Florida Ag Research
1840 Biddle Ranch Road, San Luis Obispo, CA 93401. (805) 594-1800
3001 N. Kingsway Road, Thonotosassa, FL 33592. (813) 986-5599
21602 27 ½ Mile Road, Albion, MI 49224. (517) 243-6749

Pestalotiopsis Research Update

Frank Sances, Balaji Aglave, and Mark Keeley

March 31, 2020

This report presents results from investigations by our research group to provide critical information to the Florida Strawberry Research and Education Foundation to help manage *Pestalotiopsis* disease in commercial plantings.

Pestalotiopsis clavispora is a fungal disease of several crops that has been in Florida on strawberries as early as 1972 (Howard and Albregt), and more recently in 2012 (Baggio, et al.). However, with the exception of these two occurrences, it has not been an important or reoccurring problem to Florida strawberry production until the winter of 2018, and again in 2019. At this time, many growers reported losses from early season infections to fruit, where the fungus causes darkened spotting (lesions) that render berries unmarketable. In other strawberry production areas in Europe and the Mideast, however, it has emerged as an important fungal pathogen of this crop. In Huelva Spain for example, there was an outbreak in 2012 and 2013 that caused extensive losses to Spanish strawberry growers (Chamorro, et al. 2015), and also on Chilean blueberries in 2007-2008 (Espinoza, et al. 2008). Recent epidemics in Florida began in 2018 with a single nursery plant source from North Carolina. Since that time, the fungus has infected strawberry crops throughout Central Florida, and now may be a reoccurring key pest of strawberries here during certain weather conditions. This problem is worsened by the lack of fungicide efficacy from products currently registered for use on strawberries, and hence, control by sprayable fungicides is quite limited. Our group and others are conducting extensive fungicide trials with new and existing products to find alternatives for Florida growers going forward.

<u>2019-2020 Season Assessments</u>. To begin investigations and help better understand *Pestalotiopsis* in the field, three commercial strawberry grower sites were selected in the Plant City-Dover area: Sydney Rd., Fresa Way, and Varn Rd. locations. At each site, established infestations of *Pestalotiopsis* were studied mid-season on two Florida strawberry cultivars, Sensation and Brilliance. Data collections were conducted by a team of four biologists working over ten days, who recorded disease incidence and severity from each strawberry row at 50-foot lengths ("one pixel"). Among all sites, over 6200 individual plot ratings were made to describe disease presence by documenting the accumulation of leaf fungal lesions from planting to the first week of February. These data were then used to construct "Heat Maps" (Fig. 2), where comparisons are possible

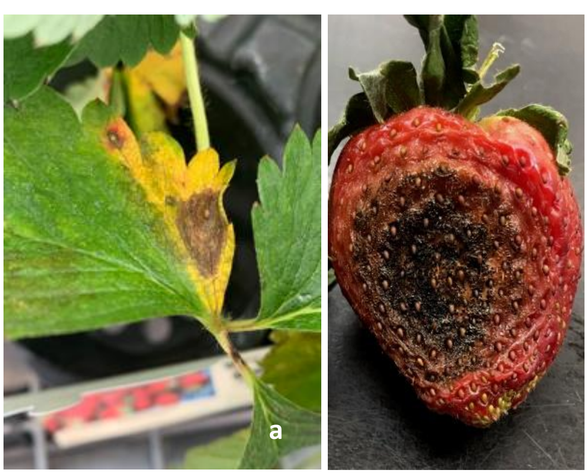


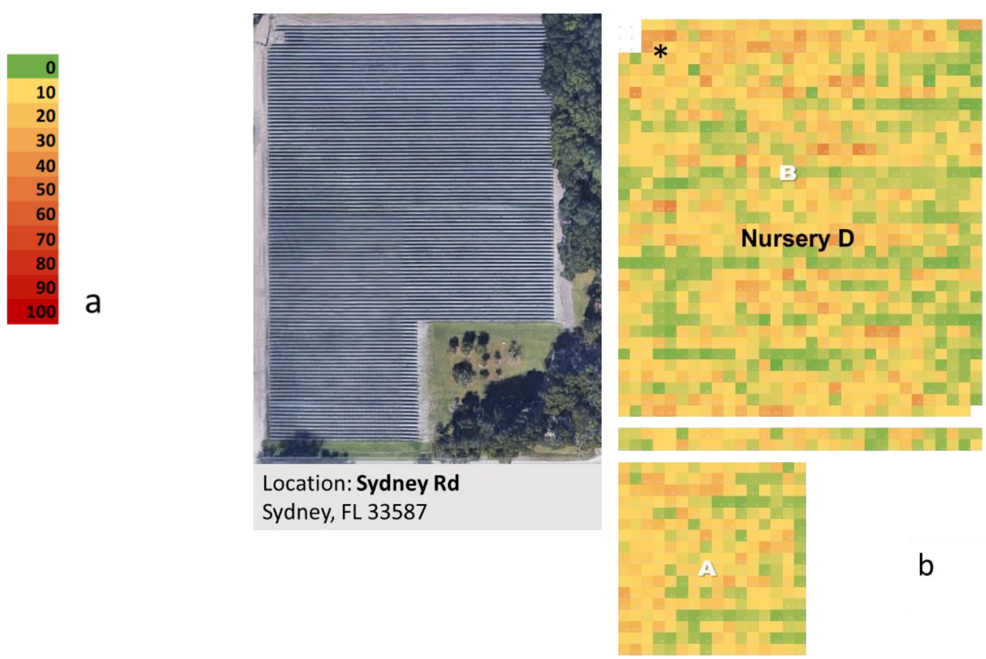
Figure 1. *Pestalotiopsis* Symptomology on (a) foliage and (b) berries. Necrotic lesions grow in a circular pattern where spores develop and are released as infectious particles dispersed into wind and water.

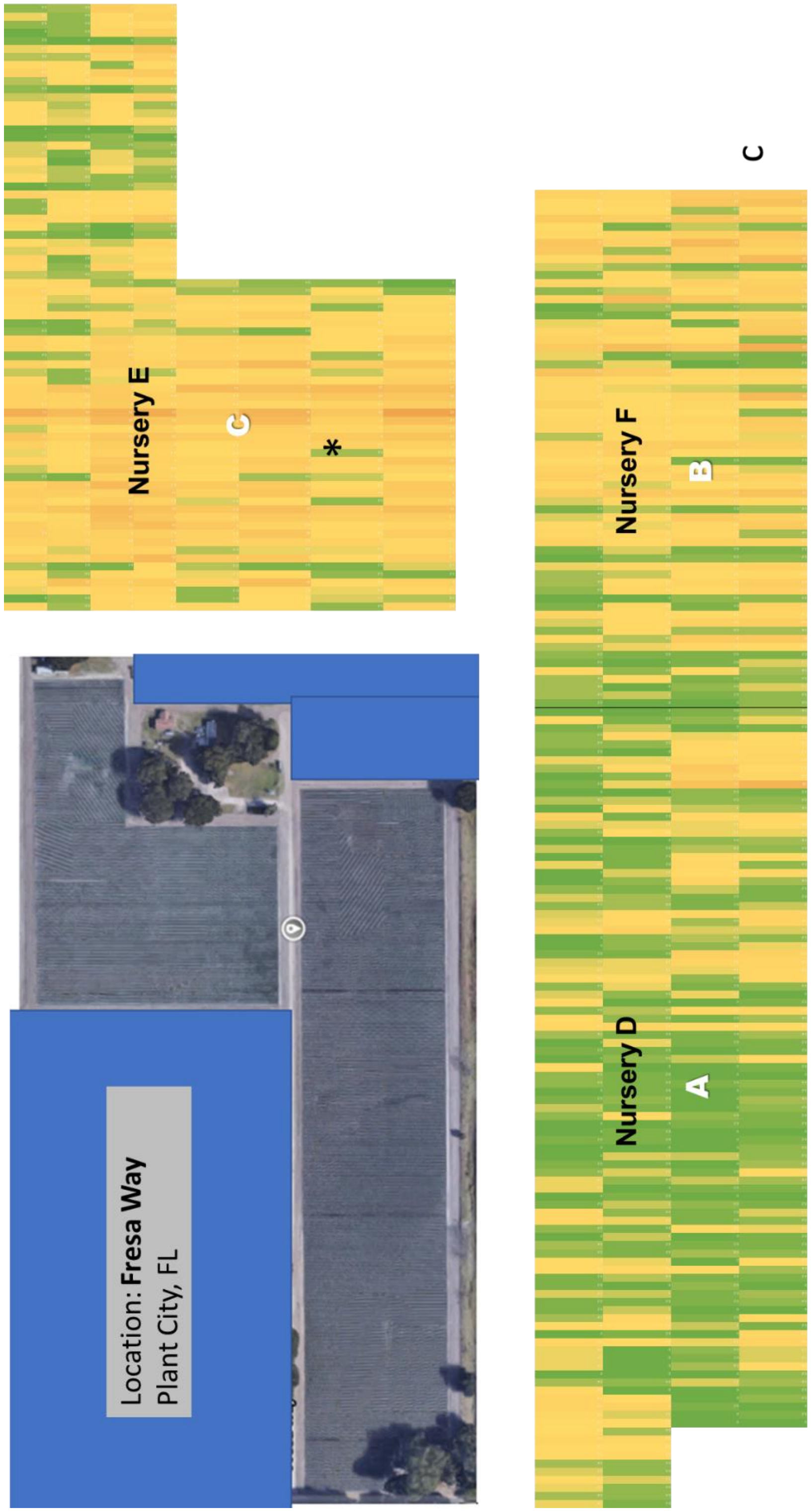
among farm sites, tractor spray patterns, plant nursery sources, strawberry varieties, interaction with other pests, and overall distribution of the disease across the focus fields. In addition to the in-field disease surveys, numerous *in vivo* greenhouse studies were conducted with alternate crops, native weeds and tree species, and common ornamentals to characterize a potential host range of *Pestalotiopsis* In Central Florida. In addition to these completed studies reported here, current studies are underway to determine survivability of fungal spores on various substrates such as plastic mulch, clothing, and machinery. In these settings, various sterilants are being tested for field sanitation programs to prepare growers for managing this disease in 2021 and beyond. A second report will be presented to FSREF when remaining research is completed next month. This report presents data collected to-date.

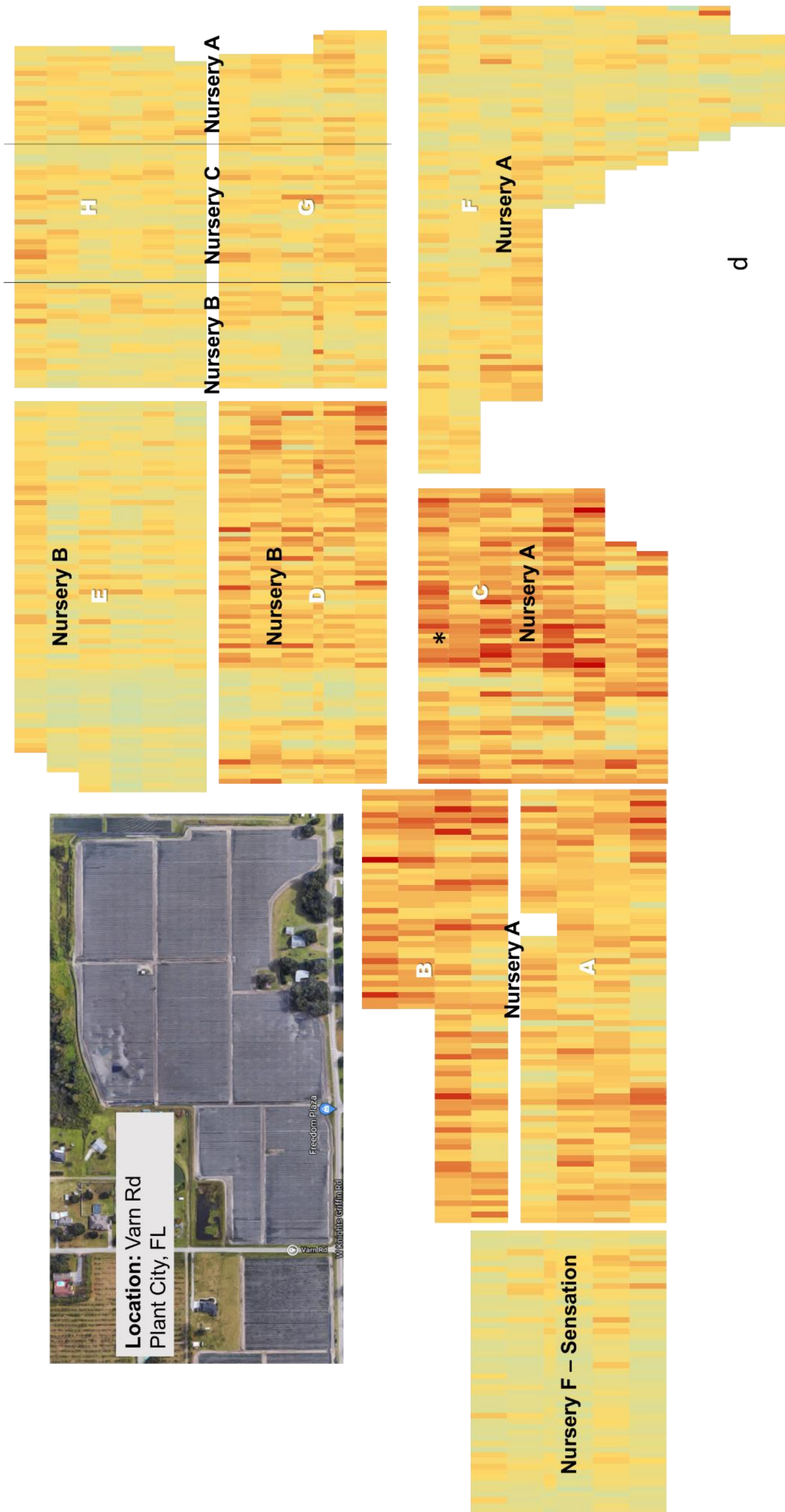
Results:

1. <u>Disease severity "Heat Maps"</u>
   Severity ratings for *Pestalotiopsis* from 10 strawberry plants in each 50-feet of row were taken using the standard disease severity rating of 0-100, based on percentage of the individual plant infected. Back at the lab, these data were then entered into a relational data base and assigned a color rating to create "Heat Maps" to describe disease magnitude across these plantings in a detailed matrix (Fig. 2). In this format, green colors indicate low disease severity (0-10 severity), red color is most severe (80-100 severity), with intermediate yellow and orange colors representing severity ratings between 20 and 70 (see Fig 2a). While each farm grew either Sensation or Brilliance varieties, seven nursery sources supplied plants among them, which are designated as Nursery A-F.

Figure 2. *Pestalotiopsis* Severity Heat maps and Site Descriptions for three strawberry farms in Central Florida. (a) Heat Map key for rating symptoms, with 0 for clean plants and 100 for plants with lethal disease severity levels. Farm sites include commercial grower operations located on (a) Sydney Rd, (b) Fresa Way, and (c) Varn Rd. Inset in bold text on each map are the Nursery Source (identified by anonymous letter) and variety. All plantings were Brilliance variety, except where noted. White letters denote block designation. Asterisks indicate location of outbreak per grower conversations. This data shows the spread of disease from the original epicenter through the plantings during the mid-December 2019 epidemic period.




Location: Fresa Way
Plant City, FL
Nursery E
Nursery F
Nursery D
A
B
C
C
*



Nursery A
Nursery C
Nursery B
H
G
F
Nursery A
d
Nursery B
E
Nursery B
D
C
Nursery A
*
B
Nursery A
A
Location: Varn Rd
Plant City, FL
Freedom Plaza
Varn Rd
Nursery F – Sensation

Some nurseries provided transplants to more than one site and one farm had a single plant source. While this analysis emphasizes disease severity, disease incidence was also recorded. Here, disease incidence was similar at Sydney Rd. and Fresa Way, 53% and 60% respectively. However, at Varn Rd., disease incidence by plot was 100%, where every plant sampled at the site had foliar or fruit *Pestalotiopsis* lesions present to a greater or lesser degree (severity).

2. Field factors and disease incidence and severity

Since *Pestalotiopsis* became widespread in Florida strawberry plantings in late 2019, many observations have been made by workers of initial establishment of field epicenters, spread within plantings from equipment and personnel movements, and edge effects related to possible alternate hosts near field borders. Although future research is required to clearly define these issues, the preliminary data presented herein provide data on the distribution of the disease within commercial fields once epicenters are present. The initial infection of strawberry plants by *Pestalotiopsis*, and its subsequent spread in fields is what growers must understand if commercial control is to be optimized. Hence, these three typical grower fields in the Plant City area provide such an opportunity for understanding how the disease developed so quickly, spreads through plantings, and is affected by field factors studied by these researchers.

a) Field distribution and spread from epicenters

Disease development at all 3 sites began in one or more epicenters in the fields from December 15 through December 30th (exact times of each establishment is not known). During this period of infectivity, daytime temperatures averaged 68.9°F with nighttime temperatures at 63.8°F. According to our interviews with growers, at Varn Rd. the pest developed in C block initially, at Fresa Way in the southern portion of the A Block, and at Sydney Rd. in the North West quadrant (See asterisks on Fig. 2).

b) Spray tractor routes and disease incidence and severity.

The illustration in Fig. 3 below shows tractor passage from an overhead perspective on one farm site. Ratings of disease incidence every 50 feet of row in relation to spray tractor movements are summarized in Fig. 4 and Table 1. Figure 5 presents disease Severity data averaged for all 3 sites in relation to one another.

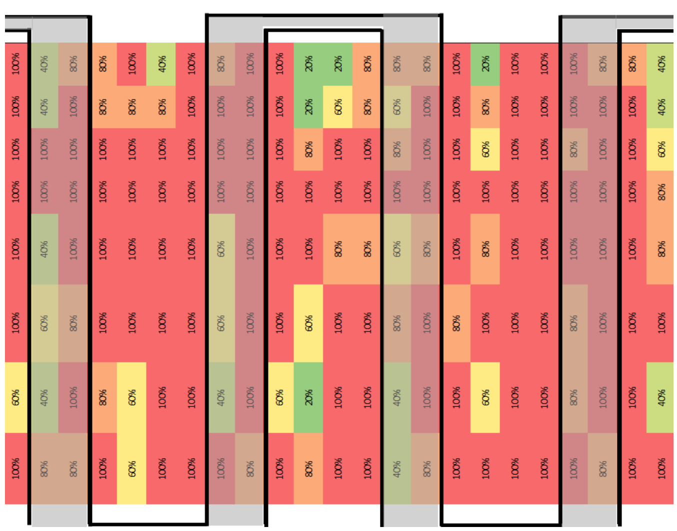


Figure 3. Close-up *Pestalotiopsis* infections in 50-foot sections of planted rows with Tractor Patterns (shaded) Average incidence of *Pestalotiopsis* infestation on Brilliance variety strawberry rated on a 0-100% scale, where 100% (red) represents all plants in 50 linear foot of bed with active *Pestalotiopsis* lesions on fruit or foliage.

Table 1. Average disease Severity between Boom and Tractor Chassis locations in fields. Comparing the increase or decrease in disease severity in under-boom or under-tractor Brilliance cv. strawberry plants from each nursery source; * indicates significant differences, p<0.05, ANOVA, Student-*t* Means comparison.

	Varn Rd.				Sydney Rd	Fresa Way		
Nursery:	**A***	**B**	**C***	**F (Sensation)**	**D***	**D**	**E***	**F* (Brilliance)**
Boom Sides	23.2	20.6	20.0	14.2	11.8	1.1	4.5	2.9
Under Tractor	29.6	19.9	17.8	13.0	10.1	1.1	3.3	4.1
% Difference (Boom vs Under Tractor)	22%	-3%	-12%	-9%	-17%	0%	-37%	31%

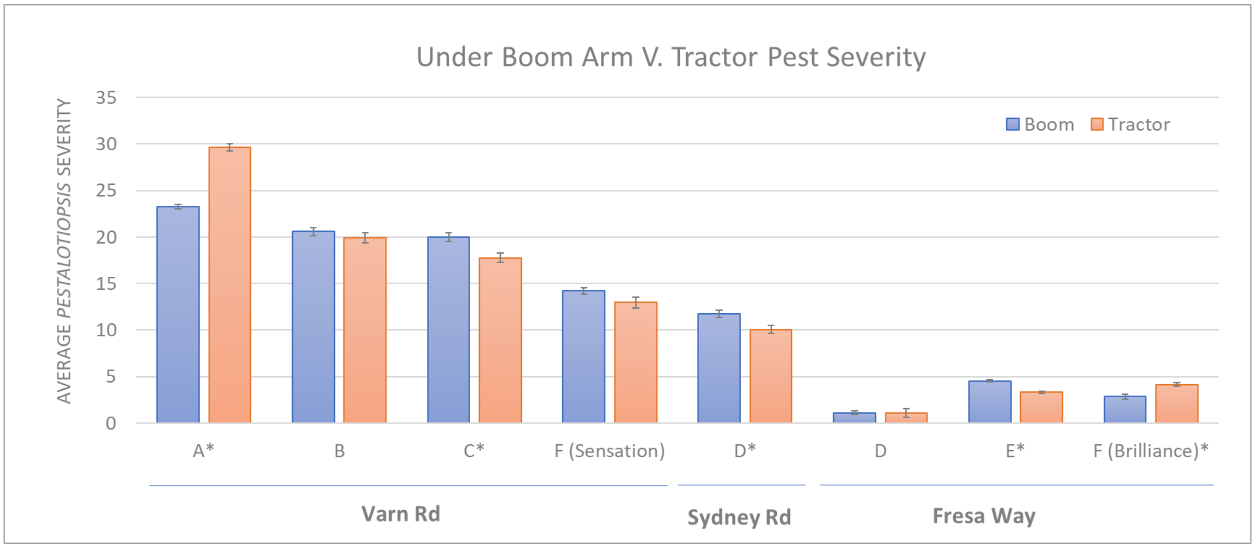


Figure 4. Under Boom vs Under Tractor *Pestalotiopsis* severity by Nursery site. All strawberries were Brilliance variety, except one planting of Sensation at Varn Rd. While numerically similar, asterisks denote where differences were statistically significant at p<0.05 for Sydney Rd. (Nursery D), and A, C, and E plant sources. Sensation variety from nursery F did not show significant differences between under boom or tractor path severity ratings. ANOVA, Student-*t* Means comparison.

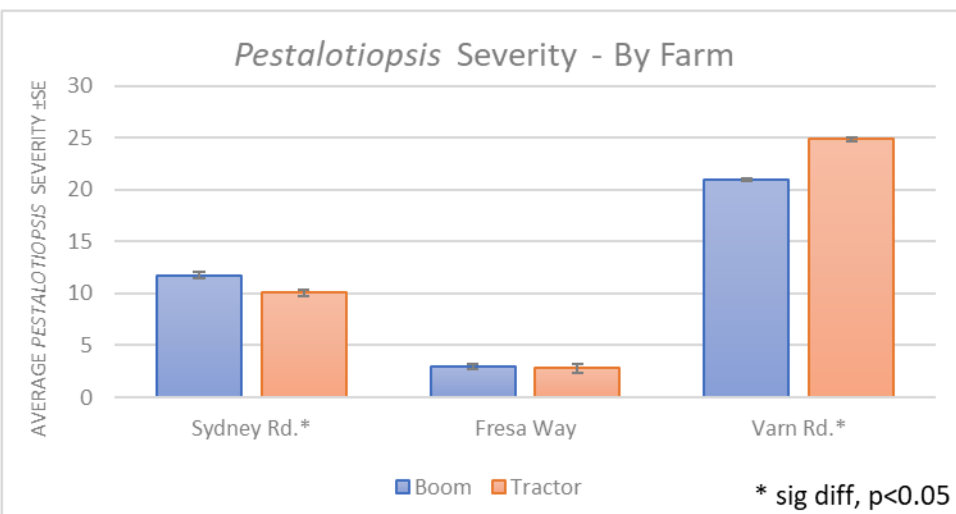


Figure 5. *Pestalotiopsis* disease severity under Spray Boom vs. Under Tractor Chassis by Farm site studied. Data represent the average disease severity from over 6000 disease ratings of strawberry rows across all planting blocks and location on the farms. ANOVA, Student-*t* for means comparison.

While a slight increase under tractor rows occurred at Varn Rd. in one plant nursery block, there were no consistent trends demonstrating *Pestalotiopsis* incidence was influenced by row position relative to equipment drive rows when comparisons are made across all strawberry rows classified.

c) <u>Disease severity comparison of commercial farms and plant sources</u>
When disease severity data presented in Figure 6 are organized according to plant source and cultivar, several relationships are indicated. These comparisons <u>do not prove cause and effect</u>, but rather describe disease findings at a given farm and field location organized by plant source. Overall, these results show differences between disease severity among the farms studied, where Varn Rd. had the most advanced infection at the time of sampling midseason. With regard to the plant source, the severity for a given plant source was dependent on where the infection epicenter was located relative to the nursery source.

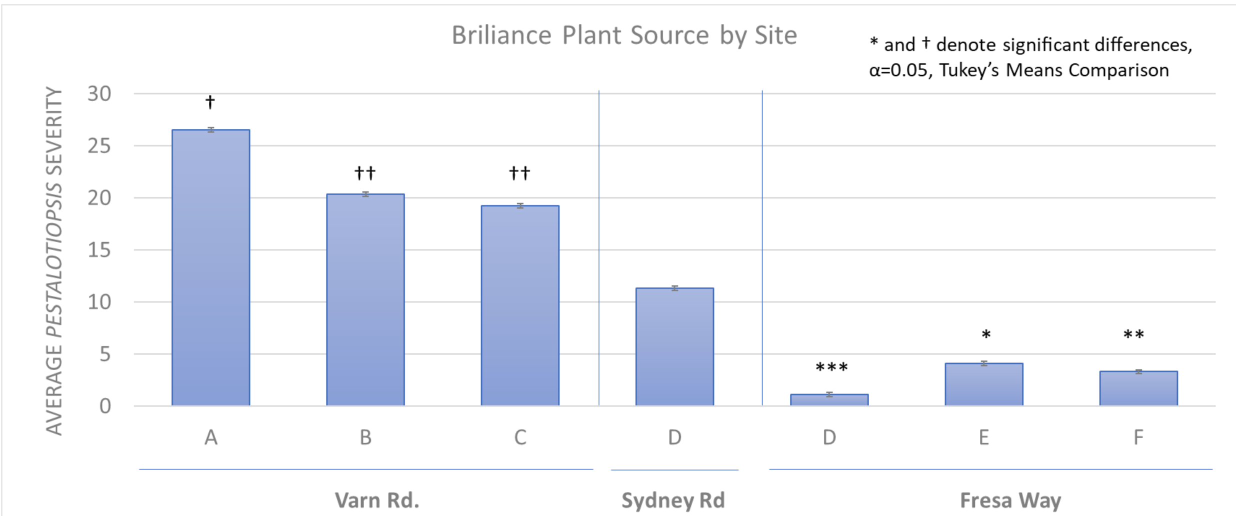


Figure 6. *Pestalotiopsis* Severity by Nursery Source and Location. Average severity ratings for *Pestalotiopsis* infestation on Brilliance variety strawberries from different nursery sources for each farm site sampled. Tukey's Means Comparison, α=0.05.

d) <u>Correlation to Mite Feeding</u>
During evaluations of the farm sites, along field edges and other isolated areas that had evidence of Two Spotted Spider Mite (*Tetranychus urticae*) feeding early season, biologists reported an apparent increase in severity of *Pestalotiopsis* infection. At Varn Rd., however, there was an opportunity to quantify this at one location in a separate planting that had been infested at various levels of spider mites' early season. From 30 plots (50 ft in length) of varying spider mite damage, an evaluation was performed by Dr. Aglave alone rating spider mite feeding damage on leaf undersides and overall plants, followed by a second sequential rating of the severity of *Pestalotiopsis* disease lesions on these same plant structures. Results from these evaluations are presented in Figure 7. These data show that *Pestalotiopsis* <u>disease severity increased proportionally with increasing spider mite damage</u> on strawberry. Further studies are in progress under controlled conditions to validate this preliminary finding described here. Nevertheless, these field data suggest with high statistical significance that Spider Mite feeding exacerbates infection by *Pestalotiopsis.*

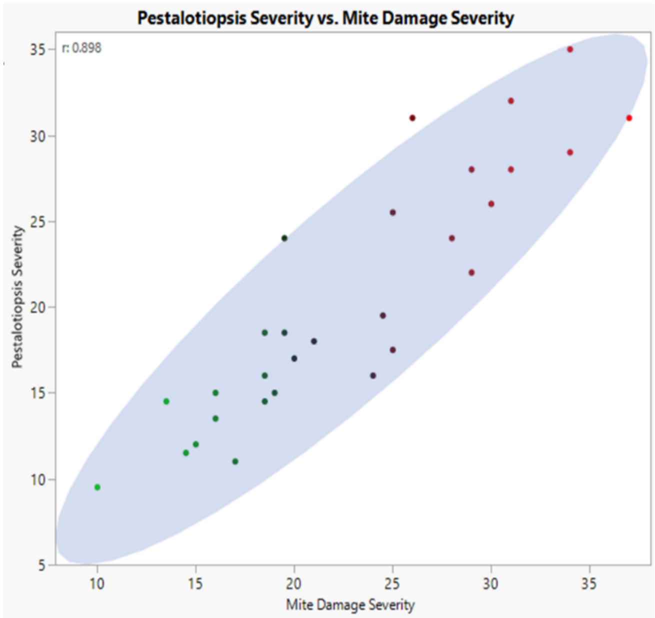


Figure 7. Correlation Between Mite Damage and *Pestalotiopsis* disease Severity. Documented at a Varn Rd. planting of Sensation, where distinct levels of Spider Mite (*Tetranychus urticae*) damaged plants ranged from very low to no damage (healthy), through several intermediate levels of damage, to very high levels where leaf undersides were fed out and dry. In these rows, the severity of *Pestalotiopsis* was notably higher as mite feeding damage increased. This interaction was found in several areas in other plantings during our evaluations but was difficult to document. At this site however, we were able to independently rate both types of damage in 30 plots of 50-foot sections of row. When data were paired in correlation analysis shown here, the disease encouragement effect of Spider Mite feeding damage was validated. Specifically, *Pestalotiopsis* disease severity was highly correlated to increasing levels of Spider Mite damage. Correlation r=0.898.

Figure 8. Strawberry plant (a) with Spider Mite infestation at Varn Rd (b) Close up of and eggs and adult female Spider Mites present on strawberry leaflet undersides.

e) <u>Sources of *Pestalotiopsis* inoculum in the field from local host plants.</u>

Identification of host plants for *Pestalotiopsis* infecting strawberry plantings in Central Florida is a primary objective of our research for FSREF. Without this basic information, field sanitation and

Figure 9. Experimental greenhouse plantings of candidate *Pestalotiopsis* host plants propagated for testing. 30 different species of Florida plants that included area crops, native weeds, and ornamentals were tested for colonization by *Pestalotiopsis* compared to Brilliance strawberry.

integrated control of this fungal pathogen cannot be optimized. This objective was investigated by propagating 30 commonly occurring Central Florida plant species comprised of crops, weeds, and ornamental plants found growing near strawberry fields (Table 2). *Pestalotiopsis* inoculum was obtained from the Sydney Rd. site in January and used for experiments where a spore suspension was applied to greenhouse grown test plant foliage, placed into an incubator, and observed for pathogenicity for a thirty-day period. Brilliance cv. strawberry was also included for comparison, totaling 31 infectivity tests, replicated on 10 plants of each species in our laboratory greenhouse in Thonotosassa. Results from these studies are shown in Figure 10. Here, 12 of the 30 possible plant species (38.7%) were found to be hosts of *Pestalotiopsis,* with Bell Pepper and Lambsquarter having the highest numerical disease severity among the group. This fungus also colonized blueberry, tomato, and watermelon. We did not observe any fungal colonization on cantaloupe, squash or cucumber. In these tests, watermelon, both seeded and seedless cultivars, were susceptible to *Pestalotiopsis*, but other cucurbits commonly used for second crops following strawberry were not hosts in our studies. Of particular concern in these host range findings are the Florida native plant species, Cabbage Palm, and Live and Water Oaks, all of which were easily colonized by the fungus. Finally, Rye Grass, commonly used for roadways in strawberry plantings showed to be a very susceptible host of *Pestalotiopsis.*

Table 2. Potential Host Plant Species tested for *Pestalotiopsis.*

Cover Crops:	Horticultural:		Weed Species:		Landscape:
Sunn Hemp	Strawberry	Cantaloupe	Wild Geranium	Common Purslane	Viburnum
Hairy Indigo	Tomato	Cucumber	Purple Nutsedge	Chickweed	Water Oak
Sorghum	Pepper	Squash	Lambsquarter	Marestail	Live Oak
Rye Grass	Blueberry	Collards	Goatweed	Bushmint	Cabbage Palm
	Watermelon (Seeded and seedless)		Dog Fennel	Goosegrass	Bahia Grass
			Wandering Cudweed	Eclipta	
			Florida Tassleflower		

Table 3. Infectivity results from host plant inoculations with *Pestalotiopsis* followed by at 80°F in temperature-controlled greenhouses. Average *Pestalotiopsis* severity (0-100 scale) and percentage of plants with symptoms (incidence) shown as percentages. Different letters indicate statistically significant differences among hosts. ANOVA, α=0.05.

Type	Host	Avg Severity	Incidence	Type	Host	Avg Severity	Incidence
Cover Crop	Hairy Indigo	0.0 gh	0%	Weed	Bushmint	0.0 fgh	0%
Cover Crop	Rye Grass	9.5 bcd	60%	Weed	Chickweed	0.0 gh	0%
Cover Crop	Sorghum	0.0 gh	0%	Weed	Common Purslane	0.0 gh	0%
Cover Crop	Sunn Hemp	0.0 gh	0%	Weed	Dog Fennel	0.0 h	0%
Horticultural	Bell Pepper	15.0 a	80%	Weed	Eclipta	0.0 gh	0%
Horticultural	Blueberry	7.0 de	60%	Weed	Florida Tasselflower	12.3 abc	62%
Horticultural	Cantaloupe	0.0 gh	0%	Weed	Goatweed	0.0 gh	0%
Horticultural	Collards	0.0 gh	0%	Weed	Goosegrass	0.0 gh	0%
Horticultural	Cucumber	0.0 gh	0%	Weed	Lambsquarter	15.0 a	75%
Horticultural	Squash	0.0 gh	0%	Weed	Marestail	7.9 cde	71%
Horticultural	Strawberry	13.0 ab	70%	Weed	Purple nutsedge	0.0 gh	0%
Horticultural	Tomato	5.5 def	50%	Weed	Wandering Cudweec	0.0 gh	0%
Horticultural	Watermelon - seeded	5.0 ef	35%	Weed	Wild Geranium	0.0 gh	0%
Horticultural	Watermelon - seedles	6.3 de	44%				
Landscape	Bahia	0.0 gh	0%				
Landscape	Cabbage Palm	8.9 bcd	57%				
Landscape	Live Oak	7.5 de	60%				
Landscape	Viburnum	0.0 gh	0%				
Landscape	Water Oak	4.0 efg	50%				

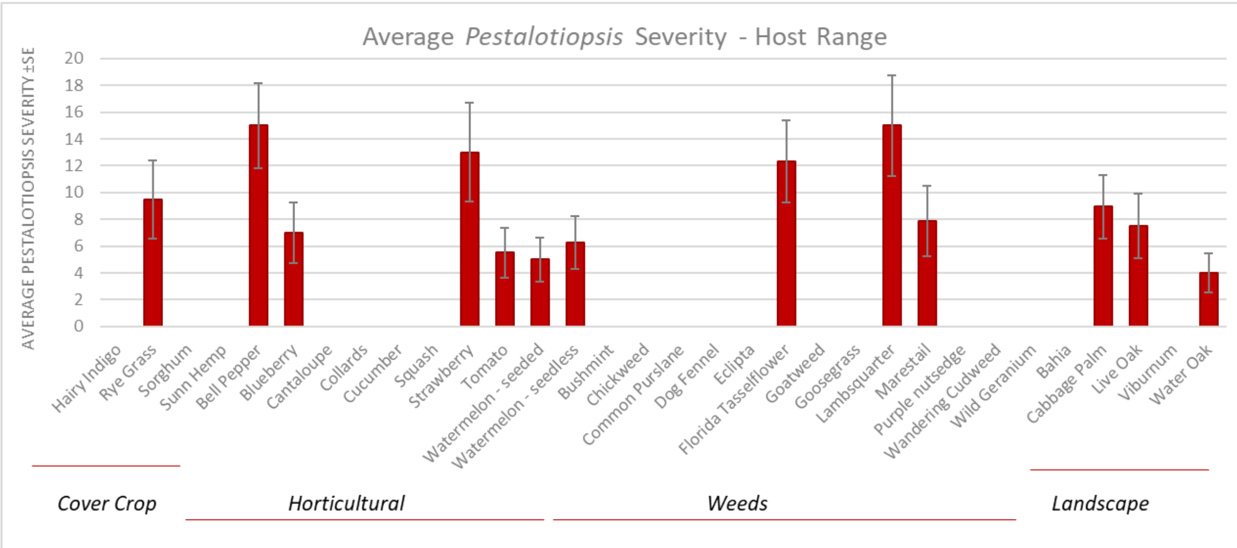


Figure 10. *Pestalotiopsis* disease Severity on a range of host plants common in the Central Florida strawberry growing district. Infectivity, described here as disease severity rated 0-100, was found to vary considerably among plant species tested. Crops such as blueberry, tomato, and watermelon were easily infected by *Pestalotiopsis*, although not as much as strawberry. However, bell pepper and the weed Lambsquarter were numerically more susceptible. Alternate hosts also include Cabbage Palm and Live and Water Oaks, as well as Rye Grass, which has been used to stabilize roadways in strawberry plantings for decades.

Discussion:

Pestalotiopsis disease ratings taken from the three farm sites show that regardless of the location and magnitude of the initial epicenters in fields, this pathogen becomes well distributed across plantings very quickly. This occurs even during rigorous multiple applications of modern fungicides using optimized equipment in high gallonage. Disease Heat Maps presented herein give field distributions at three levels

Figure 11. *Pestalotiopsis* severity on multiple host plants representing horticultural, weed, and cover crop plant species. (a) Strawberry, (b) Tomato, (c) Watermelon, (d) Lambsquarter, (e) Marestail, and (f) Rye Grass.

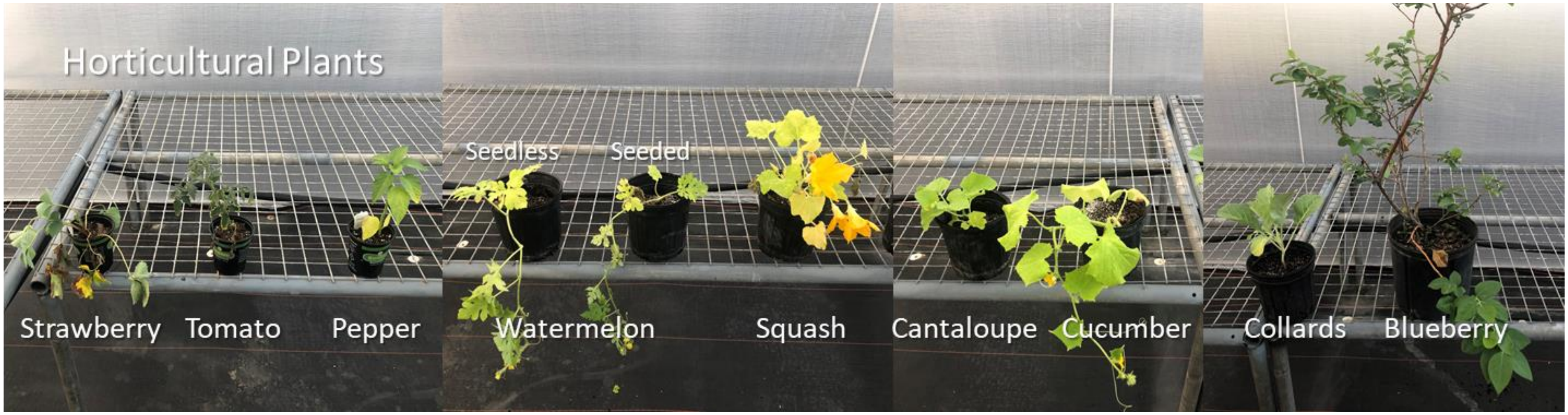
of *Pestalotiopsis* infection (Fig. 5), relative to initial epicenters from mid to late December. In all 3 cases, plant development was severely affected, and many fruits were rendered unmarketable by this fungus. Significant fruit losses occurred even at Fresa Way, where disease development was relatively low in comparison to the other sites. From these observations, the tolerance for *Pestalotiopsis* in commercial strawberry may be very low, requiring a rigorous preventative fungicide program during peak disease episodes.

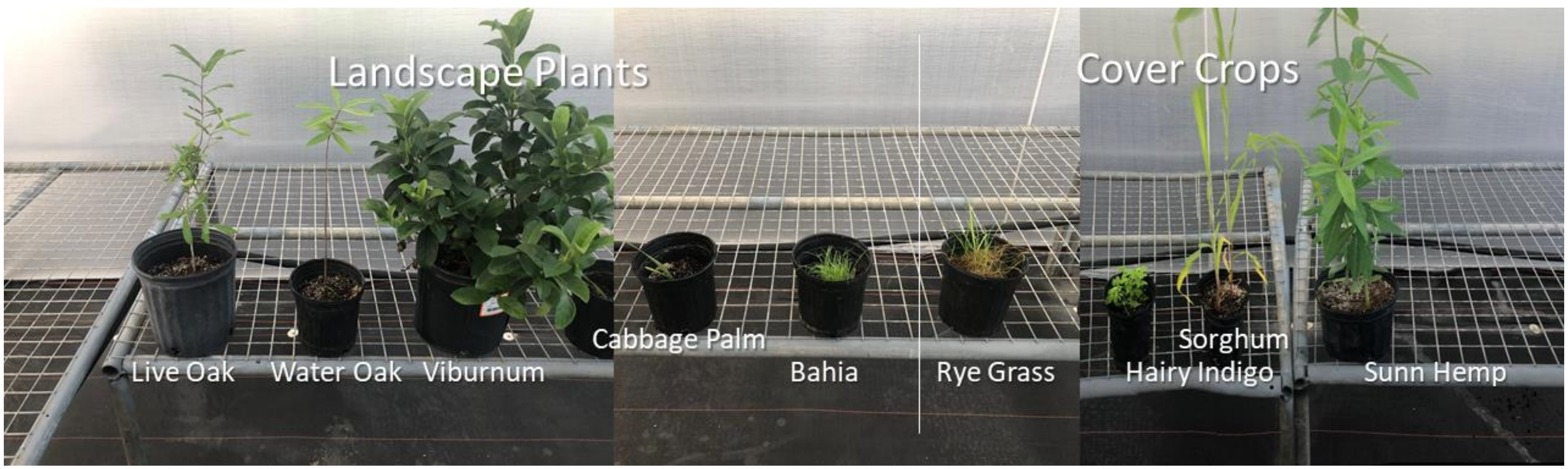
Disease severity comparisons between strawberries grown under spray booms vs under tractor chassis showed some differences in *Pestalotiopsis* infection at one site, but results were not consistent across the remaining plantings. This finding suggests there are no disease trends corresponding to row location relative to tractor mounted spray equipment design.

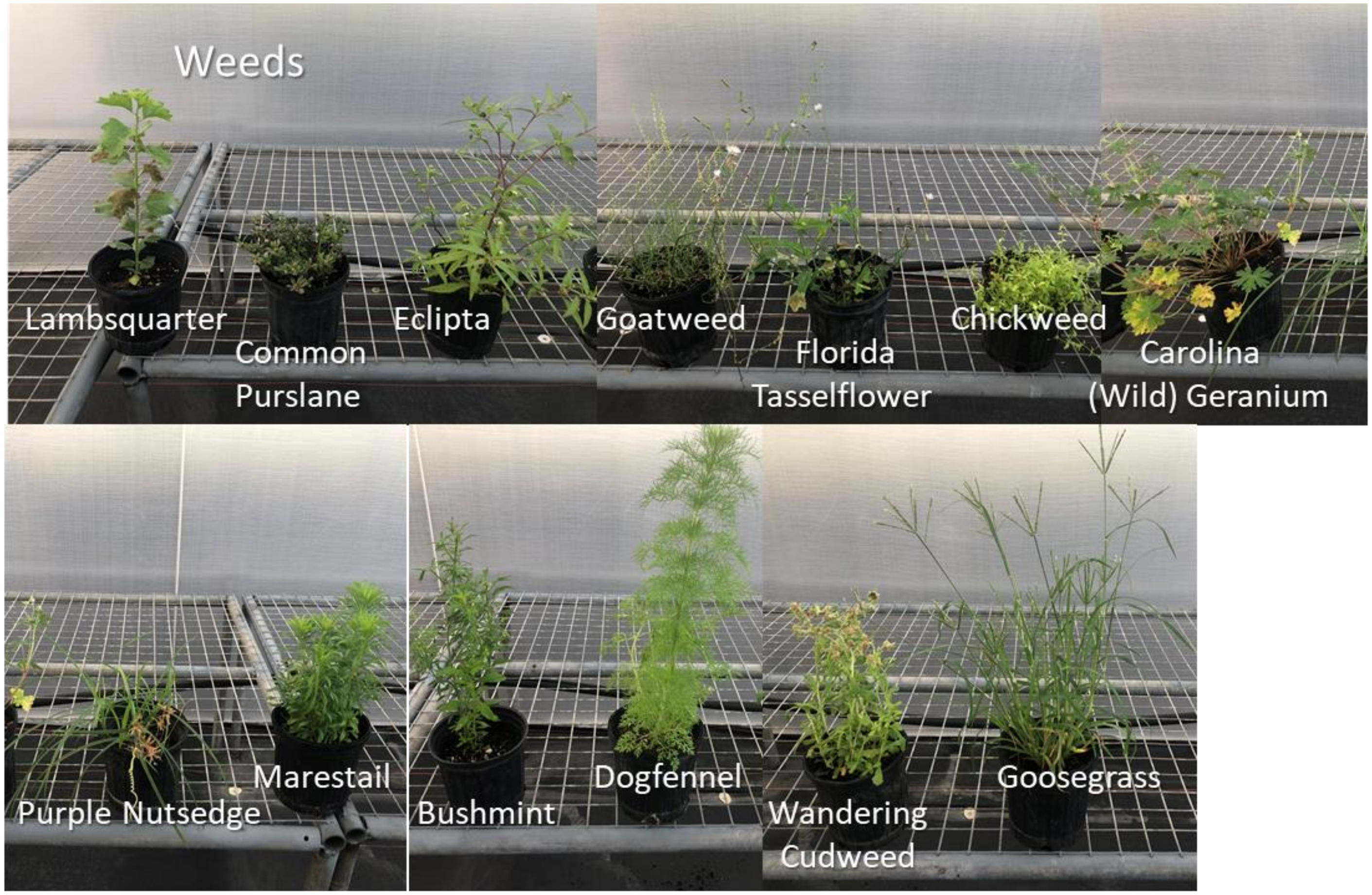
The relationship between Spider Mites and *Pestalotiopsis* severity was found to be highly correlated, given the frequent observations by our biologists while studying the disease at the farm sites, and the high correlation values ($r>0.89$) between the two pest data sets where an intense focus evaluation was performed. As the literature on *Pestalotiopsis* suggests, it is a weak pathogen with the tendency to preferentially infect previously damaged foliar and fruit tissues, including crowns on strawberry (Marshall, 2017). In the UK for example, researchers recommend dipping transplants in fungicide to avoid early season infections of *Pestalotiopsis* from root and crown wounding at planting. With regard to Spider Mite injury encouraging plant diseases, this phenomenon has also been previously reported on Apples (in the same botanical family as strawberry). In this case, Spider Mites have been shown to markedly increase the severity of *Alternaria* Blotch on foliage, similar to what we are seeing here in Florida with *Pestalotiopsis* on strawberry. From these cases, it is apparent that *Pestalotiopsis* and *Alternaria* pathogens more easily colonize plant tissues that are previously damaged from Spider Mite feeding, which demonstrate that early-season Spider Mite control is essential to minimize *Pestalotiopsis* disease establishment and spread later-season. This may be a problem in organic strawberry production, since this low threshold for Spider Mite damage prevents biological control using predators alone that require growers tolerate low levels of Spider Mite feeding (Sances et al. 1979a, 1979b). This is the case in North Carolina, where apple growers have set very low Spider Mite treatment thresholds, even though mite feeding may not be injurious to the crop at the low population levels that cause *Alternaria* infections. In many North Carolina apple orchards, very low Spider Mite thresholds prevent biological control from being implemented, which may possibly be the case with strawberry as well. In any event, it is apparent from these findings that control of Two Spotted Spider Mites is an important component of commercial management of *Pestalotiopsis* on Florida strawberry.

APPENDIX

Photo 1. Host Range test plants from 30 species compared to strawberry.






Weeds
Lambsquarter
Common Purslane
Eclipta
Goatweed
Florida Tasselflower
Chickweed
Carolina (Wild) Geranium
Purple Nutsedge
Marestail
Bushmint
Dogfennel
Wandering Cudweed
Goosegrass

REFERENCES

Baggio, J.S., Mertely, J.C., and Peres, N.A. March 2019. Is *Pestalotiopsis* a new threat to Florida strawberry production? Technical Report. UF IFAS Extension Solutions.

Chamorro, M., Aguado, A., and Delos Santos,B. 2016. First report of root and crown rot caused by *Pestalotiopsis clavispora (Neopestalotiopsis clavispora)* on strawberry in Spain. *Plant Disease*. 100(7): 1495.

Espinoza, J., Briceño, E., Keith, L., and Latorre, B. 2008. Canker and Twig Dieback of Blueberry Caused by *Pestalotiopsis* spp. and a *Truncatella* sp. in Chile. *Plant Disease*. 92. 10.1094.

Howard C.M. 1972. A Strawberry Fruit Rot Caused by *Pestalotia longisetula*. *Phytopathology*. 63:862-863.

Sances, F.V., Wyman, J.A., and Ting, I.P. 1979. Morphological Responses of Strawberry Leaves to Infesations of Twospotted Spider Mite. *Journal of Economic Entomology*. 72: 710-713.

Sances, F.V., Wyman, J.A., and Ting, I.P. 1979. Physiological Responses to Spider Mite Infestation on Strawberries. *Environmental Entomology*. 8: 711-714.