm, respectively. Average water temperature, turbidity, total dissolved solids, and total suspended solids ranged from 19.10–33.85 °C, 8.50–167.50 FAU, 29.50–804.50 ppm, and 0.0005–0.6257 ppm, respectively. These wide ranges for the physicochemical attributes of the water indicated a high variability of water being treated within and among the ponds, an indication that adequate monitoring is critical for effective treatment. The average ( $\pm$  standard deviation) coliform concentration in the untreated water samples for NaOCl and PAA were  $4.28 \pm 0.57$  and  $4.32 \pm 0.54$  log MPN/100 mL, respectively. The initial *E. coli* concentrations for NaOCl were  $1.22 \pm 1.03$  and for PAA were  $1.21 \pm 1.02$  log MPN/100 mL ( $n = 120$ ). Measured free residual NaOCl and PAA ranged from 1–5 ppm and 3.5–15 ppm, respectively. The average coliform concentration in the treated water samples for NaOCl and PAA were  $1.30 \pm 0.86$  and  $1.38 \pm 1.42$  log MPN/100 mL, respectively, while *E. coli* concentrations were  $0.12 \pm 0.37$  for NaOCl and  $0.44 \pm 0.78$  log MPN/100 mL

( $n = 96$ ) for PAA. The average coliform log reduction achieved during NaOCl and PAA treatments were  $2.96 \pm 0.78$  and  $2.95 \pm 1.46$  log MPN/100 mL, respectively. The average *E. coli* log reduction achieved during NaOCl and PAA treatment was  $1.17 \pm 0.93$  and  $0.90 \pm 0.69$  log MPN/100 mL, respectively. Analysis of variance comparing coliform and *E. coli* concentrations before and after treatment indicate that both NaOCl and PAA treatments were effective in inactivating microbial indicators in agricultural water. NaOCl and PAA significantly ( $P < 0.05$ ) reduced the concentrations of total coliforms and *E. coli* after treatment regardless of sampling event, pond, or sampling time. Both treatment methods have the potential to effectively reduce microbial populations in surface waters and can be used to help mitigate food safety risks associated with agricultural water used during produce production.

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—Scientific Note—

## Evaluation of Fungicides Applied Preharvest for Postharvest Diplodia Stem-end Rot Control on Grapefruit

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**Additional index words.** fruit decay, HLB, *Lasiodiplodia theobromae*, shelf life

Diplodia stem-end rot (SER, caused by *Lasiodiplodia theobromae*) is an important postharvest disease in Florida citrus. The presence of Huanglongbing (HLB) in citrus groves has exacerbated Diplodia SER. The discovery and implementation of improved decay control methods is essential to sustain Florida's fresh citrus industry. Evaluation of preharvest fungicides or other compounds for postharvest Diplodia SER control was conducted during the 2019–20 and 2020–21 seasons on red grapefruit (*Citrus × paradisi*). Tested compounds and formulations included: Topsin 4.5 FL (thiophanate-methyl), Quadris Top (azoxystrobin + difenoconazole), Graduate A+ (fludioxonil + azoxystrobin), Headline (pyraclostrobin), Mentor EC (propiconazole), Mertect 340-F (thiabendazole), Switch 62.5 WG (fludioxonil + cyprodinil), Miravis Prime (fludioxonil + pydiflumetofen), Miravis Top (difenoconazole + pydiflumetofen), Thyme Guard (thyme oil) and Citrus Fix (2,4-Dichlorophenoxyacetic acid or 2,4-D). During the first season, the selected compounds were hand-sprayed on individual grapefruit (replicates) within the tree canopy in two locations (groves). During the second season, the compounds were applied to each of three trees (replicates) in each of four locations (groves). The application rates were based on label instructions. For materials with no preharvest citrus label, rates/concentrations were based on their labels for other crops or their postharvest use. Control trees were sprayed with water. Fruit were harvested 2 d and 14 d after treatment. Harvested fruit were exposed to 5-d degreening conditions [5 ppm ethylene, 85 °F, and 90% relative humidity (RH)]. The fruit were then incubated at 75 °F with 90% to 95% RH for 3 weeks and Diplodia SER occurrence was recorded weekly. Fruit treated with Topsin 4.5 FL and Graduate A+ in all tests significantly reduced Diplodia SER incidence and severity compared to the controls. Mertect 340-F, Miravis Prime, Headline, and Quadris Top significantly reduced Diplodia SER in some tests. Switch 62.5WG, Thyme Guard, Mentor EC, and Citrus Fix did not significantly reduce Diplodia SER.

Topsin 4.5 FL has previously been reported to effectively control Diplodia SER (Salvatore and Ritenour, 2007; Zhang and Timmer, 2007) and served as a positive (likely best-case) control in these tests. Topsin had once been available for Florida citrus through a temporary EPA Section 18 emergency use exemption, but efforts by the registrant to obtain a full label were abandoned in 2009. The consistent effectiveness of Graduate A+ for postharvest Diplodia SER control mirrors its reported postharvest effectiveness against *L. theobromae* (Zhang, 2007; Zhang and Timmer, 2007). However, Graduate A+ is currently registered only for postharvest use and the rates used in these studies are higher than what would be allowed for preharvest use. The effectiveness of Mertect 340-F against Diplodia SER is expected since its active ingredient, thiabendazole, is commonly used commercially for effective control of citrus postharvest Diplodia SER (Zhang, 2007). Headline, Quadris Top, and Miravis Prime contain fludioxonil or strobilurin compounds, which are likely the key active ingredients leading to reduced Diplodia SER. Product formulation may also affect decay control efficacy of the compounds. For instance, Switch 62.5 WG, containing fludioxonil, did not reduce Diplodia SER in all tests in this study.

Further work is in progress to evaluate concentrations of fludioxonil, strobilurins, their combinations, and formulations and new compounds for Diplodia SER control in Florida.

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—Scientific Note—

## Determining Optimal Handling Conditions and Shelf Life for Orange- and Purple-fleshed Sweetpotatoes: Preliminary Studies

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**Additional index words.** anthocyanins, ascorbic acid, carotenoids, curing, storage

Some Florida farmers are planting alternative crops like sweetpotato to diversify their operations. Since consumers are demanding healthier, nutrient-rich foods in their diets, the purple-fleshed sweetpotato has potential as a high-value crop with suitable growing conditions in the Florida climate. Sweetpotato roots are dug mechanically which can cause damage that leads to postharvest losses due to moisture loss, unmarketable appearance and disease. Curing is a low-cost postharvest technique that is applied prior to storage to maintain quality by enhancing starch to sugar conversion, suberization (wound healing) and skin set. However, improper curing conditions can lead to excessive sprouting and increased weight loss, which renders the roots unmarketable. Although harvest and handling techniques have been developed for orange-fleshed cultivars, recommendations for purple-fleshed cultivars are needed. For this study, the objective was to measure key quality parameters of purple-fleshed and orange-fleshed sweetpotato cultivars as affected by curing conditions and storage time.

Orange-fleshed 'Covington' sweetpotatoes were hand dug at the University of Florida/IFAS Hastings Agricultural Extension Center and purple-fleshed 'Charleston Purple' sweetpotatoes were mechanically dug from a commercial farm, both 150 d after planting, and transported to the University of Florida for storage and quality analyses (Fig. 1). Sweetpotatoes from each cultivar were stored either uncured at 15 °C (59 °F) and 85% relative humidity (RH) for 3 months or first cured for 7 d at 29 °C (84 °F) and 90% RH then transferred to 15 °C (59 °F) for the remaining total of 3 months. Evaluations were conducted after 0, 7, 28, 57, and 84 days for sprouting, decay, pulp color, moisture content, soluble solids content (SSC, °Brix), and nutritional quality (total anthocyanins, carotenoids, ascorbic acid).

Decay was < 8% for both cultivars during storage. However, 'Charleston Purple' developed significantly more sprouting than



Fig. 1. 'Charleston Purple' (left) and 'Covington' (right) sweetpotatoes.

'Covington'. Sprouting for cured 'Charleston Purple' was 54% and for uncured was only 6%, while 'Covington' had no sprouting regardless of curing treatment. Pulp color remained unchanged during storage for both treatments for 'Covington' (mean hue\* angle = 60°) and for 'Charleston purple' (mean hue\* angle = 350°). Initial moisture content was lower for 'Charleston Purple' (68%) compared to 'Covington' (75%) and decreased slightly for both cultivars during storage. Initially, soluble solids content was similar between cultivars (9.4 °Brix) but increased to 11.4 °Brix in 'Covington' after 3 months storage. Ascorbic acid content was higher in 'Charleston Purple' (70 mg/100 g) compared to 'Covington' (18 mg/100 g) and decreased slightly during storage. As expected, 'Charleston Purple' had higher anthocyanin (3.3 mg/100 g) and lower carotenoid content (0.1 mg/100 g) when compared with 'Covington' in which anthocyanins were not detectable and carotenoid content was 4.3 mg/100 g. The results from this study are comparable to previous reports for both sweetpotato types; however, the sprouting that occurred during the curing period of 'Charleston Purple' should be investigated further. Future studies are planned to evaluate the potential of several purple-fleshed cultivars for Florida production related to curing, sprouting and quality during storage in order to provide detailed recommendations.

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## **Strawberry DNA Tests for Improving Fruit Quality and Disease Resistance**

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### **Abstract**

**Developing new strawberry cultivars with improved fruit quality and disease resistance will contribute to the profitability and sustainability of the U.S. strawberry industry. In the last few years, strawberry breeders and researchers have developed modern genetic and genomic tools, including diagnostic DNA tests, to facilitate DNA-informed breeding that can improve strawberry varieties with both high fruit quality and disease resistance. DNA tests allow breeders to prioritize and select offspring with higher potential, and be more efficient with available genetic resources. Currently, DNA markers for fruit quality (fruit color, flowering habit, and flavor) and disease resistance against multiple pathogens have been used to develop new strawberry cultivars in our breeding program. Each year, about 60,000 seedlings are screened to improve disease resistance and fruit quality using high-throughput marker-assisted seedling selection. We will present the recent advances in the application of DNA tests and development of new strawberry cultivars.**

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## **LB8-9 ‘Sugar Belle’ Kombucha: Alternative Citrus Product in the Huanglongbing Era**

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### **Abstract**

Huanglongbing (HLB) is a severe citrus greening disease for citrus plants with no cure. Because of HLB, the citrus production in Florida decreased by 69.8% from 2003–2004 to 2019–2020 seasons. Growing HLB-tolerant citrus plants such as LB8-9 ‘Sugar Belle’ (Sugar Belle) is one of the ways to overcome this problem. In this study, Sugar Belle juice was mixed with the initial stage of kombucha and fermented. The chemical changes during Sugar Belle kombucha fermentation were studied and compared with the regular kombucha fermentation. Samples were collected at day 0, 3, 7, and 10 during the fermentation. The amount of volatiles were identified and quantified by GC-MS and sugars, organic acids, tea polyphenols, and citrus flavonoids by LC-MS/MS. Sensory panels were conducted for sensory aspects and the overall liking. The data from the analyses above were combined and analyzed to reveal the relationship between chemical constituents and sensory attributes. This successful production of Sugar Belle kombucha with the beneficial compounds and the comparable consumer’s acceptability will have the potential to increase the demand for the LB8-9 ‘Sugar Belle’. This would get citrus farmers to grow LB8-9 ‘Sugar Belle’ as an alternative variety for the citrus industry in the circumstance of the lack of a cure for HLB.

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A full paper has been submitted to the *Journal of Food Science* and is in review.

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## **Productivity and Postharvest Quality of Strawberry Cultivars Grown in Southeast Georgia**

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### **Abstract**

Most commercial strawberry (*Fragaria × ananassa*) growers in the Southeast use an annual plasticulture production cycle, planting cultivars that have been used for years in the region such as ‘Chandler’, ‘Camarosa’, or ‘Sweet Charlie’. Though these strawberry cultivars have proven their acceptability in yield and quality, there are a number of newer, June-bearing cultivars with potential for Southeastern production, that are now widely grown in California and Florida. Some of these cultivars are photoperiod-sensitive and have not been used in Southern Georgia since warm weather conditions are expected to cause softening of the fruit, dramatically reducing their storability and acceptability. To our knowledge, there are no studies that investigate the suitability of those cultivars to southeast Georgia climatic conditions. For this study, nine June-bearing and four day-neutral cultivars, obtained as plugs, were established in a commercial U-pick operation of the Coastal Plain (Lowndes County, GA) in early November. The experimental beds were cordoned off the rest of the field. They were drip irrigated and fertilized, with frost protection provided via row covers on a need basis. This work aimed to evaluate 13 new to the area cultivars under Coastal Plain growing conditions for viability, productivity, and fruit quality at harvest and after cold storage. The first harvest occurred in late January 2020 with the last one in mid-March (the experiment was halted early due to COVID-19 restrictions). Mature, red strawberries were harvested in individual plastic bags and stored in clamshells at 1 °C with 90% relative humidity for seven days. Cultivars that showed early plant development and fruit production were noted. Fresh market fruit yield per plant and average berry size were calculated for the growing season. The differences in several quality parameters such as water loss, firmness, color, sugar and acids distribution, were evaluated on the day of harvest and after seven days of cold storage. While there were no apparent fruit decay symptoms in any of the cultivars, possibly due to the short duration of the cold storage, significant differences in other quality parameters studies were observed. The average yield per plant along and the average fruit size differed significantly among a number of cultivars. The results of this research will provide valuable information to the strawberry growers of Georgia as well as North Florida regarding appropriate cultivar selection.

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## Determining the Relationship Between Distribution Temperatures and Strawberry Quality

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### Abstract

Temperatures were monitored using real-time data loggers placed in flats for eight strawberry truck shipments from fields in Plant City, Florida, to a distribution center (DC) in Georgia, and from Salinas, California to DCs in Georgia, Maryland, Pennsylvania, Virginia, South Carolina, North Carolina, and Texas. The strawberry temperatures were further tracked from the DCs to the retail stores. In order to understand the relationship between temperature management during distribution and strawberry quality, the collected data was used to construct four, 7-d logistics scenarios for two trials using programmable temperature-humidity storage rooms at the University of Florida, Institute of Food and Agricultural Sciences Postharvest Lab. The scenarios were: 1) control (constant 1.1 °C/34 °F for 7 days); 2) warming during transport to DC (warm from 1.1 °C to 4.4 °C/40 °F during days 1–4); 3) ambient retail display (warm to 22.4 °C/72 °F on days 6 and 7); and, 4) warming during transport/ambient retail. For farm/DC/retail assessments, strawberry clamshells were subjectively evaluated using a 1–3 “Would I purchase?” scale; for at-home assessment a 1–3 “Would I eat?” scale was used. Individual fruit were also evaluated as marketable, or as bruised, mold/decay, or calyx browning based on the primary defect present. Strawberries were also objectively evaluated for color, firmness, soluble solids content, total titratable acidity, and pH. Sensory evaluations were conducted during the at-home period (final 3 evaluation days) by a panel of five strawberry experts.

The abstract was presented at the 2021 FSHS Annual Meeting.

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## **Investigating the Potential of Oxygenated and Ozonated Water as a Replacement of Chlorine for Peach Sanitation**

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### **Abstract**

Sanitation is an important postharvest step for peach packing houses aiming at reducing the microbiological load of the fruit as well as from all the surfaces they contact. It also ensures the food safety of the fruit and extends the postharvest shelf life. The most common sanitation method is the addition of chemical oxidizers such as sodium hypochlorite in the flume or hydrocooling water. A novel technology called High-Oxygen Water (HOW) is an alternative sanitation system based on the generation of stable nanobubbles of oxygen in water that can be coupled with ozone, without the need for chemicals. The suspended solution has the ability to reduce microorganism loads and could serve as an effective sanitation treatment for peaches during hydrocooling without chemical residues. The adoption of HOW could lead to a significant reduction in chemical and water consumption during the postharvest processing of peaches in a packinghouse. Based on our research, no changes in current packing lines need to be made other than the addition of a HOW generator in place of the chlorine injection system. Our team is evaluating the benefits of HOW by determining quality changes and decay incidence during storage compared to the standard sodium hypochlorite treatment. Peaches were treated using HOW at 10, 20, and 30 ppm of dissolved oxygen in a water tank for 30 minutes. This treatment was compared with a sodium hypochlorite treatment at 50 ppm of free chlorine as the standard agricultural practice in the industry. Peaches were stored at 1.5 °C and 90% relative humidity (RH) for 7, 14, 21, and 28 days plus 3 days of ambient temperature storage when postharvest quality traits were assessed. The potential for this technology to substitute for or enhance existing postharvest practices is being investigated. The results will provide valuable information for peach packinghouse operations.

The abstract was presented at the 2021 FSHS Annual Meeting.

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## Improving Botrytis Gray Mold Resistance Using CRISPR Gene Editing in Cultivated Strawberry

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### Abstract

The cultivated strawberry is an important economic fruit crop across the world. In 2019, according to the Food and Agriculture Organization, U.S. strawberry yield was approximately 1.02 million tons. Gray mold disease caused by *Botrytis cinerea* has been a serious problem to strawberry industries and growers. *B. cinerea* can reduce commercial strawberry yield by over 50% in the United States. In Florida, the fruit rot caused by *B. cinerea* is one of the most problematic diseases, but currently there is no genetic resistance available to develop new cultivars. We recently identified two transcription factor genes associated with resistance to Botrytis gray mold. CRISPR/Cas9 gene editing technology was used to modify the target gene in the main University of Florida cultivar—Florida Brilliance. A transient RNA interference assay for candidate resistance gene showed a decrease in the area under the disease-progress curve and disease frequency in strawberry fruits against *B. cinerea*. We are currently working to obtain mutants for each target gene using CRISPR/Cas9 gene editing. Our findings will be valuable for the breeding of Botrytis gray mold resistance.

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## **Investigating the Dynamic Transcript Level Changes of Senescence-associated Genes in Lettuce during Postharvest Storage**

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### **Abstract**

Lettuce (*Lactuca sativa*) is considered highly perishable and has a relatively short shelf life due to the induced leaf senescence mechanism that is influenced by both developmental and environmental factors. It is very important to understand the molecular mechanism underlying postharvest senescence in order to breed new lettuce varieties having extended shelf-life. To this end, quality measurements and gene expression studies in lettuce cultivars were carried out to estimate the variability in the lettuce accessions for determining the shelf-life. We focused on transcriptional changes of twelve senescence-associated genes (SAGs) in four lettuce cultivars, accession 60184, 'Manatee', 'Tall Guzmaine', and 'Okeechobee' during postharvest storage. Among those lettuce cultivars, accession 60184 has the shortest shelf life while 'Okeechobee' has the longest shelf life. The other two cultivars were observed to have intermediate shelf life. In this study, we observed the some of the SAGs, namely ORE15, ORE1, NAC90, LUX, NAC27, and WRKY, were differentially upregulated. Others were down-regulated during postharvest storage and had enhanced expression in lettuce, namely accession 60184. We found strong positive or negative correlation between gene expression of SAGs and shelf life of lettuce cultivars. These results indicated that lettuce SAGs can be used as markers to estimate lettuce shelf life. In summary, combining results from physiological and molecular measurements will provide genetic resources to plant breeders and researchers in establishing new markers for developing lettuce cultivars with enhanced shelf life.

The abstract was presented at the 2021 FSHS Annual Meeting.

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## **Investigate the Regulation of Senescence Associated Signaling Mechanism in Postharvest Broccoli**

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### **Abstract**

Broccoli (*Brassica oleracea* L. var. *italica*) is prone to yellowing and wilting due to the relatively high respiration rate and tissue senescence during postharvest handling, transportation, and storage. This greatly affects the quality and reduces market value and can lead to a problem of food waste and loss. Treating broccoli florets with 1-MCP and controlled atmosphere (CA) can delay senescence. However, little is known about the mechanisms at the molecular level. Here, we combined physiological, biochemical and genomics analyses on the postharvest broccoli and identified a core gene regulatory network governing both senescence-associated developmental events and activation of stress responses. These findings can provide guidance on how to extend broccoli shelf life and reduce economic losses. They also generate genetics and molecular recourses for marker-assistant breeding and expand the general scientific knowledge of regulating senescence of *Brassicaceae*.

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## Viburnum Foliar Pathogen Identification and Fungicide Efficacies

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**Additional index words:** Viburnum disease, viburnum disease management, downy mildew, leaf spots, defoliation

Central Florida nursery growers have reported disease management challenges since 2004 impacting the production of ornamental *Viburnum* spp. Reported symptoms included blighting and rapid defoliation that were historically known as downy mildew (DM). Growers indicated that common labeled fungicides failed to provide acceptable levels of disease control. In the spring of 2020, symptomatic plant samples were collected from local nurseries in Hillsborough and Manatee Counties. Identification of isolated fungi revealed the presence of multiple pathogens throughout the growing seasons (spring, summer, and fall), including *Plasmopara* sp., *Cercospora* sp., *Corynespora* sp., *Colletotrichum* sp., *Phoma* sp., *Phyllosticta* sp., and *Pestalotiopsis* sp. These diseases had symptom progression very similar to DM and were not easily identified. Two fungicide trials were conducted at a commercial nursery. The first trial, conducted during July and August, evaluated 13 fungicides available to nursery growers. The second trial, conducted during September and October, evaluated 7 fungicides. Both trials included a non-treated control, with all treatments replicated (n=6) and arranged in randomized complete blocks. Not surprisingly, fungicides that target DM oomycetes (i.e., *Plasmopara* sp.), containing ametoctradin, cyazofamid, dimethomorph, fluopicolide, mandipropamid, mefenoxam, and oxathiapiprolin, failed to reduce disease severity. Fungicides containing benzo-vindiflupyr, difenoconazole, fluxapyroxad, and pyraclostrobin that typically target true fungi, statistically reduced disease severity. Copper sulfate and mancozeb, or the systemic fungicide, flutriafol, failed to reduce disease severity, while a generic phosphite gave an intermediate level of control. Results stress the importance of an appropriate disease diagnosis to avoid making ineffective fungicide applications.

*Viburnum* sp. is an important woody ornamental crop for nursery growers in Florida. The main varieties grown in the Central Florida region are *V. suspensum* (Sandankwa viburnum), *V. odoratissimum* (sweet viburnum), and *V. obovatum* (Walter's viburnum). Viburnums are quick growing shrubs that reach an average height of about 30 ft. if left alone. They produce small white flowers and drupe fruit. They are used popularly in the landscape as hedges or screen plants. Around 2004, Central Florida nursery growers began to report severe disease management challenges impacting the production of ornamental *Viburnum* spp. especially during high humidity, mist propagation (Elwakil et al. 2021). Previously, viburnum cuttings would root with ease around 100% propagation success rate. When disease started to present itself, propagation success rates could easily drop to 0%. Reported symptoms included reddish to dark foliar spots followed by blighting and rapid defoliation of cuttings. Young plants, liners that were potted in one- or three-gallon containers also showed intense disease symptoms (Fig 1.). The problem was originally identified as downy mildew (DM) caused by the oomycete *Plasmopara viburni*. This fungus is spread with the movement of air and water. High humidity, leaf wetness, plant overcrowding, foggy days, and cooler weather favor the growth

of DM (Salgado-Salazar, 2017). Around 2014, growers indicated that common labeled fungicides targeting DM failed to provide acceptable levels of disease management. It was thought that maybe DM became resistant to fungicides rendering control unsatisfactory. Research was necessary to determine efficacies of



Fig. 1. Foliar symptoms observed during Viburnum propagation in liners (top) and recently potted plants (bottom) showing the issues of propagating from diseased plants.

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commercially available fungicide chemistries for the management of DM on *Viburnum* sp. to increase profitability and economic sustainability.

### Materials and Methods

Symptomatic foliage of Sandankwa viburnum (*V. suspensum*) and Awabuki viburnum (*V. odoratissimum* var. awabuki) was collected from local nurseries for pathogen identification throughout the growing seasons (spring, summer, and fall) in Hillsborough and Manatee Counties.

Two fungicide trials were conducted at a commercial nursery in Hillsborough County. The first trial was conducted during July–August using naturally infected *V. suspensum* plants grown in 3-gallon containers at a commercial production plant nursery. The trial was designed in completely randomized blocks with 6 replicates including 13 fungicide treatments representing 12 modes of action (MOA) and water control (Table 1). A second trial was conducted during September–October using the same setup as the first trial but focused on seven fungicides with a water control (Table 2).

All fungicide spray treatments were applied twice at a 14-day interval, except for copper sulfate, mancozeb, and a phosphite that were applied weekly, using a handheld pump sprayer, calibrated

to deliver fungicide treatments in 0.5L volume at recommended products' spray rates. In the second trial, flutriafol was applied as a soil drench per manufacturer's recommendation. Plants were fertilized and overhead irrigated according to grower production standards. The percentage of symptomatic foliage was rated weekly for six weeks to calculate the area under the disease progression curve (AUDPC).

Pathogenicity tests were performed in a growth chamber to confirm pathogenicity of isolated fungal species from collected nursery plant samples. This included isolating the fungal species and culturing them for phenotypical identification and creating mass inoculum. Healthy plants were inoculated in a growth chamber using an inoculum solution spray. Disease development of inoculated plants in addition to a water control was monitored daily.

Data analysis was conducted using a generalized mixed model analysis (PROC GLIMMIX) within SAS (version 9.4) with blocking as a random variable and fungicide treatment as a fixed effect. Mean separations were performed using Fisher's protected LSD at a 95% level of confidence.

### Results and Discussion

Identification of isolated fungi revealed the presence of multiple pathogens throughout the growing seasons (spring,

Table 1. List of fungicide treatments applied to *V. suspensum* in the first trial conducted in July through August 2020 and area under disease progression curve (AUDPC) representing disease severity.

Product	Active ingredient	FRAC	Rate/100 gal	AUDPC <sup>z,y</sup>
Protect	Mancozeb	M3	2 lb	1586 a
Cuprofix Ultra 40D	Copper sulfate	M1	1.9 lbs	1857 a
Subdue Maxx	Mefenoxam	4	2 fl oz	1277 ab
Micora	Mandipropamid	40	8 fl oz	1594 a
Orvego	Dimethomorph + ametoctradin	40 + 45	14 fl oz	1360 ab
Ryora (Topguard)	Flutriafol	3	14 fl oz	1855 a
Adorn	Fluopicolide	43	4 fl oz	1069 abc
Stature	Dimethomorph	40	12.25 fl oz	1045 abc
Segovis	Oxathiapiprolin	49	3 fl oz	1213 ab
Segway	Cyazofamid	21	6 fl oz	968 abc
Phostrol	Phosphite	33	64 fl oz	836 abc
Orkestra	Pyraclostrobin + Fluxapyroxad	11 + 7	10 fl oz	649 bc
Postiva (Miravis Top)	Benzovindiflupyr + Diffenoconazole	7 + 3	28 fl oz	521 c
Water control	--	--	--	1716 a

<sup>z</sup>Area Under the Disease Progression Curve (AUDPC), calculated using final four disease severity ratings.

<sup>y</sup>AUDPC means followed by the same letter are not significantly different at the 95% level of confidence. *P*-value: 0.0122.

Table 2. List of fungicide treatments applied to *V. suspensum* in the second trial conducted in September through October 2020 and area under disease progression curve (AUDPC) representing disease severity.

Product	Active ingredient	FRAC	Rate/100 gal	AUDPC <sup>z,y</sup>
Protect	Mancozeb	M3	2 lb	139 c
Phostrol	Phosphite	33	2 qt	193 abc
Cuprofix Ultra 40D	Copper sulfate	M1	1.9 lb	160 bc
Orkestra	Pyraclostrobin + Fluxapyroxad	11 + 7	10 fl oz	144 bc
Postiva (Miravis Top)	Benzovindiflupyr + Diffenoconazole	7 + 3	28 fl oz	90 c
Ryora (Topguard)	Flutriafol (drench applied)	3	14 fl oz	290 a
Segovis	Oxathiapiprolin	49	3 fl oz	261 ab
Water control	--	--	--	172 abc

<sup>z</sup>Area under the disease progression curve (AUDPC), calculated using final four disease severity ratings.

<sup>y</sup>AUDPC means followed by the same letter are not significantly different at the 95% level of confidence. *P*-value: 0.0312.



Fig. 2. Viburnum leaf exhibiting symptoms of downy mildew (top) caused by *Plasmopara* sp., with sporulation showing on leaf underside (bottom left), through a hand lens (bottom center), and through a microscope (bottom right).



Fig. 3. Common foliar symptoms observed on Viburnum spp. caused by one or a combination of *Cercospora* sp., *Corynespora cassiicola*, *Colletotrichum* sp., and *Phyllosticta* sp. Disease symptoms similarities highlight difficulties to accurately diagnose.

summer, and fall), including *Plasmopara* sp., *Cercospora* sp., *Corynespora* sp., *Colletotrichum* sp. and *Phyllosticta* sp. Many caused symptoms of leaf spotting, blighting and defoliation similar to DM. Repeated sampling during trials failed to detect *Plasmopara* sp., the cause of downy mildew. Additional surveys of diseased viburnum from other nursery sites also failed to identify *Plasmopara* sp. in winter and spring of 2021. Controlled inoculations confirmed pathogenicity for *Colletotrichum* sp. and *Corynespora* sp., while *Pestalotiopsis* sp. appears to be an opportunistic saprophyte. Pathogenicity tests for *Phyllosticta* sp. and *Phoma* sp. are in progress. Each fungal genus designation was confirmed based on internal transcribed spacer region sequence. Additional sequencing is in progress for proper phylogenetic placement at the species level. These results stress the importance for growers to get an appropriate disease diagnosis to avoid making ineffective fungicide applications.

At the initiation of the first trial (Table 1), the initial survey of viburnum found *Plasmopara* sp. (downy mildew) (Fig. 2), *Cercospora* sp. and *Colletotrichum* sp. as the primary pathogens present. However, subsequent sampling failed to find any sign of downy mildew. Rather, *Colletotrichum* sp., *Corynespora cassiicola*, *Phyllosticta* sp., *Phoma* sp., and a *Pestalotiopsis* sp. were recovered from symptomatic foliar tissues (Figs. 3 and 4). Not surprisingly, the fungicides containing ametoctradin, cyazofamid, dimethomorph, fluopicolide, mandipropamid, mefenoxam, and oxathiapiprolin that specifically target oomycetes (i.e., *Plasmopara* sp.), failed to reduce disease severity at a level that was statistically significant relative to the non-treated control based on AUDPC. Benzovindiflupyr, difenoconazole, fluxapyroxad, and pyraclostrobin fungicides that are typically applied for the management of true fungi, reduced disease severity at a statistically significant level.

In the second trial, seven fungicides (Table 2) were re-evaluated on a new set of younger plants. In this trial, lower disease pressure from *Cercospora* sp., *Colletotrichum* sp., *Corynespora cassiicola*, *Phyllosticta* sp. produced more variable results. Numerically, the fungicides flutriafol and oxathiapiprolin appeared to increase disease severity relative to the non-treated control. Based on AUDPC, only benzovindiflupyr + difenoconazole reduced disease severity at a statistically significant level relative

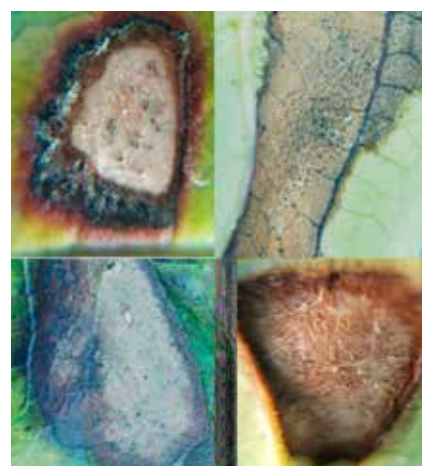


Fig. 4. Hand lens view of Viburnum leaf lesions associated with *Colletotrichum* sp. (top left), *Cercospora* sp. (top right), *Phyllosticta* sp. (bottom left), and *Corynespora* sp. (bottom right).

to the non-treated control; while pyraclostrobin + fluxapyroxad numerically also gave some control based on the final disease severity rating. At the time of submission of this paper, the trial was being repeated.

## Conclusion

Our findings align with the growers' reports of challenges with foliar disease management in viburnum while shedding a light on the components of this management puzzle. Growing season and environmental conditions play a key role in management decisions as a result of the multiple foliar diseases of *Viburnum* sp. occurring throughout the year. These results stress the importance of correct disease and pathogen diagnosis to select the appropriate fungicide treatments. Recommendations for foliar disease management of viburnum can be adjusted based on this research which includes correct disease identification, the timing of preventative broad-spectrum and pathogen-specific fungicide treatments based on environment and season, and fungicide ro-



tations. It is essential that growers selectively propagate plants from clean nursery or landscape stock and manage propagation intensely due to environmental conditions that favor disease outbreak. Future research will include pathogenicity testing of isolated fungi (*Phyllosticta* and *Phoma*), repeated fungicide efficacy testing, and additional sequencing for phylogenetic

placement to the species level.

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## **Response of Container-grown Muhly Grass to Controlled-Release Fertilizer Rate and Longevity**

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**Additional index words.** irrigation, *Muhlenbergia capillaris*, plant production, substrate nutrition

Liners of muhly grass (*Muhlenbergia capillaris*) were planted 15 February 2018 in 2.4-L containers in a pine bark-based substrate amended with four controlled-release fertilizer treatments: (T1) 0 kg·m<sup>-3</sup>, (T2) 2.8 kg·m<sup>-3</sup> and (T3) 5.5 kg·m<sup>-3</sup> of 18N–2.6P–10K (Osmocote® 18–6–12, 8–9 months longevity at 21°C), and (T4) 4.7 kg·m<sup>-3</sup> of 21N–1.7P–6.6K (Osmocote® 21–4–8, 12–14 months longevity at 21°C). Plants were grown on polypropylene ground cover under overhead sprinkler irrigation at Hibernia Nursery in Webster, FL to evaluate the impact of fertilizer rate and longevity on the plant growth response. After six months, changes in plant heights (final–initial) for T2, T3, and T4 were similar. Substrate NO<sub>3</sub>-N, P, and K decreased dramatically during the evaluation for all treatments and ranged from 19–0.2, 1.5–0.6, and 71–0.5 mg·L<sup>-1</sup>, respectively, for T2. These data indicate that muhly grass has a low nutrient requirement, likely needing less fertilizer than applied with T2. The minimal amount of fertilizer to apply to muhly grass without sacrificing growth deserves further investigation.

Hibernia Nursery, located in Webster, FL produces container plants for the southeast and mid-Atlantic markets. Muhly grass, a Florida native plant (Gilman, 1999) is grown from seed by placing dried flowers on 51-cell trays in late fall. In the following spring, clumps of seedlings in each cell are transplanted to 2.4-L containers. Plants are grown in a pine bark-based substrate amended with 4.7 kg·m<sup>-3</sup> of 21N–1.7P–6.6K controlled-release fertilizer (Osmocote® 21–4–8, 12–14 months longevity at 21°C, ICL Specialty Fertilizers, Dublin, OH). Because muhly grass requires approximately six months to reach marketable size, our objective was to determine if a fertilizer with a shorter longevity and applied at the same rate of N (1.0 kg·m<sup>-3</sup>) would result in similar plant growth compared to plants receiving the fertilizer labeled for a longevity of 12–14 months. A fertilizer with a shorter longevity could result in important environmental benefits.

### **Materials and Methods**

Liners of muhly grass (*Muhlenbergia capillaris*) were planted 15 February, 2018 in 2.4-L black containers (Landmark Plastic Corp. Akron, OH) at Hibernia Nursery in Webster, FL. The substrate (OldCastle®, Lakeland, FL) was composed of pine bark (70%), Florida peat (25%), and leaf compost (5%) amended with dolomitic limestone (8.3 kg·m<sup>-3</sup>) and Micromax® (0.9 kg·m<sup>-3</sup>). The following fertilizer treatment (T) amendments were added at the nursery: (T1) 0 kg·m<sup>-3</sup>, (T2) 2.8 kg·m<sup>-3</sup>, and (T3) 5.5 kg·m<sup>-3</sup> from 18N–2.6P–10K (Osmocote® 18–6–12, 8–9 months

longevity at 21°C), and (T4) 4.7 kg·m<sup>-3</sup> from 21N–1.7P–6.6K (Osmocote® 21–4–8, 12–14 months longevity at 21°C). T3 and T4 contained the same amount of N (1.0 kg·m<sup>-3</sup>). There were 12 plants for each of the four treatments randomly grouped together in each of four blocks on polypropylene ground cover. Muhly grass border plants were placed around each block.

Plants received overhead sprinkler irrigation as needed with Excel-Wobbler™ (Senninger, Clermont, FL) nozzles (grey #9 orifice) spaced ≈ 7.6 m apart in a triangular arrangement. Hibernia staff typically monitors container substrate moisture status once or twice a week by performing a moisture status rating for the substrate in irrigated zones. On 8 May, the irrigation application rate was measured and averaged 1.0 cm·h<sup>-1</sup> and Distribution Uniformity was 84% (Million and Yeager, 2012).

In each block, five plants from each treatment were selected and tagged. Heights of these plants were measured from substrate surface to uppermost foliage tip on 19 February, 26 April, and 7 Aug. Change in plant height was calculated as height in Aug. minus height in February. Plant height data were analyzed as a randomized block design using SAS statistical software for Windows.

One plant from each treatment for each block was also tagged for conducting pour-through (PT) extracts of the substrate on 19 February, 27 Mar., 26 April, 5 June, and 7 Aug. Substrate electrical conductivity (EC) was determined for all extracts and NO<sub>3</sub>-N, P, and K were determined for extracts collected on 19 February, 26 April, and 7 Aug. Extract pH was also determined in April. Deionized water application amounts used at each sampling for the PT extracts ranged from 140 mL in February, to 240 mL in Aug.

Border plants around each block were pruned 15 cm above the substrate on 2 April, 15 May, and 20 June so that light penetration to interior plants was not inhibited. On 19 June,

Trade names, companies, and products are mentioned for informational purposes only and do not constitute an endorsement. The authors gratefully acknowledge the assistance of Jeff Million for conducting the statistical analyses.

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plants were grouped according to treatment and on 20 June, six employees of the nursery ranked each group from best to worst. Plants for each treatment were also pruned 15 cm above the substrate on 20 June.

### Results and Discussion

Growth as the change in plant heights (final-initial) was similar for T2, T3, and T4 and greater than T1 (Table 1). The fact that T2 had similar growth to T3 and T4 indicates that the shorter longevity of T2 (8–9 months) and the lower application rate (50% less N) were sufficient when compared with T4 (12–14 months) used by the nursery. Additionally, T3 that had the same amount of N as T4, resulted in similar growth with a shorter longevity (8–9 months). Four of six nursery personnel ranked plants that received T3 the highest, further supporting the use of a shorter longevity fertilizer formulation. These growth response data indicate that production of muhly grass for 6 months in 2.4-L containers does not require fertilizer with a longevity of 12–14 months, nor does it require 1.0 kg·m<sup>-3</sup> N applied with T3 and T4 to achieve its potential growth. Container-grown nursery crops are typically grown in substrates amended with 1.2–1.8 kg·m<sup>-3</sup> N (Yeager, et al., 2010).

Substrate EC, NO<sub>3</sub>-N, P, and K measurements were taken during production to document the availability of nutrients and provide insight regarding fertilizer longevity. Substrate NO<sub>3</sub>-N, P, and K for T2, T3, and T4 decreased rapidly after the first PT in February. (Table 2) and all nutrient concentrations in April and Aug. were below the desirable levels suggested for ornamental crops (FDACS, 2014).

Substrate NO<sub>3</sub>-N concentrations for T3 were 4.5 mg·L<sup>-1</sup> in April and 0.2 mg·L<sup>-1</sup> in Aug. For T4, the concentrations were 4.8 mg·L<sup>-1</sup> in April and 4.8 mg·L<sup>-1</sup> in Aug., indicating the potential for longer-term availability or longevity with T4 compared to T3. The shorter longevity and lower rate of T2 was also evident from the lower NO<sub>3</sub>-N concentrations (Table 2).

Substrate EC exhibited a similar trend to that of nutrients, decreasing rapidly after the first PT. Average EC ranged from 1.4 d·S<sup>m</sup> for T2 and T4 in February. to 0.1 for T1, T2, and T3 in Aug. Average substrate pH in April ranged from 7.2 for T1 and T4 to 7.4 for T3.

The fact that similar plant growth was achieved with T2, T3, and T4 indicates that muhly grass has a relatively low nutrient

Table 1. Heights of muhly grass (*Muhlenbergia capillaris*) grown in 2.4-L containers at Hibernia Nursery, Webster, FL in 2018.

Treatment <sup>z</sup>	Plant height (cm)			Change (cm) Aug.–February.
	19 February.	26 April	7 Aug.	
T1	17	20	42	25
T2	17	35	68	51
T3	15	36	68	53
T4	17	35	70	53
Significance				
Treatment	0.0004	0.0001	0.0001	0.0001
P>F	***	***	***	***
LSD	1	3	3	3

<sup>z</sup>T1 = 0 kg·m<sup>-3</sup>, T2 = 2.8 kg·m<sup>-3</sup> and T3 = 5.5 kg·m<sup>-3</sup> from 18N–2.6P–10K (Osmocote® 18–6–12, 8–9 months longevity at 21 °C) T4 = 4.7 kg·m<sup>-3</sup> from 21N–1.7P–6.6K (Osmocote® 21–4–8, 12–14 months longevity at 21 °C).

Table 2. Substrate extract nutrient concentrations (mg·L<sup>-1</sup>) for muhly grass (*Muhlenbergia capillaris*) grown in 2.4-L containers at Hibernia Nursery, Webster, FL in 2018.

Nutrient	Treatment <sup>z</sup>	19 February.	26 April	7 Aug.
NO <sub>3</sub> -N	T1	0.5	0.12	0.07
	T2	19.3	0.54	0.21
	T3	31.1	4.54	0.18
	T4	10.5	4.77	4.76
P	T1	1.0	0.30	0.60
	T2	1.5	0.36	0.56
	T3	1.6	1.29	0.77
	T4	1.8	0.46	0.93
K	T1	47.3	2.51	0.11
	T2	70.9	4.61	0.54
	T3	71.2	4.41	0.14
	T4	64.3	1.57	2.66

<sup>z</sup>T1 = 0 kg·m<sup>-3</sup>, T2 = 2.8 kg·m<sup>-3</sup> and T3 = 5.5 kg·m<sup>-3</sup> from 18N–2.6P–10K (Osmocote® 18–6–12, 8–9 months longevity at 21 °C) T4 = 4.7 kg·m<sup>-3</sup> from 21N–1.7P–6.6K (Osmocote® 21–4–8, 12–14 months longevity at 21 °C).

requirement, likely requiring less fertilizer than applied with T2. These results agree with the findings of Zhao et al. (2018) that *M. filipes*, seems to have a low requirement for N. The minimal amount of fertilizer to apply without sacrificing growth deserves further investigation.

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—Scientific Note—

## **Investigating Green Industry Firms' Market Shares to Wholesalers, Landscapers, Retailers, and Direct-to-Consumer Channels**

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**Additional index words.** direct sales, fractional logit, horticultural sales, market channels

Combing four waves of the National Green Industry Survey from 2004-2019, this study explored factors that impact firms' choices of entering wholesale markets using a probit model, diversification of wholesale market channels using a zero-inflated Poisson regression model, and market shares among major market channels using a fractional logit model. Over the survey period, traditional wholesale market channels such as landscape services companies, re-wholesalers, and single-location garden centers remained mainstream channels. Supply to mass merchandisers and home centers remained low despite growing demand from big-box stores. While the number of firms selling through one major market channel remained stable, the number of firms selling through more than two channels declined significantly after 2009 and was not yet resorted. Surveyed firms also reported 30% of their 2018 sales were generated from direct sales, indicating that direct-to-consumer (DTC) channel, as a relatively new addition to the conventional channels, has the potential to gain significant market share.

Our empirical results show firms' choices of entering wholesale market and the number of wholesale market channels were positively correlated with firms' perceived importance

in production costs, labor costs and competitions. Firms with more negotiated sales or more sales to repeat customers were more likely to enter wholesale market and have more diversified market channels. Attending more trade shows increased not only the likelihood of accessing wholesale market but the number of wholesale market channels. Plant types were important factors determining firms' choices of entering a specific wholesale market channel. Particularly, we found some evidence of shifting sales between landscapers and re-wholesalers, indicating these two marketing channels could be substitute markets for similar plant types. As an indicator of potential demand for ornamental plants, increases in the housing price index increased firms' sales shares to landscapers who were closely related to the housing market.

During the COVID-19 pandemic, there was a surge in demand for houseplant categories. The coronavirus pandemic has altered many firms' business strategies and consumers' lifestyles including shopping behaviors and gardening decisions. Future studies may consider the long-run impact of the pandemic on different market channel alternatives and further explore the post-pandemic trends of DTC and conventional market channels.

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—Scientific Note—

## **Preferences for Sustainable Residential Lawns in Florida: The Case of Irrigation and Fertilization Requirements**

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**Additional index words.** Low-input lawn, water savings, generalized multinomial logit, consumer segmentation

The American landscape is well defined by the presence of turfgrass. To maintain the lush, green carpet, irrigation, fertilizer, and other necessary inputs are required. When these inputs are applied to excess, they are harmful to the natural environment. In this investigation, we evaluate the Florida homeowners' preferences of high- and low-level inputs of irrigation water and fertilizer using Generalized Multinomial Logit and Latent Class Logit regression models. Results indicated that there are heterogeneous preferences for the level of irrigation water and fertilizer application by Florida homeowners, including high-input users (33% of the sample), irrigation conscious users (27%), fertilizer conscious users (23%), and moderate input users (17%).

With approximately a third of the sample, and two classes of consumers seeking low-input turf grasses, it is clear there is a desire by some consumers for low irrigation and fertilizer

tolerant species. This overall result also suggests that there is a considerable majority of consumers who either are indifferent to the amount of water, chemicals, and maintenance they contribute to their turfgrass, as is the case for the Moderate Input consumers, or they have stronger pressures to have fit social norms and have relatively low knowledge how to care for their lawn (High Input consumers). This result is supported by previous studies where attractiveness, maintenance, and cost contribution to the consumer's prioritization of how they care for their lawn.

This was true with Irrigation Conscious and Fertilizer Conscious consumers, both environmentally conscious consumers, having higher knowledge about turfgrass than Moderate Input and High Input consumers. Florida homeowners are also willing to pay more for low-input attributes and they place high value and prefer low input into their turfgrass.

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—Scientific Note—

# Pilot Study to Evaluate Sargassum Compost for Soil Amendment Potential

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**Additional index words.** compost, Sargassum management, soil amendments

Since 2011, huge influxes of pelagic (living in the open sea) sargassum have been inundating Florida beaches, seagrass meadows, residential canals, and marinas. Sargassum is a genus of brown macroalgae, more generally referred to as seaweed. Sargassum provides numerous ecological benefits in the open ocean and ashore; however, these unprecedented accumulations are negatively affecting nearshore environments and tourism economies. Many coastal counties are developing sargassum management plans as these summer influxes are likely the “new normal.” In Monroe County alone, the Tourist Development Council estimates the economic impact from a severe sargassum year could be \$20 million and 300 lost jobs (Unpublished report. Rockland Analytics. 2020. Executive Summary, Assessment of Sargassum Activity in the Florida Keys and its Impact of Monroe County’s Economy).

As a result, there is a need to find economical methods for sargassum removal and reuse. However, there is a concern of heavy metal accumulation, specifically arsenic, that could limit the reuse of this macroalgae (Rodríguez-Martínez et. al. 2020). Composting could offer a viable option as part of a management toolbox for governments and stakeholders. The brown macroalgae is high in nitrogen content and other macro- and micronutrients. The material breaks down rapidly and Sembera et. al. (2018) found soluble salt content to be within the acceptable range for composts typically sold in the horticulture industry.

### Materials and Methods

The goal of this pilot study is to determine the viability of sargassum compost as a soil amendment as tested through a range of typical metrics measured for commercially available compost. This study is also being replicated in Sarasota and Martin Counties. For each location, twelve Geobin Composting

Systems were filled with four different compost recipes consisting of sargassum and woodchips (Table 1). The wood chips are specific to each county. For Monroe County, FL woodchips are a mixture of tropical hardwood species typically found in utility trimmings. Every 30 d the compost piles are turned by hand with shovels and samples are collected for analysis following standard operating procedures. Samples are analyzed for water content, pH, electroconductivity, and temperature. Every 60 d samples are also analyzed for total nitrogen, total phosphorus, and potassium. Arsenic (As) levels will also be measured every 60 d.

### Discussion

This pilot study will run for four months and is still underway. Our goal is to assess the quality of sargassum compost and identify the As levels. This information may help alleviate concerns people may have and help to manage the economic impact associated with sargassum. If As levels are low, composting sargassum may be part of a larger management toolbox for dealing with heavy sargassum loads on beaches and shorelines. By utilizing sargassum in a composting system, taxpayer dollars could be saved by diverting this material from landfills and create a value-added product for community reuse. The City of Fort Lauderdale has a similar large-scale, sargassum composting program that it implemented in 2010, though no analysis of the finished compost has ever been conducted.

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Table 1. Sargassum/wood chip treatments in a composting pilot study conducted in 2021 in Key West, FL.

Treatment I	Treatment II	Treatment III	Baseline control
100% sargassum by volume	50% sargassum/50% wood chips by volume	25% sargassum/ 75% wood chips by volume	100% wood chips by volume



—Scientific Note—

## Going Beyond the Grant for Community, Educational, and Volunteer Engagement

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**Additional index words.** community support, native plants, teaching gardens

Teaching gardens offer University of Florida, Institute of Food and Agricultural Sciences Extension Agents, an opportunity to reach a wider audience than a typical classroom setting, while also providing environmental benefits that can have lasting impacts in a community. Many residents in Monroe County, FL often relocate to the island chain from other regions of the country, with little knowledge of the flora and fauna that grow here. So, one of the goals of the demonstration garden was to expose residents to a wider diversity of native flora that thrive in our environment and do well in a landscape setting. Additionally, as land is cleared, and natural ecosystems are removed, less habitat is available for pollinators and beneficial organisms. Native wildflower demonstration gardens can help connect larger areas of conservation and create pathways for fauna by providing important habitat.

Monroe County Extension Service was awarded the Viva Florida Grant from the Florida Wildflower Foundation in 2019. The \$3,000 grant was the first step toward developing the public demonstration garden. Extension staff also worked with Monroe County government for additional support since the proposed site was on county property. Monroe County supplied water to the garden and enabled the pruning of the mature native trees. Extension staff and volunteers partnered with Key West Botanical Garden Nursery to provide the plant material that would be installed. The Botanical Garden is one of the recipients of the Florida Department of Agriculture's Endangered Plant Advisory Council (EPAC) grants for propagating and researching rare and endangered plants in the Keys. A part of the Garden's mission is to reestablish threatened and endangered plants in the community and the demonstration garden provided an opportunity to showcase some of these plants. Thirteen state-threatened and endangered plants were donated to help raise awareness. Keys Energy Services and Golden Bough Tree Service provided free mulch. Extension staff also worked with a local graphic designer, Local Guy Design, on developing educational signage. Other businesses and organizations donated services and financial support for the garden including Bandit Wildlife Management, City of Key West, and the Key West Garden Club.

Three educational signs were installed to help support the overall objectives of the demonstration garden—Butterflies in the Garden; Beneficial Insects: Friends of the Garden; and Threatened and Endangered Plants. A QR code was included on each sign that directs visitors to the Monroe County Extension Service's webpage on educational gardens to provide additional information about the plants growing in the garden. To date, eight local fact sheets have been written to provide gardeners with growing information that is relevant in Monroe County. Since some of the plants are very rare, little had been written about these species to support gardeners that wanted to utilize them in their landscapes. The demonstration garden has also provided an opportunity to observe, document, and write about these plant species.

Extension staff and volunteers have received overwhelmingly positive feedback for the 3600 ft<sup>2</sup> wildflower demonstration garden in the heart of downtown Key West. Over \$2,400 was received in donated services and products and just over \$2,000 was spent from the Extension Agent's programming funds, in addition to the initial grant award. Two-hundred and fifty Master Gardener Volunteer service hours have gone toward installing and maintaining the garden.

A Qualtrics survey was sent out to stakeholders, volunteers, and the public to gauge the success of the garden. Six community organization leaders, including the Mayor of the City of Key West, that provided Letters of Support for the garden, responded that we met our objectives of the demonstration garden and beautified the area. Seventy percent of the respondents to the survey said they used the website for additional information about growing plants. Twenty-eight Master Gardeners over the past two years have volunteered in the garden. All said they appreciated having an opportunity to work outside during the pandemic and that it was important for their mental health. Additionally, 80% of Master Gardeners said it was important to them to have hands-on gardening opportunities in extension sponsored demonstration gardens.

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—Scientific Note—

## **Anthocyanin Accumulation in Peppers: Potential Roles in Impacting Ornamental Value and Nutritional Qualities**

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**Additional index words.** antioxidant activity, pigments, purple peppers, vegetable breeding

Accumulation of anthocyanins leading to purple coloration is a common trait in the Solanaceae including peppers. Anthocyanins have been linked to improved plant stress tolerance and improved antioxidant qualities of the fruit. Also, the color is a good visual marker in breeding. At the University of Florida's pepper breeding program, we aim to incorporate purple color traits into breeding lines of *Capsicum annuum* so that new cultivars with both improved nutritional qualities and ornamental value could be created. Toward this, an advanced breeding line JR segregating for purple coloration-related phenotypes were generated, with 'Black Olive' as the source of the purple coloration. The objectives of the current investigation are 1) to document various purple coloration-related phenotypes in the progeny of 'JR2C' and 'JR-X' and 2) to test whether the purple fruit and the green fruit segregants significantly differed in their total antioxidant capacity.

### **Findings**

Purple fruit from lines 'JR2C88' and 'JR2C28' had  $0.192 \pm 0.016$  and  $0.165 \pm 0.011 \mu\text{mol}\cdot\text{g}^{-1}$  fwt of total anthocyanins, respectively. Green fruit from lines 'JR2C34' and 'JR2C32' had no detectable anthocyanins. The hypothesis that purple peppers have significantly increased antioxidant potential as compared to green fruit was tested. Antioxidant activities of extracts were measured by the Ferric Reducing Antioxidant Power (FRAP) assay (Benzie and Strain, 1996) and 1,1-diphenyl-2-picryl-hydrazyl (DPPH) antioxidant assay (Sharma and Bhatt, 2008), expressing antioxidant activities as ascorbate equivalents. Using FRAP assay, purple fruit from 'JR2C88' and 'JR2C28' had  $5.43 \pm 0.38$  and  $5.19 \pm 0.36 \mu\text{mol}\cdot\text{g}^{-1}$  fwt, respectively, and green fruit from 'JR2C34' and 'JR2C32' had  $3.35 \pm 0.29$  and  $2.54 \pm 0.44 \mu\text{mol}\cdot\text{g}^{-1}$  fwt, respectively. Using the DPPH assay, purple fruit from 'JR2C88' and 'JR2C28' had  $6.28 \pm 0.22$  and  $6.13 \pm 0.62 \mu\text{mol}\cdot\text{g}^{-1}$  fwt, respectively, and green fruit from 'JR2C34' and 'JR2C32' had  $4.51 \pm 0.29$  and  $9.02 \pm$

$0.84 \mu\text{mol}\cdot\text{g}^{-1}$  fwt, respectively. Purple fruit extracts had a greater antioxidant activity than green fruit on average by a factor of 1.8 as determined by FRAP assay, but with DPPH assay the two lines with green fruit differed significantly when compared to purple fruited lines.

The inheritance of purple vs. green cotyledon was studied in a segregating population of 84 'JR-X' progeny plants. The purple cotyledon phenotype segregated as a single recessive factor and was independent of the presence of purple coloration in flowers and fruit. In both populations referenced, distinct flower phenotypes ranging from white to purple were observed, consistent with results reported by Rêgo et al. (2015).

### **Suggestions for Future Study**

The relationship between the presence of anthocyanin and antioxidant activity will be further explored in green and purple fruit, from segregants in the 'JR2C' progeny population. Also, the FRAP and DPPH assays will be used to investigate antioxidant activities of different parts of the fruit tissue since the purple coloration is found in the first few cell layers of the pericarp. Further research will focus on the inheritance patterns of the four distinct flower phenotypes present in the 'JR-X' progeny namely white, purple radiant, purple glow, and purple grape.

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—Scientific Note—

## Wrong Plant, Wrong Place Costs Over One Hundred Thousand Dollars

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**Additional index words.** alkaline, diagnostics, landscape, soil test

A Central Florida city completed a huge park improvement project to the tune of \$4.5 million in 2018. The park boasts tennis and basketball courts, playgrounds, pathways, exercise equipment, a splash pad and amphitheater, pavilions, and a prominently located promenade of sixteen *Phoenix sylvestris* palms. The palms were uniformly and prominently planted but showing various stages of decline.

A former city commissioner, a landscaper himself, noticed the palms never really became established, so he and the park director contacted the Seminole County Extension Office. He described 16 silver date palms: some looking fine and some on the brink of death. The county extension agents made a site visit. Almost the entire park was planted with native plants, except for one area planted with 16 non-native *P. sylvestris* palms. The best of the group looked frazzled and had nutrient deficiency symptoms, and the worst warranted concern prompting disease diagnostics.

The three horticulture extension agents in Seminole County performed diagnostics. The agents took soil samples to test for nutrients and drilled for trunk core samples to test for disease. No disease was detected. The soil samples had a range of pH starting at 7.8 and increasing to mid-8. Compounding the issue was soil compaction. The soil where the palms were planted was noticeably hard and tolerable by only some plants.

The issues observed on the non-native and very expensive palm trees were consistent with high pH and compact soils. The leaves showed micronutrient deficiencies. After consulting with soil scientists, it was determined that it was unreasonable to drop the pH with sulfur and too expensive to amend the entire area with improved soil. A chelated iron product and good nutrient management could potentially limp the trees through, but the extension agents ultimately recommended right plant, right place. In this case, the agents' recommendations the Florida native sabal palm, which is tolerant of a wide range of pH and wet to dry conditions.

The city resisted taking the extension agents recommendation for two years. As predicted, the 16 *P. sylvestris* palms worth about \$7000 per tree before installation continued to decline. The city stayed in touch with extension agents the entire time, and in 2020, the realization that right plant, right place would lead to long term landscape resilience, the city replaced the phoenix palms with the Florida native *Sabal palmetto*, a \$400 per plant alternative.

This situation can be avoided in landscapes big and small. Test soil before planting. Select the right plant for the right place. Landscapers need to consider site and soil conditions in addition to pathological diagnostics, especially when there is no disease detected.

This paper was originally a poster presentation (P-6).

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## **Six-month and One-year Economic Prediction Models for the Environmental Horticulture Industry in Florida**

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### **Abstract**

To assist woody ornamental producers in the Florida environmental horticulture (EH) industry with budgeting and planning, a statistical prediction or forecasting model was developed using leading economic indicators and other related data that potentially affects employment and sales in the industry. The model was created using monthly reported employment from Florida EH producers as the dependent variable, used as a proxy for sales demand, along with 31 monthly independent variables and their lagged values from 2005 until 2019. The independent variables were offset six months or one year to the dependent variable to estimate two forecasting models. To improve the simplicity and ease of use of the model, variable numbers were minimized. The six-month model's predictors were reduced to four variables: S&P index from the previous quarter, Federal Funds Rate, vehicle sales, and new home sales. The reduced one-year model incorporated current employment numbers instead of the Federal Funds Rate. The simplified six-month and one-year predictor models had goodness of fit  $R^2$  values of 0.915 and 0.946, respectively. These models can be useful for EH producers to predict trends in sales and employment in the near future for market planning purposes and managing economic risk.

The abstract was presented at the 2021 FSHS Annual Meeting.

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## **Preemergence Herbicides for Weed Control in Caladium**

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### **Abstract**

Caladiums (*Caladium* spp.) are perennial ornamental plants in the family Araceae grown primarily for their colorful foliage. A substantial proportion of the tuber production of this species occurs in Lake Placid, Florida, on muck soils. Weed management can significantly reduce tuber yields and hinder harvest operations. There are limited herbicide options and as a result growers rely on hand labor for weed control. To address the lack of information on safe herbicides for caladium production, greenhouse trials were conducted at the Gulf Coast Research and Education Center, Balm, Florida, to evaluate tolerance of three caladium varieties ('Red Flash', 'White Christmas', and 'Rose Bud') to different preemergence herbicides. Aminopyralid, triclopyr, and aminocyclopyrachlor did not damage caladium at low rates if applied prior to emergence. There is significant risk of damage if the products are applied after emergence. Flumioxazin did not damage any of the caladium varieties when applied at 12–24 oz/acre. Dimethenamid-P was safe at application rates below 21 oz/acre. Flumioxazin and dimethenamid-P both appear to be viable weed management options for caladium growers with minimal crop damage risk.

The abstract was presented at the 2021 FSHS Annual Meeting.

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## Cover Crop Planting Method Influences Soil Nutrient and Water Concentrations in Citrus Alleys

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**Additional index words.** biomass, cereal rye (*Secale cereale*), conservation tillage, crimson clover (*Trifolium incarnatum*), lablab (*Lablab purpureus*), sorghum-sudangrass (*Sorghum bicolor* × *Sorghum sudanese*), sweet orange (*Citrus sinensis*)

Citrus is planted on the most acreage of any tree fruit in Florida, and recent reports describing the relationships between soil and tree health have increased interest in including cover crops (cc) as a strategy to improve tree and agroecosystems health. To assess short-term impacts of cc on crop and soil nutrient concentrations and soil moisture, a one-year study was conducted in a 16-year-old navel orange (*Citrus sinensis* Osb. var. *brasiliensis* ‘Tanaka’) orchard to compare cover crop seeding methods to the common alley management of perennial grass. Treatments were arranged in a randomized complete block, replicated four times, and included full tillage followed by cc seeding (FC), no tillage and cc seeded into existing vegetation (NC), and a control of existing vegetation with no tillage and no cc (NV). All cover crops were seeded with a no-till drill, and included a cool season cover crop mixture (Winter) of cereal rye (*Secale cereale* L. ‘FL 401’) and crimson clover (*Trifolium incarnatum* L. ‘Dixie’) seeded after the citrus harvest on 20 December 2019 and terminated when the citrus was blooming on 21 March 2020, followed by a warm-season mixture (Summer) of sorghum-sudangrass [*Sorghum bicolor* (L.) Moench × *S. sudanese* (Piper)] and lablab (*Lablab purpureus* L. ‘Sweet’) seeded when fruit were baseball-sized on 25 June 2020 and terminated by mowing on 11 September 2020 when the green fruit were nearly fully grown in size. Winter cc biomass was similar for FC and NC. Summer-grown cc biomass in FC (5890 kg·ha<sup>-1</sup>) was greater than NC (790 kg·ha<sup>-1</sup>). Orthogonal contrasts of the nutrient concentration of all biomass (weed+cc) revealed that full tillage increased magnesium (Mg) by 48% and the presence of cover crops increased nitrogen (N) and iron (Fe) by 34% and 210%, respectively. Soil samples taken after cover crop termination revealed that nutrient concentrations decreased with increasing soil depth but were not affected by these treatments. An observed 3% decrease in soil moisture in the top 12 cm (where most fibrous citrus roots are present) by the cc treatments over both cover cropping seasons may be partially explained by the increased above ground biomass of cc treatments (3725 kg·ha<sup>-1</sup>) as compared to the control (2640 kg·ha<sup>-1</sup>). Citrus leaf nutrient N remained at sufficient levels throughout the experiment. In this study, cc increased soil moisture at a depth of 70 cm in the winter (1 February to 21 March) by 180% and reduced soil moisture in the summer (25 June to 11 September) by 58% compared to NV.

Florida citrus acreage is 24 times greater than the sum of all other state-grown fruit crops combined and produced 3029 tons of fruit in 2019 (USDA NASS, 2019). The identification of huanglongbing (HLB) in 2005, a disease caused by a phloem-limiting bacterium *Candidatus Liberibacter asiaticus* and spread by the Asian citrus psyllid (*Diaphorina citri*) necessitated new production strategies. Researchers have been investigating possible remedies to HLB such as: vector management (Alvarez et al., 2016); biological control (Hall and Gottwald, 2011); slow-release fertilizers (Vashisth and Grosser, 2018); foliar fertilizers (Morgan et al., 2016); citrus under protective screen (Schumann et al., 2019); breeding (Ramadugu et al., 2016); cultivar selection (Folimonova et al., 2009); and other antibacterial agricultural sprays and injections (Ansunce et al., 2019; Hu et al., 2018; Shin et al., 2016; and Yang et al., 2018). Infected citrus trees require additional nutrients to remain at or above the current recommendations for non-HLB infected trees (Chinyukwi et al., 2020; Schumann et al., 2019). However, increasing nutrient rates without modifying practices to increase the retention time of those nutrients in sandy soils will increase the risk to water quality (Morgan, 2007; Vashisth and Grosser, 2018).

In a typical Florida citrus production system, alleys are seeded with perennial bahiagrass (*Paspalum notatum* Fluegge) and managed by mowing, no-tillage, no pesticide application, and rain-fed irrigation. Bahiagrass as a ground cover has the ecological benefits of maintaining a live root in the soil year-round, increasing soil organic matter, and increasing water holding capacity (Wright et al., 2015). The integration of cover crops (cc) in annual and perennial crop production systems also provides these benefits and can additionally provide a suitable habitat for pollinators (Bugg and Waddington, 2004), increase nutrient cycling (Maltais-Landry and Frossard, 2015; Schipanski et al., 2014), reduce nutrient leaching (Schipanski et al., 2014), provide climate change adaptation (Kaye and Quemada, 2017), and alter hydrologic and nutrient distributions in the soil due to on different rooting architectures (SARE, 2007), but may reduce water and crop nitrogen N uptake when grown as an intercrop (Celette et al., 2009). Aside from harvesting, perennial fruit growers have the opportunity to plant and grow cover crops (cc) year-round and termination of cc can coincide with times when trees increase nutrient uptake. For example, cc can be planted immediately after harvesting for early-ripening citrus in central Florida and terminated ahead of the tree N demand at spring flush.

The management, risks, and benefits of cc have been extensively documented in agronomic systems. In contrast, scientific and technical efforts to advance cc integration in horticultural systems have been diluted among a diversity of crops, production methods, rotations, and

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location. Without the rotational constraints associated with annual crops, cc in orchard alleys can be maintained year-round without sacrificing production area. Cover crop benefits are directly correlated with biomass for most soil health metrics, but land preparation prior to cover crop planting may require full-tillage. Studies conducted in agronomic systems have demonstrated the negative effects of full-tillage on soil compaction, water infiltration, available water, soil microbial respiration, and organic matter retention (Blanco-Canqui and Ruis, 2018; Nunes et al., 2018). Reducing tillage can improve soil conditions by increasing soil organic carbon (C), soil N concentration, wet aggregate stability, water infiltration, and plant available water in most systems (Alam et al., 2016; Blanco-Canqui and Ruis, 2018; Harramato and Brainard, 2012; Morugán-Coronado et al., 2020; Nunes et al., 2018). The objective of this project was to evaluate cc seeding strategies on cc establishment and growth, seasonal responses to soil moisture and nutrient content, and tree leaf nutrient status following cc termination.

### Materials and Methods

**EXPERIMENTAL SITE.** The weather conditions during the experiment were moderate for the region with a total of 815 mm of rainfall and an average temperature of 22.2 °C. The experiment was conducted at the University of Florida Institute of Food and Agricultural Sciences (UF/IFAS Plant Science and Research Center (PSREU) in Citra, FL (29.411261°N; -82.149435°W) on 16-year-old navel orange (*Citrus sinensis* Osb. var. *brasiliensis* 'Tanaka') trees on Swingle citrumelo rootstock [*C. paradisi* Macf. × *P. trifoliata* (L.) Raf.] in a Tavares sand (Hyperthermic, uncoated Typic Quartzipsamments) with a pH of 6.6. Trees were previously documented to have HLB but were generally vigorous, with typical yields of 21,941 kg·ha<sup>-1</sup> (145 trees/acre) (B. Nelson, UF/IFAS PSREU Research Coordinator, personal communication). Tree alleys are 2.4 m wide, and prior to this study, were managed by mowing existing vegetation, mostly bahiagrass sod, to a height of 11 cm. Trees received fertilizer applications according to UF/IFAS recommendations and are hand harvested and pruned annually. Trees were irrigated by micro jet sprinklers positioned near the trunk of each tree. Irrigation water was distributed to a diameter of < 1.8 m and did not irrigate the alleys.

**EXPERIMENTAL DESIGN.** The treatments were arranged in a randomized complete-block design with four replicates and included existing vegetation, no-tillage, and no cc (NV) that served as the modified grower standard; no-till with cover crops (NC); and full-till with cover crops (FC). Plots consisted of three measurement trees and the width of one alley on the both sides of the tree row (231.8 m<sup>2</sup>). Plots were separated by an 8.2 m buffer between blocks and 3.7 m between treatments.

**TREATMENT ESTABLISHMENT.** A cool season cover crop mixture (Winter) of cereal rye (*Secale cereale* L. 'FL 401') and crimson clover (*Trifolium incarnatum* L. 'Dixie') were seeded with a no-till drill (Sukup 2100, Sukup Manufacturing Co., Sheffield, IA) on 20 Dec. 2019 and terminated by mowing on 21 Mar. 2020, followed by a warm-season mixture (Summer) of sorghum-sudangrass [*Sorghum bicolor* (L.) Moench × *S. sudanese* (Piper)] and lablab (*Lablab purpureus* L. 'Sweet') on 25 June 2020 and mowed on 11 Sept. 2020. Species were selected because they were adapted for local conditions, were commercially available, and in use by area growers. All plots (FC, NC, and NV) were mowed prior to seeding cover crops on 18 Dec. 2019. In the FC plots, bahiagrass and weeds in alleys were treated with glyphosate herbicide and rototilled to a depth of 15 cm within a week of seeding winter and summer cover crops. Trees received 1600 kg·ha<sup>-1</sup> of granular fertilizer 14N-3.1P-11.6K plus minor elements divided into four applications between February and September. An additional 22.4 kg·ha<sup>-1</sup> N was applied to alleys on 24 Dec. 2020 after the cc planting to support rye establishment.

**BIOMASS AND TISSUE NUTRIENT ANALYSIS.** Biomass (cc and weed) was clipped at ground level and collected from a randomly selected 0.25 m<sup>2</sup> quadrat within each plot and separated by cc species, broadleaf weeds, and monocot weeds. At the end of each cc season, 30 of the most re-

cently developed mature citrus leaves without visible HLB symptoms were randomly selected from the three trees within each plot on the same day as total biomass collection. Biomass samples were dried to constant weight in a forced air drier (63 °C), weighed, and ground to a 1 mm diameter and submitted for nutrient analysis (Waypoint Analytical Laboratory, Mulberry, FL).

**SOIL NUTRIENT ANALYSIS.** Soil samples were collected using two methods, a standard hand-held probe (2.5 cm diameter probe to 15 cm deep) before the treatments were imposed and a 6.4 cm diameter core collected with a gas-powered auger (Echo Earth Auger Model EA-410, AMS Inc., American Falls, ID) at cover crop termination (12 Mar. and 11 Sept. 2020). The soil core was removed in 15-cm depth increments to 75 cm deep and divided into three sections: a) 0–15 cm; b) 15–45 cm; and c) 45–75 cm deep and air dried to a constant weight. Soil samples were filtered through a 2 mm screen and submitted to the UF/IFAS Analytical Research Laboratory (Gainesville, FL) for analysis with a Mehlich-3 extraction. Organic matter was measured at the 0-15 cm depth by loss on ignition. Ammonia (NH<sub>4</sub>N) (EPA 350.1), nitrate (NO<sub>3</sub>N) (EPA 353.2), and total Kjeldahl N (TKN) (EPA 351.2) were measured at all depths.

**SOIL VOLUMETRIC WATER.** Soil water content was measured using two different methods, each at different time scales and depths. Three capacitance sensors (EnviroSCAN, Sentek PTY Ltd. Kent Town, SA, Australia) were installed (BMP Logic, Trenton, FL) in a representative plot for each treatment. Each sensor was removed temporarily for cc seeding and reinstalled in a different location within the same plot. Measurements were collected every 30 min and centered at five depths (10.2 cm, 20.3 cm, 40.6 cm, 71.1 cm, and 101.6 cm). Volumetric soil water content was measured monthly with a time domain reflectometry (TDR) handheld probe (HydroSense II, Campbell Scientific, Logan, UT) to 12 cm.

**STATISTICAL ANALYSIS.** Treatment response variables were analyzed using the mixed model procedure PROC GLIMMIX (SAS v 9.4, SAS Institute, Cary, NC) for main effects and interactions. When relevant, means were separated using the LSD method at a significance level of  $P \leq 0.05$ . Orthogonal contrasts with both seasons combined were conducted to test a priori hypotheses that tillage soil disturbance (FC vs. NC and VC) and cover crop presence (FC and NC vs. VC) affected the parameters studied at a significance level of  $P \leq 0.05$ . TDR soil moisture data were arcsin transformed for normality and modeled with a heterogeneous compound symmetry variance-covariance structure using repeated measures ANOVA. Results are presented as untransformed data using statistical conclusions drawn from the transformed data. Soil volumetric water data measured by the capacitance probes were not statistically analyzed due to the lack of replication.

### Results

**BIOMASS.** Winter-grown cc biomass was similar between FC and NC, but both treatments reduced weed biomass compared to NV (Table 1). Winter cc biomass exceeded weed biomass by a ratio of 15:1 in FC and 3:1 in NC. Summer-grown cc biomass was greatest in FC (5890 kg·ha<sup>-1</sup>) compared to NC, and weed biomass was lowest in FC (590 kg·ha<sup>-1</sup>) compared to NC and NV. Contrasts revealed that full-tillage increased total biomass production (cc+weed) ( $P < 0.001$ ) and decreased dicot and total weed biomass ( $P \leq 0.048$  for each) compared to NC and VC. Additionally, the inclusion of cover crops (FC and NC) increased total biomass ( $P = 0.010$ ) and decreased total weed biomass ( $P = 0.014$ ) compared to NV.

**TISSUE NUTRIENTS.** In both winter and summer, legume cc species had a greater concentration of nutrients than grass cc species in FC and NC, except for a reversed pattern for copper (Cu) and zinc (Zn) in the summer under NC management (Table 2). Citrus leaf nutrient concentrations were not affected by tillage treatment ( $P \geq 0.144$ ) for the same nutrients presented in Table 2.

**TISSUE NUTRIENT ESTIMATES BY WEIGHT.** Tissue nutrient content estimates were greater in the summer due to increased biomass compared to the winter ( $P \leq 0.013$  for all nutrients listed in Table 3).

Table 1. Dry weight of weed and cover crop biomass under the treatments of no-till with existing vegetation (NV), no-till with cover crops (NC), and full-till with cover crops (FC).

Season	Weed			Cover crop			Total biomass
	Dicot	Monocot	Total	Legume	Grass	Total	
----- (kg·ha <sup>-1</sup> ) -----							
Winter <sup>z</sup>							
FC	150 b <sup>y</sup>	20	170 b	210	2180	2390	2560 a
NC	309 b	140	449 b	180	1420	1600	2049 a
NV	840 a	160	1000 a	--	--	--	1000 b
SE <sup>x</sup>	120	60	130	60	290	330	0.26
<i>P</i> value	0.018	0.190	0.011	0.753	0.165	0.192	0.012
Summer							
FC	300	290	590 b	1390	4500 a	5890 a	6480
NC	3170	1470	4640 a	360	430b	790 b	5430
NV	2580	2100	4680 a	--	--	--	4680
SE	900	630	530	400	210	390	0.57
<i>P</i> -value	0.137	0.200	0.002	0.165	< 0.001	0.003	0.157

<sup>z</sup>Winter biomass was harvested on 21 Mar. 2020 (92 d of growth); Legume cover crop (cc) = Crimson clover; grass cc = Cereal rye. Summer biomass was harvested on 11 Sept. 2020 (78 d of growth); Legume cc = Lablab; grass cc = Sorghum-sudangrass.

<sup>y</sup>Statistical analysis was conducted within each season and treatments with the same letter besides the mean are not significantly different at  $P \leq 0.05$  as calculated by LSD.

<sup>x</sup>SE represents the standard error of the mean of the sample.

Table 2. Nutrient analysis for cover crops grown after full-till (FC) or no-till (NC).

Season	Tillage	Species	Ca <sup>z</sup>	K	Mg	N	P	S	B	Cu	Fe	Mn	Zn
			----- (%) -----						----- (ppm) -----				
Winter <sup>y</sup>	FC	CR	0.4	1.7	0.2	1.9	0.4	0.1	4.3	5.5	55.5	36.5	41.0
		CC	1.4	1.4	0.5	3.1	0.3	0.2	30.3	12.3	274.5	64.0	80.8
SE <sup>x</sup>			0.1	0.2	< 0.01	0.2	< 0.01	< 0.01	1.3	0.4	22.6	5.1	2.5
<i>P</i> value			0.001	0.191	0.002	0.007	0.404	0.003	0.000	0.001	0.006	0.031	0.002
	NC	CR	0.4	1.6	0.2	1.7	0.3	0.1	3.3	4.8	40.8	45.3	40.0
		CC	1.2	1.5	0.5	3.0	0.4	0.2	29.3	12.0	176.5	64.5	86.5
SE			< 0.01	0.2	0.0	0.2	< 0.01	< 0.01	1.1	1.1	35.2	10.3	7.4
<i>P</i> value			0.000	0.620	0.000	0.003	0.159	0.007	0.000	0.012	0.072	0.064	0.005
Summer	FC	SS	0.4	1.1	0.3	1.2	0.3	0.16	2.3	5.0	50.0	23.0	59.0
		LL	1.2	1.8	0.3	2.7	0.4	0.24	27.3	6.5	91.5	37.3	39.8
SE			< 0.01	< 0.01	< 0.01	0.1	< 0.01	< 0.01	0.7	0.2	13.3	2.5	2.2
<i>P</i> value			0.000	0.000	0.301	0.001	0.011	0.012	0.000	0.014	0.114	0.029	0.003
	NC	SS	0.4	1.6	0.4	1.5	0.5	0.20	2.5	8.5	118.3	26.3	82.5
		LL	1.3	1.6	0.3	2.3	0.4	0.23	24.0	5.3	57.3	29.3	37.5
SE			0.1	0.2	< 0.01	0.2	< 0.01	< 0.01	2.4	0.4	30.5	5.8	2.3
<i>P</i> value			0.002	0.973	0.194	0.048	0.496	0.035	0.008	0.010	0.252	0.647	0.001

<sup>z</sup>Nutrients calcium (Ca), potassium (K), magnesium (Mg), nitrogen (N), phosphorous (P), and sulfur (S) are reported as a percent (%) of total tissue. Nutrients boron (B), copper (Cu), iron (Fe), manganese (Mn), and zinc (Zn) are reported in parts per million (ppm).

<sup>y</sup>Winter season = 20 Dec. 2019 to 21 Mar. 2020; Cover crop species: CR = Cereal rye (grass), CC = Crimson clover (legume). Summer season = 25 June 2020 to 11 Sept. 2020; Cover crop species: SS = Sorghum-sudangrass (grass), LL = Lablab (legume).

<sup>x</sup>SE represents the standard error of the mean of the sample.

Cover crops accumulated more magnesium (Mg) in FC compared to NV, and both N and iron (Fe) were greater in FC and NC than NV (Table 3). Tillage and cc contrasts demonstrated that both full-tillage and the inclusion of cover crops increased the nutrient concentrations for Mg ( $P \leq 0.029$  for both) and N ( $P \leq 0.031$  for both) compared NV. Full tillage increased sulfur (S) ( $P = 0.051$ ) and the inclusion of cover crops increased Fe ( $P = 0.018$ ) and phosphorus (P) ( $P = 0.035$ ).

**SOIL NUTRIENT CONCENTRATIONS.** Soil organic matter content in the top 15 m was not affected by tillage, season, or an interaction and observed values ranged from 1.5–2.1 mg·kg<sup>-1</sup>. The majority of soil nutrient interactions were influenced by the decline of nutrient concentration

with increasing soil depth, excluding sodium (Na) and inorganic N (NH<sub>4</sub>N and NO<sub>3</sub>N) (Table 4). In addition, TKN ( $P = 0.03$ ) and Zn ( $P = 0.04$ ) were increased at the summer sampling date, whereas NH<sub>4</sub>N ( $P \leq 0.001$ ) was reduced. Contrasts comparing all depths combined did not demonstrate any differences for tillage or cc, but applying a contrast analysis focused on the 0–15 cm profile indicated that full-tillage and cover crops decreased soil nutrient concentrations for most nutrients. At 0–15 cm, full-tillage and cc inclusion decreased the soil nutrient concentration of aluminum (Al) ( $P \leq 0.043$  for both), manganese (Mn) ( $P \leq 0.022$  for both), and P ( $P \leq 0.007$  for both). Full-tillage decreased Fe ( $P = 0.028$ ) and increased Mg ( $P = 0.040$ ).

Table 3. Biomass (weed+cover crop) for winter and summer seasons and estimated nutrient concentration by weight for treatments of no-till with existing vegetation (NV), no-till with cover crops (NC), and full-till with cover crops (FC).

Tillage	Biomass (kg·ha <sup>-1</sup> )	Nutrient concentration (g·ha <sup>-1</sup> )										
		Ca <sup>z</sup>	K	Mg	N	P	S	B	Cu	Fe	Mn	Zn
NV	2640 b <sup>y</sup>	30.0	69.2	9.8 b	48.8 b	9.9	5.2	40.0	24.7	386.5 b	151.9	217.5
FC	4100 a	35.9	65.2	14.5 a	74.0 a	13.1	7.1	44.7	30.1	1033.9 a	165.7	221.8
NC	3350 ab	33.9	71.9	12.7 ab	56.9 a	13.6	5.7	56.0	30.9	591.2 a	137.3	187.7
SE <sup>x</sup>	0.31	4.34	9.21	0.92	5.74	1.31	0.61	10.29	2.51	86.36	14.09	13.23
<i>P</i> -value	0.025	0.909	0.550	0.006	0.040	0.086	0.095	0.464	0.133	0.048	0.665	0.140

<sup>z</sup>Nutrients calcium (Ca), potassium (K), magnesium (Mg), nitrogen (N), phosphorous (P), and sulfur (S) are reported as a percent (%) of total tissue. Nutrients boron (B), copper (Cu), iron (Fe), manganese (Mn), and zinc (Zn) are reported in parts per million (ppm).

<sup>y</sup>Values above represent the mean and treatments with the same letter besides the mean are not significantly different at  $P \leq 0.05$  as calculated by LSD.

<sup>x</sup>SE represents the standard error of the mean of the sample.

Table 4. Soil nutrient concentration at multiple depths for treatments of no-till with existing vegetation (NV), no-till with cover crops (NC), and full-till with cover crops (FC).

Tillage	Depth (cm)	Nutrient concentration (mg kg <sup>-1</sup> )												
		Al <sup>z</sup>	Ca	Cu	Fe	K	Mg	Mn	Na	NH <sub>4</sub> N	NO <sub>3</sub> N	TKN	P	Zn
NV	0–15	653 b <sup>y</sup>	392 a	2 a	156 a	23	43 a	8 a	13	3	1	477 a	248 a	7 a
	15–45	805 a	138 b	1 b	122 b	17	20 b	4 b	13	2	2	162 b	176 b	2 b
	45–75	653 b	142 b	0.4 c	111 b	22	21 b	3 b	13	2	1	108 b	101 c	2 b
SE <sup>x</sup>	58	37	0.1	7	3	5	1	0.6	0.2	<0.1	29	16	0.5	
<i>P</i> -value	0.012	<0.001	<0.001	0.007	0.103	0.001	0.004	0.565	0.346	0.604	<0.001	0.004	<0.001	
FC	0–15	528 b	507 a	2 a	138 a	19 a	64 a	13 a	13	2	1	524 a	203 a	8 a
	15–45	681 a	210 b	1 b	110 b	14 b	23 b	5 b	13	2	2	184 b	159 a	3 b
	45–75	648 a	112 c	0.3 c	105 b	12 b	18 b	3 b	13	2	2	111 b	95 b	1 c
SE <sup>x</sup>	55	34	0.1	7	3	4	1	0.6	0.2	<0.1	26	15	0.5	
<i>P</i> -value	0.038	<0.001	<0.001	0.027	0.011	<0.001	<0.001	0.594	0.62	0.968	<0.001	0.011	<0.001	
NC	0–15	588 b	402 a	2 a	155 a	19	37a	9 a	13	2	1	457 a	226 a	6 a
	15–45	722 a	231 b	1b	113 b	13	20 b	5 b	13	2	1	173 b	167 b	2 b
	45–75	651 ab	117 b	0.4 b	112 b	13	16 b	4 b	14	2	1	95 b	90 c	1 b
SE	55	34	0.1	7	3	4	1	0.6	0.2	<0.1	26	15	0.5	
<i>P</i> value	0.045	0.007	<0.001	<0.001	0.057	0.006	0.01	0.655	0.33	0.819	<0.001	<0.001	0.002	

<sup>z</sup>Nutrients are defined as aluminum (Al), calcium (Ca), copper (Cu), iron (Fe), potassium (K), magnesium (Mg), manganese (Mn), sodium (Na), ammonia N (NH<sub>4</sub>N), nitrate N (NO<sub>3</sub>N), total Kjeldahl N (TKN), phosphorus (P), and zinc (Zn).

<sup>y</sup>Values above represent the mean ± SE and treatments with the same letter besides the mean are not significantly different at  $P \leq 0.05$  as calculated by LSD.

<sup>x</sup>SE represents the standard error of the mean of the sample.

**SOIL MOISTURE.** Soil moisture measured by TDR from 0-12 cm deep ranged from 0.1% to 6.3% and decreased throughout the winter and summer cc seasons. Soil moisture in the VC plots was 3% greater than FC or NC ( $P = 0.002$ ). The trends were consistent within each season, and a means separation was observed during the drier winter season (Fig. 1). Contrasts revealed that full-tillage reduced soil moisture ( $P < 0.001$ ), but the inclusion of cc did not ( $P = 0.318$ ).

Capacitance soil moisture readings from 1 Feb. to 24 June (Winter) indicate a general trend of increased moisture below 10 cm for FC and NC compared to VC (Table 5). In February and March, the average daily soil moisture in FC and NC increased immediately after rainfall events to a depth of 40 cm, but in VC the water infiltration to 40 cm was observed only after a 2-d rainfall event that exceeded 2.8 cm. The increased rainfall from June to September resulted in water infiltration below the 100 cm profile after these rainfall events. The cc treatments (FC and NC) reduced moisture at and below the 70 cm profile compared to VC.

## Discussion

Results of this study suggest that tillage may not impact cover crop biomass accumulation in the winter, but it should be noted that the

winter season was the first time the plots were tilled, and the cumulative effect of tillage may not have become evident until the summer season after the FC plots had been fully tilled twice. Additionally, the use of strip tillage to create a narrow prepared seedbed in the summer may increase cc biomass while retaining some existing vegetation, and further evaluation is needed to measure offsetting impacts to soil health due to increased soil disturbance.

The increase in legume percent N compared to grass percent N on a dry weight basis is well documented, but the overall N contribution from legume residues in this study was offset by lower yields. Granatstein and Mullinix (2008) reported nutrients from cc could increase tree leaf N in apple orchards, but those same nutrients could be lost by volatilization or leaching events. Although citrus leaf N was sufficient at both measurement times, a longer-term study is needed to determine whether cc and/or tillage affect citrus leaf N. The increase in total tissue nutrient N during cc growth is promising, considering the potential for future N nutrient cycling within the system (Li et al., 2015; Ranells and Waggener, 1997). The additional requirement of micronutrients for HLB-infected trees may be satisfied in part through cc residue decomposition and micronutrient cycling. The increased uptake of Mg, Fe, and P in cc tissues as compared to existing vegetation is also promising, but the observed

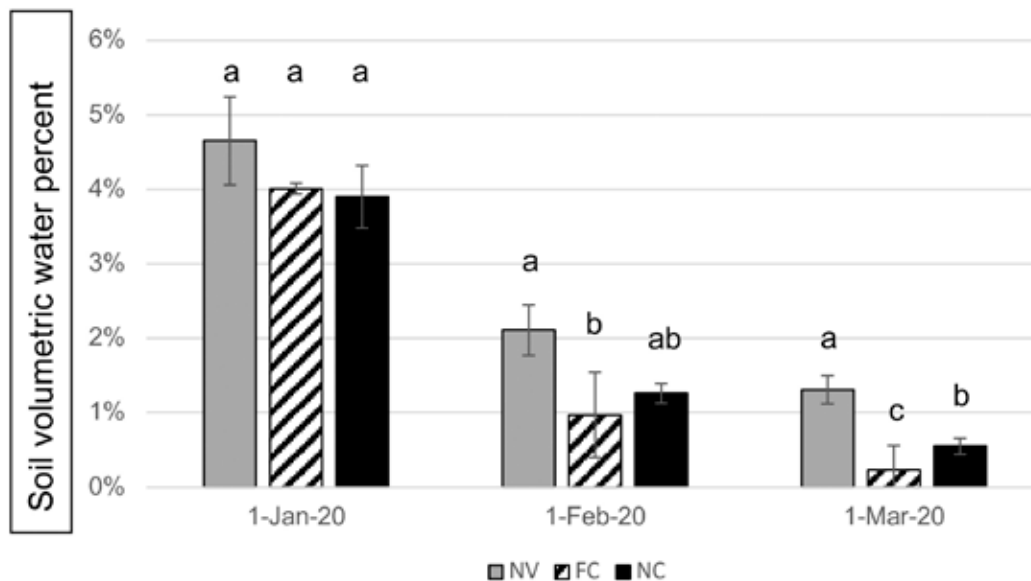


Fig 1. Winter season soil volumetric water percent at soil depth of 0–12 cm (bars represent STD) measured by a TDR instrument in citrus alleys from treatments of no-till with existing vegetation (NV), no-till with cover crops (NC), and full-till with cover crops (FC). Within each date, percentages with the same letter above the bar are not significantly different at  $P \leq 0.05$  as calculated by LSD.

Table 5. Average soil volumetric water measured by a capacitance soil moisture probe at multiple depths for tillage treatments of no-till with existing vegetation (NV), no-till with cover crops (NC), and full-till with cover crops (FC).

Season <sup>z</sup>	Tillage <sup>y</sup>	Soil volumetric water (%)					Avg.
		10 cm	20 cm	40 cm	70 cm	100 cm	
Winter	NV	4.21	2.94	2.41	2.47	2.58	2.92
	FC	3.76	5.10	6.10	4.47	3.65	4.61
	NC	4.40	6.12	6.26	4.42	4.75	5.19
Summer	NV	4.51 <sup>y</sup>	8.88 <sup>y</sup>	6.16	7.42	7.95	6.98 <sup>y</sup>
	FC	6.34	6.52	6.34	3.00	3.42 <sup>y</sup>	5.12 <sup>y</sup>
	NC	3.24	7.71	8.38	5.56	5.15	6.01
Yearly Avg.	NV	4.64 <sup>x</sup>	4.90 <sup>x</sup>	3.61	4.09	4.37	4.32 <sup>x</sup>
	FC	4.96	5.70	6.09	3.97	3.56 <sup>x</sup>	4.86 <sup>x</sup>
	NC	4.44	6.71	6.66	4.56	4.73	5.42

<sup>z</sup>Season: All measurements occurred in 2020. Fall = 1 Feb. to 21 Mar. (46 mm rainfall; 31 mm rainfall during preceding 38 d); Summer = 25 June to 11 September (389 mm rainfall).

<sup>y</sup>Validity of the values shown are questionable and are directly reported from the sensor. Patterns of a similar or increased soil moisture from the 10 cm to 20 cm range were not observed in the control group and no response at the 100 cm was observed until the later season in the Full group, which is inconsistent with the other treatments.

<sup>x</sup>Average amounts include questionable results as noted above.

decrease in soil P may result in an offsetting nutrient cycling benefit.

The reduced soil nutrient concentrations below 15 cm for most of the nutrients measured in this study was expected due to the decreased amount of organic matter below 18 cm (NRCs, 2018). Results of previous studies that were not consistent with this study include: cereal rye translocated potassium (K) from deep to shallow soil profiles (Eckert, 1991); crimson clover increased soil organic matter and soil N compared to winter fallow (Neely et al., 2018); and a mixture of annual ryegrass [*Lolium perenne* L. ssp. *multiflorum* (Lam.) Husnot], red clover (*Trifolium pratense* L.), crimson clover, and hairy vetch (*Vicia villosa* Roth) increased Fe and Zn (Nunes et al., 2018), and in the same study, increased P under no-till versus full-till operations.

Cover crops reduced the volumetric soil water at the 0–12 cm depth, but the lack of nutrient change in the citrus tree leaves suggests that the observed water reduction may be offset by uptake at lower depths or during times of ample moisture. These results are consistent for research conducted in wheat and corn (McGuire et al., 1998; Sanders et al., 2018)

and inconsistent for pear (Brooks et al., 2018). Orthogonal contrasts revealed that tillage also reduced soil water, which is consistent with Blanco-Canqui and Ruis (2018) and Nunes et al. (2018).

Deep-rooted cover crops planted and terminated before row cropping seasons store soil water by creating channels and increasing porosity within the soil profile (Brooks et al., 2018; SARE, 2007; Rankoth et al., 2019). Perennial systems that include cover crops can increase leaching through similar mechanisms, but rainfall, soil nutrient dynamics, and plant water uptake need to be considered as a whole to assess impacts. Citrus grown in Florida is fertilized during the periods of increased rainfall (spring/summer) and it is promising that treatments with cover crops reduced the average soil moisture by 58% compared to modified grower standards below 70 cm. The combination of increased soil nutrient loading from fertilizers and biologically mediated decomposition, combined with a reduction of water below the citrus root zone suggests that cover crops may alleviate nutrient leaching, possibly due to cover crop water uptake from the deeper rooting architecture.



## Conclusion

The results of this study demonstrate that the method of tillage during cc planting has season-long impacts on soil moisture, cc and weed biomass accumulation, and nutrient cycling. Full-tillage and cc inclusion did not affect citrus leaf nutrient levels during this study, but full-tillage increased aboveground biomass in the summer. Total nutrient concentration of the aboveground biomass was driven by an increase in biomass as compared to cover crop type (legume cc vs. grass cc). An increase in cc biomass will increase the overall benefits and the persistence of some undisturbed existing vegetation may improve soil moisture where most citrus roots are present. Full-tillage and cc inclusion reduced the amount of water in the shallow rooting zone (0–12 cm) but the deeper-rooted cc may also reduce leaching below most citrus roots (below 70 cm). Further refinements in management (strip tillage, planting date, species, termination, and ratooning potential) that are paired with seasonal activities, such as harvesting and pruning, are needed for each system that has different maturing cultivars. Species and phenology of cc may also impact the growth and reproductive cycle of known pests, including the Asian citrus psyllid, which is a known vector for HLB.

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## The Palmeras: A Natural Reserve, Cubarral, Colombia

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The Palmeras Natural Reserve (PNR) is located in the Municipality of Cubarral, Department of Meta, Colombia. It has 250 hectares (618 acres) of primary and secondary forests. Its elevations range between 5413 and 6233 feet above sea level, with temperatures ranging from 53 to 74 °F, rainfall above 4000 mm per year and average relative humidity of 90%. This ecosystem, within the humid premontane forest, is characterized as a transition zone between the regions of Orinoquia, Amazonia and the Andes, a habitat with high biological diversity. The Las Palmeras Reserve has been an important research center for biodiversity, as demonstrated in the findings of birds, mammals, and plant species. The dominant plant species include the Choapo palm (*Dictyocaryum lamarckianum*), and the most abundant is Colorao (*Croizatia brevipetiolata*). Other important plant species include Half cumin (*Licaria canella*), Matapalo (*Clusia hachensis*), Palomo (*Alchornea glandulosa*), Chuguaca (*Hieronyma oblonga*), Manzano (*Billia rosea*), Laurel (*Aniba robusta*), Quino (*Ladembergia oblongifolia*), Drago (*Croton smithianus*), and Palma macana (*Wettinia fascicularis*).

Colombia is recognized as the richest country in terms of palm diversity in the Americas, and the third richest in the world with 212 species spread over 44 genera. The Cubarral municipality is in the foothills area and is undergoing continuous development, as it is one of the most economically productive areas in the region. This causes strong pressure on the territory and changes in the structure of the forest by anthropic intervention, even in the high elevation areas, where some species are threatened with extinction. Logging is frequently driven by local people's need for income and the local economies' need for wood. Logging causes significant impacts to forest ecosystems and local communities, biodiversity loss, habitat loss of fauna and flora, erosion, sedimentation of water resources, ecosystem fragmentation, and changes in land use.

The Palmeras Natural Reserve (PNR) was established as a conservation and rehabilitation center of biodiversity. The PNR is a valuable resource available for national and international researchers. This paper includes a list of the most valuable plant species in the reserve and highlights some of the reserve's outgoing projects.

**LOCATION.** The PNR is in the Municipality of Cubarral Department of Meta (E623500;N422886) and has an area of 250 ha (635 acres). The reserve is located 160 km (99 miles) from Bogota, 70 km (43 miles) from Villavicencio and 12.5 km (7.8 miles) from the urban area of Cubarral. Accessing the reserve is an adventure; once in Cubarral it can be reached by road, then by hiking along the mountain or by mule.

**CLIMATIC CONDITIONS.** The reserve is located at elevations between 5413 and 6233 ft above sea level, with temperatures ranging from 53 to 74 °F. The precipitation average is 4000 mm

per year, with a relative humidity of 94%, corresponding to the very humid premontane forest life zone.

**HABITAT AND THE FOREST.** The PNR has primary and intervened forest. The intervened forest had been the object of selective exploitation on valuable timber species, and these species have been in the process of natural restoration for over 10 years. The primary forest is located at the top of the property. Among the most representative tree species are the Choapo palm (*Dictyocaryum lamarckianum*), Colorao (*Croizatia brevipetiolata*), Medio cumin (*Licaria canella*), Matapalo (*Clusia haughtii*), Palomo (*Alchornea glandulosa*), Chuguaca (*Hieronyma oblonga*), Manzano (*Billia rosea*), Laurel (*Aniba robusta*), Quino (*Ladembergia macrophylla*), Drago (*Croton smithianus*), Comino Real (*Aniba perutilis*), and Macana palm (*Wettinia fascicularis*) (Carvajal, 2020).

Research conducted in the past decade in the PNR identified forest species directly associated with the survival of birds. One of the most important is the Choapo palm (*Dictyocaryum lamarckianum*) (Fig. 1) that is important to a conservation project of the endangered yellow-eared parrot (*Ognorhynchus icterotis*) in Colombia (Fig. 2). The Choapo palm is vital to the existence of the yellow-eared parrot and this bird depends on it for reproduction, food, and shelter (Murcia et al., 2019).

### Management Development

The different land uses, land management practices, and population pressures have resulted in the destruction of most of the primary forest. The main cause of deforestation is the illegal use of timber, and livestock in hillside areas that causes problems of erosion, sedimentation, and mass removal. The following actions have been conducted:

**PRELIMINARY RESEARCH.** The PNR was officially established in 2010. Since then, research partnerships have been formed with CORMACARENA, University Distrital Francisco Jose

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Fig. 1. Panoramic view of the forest showing the Choapo palm (*Dictyocaryum lamarckianum*).



Fig. 2. Yellow-eared parrots (*Ognorhynchus icterotis*) nesting in a Choapo palm.

de Caldas, the Cañon del Guatiquia Foundation, group ecology Cabildo Verde de Cubarral and other ecological groups. Efforts have been made targeting an inventory of the species found and evaluation of the existing forest.

More than 200 plant species have been identified including palms, orchids, trees, and shrubs; some of which some are listed as endemic to the region and to Colombia, some of which are endangered. The inventory includes complete photographic material for species identification, plus botanical and taxonomic descriptions. The authors are working on getting funding for publication (Carvajal, 2020).

Some of the palm species have potential uses for economic development of the region. Three species of palms are used by the Iriarteae indigenous people for the manufacture of crafts for local and international commerce (Paz López, 2019).

**MONITORING THE DYNAMIC OF THE FOREST.** A study has been conducted since 2009 to evaluate the dynamics of the forest at the PNR, including its floristic composition and biomass. A permanent 1-ha plot was used to measure the number of individuals, number of species, families, and dominant species with interesting results: number of plants = 607; number of species = 41; number of families = 21; dominant species = *Croizatia brepetiolata* (AB: 24.6 m<sup>2</sup>); and carbon storage = 91.69 t/ha.

**CONSERVATION OF THE CHOAPO PALM (*DICTYOCARYUM LAMARCKIANUM*).** A study is taking place to evaluate the population dynamic of the Choapo palm on a permanent plot of one hectare in the reserve, in which measures are taken annually (seedlings, juveniles, and mature plants). More than 8500 individuals/ha have been found at the PNR. This palm produces up to three clusters measured, two m. in height (2000–2500 fruits each). In this 1-ha plot the study has found 6891 seedlings, 640 juvenile plants and 48 mature plants (Alvares and Cárdenas, 2017; Carvajal, et al., 2015; Pedroza Padilla, 2016; and Torrejano Munevar, et al., 2018).

**NEW PLANTINGS OF CHOAPO PALM (*DICTYOCARYUM LAMARCKIANUM*).** The PNR has repopulated with new plantings of the Choapo palm since 2016. The reforestation involved the local community and over 2500 palms have been established.

**CONSERVATION OF YELLOW-EARED PARROT (*OGNORHYNCHUS ICTEROTIS*).** Two-hundred-seventy-six species of resident and migratory birds have been recorded at the reserve, including *Ognorhynchus icterotis*, *Aegolius harrisi*, *Catharus dryas*, *Falco deiroleucus*, *Grallaria hypoleuca*, *Machaeropterus regulus*, *Malacoptila fulvogularis*, *Piranga olivacea*, *Piranga rubra*, *Setophaga ruticilla*, *Tangara parzudakii*, *Thlypopsis ornata*, *Vireo flavoviridis*, *Wilsonia canadensis*, *Catharus minimus*, *Dendroica fusca*, *Dendroica striata*, *Dendroica cerulea*, *Catharus ustulatus*, *Seiurus noveboracensis*, *Contopus virens*, *Pheucticus ludovicianus*, and *Elanoides forticatus*, among others (Ruiz, 2017; Carvajal et al., 2012; Murcia et al., 2011 and Murcia et al., 2019).

The yellow-eared parrot has been designated as an endangered species in Ecuador and Colombia. This bird has been associated with the Choapo palm, and other studies relate the survival of the yellow-eared parrot with the wax palm (*Ceroxylon quindiuense*). This discovery is unique and shows the bird has been able to adapt to other ecosystems. The bird moves in the area approximately 500 ha, (60 and 80 individuals). Due to the small number of natural nests, artificial nests have been established to help ensure conservation of the parrot population. Establishing nests for the parrots will minimize the pressure on the forest. Artificial wooden nests have been placed in the Choapo palms up to 25 m high to facilitate the acceptability of precious parrots. Trained personnel were required for the installation. The project effectiveness was successful with 100% of bird colonizing the nests (Ruiz, 2017).

More research needs to be done to understand population density, eating habits, species propagation, reproductive habits, inter-specific relationships and local migrations. More artificial nests also need to be placed to help the yellow-eared parrot. Funding is required to continue with the project.

**EDUCATION EFFORTS.** The PNR has been hosting students from local schools and universities. One of the recent popular educational events has been the Parrot Festival. The festival takes place in Cubarral where students from the community participate in a parade proclaiming the importance of the endangered habitat in the area. Educational presentations include talks about the yellow-eared parrot and its needs for survival.

## Conclusions and Future Tasks

The PNR is an important resource to help preserve over 200 species of palms, orchids, trees, and shrubs—of which some are listed as endemic to the region and the country or are endangered.

Future projects will include the protection of the natural resources that are present in the reserve with focus on most sensitive plant and animal species, including:

1. Habitat area—Land on which the primary objective will be to protect natural resources essential to the continued existence of native plants and resident and migratory wildlife.
2. Management area/ trail corridor—Lands which includes ecological trails, including developed hiking trails that run through the forest with educational interpretation.
3. Improve facilities—Establishment of a classroom and dormitory housing to support researchers and possible ecotourism. Active management of land in these areas would be required to facilitate activities while protecting valuable natural resources.
4. Outreach—Outreach is required to increase public interest and participation. Efforts have been made to involve the local schools and should be continued and extended to all members in the community.
5. Non-consumptive and ecotourism operations—PNR is a place with high potential for ecotourism, a place to conserve native fauna.
6. Interest group studies—Invite specific partners to contribute with the project.
7. External funding—Funding is crucial to keep developing the project. This support includes national and international organizations and individuals that agree with the goals of the PNR.
8. Establishment of a nursery to produce plant material for ecological restoration purposes in the PNR.
9. Recruitment of a ranger for the care, maintenance and protection of the PNR.

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## Encouraging Biodiversity with Wildflower Demonstration Plots

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**Additional index words.** pollinators, biodiversity, ecological landscape, Florida–Friendly

**Biodiversity is under threat in our landscapes as natural lands are converted into urban areas. Wildflowers have the potential to support insect pollinators, which can provide valuable ecosystem services. Many wildflowers are fairly low maintenance in the landscape, are easy-to-grow from seed and can be sustained year after year. Extension demonstration gardens are ideal locations to showcase new practices and plant selections. Objectives of this study were to 1) determine species and flower mixes to maximize flowering time, and 2) educate the public about wildflower species selection and growing techniques. Demonstration plots of wildflower species and mixes were direct seeded at the University of Florida/IFAS Orange County Extension center on 1 October 2020. Flowering time was recorded, and field days were conducted both in-person and virtually to educate the public. Cosmos, zinnias, and phlox were the earliest blooms starting less than 60 days after planting (DAP). Blanketflower, sweet alyssum, calendula, lanceleaf coreopsis and Leavenworth's tickseed flowered 90 DAP or later. After viewing our wildflower plots, 100% of survey respondents (n = 104) indicated they were likely to plant wildflowers. Two main reasons reported to plant wildflowers were to attract pollinators and use native plants. Responses indicated an increase of knowledge in seed planting (85%) and seed saving (84%). Wildflowers can be easily started from seed and are a great way to increase biodiversity of our landscapes. Extension centers are a trustworthy places for people to learn about species suitable for their location and initiate adoption of environmentally-friendly practices.**

Landscapes in new developments of housing and retail spaces are often planted with perennial shrubs, some of which may be low maintenance, but they also have low wildlife value. Biodiversity is important to having an ecological landscape where the beneficial insects prey on garden pests (Dale et al., 2020). Many wildflowers are low maintenance, are easy to grow from seed and provide food in the form of pollen and nectar, for insects. Wildflowers have the potential to support insects, which can provide valuable ecosystem services such as biological pest control and pollination of food crops. In addition, wildflower gardens can be sustained year after year as seeds can be saved or allowed to reseed in place. Home gardeners face many barriers to adopting new practices, such as planting wildflowers in their yards. Demonstration gardens at extension centers are an ideal location to showcase species to enable the public to learn characteristics of plant selections. Extension centers are trustworthy places for people to get information and see practices in action (Glen et al., 2013). Extension programs can also assist people by supplying them with seeds. Seeds can also be easily obtained from local plant nurseries, seed suppliers and gardening groups. Objectives of this study were to 1) determine species and flower mixes to maximize flowering time and 2) educate the public about wildflower species selection and growing techniques.

### Materials and Methods

Demonstration plots of seven wildflower species and mixes were direct seeded at the University of Florida/IFAS Orange

County Extension center on 1 Oct. 2020. Plantings included two species of tickseed (*Coreopsis lanceolata* and *Coreopsis leavenworthii*), blanketflower (*Gaillardia pulchella*), phlox (*Phlox drummondii*), blanketflower + phlox, *Coreopsis lanceolata* + phlox, and Ferry-Morse wildflower seed mix (Ferry-Morse, Norton, MA). The purpose of the wildflower seed mixes was to extend the flowering time, as some of the native species take many months to bloom. Plots were 1.2 × 1.2 m in size and were prepared by loosening the soil with a hoe, leveling it out with a rake, and sprinkling the seeds on the ground and patting them in. The experimental design was set up as a randomized complete-block design with four replications. Plots were maintained with hand weeding and watered twice a week for establishment during the first month and once per week after establishment. No fertilizer or pesticides were used. Flowering time was recorded when blooms first appeared for each species or flower mix. Field days were conducted both in person and virtually via Facebook live video to show the public the wildflower demonstration plots.

### Results

Cosmos and zinnias in the Ferry Morse seed mix were the earliest blooms starting at 30 days after planting (DAP) and phlox began flowering 62 DAP as shown in Fig. 1. Blanketflower, sweet alyssum, and calendula were flowering 96 DAP. Lanceleaf coreopsis blooms appeared 147 DAP (Fig. 2) and Leavenworth's tickseed did not flower until April 2021, at 196 DAP. Survey responses (n = 104) were collected from in person (n = 17) and online viewers (n = 87). After viewing our wildflower demonstration plots, 100% of survey respondents indicated they were likely to plant wildflowers in their garden.

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Fig. 1. Cosmos and zinnias in bloom at 62 days after planting at the Orange County Extension Center, Orlando, FL. Photo credit: Tiare Silvasy.



Fig. 2. Tickseed and phlox in bloom at 147 days after planting at the Orange County Extension Center, Orlando, FL. Photo credit: Tiare Silvasy.

Survey respondents reported the main two reasons to plant wildflower plots were to attract pollinators and to use native plants. Other reasons included to conserve water, reduce fertilizer and pesticide use. Survey respondents indicated an increase of knowledge in seed planting (85%) and seed saving (84%).

### Conclusion

Wildflowers can be easily started from seed and are a great way to increase biodiversity of our landscapes. This could result in more diverse wildlife and pollinator populations. Wildflower seeds are cheap and can be easily obtained by mail order, they are adapted to low inputs of fertilizer and can withstand rainfall and drought. Wildflower gardening is sustainable; after the initial purchase of seeds, gardeners can save seeds and reseed the plot or share seeds with others. The University of Florida Florida-Friendly Landscaping™ program recommends both native and nonnative species. In this trial we found that native wildflowers bring unique insects, while non-native wildflowers are earlier to bloom. Extension centers are a trustworthy places for people to learn about species suitable for their location, learn relevant horticultural techniques and initiate adoption of environmentally friendly gardening practices.

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## Florida Producers' Perceptions of Nutrient Agricultural Best Management Practices Profitability

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**Additional index words.** best management practice adoption, perceptions, profitability

**Agricultural best management practices (BMPs) have public and private benefits. Public benefits include maintaining or improving water quality, while private benefits include reducing agricultural inputs or increasing yields. BMPs that help improve fertilizer use efficiency and reduce leaching events can have direct impact on both water quality and farm profitability. The dynamics of crop growth and the nutrient cycle make timely and adequate applications of fertilizers important. Though cost share exists for BMP adoption, producers largely absorb the cost and risks of using BMPs. We conducted a statewide survey of Florida producers to learn their perceptions of nutrient BMPs profitability and barriers to use. The survey examined agronomic crops, citrus, and fruits and vegetable producers' use of controlled release fertilizer, calibrating fertilizer equipment, and cover crops. We found that an average of 73%, 65%, and 40% of agronomic crops, citrus, and fruits and vegetable producers agree that these BMPs are profitable. Also, an average of 31%, 16%, and 14% of respondents reported that they are not implementing BMPs because the practices are too expensive, not applicable on their farms, and they do not expect a yield benefit. We found that BMP use varies by crop category. Interestingly, we also found that few respondents think that they do not have enough time to learn about new practices. Understanding producers' perceptions of BMP profitability and barriers to adoption is important to designing effective conservation programs.**

Population growth and crop production are the largest drivers of freshwater demand in Florida. The demand for water resources increases as the population increased by more than 15 million people in the past 80 years (Fletcher and Borisova 2017). Also, the number of farms and the average farm size increased during this period (Her et al. 2017). In the agriculture sector, water use is expected to increase by 3.65% between 2020 and 2040. The state is therefore expected to spend between \$0.31 billion and \$1.77 billion by 2035 to satisfy water needs (EDR 2020).

Several technologies or practices are utilized to meet the demand for clean water in Florida. Agriculture best management practices (BMPs) are designed to mitigate agriculture's effect on the environment by controlling water runoff and nutrient leaching (Braune and Wood, 1999; Lam et al., 2011). BMP adoption also has private benefits, as improving nutrient management efficiency can increase yield and decrease input costs. For example, BMPs that help improve fertilizer use efficiency and reduce leaching events can have direct positive impacts on farm profitability. The dynamics of crop growth and the nutrient cycle make timely and adequate applications of fertilizers important in increasing both public and private benefits.

While these practices have both private and public benefits, producers voluntarily adopt BMPs that are applicable to their production systems, sometimes without fully understanding the suite of costs and benefits. State and Federal agencies offer finan-

cial incentives, such as cost share, to help shoulder the burden of BMP costs but producers largely adopt BMPs at their own cost and risk to their operations. To better understand the socioeconomic factors that affect adoption we conducted a statewide survey that asks producers to list specific nutrient BMPs they have adopted and to provide their perceptions on profitability and barriers to adoption. These survey results can be used to develop strategies for increasing BMP adoption in Florida.

### Survey Design and Methodology

We conducted a statewide survey of specialty crop producers to better understand their adoption and perceptions of specific nutrient BMPs, including controlled release fertilizer (CRF), calibration of fertilizer equipment (CFE) and use of cover crops (CC). These are a few of many BMPs which may have substantial effects on water quality. The survey has three primary sections: costs and benefits of CRF, CFE, and CC, barriers to adoption, and farm demographics. The instrument was designed with input from University of Florida/IFAS horticulture faculty, extension faculty, citrus and vegetable specialists, and producers. The survey was administered online by the Florida Survey Research Center ([fsurveyresearch.center.ufl.edu](http://fsurveyresearch.center.ufl.edu)), emailed to UF/IFAS extension agents and grower association listservs, in person, and via the postal mail from March 2018 to June 2019. This method is appropriate given the diversity of Florida crops.

For producers' two most widely grown crops, the questionnaire asks what crops they grow, on how many acres the BMPs are being applied, when they were first implemented, the additional cost of implementing the BMPs, and the estimated yield effect from using the BMPs. In addition to these three BMPs,

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researchers asked respondents to identify other BMPs they are adopting. Barriers to adopting all BMPs are revealed with questions asking if they did not implement the practice because of lack of knowledge, lack of experience, the practices are too labor intensive, the practices are too expensive, they do not perceive a yield benefit from using the practice, they do not have time to learn a new practice or the practices are too data intensive.

We received 151 completed surveys of which 80 reported growing a second crop. Because practice adoption in this sample varies by crop, we assume that each producer makes separate adoption decisions for each crop and treat those 80 responses as separate decision makers (or producers). Doing this allows us to increase the study sample to 231 respondents representing 386,900 acres where the average farm occupies 1675 acres. Our

respondents grow citrus, agronomic crops, fruits and vegetables, forages and other crops. Our analysis focusses on producers who grow agronomic crops, citrus, and fruits and vegetables, leaving 146 respondents representing 299,958 acres where the average farm is 2054 acres.

## Results

Figure 1A–C display the respondent’s perceptions of BMP use on profitability for each crop category. We see that 50 to 89% of the agronomic crop producers and 28 to 56% of vegetable produces who adopted CRF, CC, and CFE agree that these BMPs are profitable. Also, 50% and 82% of citrus producers agree that CFE and CRF are profitable. The figures also show that for those practices where benefits may be harder to quantify, like CFE and CC, up to 45% are not sure how the practices affect profitability (Fig. 1C). That most respondents indicate that these BMPs are

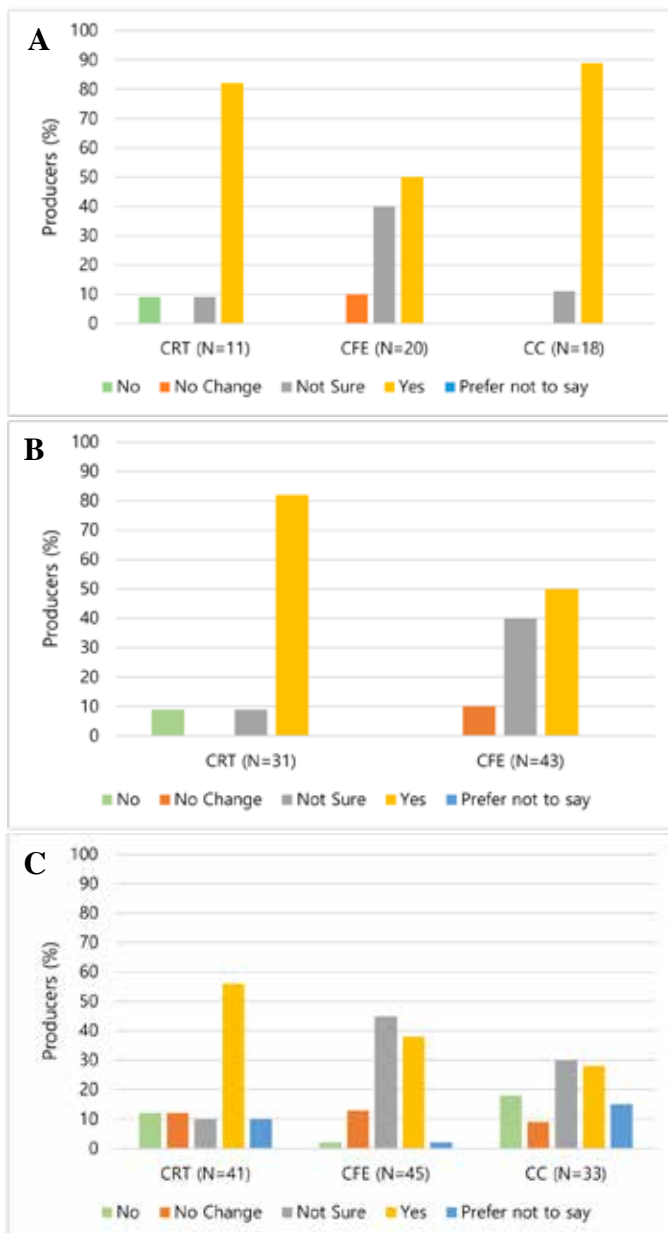


Fig. 1. (A) Florida agronomic crop producer’s perceptions of best management practices profitability. (B) Florida citrus producer’s perceptions of best management practices profitability. (C) Florida fruit and vegetable producer’s perceptions of best management practices profitability. CRT = controlled release fertilizer, CFE = calibration of fertilizer equipment, and CC = use of cover crops.

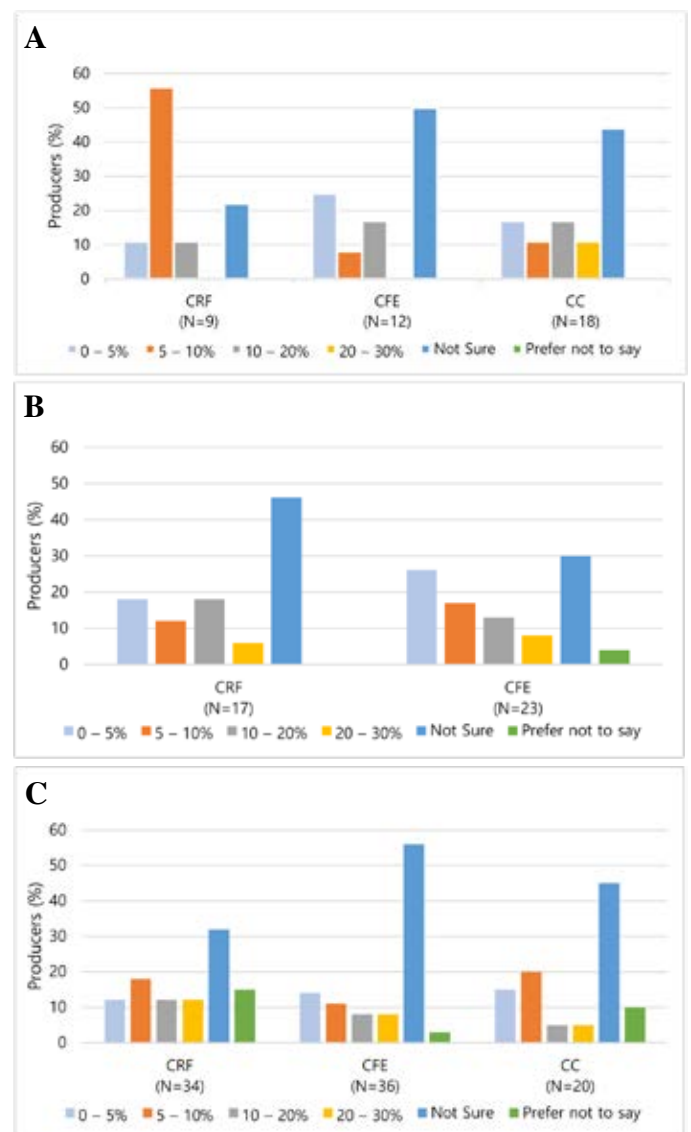


Fig. 2. (A) Florida agronomic crop producer’s perceptions of yield increase with best management practices use. (B) Florida citrus producers’ perceptions of yield increase with best management practices use. (C) Florida fruit and vegetable producer’s perceptions of yield increase with best management practices use. CRF = controlled release fertilizer, CFE = calibration of fertilizer equipment and CC = use of cover crops.

profitable may also be due to selection bias (which we did not address in our methods). Most respondents use BMPs and likely do so because of measured or perceived private benefits.

Respondents were also asked to indicate the percent yield increase attributed to the use of nutrient BMPs (Fig. 2 A–C). Figure 2A shows that 56% of agronomic crop producers indicate the CRF increased yields by 5-10%. However, 50% and 44% of agronomic crop producers indicate that they are not sure how CFE and CC affects their yields. Similarly, “Not Sure” is the largest category for citrus and fruit and vegetable producers (Fig. 2 Band C, respectively). While the large number of “Not Sure” responses leaves questions about whether the need for more information on how to calculate the benefits from CFE and CC,

most respondents indicate no or positive increases from the use of these BMPs.

In addition to CRF, CFE, and CC, respondents were asked to identify other nutrient BMPs they used and their barriers to BMP adoption (Figs. 3 and 4). In Fig. 3, we see that BMPs related fertilizer use (i.e., use University of Florida/IFAS [UF/IFAS] recommended fertilizer rates, base fertilizer applications on soil or tissue tests and keep nutrient application records) are more widely adopted than some other BMPs. The most widely adopted BMP is to keep nutrient application records, which is adopted by 56%, 84%, and 78% of fruits and vegetables, agronomic crops and citrus producers, respectively, followed by using UF/IFAS recommended fertilizer rates. Figure 4 shows reasons

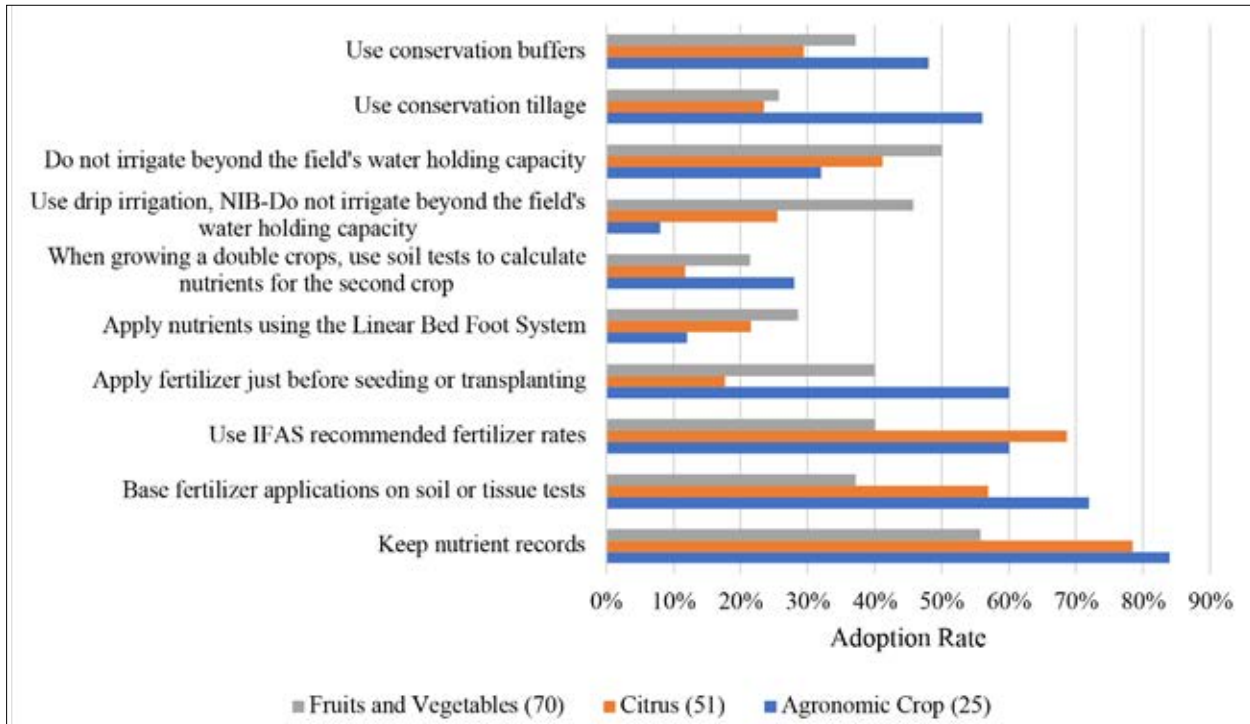


Fig. 3. Florida crop producer's use of other best management practices, besides controlled release fertilizer, calibration of fertilizer equipment and the use of cover crops.

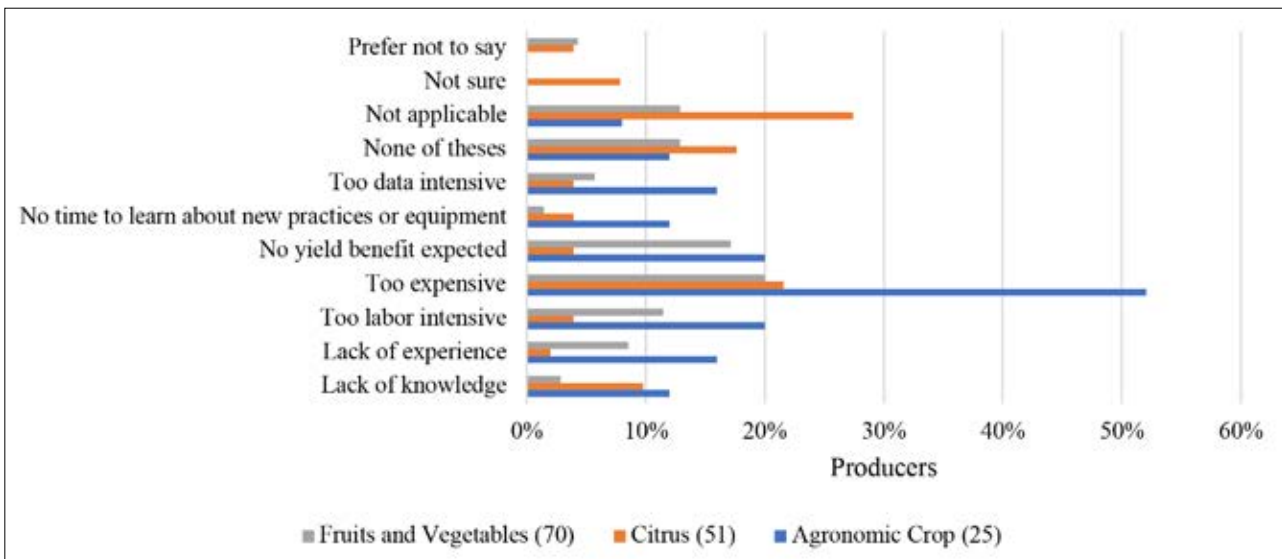


Fig 4. Florida crop producer's reasons for not implementing best management practices.

for not implementing BMPs, in general. Here, an average of 31% of respondents said that BMPs are too expensive to implement and only 6% indicated that they did not have time to learn a new practice. Also, an average of 16% and 14% of producers think that BMPs are not applicable on their farm and no yield benefit expected, respectively.

### Conclusion

These findings reveal that while producers think CC, CRF, and CFE are profitable, they are uncertain about the effect on yield. This identifies producers' information gaps in calculating the costs and benefits of BMPs. While BMP adoption has clear public (or environmental) benefits, producers should understand the full suite of costs and benefits to them when making adoption decisions. Understanding the barriers to adoption will help to identify growers' needs and highlights other areas where more education is needed. These results identify barriers to adoption but also show areas of opportunity for extension and conservation programs interested in increasing BMP adoption.

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—Scientific Note—

## University of Florida, Institute of Food and Agricultural Sciences Pilot Project for Industrial Hemp: On-Farm Trials with Extension

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**Additional index words.** CBD, hemp, pilot project

Industrial hemp is the same species as marijuana (*Cannabis sativa*); however, industrial hemp is legally distinguished from marijuana by its total delta-9-Tetrahydrocannabinol (THC) level. Industrial hemp's total THC content does not exceed 0.3% dry weight, whereas *C. sativa* is categorized as marijuana at any higher percentage.

Hemp is used for fiber, seed, oil, non-THC cannabinoids, and various other derivatives. The plant historically has been cultivated as a fiber and grain crop. In addition to these, modern hemp crops could be used for building materials, forages to feed cattle, food products for people, and essential oil extraction for cannabidiol (CBD) and other cannabinoids. CBD is one of over 100 cannabinoid compounds found in *Cannabis* that is continuing to be explored for medicinal use for a variety of ailments.

Research scientists and extension educators are now tasked to “relearn” hemp for modern production and to prepare for industry development in Florida. Much of the production knowledge has been lost due to hemp being classified as a Schedule I controlled substance by the United States Drug Enforcement Agency until recent changes in law. In other countries where hemp has been legal since the early 1990s, research regarding production is available but not necessarily applicable to Florida's environmental conditions and agricultural systems.

Specialists and extension educators must fill knowledge gaps that include best cultivation practices, adapted varieties, control for insect and weed pests, profitability potential for Florida farmers, and invasion risk of hemp to other farm fields and natural areas. In order to generate information needed by growers, the University of Florida, Institute of Food and Agricultural Sciences (UF/IFAS) Industrial Hemp Pilot Project was launched. Ongoing years of research will validate the scientific findings and help provide producers and allied stakeholders with sound, reliable information. As more research is generated, extension personnel will eventually be able to make confident recommendations regarding the production of industrial hemp in Florida.

The UF/IFAS Industrial Hemp Pilot Project is working under the long-term goal to establish hemp variety and production rec-

ommendations, while also remaining environmentally conscious. During pilot project research, hemp varieties have been assessed for plant growth, production, and health. A 14-member advisory group selected to represent diverse backgrounds in agriculture, law, and medicine was established to help guide the project. Group members were also selected to act as public representatives for the diverse interests and stakeholder groups related to the hemp industry in Florida.

The pilot project seeks to identify hemp varieties with resilience to environmental, ecological, and economic threats that could arise in Florida. Each variety was evaluated for THC levels, to meet the regulatory threshold, as well as for CBD content, to establish candidates for oil production.

The next phase in the hemp pilot project was to use the information collected in the first phase of research to implement on-farm trials, in tandem with continued trials at various UF/IFAS research and education centers.

Extension agents worked with farmers to establish farm plots that represent environmental conditions in Florida's diverse climate across the various land, soil and cropping systems. Each farm planted hemp varieties with genetics that reflected current availability for production and represented different hemp types.

Both UF/IFAS and growers obtained permits allowing them to produce hemp for research through the Florida Department of Agriculture and Consumer Services (FDACS). Like the pilot project, on-farm trials also evaluated the effects of cultivation practices on the development of hemp, monitored potential pest and disease issues, and collected both management and economic information. In total, 18 farms were selected for the on-farm trials after the selection process that took place earlier in 2020.

As the local interest of industrial hemp topics has continued to surge from the onset of commercialization of hemp, the UF/IFAS Industrial Hemp Pilot Program research leader, Dr. Zachary Brym, initiated the development of a statewide extension team. Participating extension agents within the team are housed in locations across Florida with expertise that represents various agricultural systems. These agents have been trained to provide information to producers and the general public covering a range of basic hemp related topics, as well as preliminary research findings and extension resources.

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—Scientific Note—

## Engaging Growers and Agricultural Service Providers in a Diverse Advisory Panel on Organic High Tunnel Research

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**Additional index words.** crop, engagement, empowerment, high tunnel, nutrient management, organic, vegetable production

Expert input from the end users of research provides valuable information that engages and empowers project stakeholders and steers future research in directions informed by those end users. These are primary functions of advisory panels, which are important components of a multi-institution, interdisciplinary research study of organic vegetable production in high tunnels in the Southeastern U.S. The advisory panel also plays an active role in project decision-making and in identifying challenges to organic high tunnel production distinctive to the Southeastern U.S. Recommendations that emerge from the advisory panel can be particularly important in assessing the potential viability of organic high tunnel production systems in this region. The members of the advisory panel include growers and agricultural industry professionals, such as representatives from the United States Department of Agriculture and University of Florida Cooperative Extension.

We conducted three advisory panel meetings in March 2019, 2020, and 2021. Project researchers and advisory panel members interacted during a facilitated semi-structured discussion. Researchers presented their findings from the previous year and

pose questions to the panel members based on their research objectives. Panel members then provided recommendations for changes in the current research project, which may include changes in focus, protocol and nature of data collected. The panel is also charged with helping craft future research.

The content of the discussion varied in the three advisory panel meetings held, although there were consistently identified areas of focus for future research. Pest and disease management was a common theme in panel discussions, likely due to the warmer climate in the south that lends itself to considerable pest and disease pressure. Panel members expressed interest in more research about temperature control during periods of high temperature. Crop rotation, including type of crop and timing, was another focus area repeated in the advisory panel meetings. Finally, nutrient management remained an important area of discussion throughout the meetings. Growers and agricultural service providers that served on the panel agreed these are the critical areas of focus for future efforts investigating organic high tunnel production systems in the Southeastern U.S.

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—Scientific Note—

## The Birds, the Bees, the Turf, and the Trees: Improving the Sustainability of Golf Courses with Wildlife Habitat Enhancements

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**Additional index words.** conservation, wildlife habitat

It is estimated that the population of Florida will reach 33.7 million people by the year 2070 with even more development likely to sprawl into the state's agricultural and natural lands. With urban sprawl proliferating, Florida land is shrinking, and it seems houses and development are growing faster than oranges and turfgrass. And wildlife is taking a major hit from urban sprawl. The diverse habitats across Florida, from the Panhandle down to the Keys, are home to an impressive amount of wildlife—from black bears, butterflies, deer, to panthers and bobcats, armadillos and turtles and, of course, alligators, to the water birds along the riverbanks and shorelines of Florida's magnificent coasts. They need a place for shelter, food and water, and golf courses are becoming havens for wildlife in the face of urban sprawl. If managed carefully, golf courses can represent a compatible land for the state's native wildlife and help preserve the biodiversity of Florida's quickly shrinking lands. In addition, golf course superintendents can receive certification for wildlife conservation efforts through Audubon International and potentially demand higher greens fees than those without such certification. Enhanced wildflower habitats have shown to increase pollinator populations and other beneficial insects that help with pest management. Therefore, golf courses can improve their sustainability by implementing

wildlife habitat enhancements in out-of-play areas, landscaping with native trees and vegetation, encouraging wetland areas and minimizing chemical applications.

Realizing the need for wildlife conservation and the opportunity for golf course superintendents, I created a program objective to improve the sustainability of golf courses through education and on-course wildlife habitat enhancements.

To date, I have delivered five presentations and one workshop on the topic, reaching 243 turf managers. Post-class survey results (n = 199) indicate a knowledge increase in the benefits and strategies for wildlife conservation and an increased intent to adopt at least one of the strategies presented. I also assisted golf course superintendents with pollinator and blue-bird habitat enhancement projects and littoral zone plant modification projects on several golf course ponds. As a result, three Brevard County golf courses received Audubon International certification for their conservation efforts in 2020–21.

This program is ongoing and adaptable to other counties in Florida. Adoption of wildlife conservation on golf courses in other counties would expand and strengthen the wildlife corridors across the state.

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## Concepts for Sustainable Landscape Mosaics<sup>1</sup>

Tina McIntyre, Rachel Gutner, and Sandra Wilson<sup>2</sup>

### Introduction

Florida is rapidly urbanizing, and with more and more new residents and businesses arriving daily, Florida's water resources may become limited in the future. If Florida continues to develop on trend, more than one-third of Florida's land will be developed by 2070 (UF GeoPlan Center et al. 2017). Nationwide, landscape irrigation is estimated to account for nearly one-third of all residential water use (EPA 2017). In order to be a steward of Florida's natural resources, it is important to create landscapes that do not use excessive amounts of water, fertilizer, pesticides and other resources. The concepts of Florida-Friendly Landscaping™ have been around since 1991 and continue to offer sustainable landscaping principles, including what is outlined in this document. This EDIS publication is for Florida homeowners, residential and commercial property managers, and landscape architects interested in creating aesthetically pleasing landscapes and to help individuals choose the right plant for the right place.

This document considers the need for sustainable landscaping in Florida, as well as the desire for aesthetically pleasing landscapes. As proposed by Piet Oudolf, a prominent Dutch garden designer, there is beauty in all seasons, and the dieback of a plant, if placed properly, can add to the overall effect of the garden. In this publication, we define a mosaic as a landscape composed of plants that bloom at specific times, have seasonality (including dieback), and exhibit different textures, shapes, and sizes. One can create a mosaic image of sustainable landscaping by selecting and utilizing Florida-friendly and native plants.



Figure 1. *Pentas lanceolata* (red) is interspersed with *Helianthus debilis* (yellow) and *Stachytarpheta jamaicensis* (purple) in the front yard of a residential home.

Credits: Tina McIntyre, UF/IFAS

Landscaping offers a great opportunity for homeowners to mimic Florida's beautiful natural environments and incorporate water-wise plant selections into their designs. We've created two primary mosaic concepts that embrace and highlight a certain theme. They include the pollinator concept and the seasonal concept, which can be used separately or combined to help landscapers, homeowners,

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2. Tina McIntyre, Florida-Friendly Landscaping™ Agent, UF/IFAS Extension Seminole County; Rachel Gutner, undergraduate student, University of Central Florida; and Sandra Wilson, professor, Environmental Horticulture Department, UF/IFAS Extension, Gainesville, FL 32611.

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landscape architects, and HOAs (homeowners' associations) responsibly choose appealing plants to meet that conceptual goal.



Figure 2. Scorpion tail, *Heliotropium angiospermum*, in the front of the mosaic garden, with *Calliandra hematocephala* (pink), *Stachytarpheta frantzii* (purple), and *Senna mexicana* var. *chapmanii* (yellow) in the background.

Credits: Rachel Gutner, UF/IFAS

Green spaces, such as residential yards, commercial properties, and shared communal parks, enrich our lives in many different ways. Horticulture is linked to improved mental health, improved human performance and energy, reduced stress, and much more. There are also economic benefits to investing in green spaces, such as reduced healthcare costs and increased property values. Green spaces reduce noise pollution and stormwater runoff and help to improve water quality by absorbing nutrients from pet waste, excess landscape fertilizers, and toxic organic chemicals such as pesticides before they enter surrounding waterways. They also reduce heat and cold damage in urban areas (International Society for Horticultural Science 2012). By embracing these plant concepts and proposed species in your yard, managed area, or shared space, you and your community can actualize these mental, physical, and financial benefits.

## Site Preparation and Management

The best way to control weeds is to prepare your land effectively prior to installation. With a little time and planning, you can manage weeds by solarizing the area (McSorley and Gill 2019). Solarize your garden space by removing as much of the existing undesired plants as you can, then wet the soil thoroughly. Cover the area with 1 ml thick polyethylene (PE) heavy-duty clear plastic and secure the edges with landscape ties or soil to trap heat, prevent photosynthesis, and smother the remaining unwanted

plants or seeds. Leave this covering on for about six to eight weeks. When you remove the plastic, all the roots of the weeds in the upper 4"–6" of soil should be dead. Because this treatment only targets the upper part of the soil, deeper soil may still be affected by pests, and after 3–4 months, the effects of solarization diminish (McSorley and Gill 2019).

Soil tests, which can be sent to the UF/IFAS Extension Soil Testing Laboratory (ESTL), will help determine the site conditions most accurately. See the pH column in Table 1 below to determine if the selected plants will do well in your site. Most plants will benefit from soil enhancement, but native plants are particularly well suited to sandy soil and acidic conditions. If you wish to enhance your soil, we recommend mixing organic material into the existing soil during this phase (Treadwell et al. 2019).

Although native plants typically require less maintenance and fewer resources, it is virtually impossible to create a landscape with no maintenance. Weed control (including invasive species removal) is important to maintaining the aesthetics of a landscape. However, before weeds can be controlled, they must be identified. Landscape managers or property owners should identify the weed and determine if it is problematic before removing it. Some weeds, if not invasive, might be a good addition to the landscape, depending on its purpose. To identify a plant you are unfamiliar with, visit the [UF/IFAS Weed Identification Guide](#), submit a specimen to your local Master Gardener Volunteer program, or submit a specimen to the [Distance Diagnostic and Identification System](#).

After planting, two to three inches of mulch should be applied to the landscape to suppress weed germination, hold moisture in the root zone, prevent erosion and provide aesthetic uniformity. Pine needles and other leaves are a sustainable selection while being readily available most of the year. Lightly pruning plants at certain times of the year will help regenerate growth and promote blooms. Plant replacement might also be necessary if some plants fail to thrive due to natural causes.

## Pollinator Concept

A pollinator is any animal that helps carry pollen from the male part of the flower to the female part of the same or another flower. Pollination must occur for the plants to become fertilized and produce fruits, seeds, and young plants. The relationship between pollinators and plants is one of the oldest and most striking symbiotic relationships that still exists today. This symbiosis can be observed in your yard: hummingbirds are naturally drawn to the



reddish flowers of coral porterweed, *Stachytarpheta mutabilis*, and their long tongues are perfectly adapted to the long broad tubes of the flower, which lead to a high nectar reward. Hummingbirds pollinate by tending to plants with long nectaries: as they forage for nectar, pollen sticks to their beaks and faces and spreads to other plants. Through this relationship, pollinators feed and the female plant parts are fertilized. If successful, plant pollination leads to seeds that, when germinated, differ genetically from the parent. Further, the leaves of some pollinator plants may serve as food for the larval stages (i.e., caterpillars) of certain butterflies and some insects. Plants that also function as larval host plants are designated in the plant matrix. This dance and relationship that is mutually beneficial and reciprocal carries the species involved through time and space.

Pollinators are essential to human existence; their efforts contribute to a third of all food that humans eat. Even if pollinators are not directly pollinating human food crops, those plants often feed other organisms that we depend on. Pollinators also contribute greatly to healthy ecosystems because they transport pollen to plants that stabilize our soil, clean our air, supply oxygen, and support wildlife.

It is important to provide a habitat oasis to native and nonnative pollinators, such as bees, butterflies, and wasps. Some birds and hummingbirds are also effective pollinators. Many birds consume the fruits and excrete the seeds, which spreads the plant offspring. By creating a pollinator-focused landscape, you create a refuge for these vital creatures. Table 1 outlines many plant species that are suitable for pollinator gardens, with host species denoted by a dagger. Additionally, mobile web applications, such as the [FFL Plant Guide](#), are available to assist with choosing native and nonnative pollinator plants and to learn about Florida's diverse butterfly (<https://ffl.ifas.ufl.edu/butterflies>) and bee pollinators (<https://ffl.ifas.ufl.edu/bees>).

Because this concept focuses on wildlife, it is important not to use harsh chemicals when managing weeds. Some chemicals and common weed killers can also kill some species of bees, particularly honeybees (McSorley and Gill 2019).

## Seasonal Concept

Florida's natural ecosystems typically have a colorful display in fall and spring. Table 1 offers a list of plants that bloom by season. The "Year-Round" section contains plants that bloom throughout the year.

Because the list is primarily organized by bloom period, landscapers and homeowners also have the option of creating a colorful seasonal landscape. Florida's unique, semitropical environment creates a hotbed of plants that respond to seasonal changes. These plants can make for an eye-catching yard full of blooms up to 6 months out of the year. We encourage landscapers and homeowners to explore the idea of nature-inspired, seasonally based landscapes because they highlight Florida's many attractive plants. With the tendency for seasonally interesting plants to die back, many plants that are listed provide strong structural integrity with evergreen foliage.

## Plant Matrix

Listed here are Florida-friendly plants organized by seasonal bloom period. All plants in this list are pollinator plants, meaning that their blooms provide a significant source of either nectar or pollen. Some of the species also function as host plants, providing food and resources for the larval stages of adult pollinators. The growing information for each plant is included, such as USDA hardiness zones, pH preference, light and moisture preferences, native status, and perennial/annual condition.

Because all annuals selected reseed, the homeowner/groundskeeper does not have to worry about replacing the entire section when the plant dies back. Some perennials, marked with an asterisk, also reseed, but not as prolifically.

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Figure 3. Colorful bracts of spotted bee balm (*Monarda punctata*) and floral visitation by a wasp pollinator. Credits: Tina McIntyre, UF/IFAS



Figure 4. Pollinator on *Bidens alba*, which is typically targeted as a weed but can be a good source for pollinators. Also pictured, *Pentac lanceolata*. Credits: Tina McIntyre, UF/IFAS



Figure 5. *Gaillardia pulchella* providing great ground coverage for soil and habitat for pollinators.  
Credits: Tina McIntyre, UF/IFAS



Figure 6. Rapidly growing dune sunflower, *Helianthus debilis*.  
Credits: Tina McIntyre, UF/IFAS



Figure 7. Berries and leaves of the native Simpson's stopper, *Myrcianthes fragrans*.  
Credits: Tina McIntyre, UF/IFAS



Figure 8. Butterfly on *Pentas lanceolata*.  
Credits: Tina McIntyre, UF/IFAS



Figure 9. Bumble bee on hairy chaffhead, *Carphophorus paniculatus*.  
Credits: Tina McIntyre, UF/IFAS



Figure 10. *Rudbeckia* sp. cultivated in a backyard.  
Credits: Tina McIntyre, UF/IFAS



Figure 11. *Phlox* sp. growing after reseeding itself from a previous year.  
Credits: Tina McIntyre, UF/IFAS



Figure 14. American beauty berry, *Callicarpa americana*, showcasing its beautiful purple berries.  
Credits: Vincent Marcucci



Figure 12. *Rudbeckia fulgida* growing in a cultivated governmental landscape.  
Credits: Vincent Marcucci



Figure 15. Purple porterweed, *Stachytarpheta frantzii*, a common spring bloomer of Florida's natural areas.  
Credits: Vincent Marcucci



Figure 13. *Lantana involucrata*.  
Credits: Vincent Marcucci



Figure 16. *Stachytarpheta jamaicensis* (purple) is showcased with *Pentas lanceolata* (red) and *Helianthus debilis* (yellow).  
Credits: Tina McIntyre, UF/IFAS

Table 1. This table lists selected Florida-friendly plants in order of bloom period: fall, spring, or year-round. Native status, USDA Zone, and pH are listed as well. N/A in the pH column means that the preferred pH was not available. An asterisk (\*) on the binomial denotes a reseeding perennial. A dagger (†) on the binomial name of the plant signifies that the species serves as a host plant.

Binomial	Common Name	Native	USDA Zone	pH	Wet	Dry	Sun	Part-Shade	Shade	P/A
<b>Fall Bloom</b>										
<i>Ampelaster carolinianus</i> (a.k.a. <i>Symphotricum carolinianum</i> )	Climbing aster	Yes	8a–11	5.1–6.5	x	x	x			P
<i>Asclepias incarnata</i> †	Swamp milkweed	Yes	3b–11	6.5–8.0	x		x			P
<i>Asclepias perennis</i> †	Aquatic milkweed	Yes	6a–9b	5.1–7.5	x		x	x		P
<i>Caesalpinia pulcherrima</i>	Dwarf poinciana	No	9a–11	6.1–7.8		x	x			P
<i>Calliandra haematocephala</i>	Powderpuff tree	No	9a–11	6.0–7.5	x	x	x	x		P
<i>Callicarpa americana</i> (Figure 14)	American beautyberry	Yes	6–10b	5.2–7.0		x	x	x		P
<i>Carphephorus paniculatus</i> (Figure 9)	Deer tongue/hairy chaffhead	Yes	8a–9b	5.8–7.0	x	x	x			P
<i>Chamaecrista fasciculata</i> †	Partridge pea	Yes	3–9b	6.0–8.0		x	x			A
<i>Chrysopsis floridana</i>	Florida goldenaster	Yes	9a–9b	5.5–7.0		x	x			P
<i>Chrysopsis mariana</i>	Maryland goldenaster	Yes	4a–10b	5.1–7.5	x		x			P
<i>Chrysopsis subulata</i>	Scrubland goldenaster	Yes	8a–11	N/A		x	x			P
<i>Conradina canescens</i>	False rosemary	Yes	8a–9b	<6.0		x	x			P
<i>Echinacea purpurea</i> †	Purple coneflower	Yes	3–8b	6.5–7.2		x	x	x		P
<i>Heliotropium curassavicum</i>	Seaside/salt heliotrope	Yes	3a–11	6.5–8.5	x	x	x	x		P
<i>Liatis spicata</i> †	Slender head blazing star	Yes	8a–10b	5.6–7.5	x	x	x			P
<i>Monarda punctata</i> (Figure 3) †	Spotted bee balm; horsemint	Yes	3a–8b	6.1–7.8		x	x			P
<i>Muhlenbergia capillaris</i>	Pink muhly grass	Yes	7a–11	5.8–6.8		x	x	x		P
<i>Ocimum basilicum</i>	Sweet basil	No	2a–11	5.1–8.5		x		x	x	P
<i>Oenothera lindheimeri</i>	Whirling butterflies/ beeblossom	No	5–9b	6.1–7.8		x	x			P
<i>Palafoxia integrifolia</i>	Coastal plain palafox	Yes	9a–10b	4.5–7.5		x	x			P
<i>Passiflora incarnata</i> †	Purple passion vine	Yes	5a–10b	6.1–7.5		x	x			P
<i>Pityopsis graminifolia</i>	Narrow leaf silk grass	No	8a–11	5.8–7.0		x	x			P
<i>Polygonella polygama</i>	Jointweed	Yes	9a–10b	4.5–5.5		x	x	x		P
<i>Psychotria nervosa</i>	Wild coffee	Yes	9a–11	6.1–7.5		x	x			P
<i>Silphium asteriscus</i> *	Starry rosinweed	Yes	8a–10b	5.1–7.6		x	x	x		P
<i>Rudbeckia hirta</i> (Figure 10)	Black-eyed Susan	Yes	8a–10b	6.0–7.0		x	x			A
<i>Rudbeckia laciniata</i>	Green headed/cutleaf coneflower	Yes	8a–8b	4.5–7.0	x		x	x		P
<i>Ruellia caroliniensis</i> †	Carolina wild petunia	Yes	6a–10b	7.9–8.5	x	x	x	x	x	P
<i>Salvia azurea</i>	Azure blue sage	Yes	5–9b	6.1–7.8		x	x			P
<i>Salvia miniata</i>	Belize sage	No	9a–11	6.1–7.8		x	x			P
<i>Senna ligustrina</i> †	Privet senna	Yes	9a–11	6.1–7.8		x	x			P
<i>Senna mexicana</i> var. <i>chapmanii</i> † (Figure 2)	Bahama senna	Yes	10a–11	>7.0		x	x			P
<i>Serenoa repens</i> †	Saw palmetto	Yes	6a–11	5.6–6.5	x	x	x			P

Binomial	Common Name	Native	USDA Zone	pH	Wet	Dry	Sun	Part-Shade	Shade	P/A
<i>Solidago odora</i> *†	Sweet goldenrod	Yes	8b–11	5.6–7.8		x	x			P
<i>Solidago sempervirens</i> *†	Seaside goldenrod	Yes	3a–8a	5.5–7.5		x	x	x		P
<i>Sorghastrum secundum</i> †	Lopsided Indiangrass	Yes	8a–9b	6.1–6.5		x	x			P
<i>Spartina bakeri</i>	Sand cordgrass	Yes	8a–10b	6.8–7.2		x	x			P
<i>Stokesia laevis</i>	Stoke's aster	Yes	8a–10b	5.6–7.0		x	x	x		P
<i>Trichostema dichotomum</i>	Blue curls	Yes	8a–11	N/A		x	x			A
<i>Tripsacum floridanum</i> †	Dwarf fakahatchee grass	Yes	8a–10b	7.9–8.5		x	x	x		P
<i>Vernonia angustifolia</i>	Narrow-leaf ironweed	Yes	8a–9b	5.1–7.5		x	x	x		A
<b>Spring Bloom</b>										
<i>Asclepias incarnata</i> †	Swamp milkweed	Yes	3b–11	6.5–8.0	x		x			P
<i>Asimina obovata</i> †	Flag pawpaw	Yes	8a–10b	6.0–6.8		x	x	x		P
<i>Asimina pygmaea</i> †	Dwarf pawpaw	Yes	7a–10b	5.0–7.0	x	x	x	x		P
<i>Caesalpinia pulcherrima</i>	Dwarf poinciana	No	9a–11	6.1–7.8		x	x	x		P
<i>Calamintha ashei</i>	Ashe's calamint	Yes	8a–9b	6.1–7.8		x	x			P
<i>Calliandra haematocephala</i>	Powderpuff tree	No	9a–11	6.0–7.5		x	x			P
<i>Callicarpa americana</i>	American beautyberry	Yes	6–10b	5.2–7.0	x	x	x	x		P
<i>Coccoloba uvifera</i>	Seagrape	Yes	9a–11	4.5–7.2		x	x	x		P
<i>Coreopsis lanceolata</i>	Large-flower tickseed	Yes	4–9b	6.0–7.0	x	x	x	x	x	A
<i>Duranta erecta</i>	Sapphire showers duranta	No	9b–11	5.6–7.5		x	x	x	x	P
<i>Dyschoriste oblongifolia</i> †	Oblong snakeherb	Yes	8a–11	6.0–7.2	x	x	x	x		P
<i>Heliotropium curassavicum</i>	Seaside/salt heliotrope	Yes	3a–11	6.5–8.5		x	x	x		P
<i>Ilex glabra</i> †	Gallberry	Yes	8–10a	4.5–6.5		x	x	x	x	P
<i>Lonicera sempervirens</i> †	Coral honeysuckle	Yes	8–9b	4.5–7.2	x			x		P
<i>Magnolia grandiflora</i>	Southern magnolia	Yes	8–9b	4.5–7.2	x	x	x	x		P
<i>Magnolia virginiana</i>	Sweet bay magnolia	Yes	8–9b	4.5–6.5		x	x	x	x	P
<i>Myrcianthes fragrans</i> (Figure 7)	Simpson's stopper	Yes	10a–11	7.9–8.2		x	x			P
<i>Oenothera simulans</i> †	Southern beeblossom	Yes	7a–11	N/A		x	x	x		P
<i>Passiflora incarnata</i> †	Purple passion vine	Yes	5a–10b	6.1–7.5		x	x			P
<i>Phlox divaricata</i>	Wild blue phlox/phlox woodland	Yes	3a–8b	6.8–7.2		x	x	x		A
<i>Phlox drummondii</i>	Annual phlox	No	2a–11	6.1–7.2		x	x			A
<i>Psychotria nervosa</i>	Wild coffee	Yes	9a–11	6.1–7.5		x	x	x		P
<i>Rhododendron austrinum</i> or <i>Rhododendron canescens</i>	Azalea	Yes	8–10b	4.5–6.5		x	x	x		P
<i>Rudbeckia hirta</i>	Black-eyed Susan	Yes	8a–10b	6.0–7.0		x	x	x	x	A
<i>Ruellia caroliniensis</i> †	Carolina wild petunia	Yes	6a–10b	7.9–8.5	x		x			P
<i>Scutellaria integrifolia</i> *	Skullcap	Yes	6b–9a	4.8–5.2		x	x	x		P
<i>Silphium asteriscus</i> *	Starry rosinweed	Yes	8a–10b	5.1–7.6		x	x			P
<i>Stachytarpheta frantzii</i> (Figure 15)	Large-flowered purple porterweed	No	9b–11	6.1–7.8		x	x	x		P
<i>Stachytarpheta jamaicensis</i>	Porterweed	Yes	8b–11	6.1–7.8	x	x	x	x	x	P
<i>Trichostema dichotomum</i>	Blue curls	Yes	8a–11	N/A		x	x	x		P
<i>Tripsacum floridanum</i> †	Dwarf fakahatchee grass	Yes	8a–10b	7.9–8.5		x	x			A

Binomial	Common Name	Native	USDA Zone	pH	Wet	Dry	Sun	Part-Shade	Shade	P/A
<i>Vaccinium darrowi</i>	Little blueberry	Yes	8–10b	<7.0		x	x	x		P
<i>Vaccinium myrsinites</i>	Shiny blueberry	Yes	8a–11	<7.0		x	x	x		P
<i>Viburnum obovatum</i>	Walter's viburnum	Yes	8–10b	4.5–8.0		x	x	x		P
<i>Viburnum odoratissimum</i>	Sweet viburnum	No	8b–10a	4.5–8.0		x	x	x		P
<b>Year-Round Bloom</b>										
<i>Berlandiera subcaulis</i>	Florida greeneyes	Yes	8b–11	5.1–7.5	x	x	x	x		P
<i>Calliandra emarginata</i>	Dwarf powderpuff	No	9a–11	7.9–8.5		x	x	x		P
<i>Funastrum clausum</i> †	White twinevine	Yes	9b–11	6.0–8.5	x	x	x	x		P
<i>Gaillardia pulchella</i> (Figure 5)	Indian blanket flower	Yes	8a–11	7.0–8.5		x	x			A
<i>Glandularia maritima</i>	Beach verbena	Yes	9a–11	Calcareous but adaptable		x	x	x		P
<i>Hamelia patens</i>	Firebush	Yes	9a–11	5.5–8.2		x	x	x		P
<i>Helianthus debilis</i> (Figures 1, 6, and 16)	Dune sunflower	Yes	8b–10	5.5–8.2		x	x	x		P
<i>Heliotropium angiospermum</i> * (Figure 2)	Scorpion tail	Yes	8a–9b	7.9–8.5		x	x	x		P
<i>Heliotropium polyphyllum</i>	Pineland heliotrope	Yes	8a–11	6.1–6.8		x	x	x		P
<i>Lantana depressa</i>	Florida lantana or gold lantana/cream carpet	Yes	10a–11	>7.2		x	x			P
<i>Lantana involucrata</i> (Figure 13)	Buttonsage, wild lantana	Yes	8a–11	6.6–7.3		x	x			P
<i>Passiflora suberosa</i> †	Corkystem passionflower	Yes	9a–11	7.5–8.5		x	x			P
<i>Pentas lanceolata</i> (Figures 4 and 8)	Egyptian starcluster	No	10a–11	6.5–7.1		x	x	x		P
<i>Phyla nodiflora</i> †	Frogfruit	Yes	6a–11	4.5–8.0	x	x	x	x	x	P
<i>Rivina humilis</i> *	Rouge plant, pigeon berry	Yes	8a–10b	6.1–7.8		x	x	x		P
<i>Salvia leucantha</i>	Mexican sage	No	4a–12	7.6–9.0		x	x			P
<i>Salvia longispicata</i> × <i>farinacea</i>	Mystic spires blue sage	No	7–10a	6.1–7.8		x	x	x	x	P
<i>Stachytarpheta jamaicensis</i>	Blue porterweed	Yes	8b–11	6.1–7.8		x	x	x		P
<i>Zamia pumila</i> †	Coontie	Yes	8b–11	4.5–8.0		x	x	x	x	P

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## Phytotoxic Effects of Acetic Acid and d-limonene on Four Aquatic Plants

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**ADDITIONAL INDEX WORDS.** *Azolla pinnata*, cattail, *Eleocharis cellulosa*, feathered mosquitofern, invasive species, natural herbicides, organic herbicides, *Salvinia minima*, spikerush, *Typha latifolia*, vinegar

**SUMMARY.** Herbicides that are labeled for aquatic use are often the foundation of aquatic vegetation management programs in the United States because many of these products, which are registered by the U.S. Environmental Protection Agency, are effective, selective, and relatively inexpensive. Resource managers are interested in reducing the use of synthetic herbicides and are considering alternative methods for aquatic weed control. We evaluated the effects of acetic acid and d-limonene on growth of the invasive small floating species feathered mosquitofern (*Azolla pinnata*) and common salvinia (*Salvinia minima*), as well as on the native emergent wetland plants cattail (*Typha latifolia*) and gulf coast spikerush (*Eleocharis cellulosa*). Acetic acid and d-limonene (alone and in combination) were applied once as foliar treatments to healthy plants, which were grown for 8 weeks after treatment to allow for development of phytotoxicity symptoms. All experiments also included diquat dibromide at three concentrations as “industry-standard” treatments for comparison. A 0.22% concentration of diquat dibromide eliminated all vegetation of all species. Most single-product treatments provided good control of invasive feathered mosquitofern with acceptable levels of damage to native gulf coast spikerush, but only 15% and 20% d-limonene treatments were effective on invasive common salvinia and selective for native cattail. Some combinations of acetic acid and d-limonene provided acceptable control of both floating weeds and selectivity for gulf coast spikerush, but all mixes caused unacceptable levels of damage to cattail. Treating these small floating weeds with acetic acid and d-limonene instead of diquat dibromide would increase material costs by 15- to 27-fold. Although these natural products may be useful in some areas where synthetic herbicides are discouraged, they are unlikely to be affordable options for most resource managers.

**R**esource managers are responsible for ensuring that aquatic vegetation does not interfere

with navigation, flood control efforts, and other uses of state waters. Aquatic weeds are most often managed with herbicides that have been approved by the U.S. Environmental Protection Agency (USEPA) for use in aquatic systems. In Florida, statewide oversight and coordination of aquatic weed management programs are provided by the Florida Fish and Wildlife Conservation Commission (FWC), which oversees tens of millions of dollars in federal and

state funds to control aquatic plants in Florida’s public water bodies (Florida Fish and Wildlife Conservation Commission, 2018, 2019). The bulk of funding is used to manage the submerged weed hydrilla (*Hydrilla verticillata*), with ≈25% of those monies spent for floating plant control, which primarily comprise waterhyacinth (*Eichhornia crassipes*) and waterlettuce (*Pistia stratiotes*).

Floating plants can block the air–water interface, thus reducing the penetration of oxygen and light into the water column, and interfere with flood control operations by creating mats that obstruct canals and clog water movement structures (Gettys, 2019). Although waterhyacinth and waterlettuce are the most problematic floating plants in Florida, there are a number of other floating species that are intensively managed in the state, including the diminutive but invasive feathered mosquitofern (*Azolla pinnata*) and common salvinia (*Salvinia minima*). Feathered mosquitofern, a federal noxious weed, is an Australian native that has been used primarily in Asia for improving rice (*Oryza sativa*) production because of its symbiotic relationship with a nitrogen-fixing bacterium (Bodley, 2008; Madeira et al., 2013). Common salvinia, a South American species that was first reported in Florida in the 1920s, is less aggressive than feathered mosquitofern, but it has the potential for explosive growth and is frequently targeted for management (Jacono et al., 2001; Tipping et al., 2012). Both species are attractive ferns that are thought to have arrived in Florida’s waters after escaping cultivation in fish tanks and water gardens.

Herbicides are only labeled for aquatic use by the USEPA if they “will not generally cause unreasonable

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Units			
To convert U.S. to SI, multiply by	U.S. unit	SI unit	To convert SI to U.S., multiply by
0.4047	acre(s)	ha	2.4711
29.5735	fl oz	mL	0.0338
3.7854	gal	L	0.2642
9.3540	gal/acre	L·ha <sup>-1</sup>	0.1069
2.54	inch(es)	cm	0.3937
25.4	inch(es)	mm	0.0394
0.4536	lb	kg	2.2046
1.1209	lb/acre	kg·ha <sup>-1</sup>	0.8922
28.3495	oz	g	0.0353
70.0532	oz/acre	g·ha <sup>-1</sup>	0.0143
(°F – 32) ÷ 1.8	°F	°C	(°C × 1.8) + 32



adverse effects on the environment ... taking into account the economic, social, and environmental costs and benefits of the use of any pesticide” (U.S. Environmental Protection Agency, 1996). However, public concerns regarding aquatic herbicide use have driven efforts to find new ways for managers to practice aquatic weed control while reducing reliance on synthetic herbicides. These efforts include exploring the effects of “natural” herbicides that are sometimes used in home gardens and organic farming. Gettys et al. (2021) reviewed the literature regarding the use of these types of products for terrestrial weed control and evaluated the effects and selectivity of acetic acid, d-limonene, and combinations of the two on waterhyacinth, waterlettuce, pickerelweed (*Pontederia cordata*), and broadleaf sagittaria (*Sagittaria latifolia*). They reported that some combinations of acetic acid and d-limonene had acceptable effects on the invasive species and selectivity on pickerelweed and broadleaf sagittaria, but that product and labor costs would be significantly higher than those incurred with synthetic herbicides (Gettys et al., 2021). Additionally, acetic acid and d-limonene are not labeled for use as aquatic herbicides at the concentrations evaluated; therefore, environmental fate, ecological toxicity, and other information needed for USEPA approval may be unavailable (Stubbs and Layne, 2020).

Based on previous work reported by Gettys et al. (2021), the primary goals of these experiments were to evaluate efficacy and selectivity of acetic acid and d-limonene (alone and in combination with each other) on two floating invasive target species and two emergent desirable nontarget species, and to compare the costs of using these products vs. the synthetic USEPA-approved aquatic herbicide diquat dibromide.

## Materials and methods

**EFFICACY STUDIES.** Target (weed) species were feathered mosquitofern and common salvinia, and nontarget (desirable) species were gulf coast spikerush (*Eleocharis cellulosa*) and cattail (*Typha latifolia*). Plants were treated in pairs of one invasive floating species and one native emergent species. “Run 1” focused on feathered mosquitofern and gulf coast spikerush,

whereas “Run 2” focused on common salvinia and cattail.

Target species were field-collected or pulled from cultures maintained at the University of Florida Fort Lauderdale Research and Education Center (FLREC) in Davie, FL, and moved to 18-gal plastic tubs filled with well water. All tubs were amended with 10 g of crushed 15N–3.9P–10K controlled-release fertilizer formulated for 6-month release in Florida (Osmocote Plus; ICL Specialty Fertilizers, Dublin, OH), 1.2 g of 7N–0P–0K iron chelate micronutrient (Sprint 330; BASF Corp., Research Triangle Park, NC), and 3.4 g of 24N–3.5P–13.3K water-soluble fertilizer (Miracle-Gro Water Soluble All Purpose Plant Food; Scotts Miracle-Gro Products, Marysville, OH). Each tub was initially “seeded” with enough plants of a target species to cover  $\approx 25\%$  of the water’s surface; then, plants were grown for 4 to 6 weeks to allow the development of  $>80\%$  surface coverage.

Nontarget species were purchased from an aquatic nursery (Aquatic Plants of Florida, Myakka City, FL) and transported to a greenhouse at FLREC, where individual plants were transplanted to 2-L plastic pots without holes that were filled with sand [grain diameter 0.25–0.5 mm (Multi-Purpose Sand; Sakrete, Charlotte, NC)] amended with 4 g of the same controlled-release fertilizer used in the tubs. Plants were grown on greenhouse benches and irrigated twice per day (10:00 AM and 4:00 PM) with the equivalent of 0.5 inches of water per irrigation before being used in experiments. New shoots were cut back during this culture period to ensure that each 2-L pot contained a single nontarget plant. When target plant coverage reached  $>80\%$  of the surface of the water, one potted nontarget plant was introduced to each tub (water depth  $\approx 20$  cm above the surface of the pots) and all plants were then subjected to treatment.

Treatments were applied as single spot “spray to wet” foliar applications (50 mL solution per mesocosm) to above-water foliage, and all treatments included 1% v/v of a nonionic surfactant (Induce; Helena Agri-Enterprises, Collierville, TN) to aid in penetration and emulsification. Nine single-product treatments (5%, 7.5%, 10%, 15%, and 20% acetic acid; 10%, 15%, 20%,

and 30% d-limonene), 20 combination treatments (all combinations of single acetic acid and d-limonene treatments), three synthetic standard practice treatments (0.22%, 0.45%, and 0.89% diquat dibromide), and an untreated control were evaluated, with four replicates of each treatment. Base materials were 30% acetic acid (Green Gobbler 30% Vinegar Home and Garden; Eco-Clean Solutions, Copiague, NY), 100% d-limonene (100% Pure Technical Grade D-Limonene; EcoClean Solutions), and 37.3% diquat dibromide (Tribune Herbicide; Syngenta Crop Protection, Greensboro, NC). Treatments were applied to Run 1 and Run 2 plants on 10 Sept. 2020 and 28 Jan. 2021, respectively.

Plants were monitored weekly for 8 weeks after treatment and then assigned a numerical value of 0 through 10 to describe the visual quality (0 = dead; 5 = fair quality, acceptable, somewhat desirable form and color, little to no chlorosis or necrosis; 10 = excellent quality, perfect condition, healthy and robust, excellent color and form). We recorded visual quality, which has been used to describe the plant response to differing culture conditions (Gettys and Moore, 2018, 2019; Gettys et al., 2021), herbicides (Gettys and Haller, 2009, 2010, 2012; Smith et al., 2014), salt stress (Tootoonchi et al., 2020), and other experimental factors, although some researchers (Cutelle et al., 2013; Koschnick et al., 2005; Mudge et al., 2007) have reported visual injury or damage resulting from herbicide treatments. After visual scoring, a destructive harvest was conducted to collect all live biomass of floating species and all live aboveground shoots of emergent species; harvested materials were placed in paper bags and moved to a forced-air oven maintained at 65 °C for 2 weeks before being weighed. Visual evaluations and destructive harvests occurred 5–7 Nov. 2020 (Run 1) and 25–27 Mar. 2021 (Run 2).

While conducting destructive harvests, we realized that visual quality alone might not be a good indicator of treatment efficacy on feathered mosquitofern and common salvinia. For example, some mesocosms had very few live plants remaining, but the plants that were still alive were in excellent condition. We added a record of percent coverage of these small floating species in each mesocosm to describe

this observation. Then, we developed an additional metric—“VC”—to better describe treatment effects on both target species, where VC is visual quality × percent coverage. Visual quality and VC data were arcsin-transformed before analysis to normalize distribution. Data within each species were evaluated using a generalized linear model (SAS version 9.4; SAS Institute, Cary, NC) to determine whether treatment means differed from those of untreated plants at  $P = 0.05$ . Treatment means of visual values, VC, and dried biomass were then compared with those of untreated controls. Haller and Gettys (2013) reported that an ideal herbicide treatment should cause a >90% reduction in these parameters in target weeds and a <50% reduction in nontarget native plants. Therefore, we used these values as benchmarks for efficacy on the floating weeds feathered mosquitofern and common salvinia and selectivity on the emergent native plants gulf coast spikerush and cattail.

**COST COMPARISONS.** Most diquat dibromide used by FWC in fiscal year 2018–19 was applied as Tribune (Syngenta Crop Protection) (Clark and Dew, 2019). FWC’s negotiated contract price was \$35.50/gal, which was used for cost comparisons. As mentioned previously, the base “natural” products used in these experiments were 30% acetic acid and technical-grade d-limonene. Bulk purchase prices for these were \$8.00/gal (30% acetic acid, 275-gal tote) and \$31.82/gal (technical grade d-limonene, 4 × 55-gal drums) (Factory Direct Chemicals, 2019a, 2019b). Cost comparisons used a purchase price of \$8.00/gal for 30% acetic acid and \$31.82/gal for technical grade d-limonene.

## Results and discussion

**SINGLE PRODUCTS.** Diquat dibromide at 0.22%, 0.45%, and 0.89% completely eliminated all live biomass of both floating weeds and both nontarget native plants. Thus, as with

Gettys et al. (2021), diquat dibromide treatments were removed from datasets before further statistical analyses were conducted because most natural treatments were much less effective than diquat dibromide, and comparisons between natural treatments and untreated controls would be more informative.

Most single-product treatments provided good control of feathered mosquitofern in respect to biomass, and the only single-product natural herbicide treatments that failed to reduce biomass by at least 90% were 5% and 7.5% acetic acid [ $P < 0.01$  (Fig. 1A)]. Only three single-product natural treatments (d-limonene at 15%, 20%, or 30%) reduced visual quality of feathered mosquitofern by 90% [ $P < 0.01$  (Fig. 2A)]. As with biomass, the only single-product natural herbicide treatments that failed to reduce VC by at least 90% were 5% and 7.5% acetic acid [ $P < 0.01$  (Fig. 3A)]. Gulf coast spikerush was less affected by these

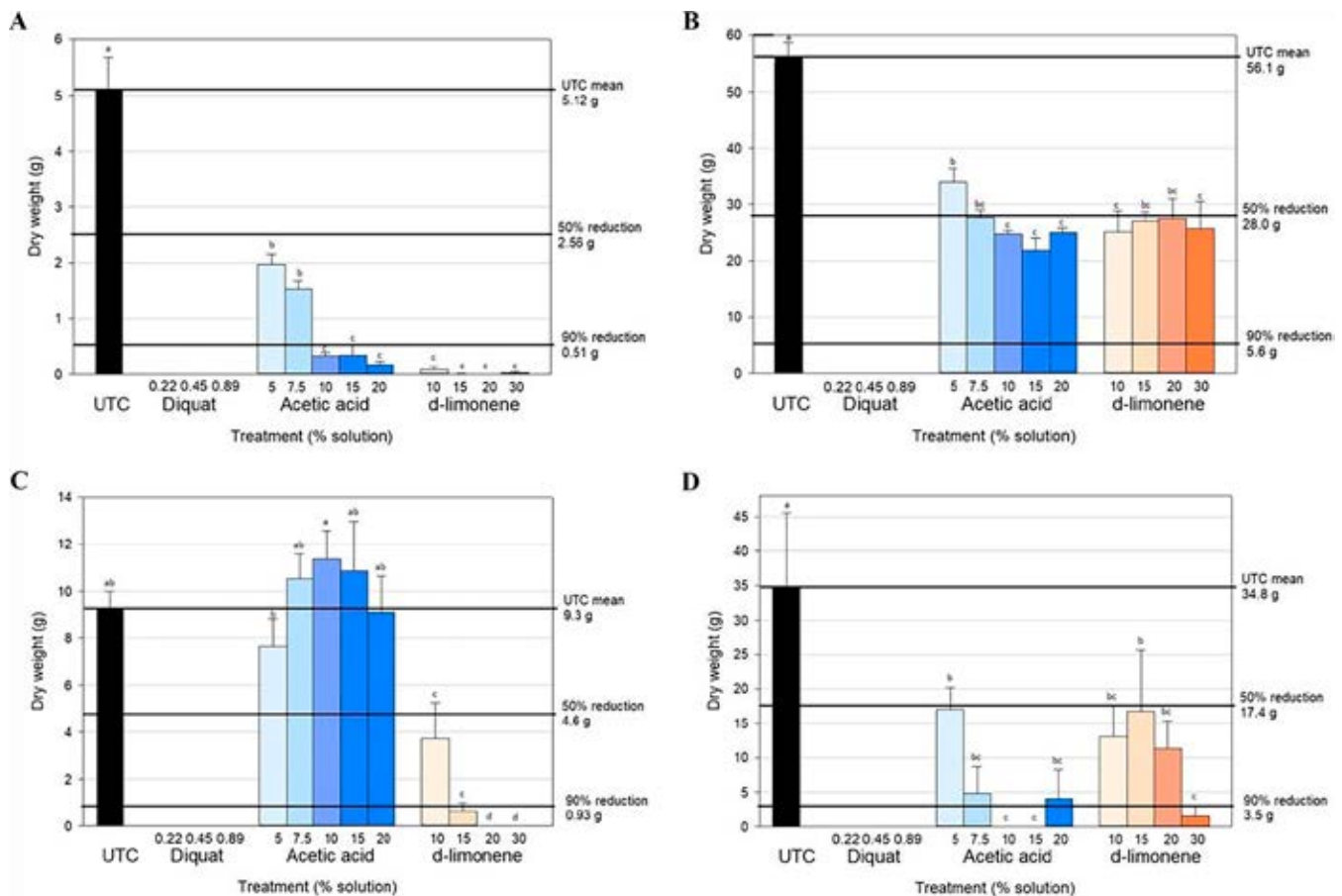


Fig. 1. Biomass of (A) feathered mosquitofern, (B) gulf coast spikerush, (C) common salvinia, and (D) cattail 8 weeks after single-product treatment. Bars are the mean of four replicates and error bars are 1 sd from the mean. Treatments coded with the same letter are not different at  $P = 0.05$ . The upper bold horizontal rule indicates the mean of untreated control (UTC) plants, whereas the central and lower bold horizontal rules indicate 50% and 90% reductions compared with UTC plants; 1 g = 0.0353 oz.

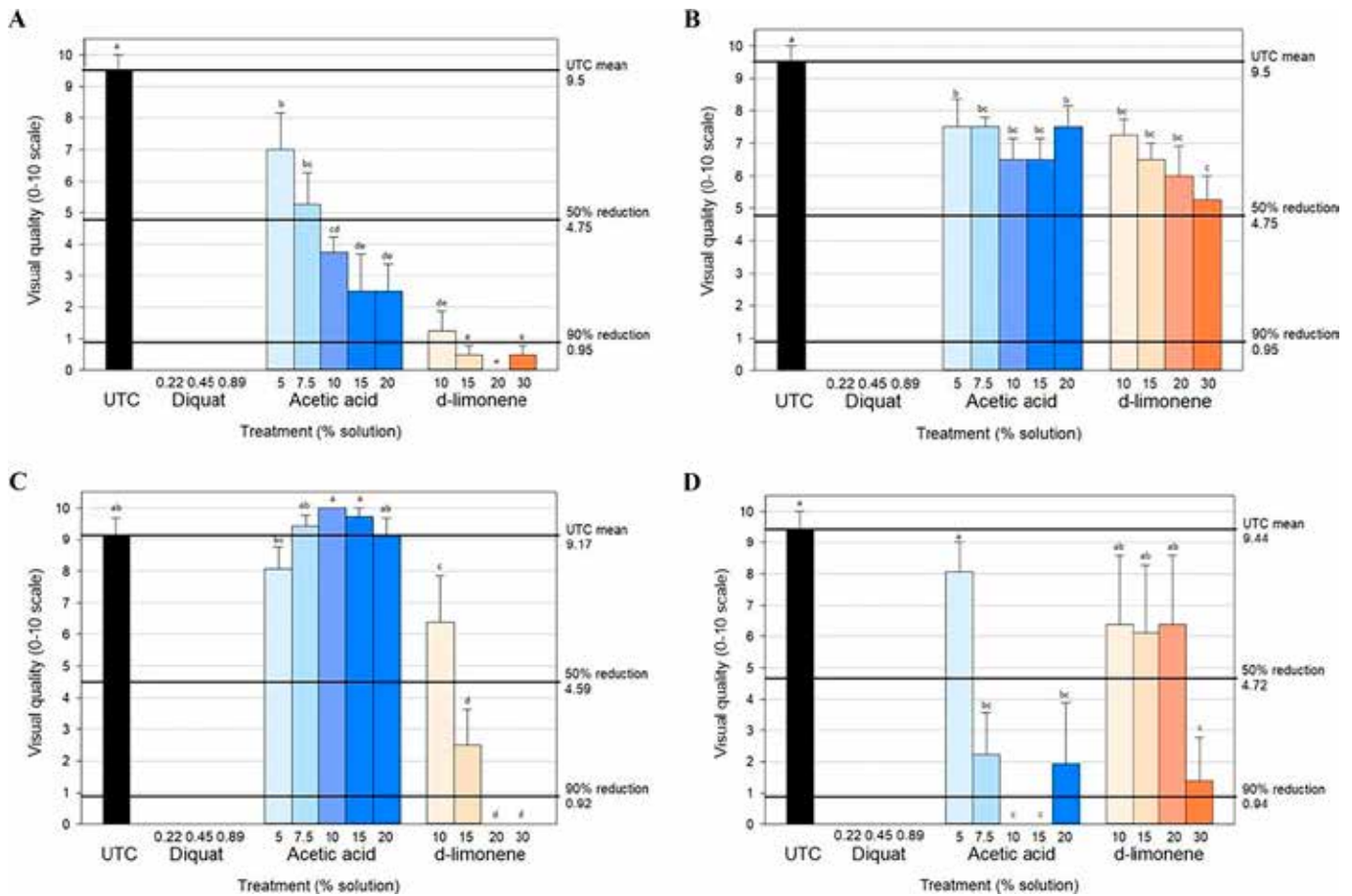


Fig. 2. Visual quality of (A) feathered mosquitofern, (B) gulf coast spikerush, (C) common salvinia, and (D) cattail 8 weeks after single-product treatment. A numerical scale of 0 through 10 is used to describe visual quality, where 0 = dead; 5 = fair quality, acceptable, somewhat desirable form and color, little to no chlorosis or necrosis; and 10 = excellent quality, perfect condition, healthy and robust, excellent color and form. Bars are the mean of four replicates and error bars are 1 SD from the mean. Treatments coded with the same letter are not different at  $P = 0.05$ . The upper bold horizontal rule indicates the mean of untreated control (UTC) plants, whereas the central and lower bold horizontal rules indicate 50% and 90% reductions compared with UTC plants.

treatments than feathered mosquitofern. Most single-product natural herbicide treatments reduced biomass by  $\approx 50\%$  [ $P < 0.01$  (Fig. 1B)], whereas reductions in visual quality ranged from  $\approx 20\%$  to 45% [ $P < 0.01$  (Fig. 2B)]. This level of damage is acceptable for nontarget species, and it is likely that gulf coast spikerush would completely recover from these treatments if given more time.

In contrast to feathered mosquitofern, most single-product treatments failed to provide good control of common salvinia in respect to biomass [ $P < 0.01$  (Fig. 1C)], visual quality [ $P < 0.01$  (Fig. 2C)], and VC [ $P < 0.01$  (Fig. 3B)], and the only single-product natural herbicide treatments that reduced these parameters by at least 90% were 20% and 30% d-limonene. Also, dry weight, visual quality, and VC of common salvinia treated with any concentration of acetic acid

were equal to (or greater than) those of untreated plants. Cattail was much more sensitive to acetic acid and less sensitive to d-limonene than common salvinia, and all single-product treatments reduced cattail biomass by at least 50% compared with untreated plants [ $P < 0.01$  (Fig. 1D)]. Applications of  $\geq 7.5\%$  acetic acid reduced biomass by  $>80\%$ , whereas only the highest d-limonene concentration (30%) reduced biomass by at least 90% compared with that of untreated plants, although visual quality [ $P < 0.01$  (Fig. 2D)] was less affected by all treatments. If selective management of common salvinia with low levels of damage to nontarget cattail is desired, then applications of 15% or 20% d-limonene may be viable options.

**ACETIC ACID AND D-LIMONENE MIXES.** All combinations of acetic acid and d-limonene had good efficacy on feathered mosquitofern. Biomass

[ $P < 0.01$  (Fig. 4A)], visual quality [ $P < 0.01$  (Fig. 5A)], and VC [ $P < 0.01$  (Fig. 6A)] were reduced by  $>90\%$  compared with untreated controls. Most treatments reduced biomass of gulf coast spikerush by between 50% and 75% [ $P < 0.01$  (Fig. 4B)], but only two combinations reduced visual quality by  $>50\%$  [ $P < 0.01$  (Fig. 5B)]. As with the single-product treatments, this level of damage is acceptable for nontarget species, and it is likely that gulf coast spikerush would completely recover from these treatments if given more time.

Virtually all combinations of acetic acid and d-limonene had good efficacy on common salvinia. With the exception of plants treated with 5% acetic acid plus 10% d-limonene, biomass [ $P < 0.01$  (Fig. 4C)] and VC [ $P < 0.01$  (Fig. 6B)] were reduced by  $>85\%$  compared with untreated common salvinia. Unfortunately, cattail

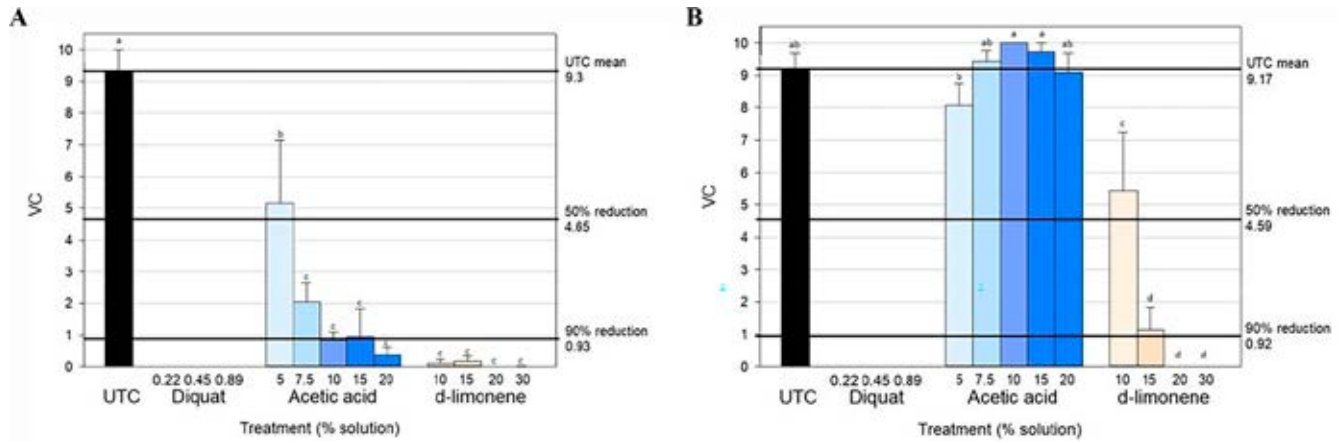


Fig. 3. VC (visual quality × percent coverage) of (A) feathered mosquitofern and (B) common salvinia 8 weeks after single-product treatment. Bars are the mean of four replicates and error bars are 1 SD from the mean. Treatments coded with the same letter are not different at  $P = 0.05$ . The upper bold horizontal rule indicates the mean of untreated control (UTC) plants, whereas the central and lower bold horizontal rules indicate 50% and 90% reductions compared with UTC plants.

biomass was affected by treatments in a similar manner [ $P < 0.01$  (Fig. 4D)], although visual quality was less affected

[ $P < 0.01$  (Fig. 5D)]. Therefore, these combinations of acetic acid and d-limonene should not be used for selective

control of common salvinia because of the unacceptable levels of damage to cattail.

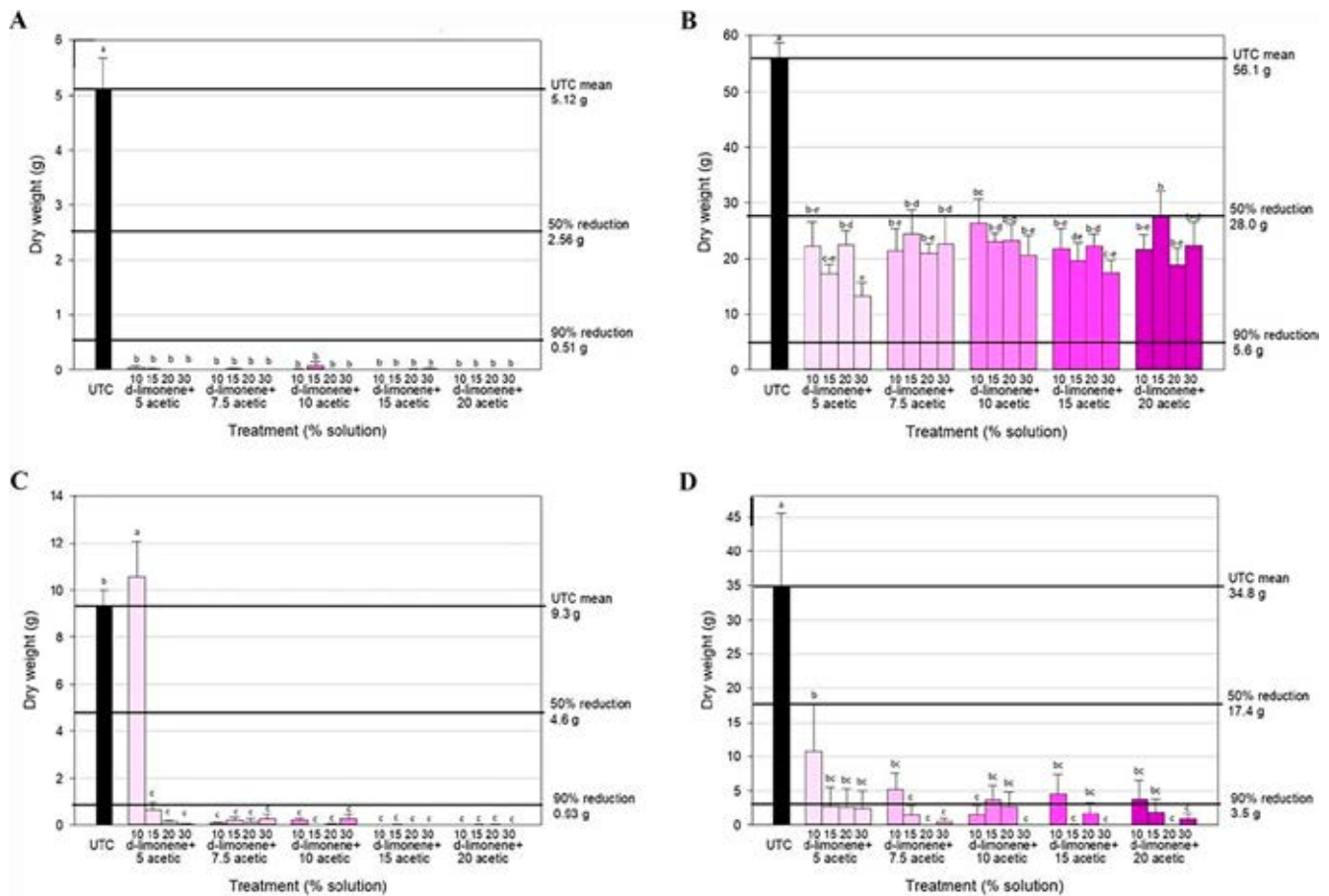


Fig. 4. Biomass of (A) feathered mosquitofern, (B) gulf coast spikerush, (C) common salvinia, and (D) cattail 8 weeks after treatment with combinations of acetic acid and d-limonene. Bars are the mean of four replicates and error bars are 1 SD from the mean. Treatments coded with the same letter are not different at  $P = 0.05$ . The upper bold horizontal rule indicates the mean of untreated control (UTC) plants, whereas the central and lower bold horizontal rules indicate 50% and 90% reductions compared with UTC plants; 1 g = 0.0353 oz.

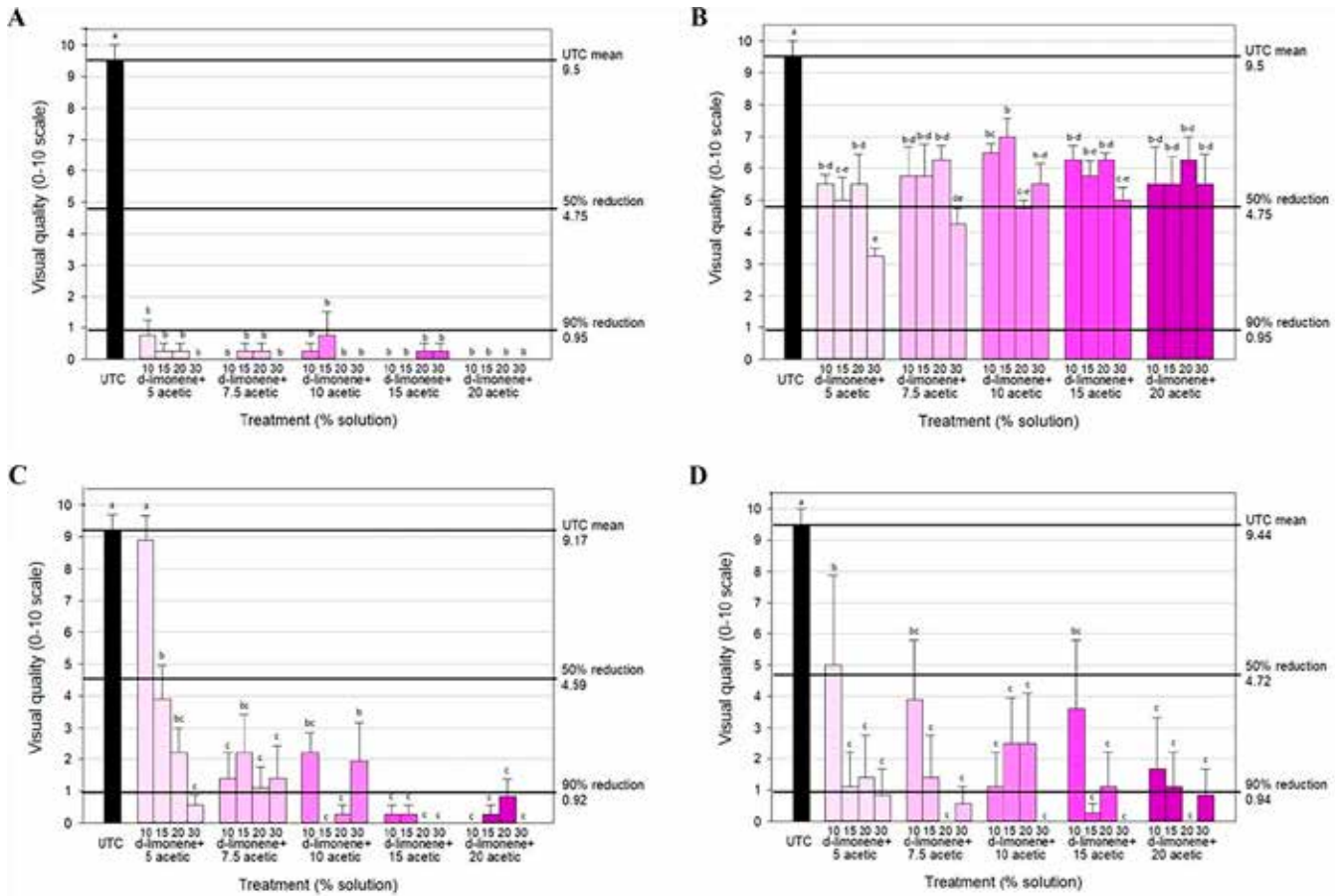


Fig. 5. Visual quality of (A) feathered mosquitofern, (B) gulf coast spikerush, (C) common salvinia, and (D) cattail 8 weeks after treatment with combinations of acetic acid and d-limonene. A numerical scale of 0 through 10 is used to describe visual quality, where 0 = dead; 5 = fair quality, acceptable, somewhat desirable form and color, little to no chlorosis or necrosis; and 10 = excellent quality, perfect condition, healthy and robust, excellent color and form. Bars are the mean of four replicates and error bars are 1 SD from the mean. Treatments coded with the same letter are not different at  $P = 0.05$ . The upper bold horizontal rule indicates the mean of untreated control (UTC) plants, whereas the central and lower bold horizontal rules indicate 50% and 90% reductions compared with UTC plants.

These results suggest that treatments using acetic acid, d-limonene, or combinations of the two may be useful for managing populations of invasive feathered mosquitofern and common salvinia while allowing selectivity with reduced damage to the native plants gulf coast spikerush and cattail.

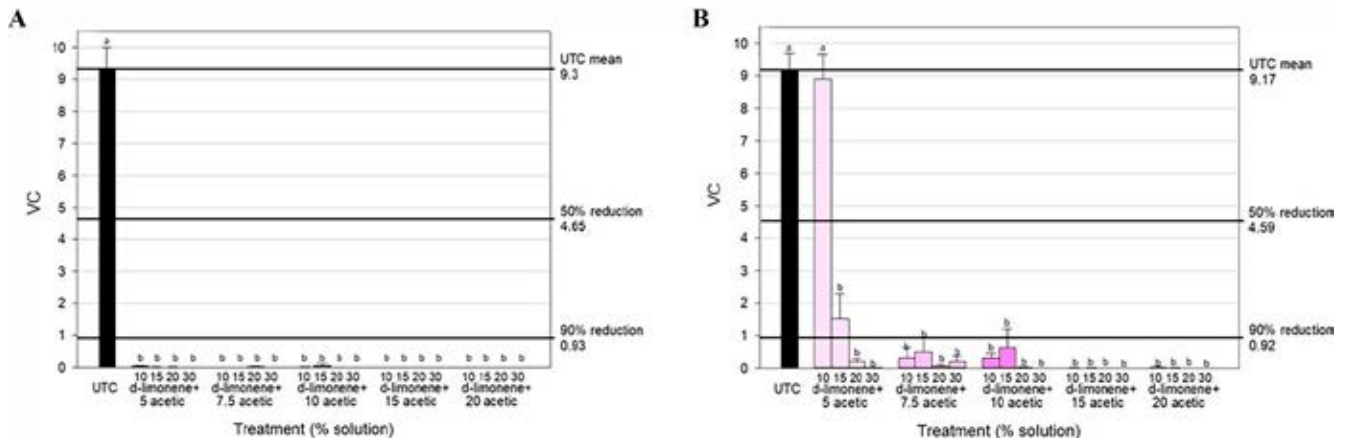


Fig. 6. VC (visual quality × percent coverage) of (A) feathered mosquitofern and (B) common salvinia 8 weeks after single-product treatment. Bars are the mean of four replicates and error bars are 1 SD from the mean. Treatments coded with the same letter are not different at  $P = 0.05$ . The upper bold horizontal rule indicates the mean of untreated control (UTC) plants, whereas the central and lower bold horizontal rules indicate 50% and 90% reductions compared with UTC plants.

**COST ANALYSIS OF ACETIC ACID AND D-LIMONENE MIXES FOR SMALL FLOATING WEED MANAGEMENT.** The synthetic standard practice treatments in these experiments used diquat dibromide and simulated spot foliar field treatments. Although we evaluated three concentrations (0.22%, 0.45%, and 0.89%) of diquat dibromide, the lowest concentration completely eliminated all plant material; therefore, calculations are based on spot treatments using a 0.22% solution. FWC's negotiated contract price for 37.3% diquat dibromide was \$35.50/gal; therefore, after dilution to a 0.22% concentration, the final cost of ready-to-use (RTU) mix is \$0.1775/gal. If all treatments are mixed to a concentration of 0.22% diquat dibromide and applied in a carrier volume equivalent to 100 gal/acre as indicated on the herbicide label (Syngenta Crop Protection, 2011), then the material cost per acre treated with the synthetic standard diquat dibromide is \$17.75. As mentioned in the Materials and Methods, calculations for the natural products in these trials are based on purchase prices of \$8.00/gal for 30% acetic acid and \$31.82/gal for technical grade d-limonene.

The FWC National Pollution Discharge Elimination System (NPDES) report for 2018 lists diquat dibromide as one of four products that were used specifically for mosquitofern (*Azolla* sp.) management (Clark and Dew, 2019). Although the most commonly used product for feathered mosquitofern control was 51% water-dispersible granule flumioxazin (217.14 lb purchased), we used diquat dibromide for synthetic standard practice treatments to maintain consistency within the trials we report here and those conducted by Gettys et al. (2021). We did, however, use the amount of flumioxazin purchased to estimate the area of feathered mosquitofern treated. Recommended use rates for surface treatments with 51% water-dispersible granule flumioxazin are 6 to 12 oz/acre formulated product (0.19 to 0.38 lb/acre a.i.) (Valent USA Corp., 2012); therefore, 217.14 lb would be used to treat 579.04 acres at 6 oz/acre or 289.52 acres at 12 oz/acre. The material cost to treat the same area with 0.22% diquat dibromide would be \$10,277.96 (579.04 acres) or \$5138.98 (289.52 acres).

Several natural treatments provided good control of feathered mosquitofern and acceptable selectivity of gulf coast spikerush. Acetic acid at  $\geq 10\%$ , any rate of d-limonene, and any combination of the two reduced biomass and VC by  $>90\%$  compared with that of untreated controls of feathered mosquitofern. The material costs to make 1 gal of RTU 10% acetic acid, 10% d-limonene, or 5% acetic acid plus 10% d-limonene are \$2.65, \$3.18, and \$4.51, respectively. If these treatments are applied in a carrier volume equivalent to 100 gal/acre (similar to diquat dibromide), then the product costs to treat 1 acre of feathered mosquitofern with 10% acetic acid or 10% d-limonene would be \$265.00 or \$318.00, respectively, whereas the product cost to treat 1 acre with 5% acetic acid plus 10% d-limonene would be \$451.00. If 10% acetic acid (the least expensive of these treatments) were used to treat feathered mosquitofern, then the material cost to treat 579.04 or 289.52 acres would be \$153,445.60 or \$76,722.80, respectively, representing a 15-fold increase in the cost to treat the same areas with diquat dibromide.

The FWC's NPDES report for calendar year 2020 does not list products used specifically for managing common salvinia. However, the report indicates that 19.13 gal of 37.3% diquat dibromide were used to manage the related giant salvinia (*Salvinia molesta*) in 2020 (Florida Fish and Wildlife Conservation Commission, 2021). If all treatments were mixed to a concentration of 0.22% diquat dibromide and applied in a carrier volume equivalent to 100 gal/acre as indicated on the herbicide label (Syngenta Crop Protection, 2011), then a total of 3826 gal of RTU mix were made from the 19.13 gal of concentrate purchased, with a calculated treatment area of 38.26 acres. The total material cost was \$679.12 (\$17.75/acre material cost). The only single-product "natural" treatments used during these trials that resulted in good control of common salvinia ( $>90\%$  reduction in biomass) and acceptable selectivity of cattail ( $<40\%$  reduction in biomass) were 15% and 20% d-limonene. The material costs to make these concentrations of RTU d-limonene are \$4.77/gal (15%) and \$6.36/gal (20%). If d-limonene is applied in a carrier volume equal

to 100 gal/acre (as with diquat dibromide), then the material cost for treating a similar area (i.e., 38.26 acres) with 15% d-limonene would be \$18,261.50 (\$477.30/acre), representing a 27-fold increase from the \$679.12 (\$17.75/acre) spent to treat with diquat dibromide. Common salvinia and cattail had similar responses to mixes of acetic acid and d-limonene, and no treatment provided good control of common salvinia and good selectivity of cattail; therefore, cost comparisons of mixes to the synthetic standard diquat dibromide are not warranted.

Based on this information, it is clear that material costs would increase if synthetic herbicides such as diquat dibromide are replaced with "natural" products such as acetic acid and d-limonene. However, other costs are likely to increase as well. As reported by Gettys et al. (2021), applications using "natural" products would require more time because of the need to transport very large volumes of base material. For example, a typical spray boat is equipped with a 100-gal tank and uses carrier water drawn from the system being treated to dilute concentrated herbicide. Filling the tank once for a synthetic treatment would require the transport of  $\approx 64$  fl oz of 37.3% diquat dibromide; as such, a spray boat operator could set out with a single 2.5-gal jug of herbicide and have enough concentrate to make 500 gal of RTU mix. In contrast, filling the tank once with the least expensive efficacious and selective "natural" treatment for feathered mosquitofern (10% acetic acid) would necessitate transporting 33 gal of 30% acetic acid, or  $\approx 330$  lb of material (without factoring in the weight of the containers used to transport the materials). Using 15% d-limonene to treat common salvinia would require transporting 15 gal ( $\approx 150$  lb) of technical-grade d-limonene. Because of the added weight of the concentrated materials needed and limited space on the spray boat, applicators would likely "mix at the ramp" and, thus, would have to return to shore for reloading after applying 100 gal of RTU natural mix.

## Conclusions

The "natural" products evaluated in these studies may have some utility for selectively managing the small floating weeds feathered mosquitofern

and common salvinia without causing unacceptable levels of damage to the desirable native plants gulf coast spikerush and cattail. Most single-product treatments provided good control of feathered mosquitofern with acceptable levels of damage to gulf coast spikerush, but only 15% and 20% d-limonene treatments were efficacious on common salvinia and selective for cattail. Some combinations of acetic acid and d-limonene provided acceptable control of both floating weeds and selectivity on gulf coast spikerush, but all mixes caused unacceptable levels of damage to cattail. Although the products examined in these studies act as contact herbicides and are not translocated, we observed that cattails struggled to recover after above-water foliar burn-back caused by treatment applications. Timmons et al. (1963) reported that cattail often fails to recover if aerial shoots are severed below the water line, a condition that could be mimicked by contact herbicide treatments such as those used in these studies. It is possible that plants in shallower (e.g., <20 cm deep) water would be able to recover and regrow after treatments, but further investigations are needed to confirm this.

Replacing the currently used synthetic herbicides such as diquat dibromide with “natural” products such as acetic acid and d-limonene would result in significant increases in management costs. Treatment of feathered mosquitofern with 10% acetic acid instead of 0.22% diquat dibromide would increase material costs alone by 15-fold, and switching from diquat dibromide to 15% d-limonene for common salvinia control would result in a 27-fold increase in product costs. As noted by Gettys et al. (2021), natural products such as acetic acid and d-limonene may be useful in some areas where synthetic herbicides are discouraged, but it is unlikely that broad-scale adoption of natural products for aquatic weed control would be an affordable option for most resource managers.

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## **Gardening in the Panhandle: LIVE! A Lesson in Harnessing Teamwork and Technology to Better Reach Clientele**

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As the COVID-19 pandemic unfolded in 2020, University of Florida/IFAS (UF/IFAS) Extension Agents were unable to perform traditional in-person educational programs and needed an innovative way to reach residential horticulture clientele. Inspired by Agents in south Florida offering “Ask an Agent Anything” online seminars, but wanting to focus more on specific, timely topics, the Northwest District Horticulture Program Implementation Team (PIT) collaborated to create a similar program series called *Gardening in the Panhandle LIVE!* Each session covered a seasonally relevant topic with knowledgeable panelists based on their individual specialties. The series was broadcast using both Zoom Webinar videoconferencing technology and Facebook Live to capture the largest possible audience. To comply with ADA guidelines for hearing disabled clientele, episode record-

ings were edited with closed captioning for YouTube. Delivering each episode required a team of 7–9 agents in the following roles: 3–4 panelists; an episode “host”; a Zoom technician; and several “behind the scenes” moderators. While the episode’s host and panelists were answering questions, the moderators were adding resource links to chats, answering pop-up questions, and filtering and forwarding potential on-air questions to the emcee. The innovative collaboration created 13 episodes and engaged a total of 871 live viewers in 2020 on various social media platforms. 22% of participants (191/871) answered follow up surveys. 97% (186/191) of respondents reported knowledge gain in at least one Florida-Friendly Landscaping Principle. 88% (168/191) of respondents reported a plan to adopt at least one Florida-Friendly Landscaping Principle as a result of participating in the program.

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## **Starting a New Job with Extension during the COVID 19 Pandemic: Overcoming Challenges**

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Beginning a career as an Extension agent is always challenging but to begin in a rural county, where the position was vacant over a year, the decline in Master Gardener participation, maintaining compliance from pandemic conditions, made the task of learning the job, extraordinarily challenging. Adaptation for this position required quick planning, flexibility, and creativity. This case study seeks to share experiences, trials, and tribulations, 1) performing a needs assessment, 2) developing community relations, and 3) retaining Master Gardener Volunteers. Because of statewide shutdowns, my first day was establishing a home office, using a personal computer. Given the timely popularity, I created my own backyard Victory Garden and promoted its benefits through a series of videos (2861 views), during a global pandemic. The virtual "Lets Walk Florida 2020" program, a 10-week journey of health and wellness, setting goals, and reporting activities, I

treated as required work task. Assisted in the development of A-Maize-ing Corn Camp for the first virtual 4-H statewide summer camp. Motivation to learn virtual programing was crucial for my success under pandemic conditions. Integration of new technology with old, enhanced my communications with the community. Nick Place, Dean for Extension, complimented how he learned of this new agent, not in person, but from a video on Extension's Facebook page (over 600 viewers). Victory Garden videos raised my awareness for the need of horticulture outreach. The Let's Walk Florida program gave me a sense of self-confidence, both personally and professionally. Weekly e-newsletters, newspaper articles about Florida-Friendly Landscaping™, and 12 new Master Gardener graduates all happened despite the Covid19 pandemic.

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## **Hurricane Season Disaster Preparedness of Plant Nurseries**

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Hurricanes and tropical storms can cause short- and long-term economic losses to agricultural operations. In 2017, the Florida Department of Agriculture and Consumer Services, announced a \$2.5 billion economic impact (\$2,558,598,303) to Florida agriculture had been caused by Hurricane Irma. Twenty-four percent

(\$624,819,895) of these losses came from the Greenhouse, Nursery and Floriculture industry. A preparation checklist was created to allow producers to remain resilient and minimize losses in their nurseries. This was in collaboration with producers, who are members of the Extension agent's advisory committee.

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## **Florida–Friendly Landscaping Facelift—Extension Collaboration with County Parks and Recreation**

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Indian River County Parks and Recreation Department (PRD) invested over \$12 million dollars into the interGenerational Center (iG Center). A beautiful modern building with many amenities. However, the landscape has been a disappointment; plant selection and placement conflicted with soil, sun, and irrigation conditions.

Indian River County Master Gardeners (IRCMGs) have been providing Florida–Friendly Landscaping (FFL) educational outreach to participants but without a physical, in-ground illustration of these management techniques via a demonstration landscape site.

Objectives included: 1) provide PRD with a solution for the unappealing and difficult to manage landscape and 2) obtain space where IRCMGs can provide visual and experiential learning opportunities for FFL programming participants.

The agent conducted a site visit and submitted a project proposal based on utilizing FFL methods of management, with emphasis on “Right Plant, Right Place” that would remedy current landscaping challenges. Project implementation incorporated practical skills of veteran volunteers and the 2020 trainee class. Signage illustrating the multi-faceted components of FFL educated visitors to the iG Center.

Three beautifully landscaped garden beds welcome visitors to the iG Center. Master Gardeners use the gardens to supplement teaching FFL concepts with educational program participants. The newly landscaped beds, totaling 1176 ft<sup>2</sup>, are worth approximately \$5,600 in labor and materials.

Due to the curb appeal with the public and its success as a demonstration landscape, we have been asked to rehabilitate another six beds at the same location.

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## FRONT AND BACK COVERS (CONTINUED)

### Front cover, upper left.

Close up of *Dracaena* ‘Waikiki’ which was discovered in Mauritius in 2004, although it is not currently listed in its flora. The actual species remains unidentified. The cultivar name was conferred by Hawaii Export Nursery Association (HENA) when it was released for production in 2010. The broad maroon leaf edge differentiates this cultivar from other available varieties.

### Front cover, upper right.

*Dracaena fragrans* ‘Massangeana’ (or ‘Mass cane’), one of the most commonly grown cultivars of dracaena produced in Florida. Large stem cuttings, like in this photo, are often imported from Central America. Florida growers then root the stem cuttings and grow the plants until several buds produce new shoots.

### Front cover, lower left.

*Dracaena sanderiana* is generally marketed as “Lucky bamboo” and stem cutting by the millions are imported into Florida each year from China and other places. The stems of varying lengths are often clustered into a wide variety of interesting arrangements like the ones shown here. It is often cited as being good for *Feng Shui*.

### Front cover, lower right.

Many *Dracaena* cultivars produced by Florida growers are on display each year at the Tropical Plant International Expo (TPIE) sponsored by Florida Nursery Growers and Landscape Association (FNGLA). In this photo, the brightly colored cultivar in the center is *Dracaena fragrans* ‘Lime-light’ with two additional selections above.

### Back cover, upper left.

Dracaenas are produced in many different sizes, from small pot plants to relatively large tree forms. The best way for a nursery to get a good start on larger plants is to plant taller cane cutting, often several per pot. This nursery is producing *Dracaena* ‘Lisa’ plants that are going to market at five to six feet tall.

### Back cover, upper right.

Another photo of assorted dracaenas from TPIE. The brightly colored selection to the right of the photo with a

dark green central stripe and light green outer stripes on the leaves is *Dracaena fragrans* (Deremensis group) ‘Lemon Lime’. The central plant is *Dracaena* ‘Art Carmen’ and there is a *Dracaena marginata* cultivar at the upper left side.

### Back cover, center left.

One of the most popular small dracaenas is this highly variegated form of *Dracaena reflexa* known as ‘Song of India’. The plant is a vigorous grower that may require pruning to keep it at a manageable size. Florida growers produce several popular cultivars of *D. reflexa*.

### Back cover, center right.

An uncommon selection of *Dracaena reflexa* ‘Haka’. Unlike *D. reflexa* ‘Song of India’ with recurved (reflexed) leaves and twisty stems, this cultivar has flat, straight leaves that seem very sharp (they are not), especially when the plant grows larger. The clasping leaf bases are also very showy when the plant is grown in higher light. Larger specimens are excellent replacements for yucca plants, since they give a similar appearance, but can be grown in much lower light.

### Back cover, bottom left.

This finely textured, smaller plant is *Dracaena reflexa* ‘Ruth Luka’ which was collected in Mauritius in 2004, but is also native to Madagascar. The plant is free-branching when trimmed back as in the photo, but the stems can also grow quite long and straight. It has potential as a cut foliage crop since the plant grows very rapidly. Although this selection is solid green, the plants have also produced sports with striped leaves. *Dracaena reflexa* is one of the most variable species of the dracaenas, many of the selections are quite different in appearance from each other.

### Back cover, bottom right.

Another photo of assorted dracaenas from TPIE. Some are quite tall. The tallest plants in the back row of the photo are *Dracaena marginata*, the dragon tree. The center features several striped-leaf dracaena cultivars, while several of the plants at the lower level are different cultivars of *Sanseveria*. Plant taxonomists have rather recently moved all of the *Sanseverias* into the genus *Dracaena*, but so far, the market has not followed.

