20/20 RESEARCH UPDATE

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Almond Board of California

Advancing California Almonds Through Innovation

With a record harvest estimated at 3 billion pounds in 2020, the Almond Board's research program delivers innovations to keep almonds profitable to farm while advancing the sustainability of how we grow and demonstrating the health and nutritional value of almonds to consumers. This report summarizes current research across almond production, environmental aspects of production, human nutrition, almond quality, and food safety.

The Almond Board, together with partners in industry and the University of California's Cooperative Extension, translate this research into practical tools to accelerate adoption of industry best practices from the farm across through the industry to handlers and food companies. Research addresses the challenges of today while developing innovations that will take the industry into the future.

This year marks twenty-five years of nutrition research, which had delivered a health halo for almonds that spurs consumer demand. Beginning with heart health, the Almond Board's nutrition research has grown to address diabetes, weight management, satiety, and gut health, to almond's contributions to skin health, cognition, and a healthy lifestyle.

ABC research is directed by over 100 industry stakeholders who share their expertise through service on the Strategic Agricultural Innovation Committee and its specialized Working Groups, the Almond Quality and Food Safety Committee, and the Nutrition Research Committee. These volunteers ensure research targets industry priorities and delivers practical and relevant information. We are grateful for Committee members sharing their time and experience, inviting others in the industry to consider service on Almond Board committees as well.

To learn more detail, forty years of project reports can be accessed in the Almond Board's Research Database on almonds.com.

For the Almond Board of California,

Richard Waycott
President and CEO
rwaycott@almondboard.com

Josette Lewis
Chief Scientific Officer, Research, and Innovation
(Strategic Ag Innovation Committee; Pollination Bee Health Workgroups)
jlewis@almondboard.com

Gabriele Ludwig
Director, Research and Innovation
(Sustainability Workgroup)
gludwig@almondboard.com

Sebastian Saa
Associate Director, Research and Innovation
(Orchard, Tree, Rootstock; Irrigation, Nutrients, Soil Health; Harvest; Workgroups)
ssaa@almondboard.com

Jesse Roseman
Principle Analyst, Research and Innovation
(Pest Management Working Group)
jroseman@almondboard.com

Tim Birmingham
Director, Quality Assurance and Industry Services
(Almond Quality & Food Safety Committee)
tbirmingham@almondboard.com

Swati Kalgaonkar
Associate Director, Research and Innovation
(Nutrition Research Committee)
skalgaonkar@almondboard.com

Guangwei Huang
Associate Director, Research and Innovation
(Biomass; Harvest Workgroups)
ghuang@almondboard.com

Ag and Environmental Affairs

Nutrition Research

Biomass

Almond Quality

Food Safety

Nickels Soil Lab Projects

Project Leaders: Franz Niederholzer[1]
[1]UCCE Colusa, 100 Sunrise Blvd, Suite E, Colusa, CA 95932 (530) 218-2359, fjniederholzer@ucanr.edu

Objectives

In support of two ongoing series of projects on almond production at Nickels Soil Laboratory:

- Continue to evaluate the economics of organic almond production methods suitable for California conditions and in compliance with U.S. Department of Agriculture and CCOF (California Certified Organic Farmers) certification requirements and compare the results with those for standard production methods.
- Planting density trial, evaluating 4 different tree spacings down the tree row: (12', 14', 16', or 18') planted in 2017. Replicated 5x times, each, on Titan peach/almond hybrid and Rootpac-R almond/plum hybrid rootstock.

Background

Like other food producers, more almond growers are shifting to organic production methods, thanks to a growing market.

Making such a transition, though, poses challenges for the growers. Among them would be how to deal effectively with such critical issues as disease control, insect control, weed control, and providing adequate nutrition, especially nitrogen.

Furthermore, although anecdotal information abounds on organic almond growing practices, there remains a lack of science-based information relevant to organic almond production.

To objectively explore what's involved in organic production, the Nickels Soil Lab is being used as the site of a research / demonstration orchard.

Since it was planted in 2006, this orchard has been being used to monitor and evaluate all aspects of producing organic almonds, including costs. The outcome will likely be a well-documented process—and possibly the basis for a viable commercial operation.

Another current and key topic of interest to almond growers is planting density- balancing cost with returns. A planting density x rootstock x pruning trial has been conducted in the northern San Joaquin Valley for more than a decade, but we are not aware of a similar trial in the Sacramento Valley. A spacing trial, without replicated rootstock or pruning treatments in 2017. Across the row spacing was held at 21', while down the row spacings of 12', 14', 16', or 18' were planted. Titan (peach/almond hybrid) and Rootpac-R (almond/plum) hybrid rootstocks were used with Nonpareil (50%), Aldrich (25%) and Kester (25%) scions, but not in a pattern to statistically compare the two rootstocks.

Discussion

We are hoping for better yield from the study block in 2020. Bloom weather was excellent.

Maintaining adequate nitrogen in the organic block remains the biggest challenge at this time. Cost of liquid organic fertilizer and incorporating dry, less expensive organic N sources have been some of the issues faced going into the 2020 crop year. In 2019, we were unable to uniformly apply 12-0-0 (dry) pelletized feather meal fertilizer due to plugging problems in the applicator. An updated machine (new AgPro 160-D) was used in 2020 and 100 lb of N/acre as 12-0-0 was delivered – shanked into the ground at 3-4" under the surface -- in late April. We are hoping to see a significant improvement in leaf N levels with this application.

Fourth leaf Nonpareil/Titan and third leaf Nonpareil /Rootpac-R yield data from the spacing trial will be presented at the conference. There was no difference in NP/Titan yield between the spacings in 2019, a year with terrible bloom weather (7.2 bee hours during the week of NP bloom). In 2020, bloom weather was excellent, with no rain and 64 hours of good bee flight during 7 days of NP bloom. It will be interesting to see yield after another year of growth plus good bloom weather.

Project Cooperators and Personnel:
John Edstrom, UCCE-Colusa County (retired); Bill Krueger, UCCE-Glenn County (retired); Bruce Lampinen, UC Davis; Stan Cutter, Nickels Soil Lab, Arbuckle, C

Documenting the Effects of Annually Applied Green Waste and Manure Composts on Almond Tree Performance

Project Leader: Roger Duncan
UC Cooperative Extension, Stanislaus County. 3800 Cornucopia Way, #A, Modesto, CA 95358. (209) 525-6800, raduncan@ucdavis.edu

Objectives

- Determine if the annual application of composted organic material improves almond orchard performance

Background

Many studies have shown that soil applications of composted organic material can increase the diversity and activity of soil microorganisms, soil water holding capacity, soil nutrients such as potassium and nitrogen, humic acid, organic matter and carbon sequestration. Despite these published reports on improvements in "soil health", few if any data have demonstrated enhancements in orchard performance. Current costs for purchase, delivery and application of composted green waste in the Modesto area is approximately $27 per ton. Common application rates range between five & ten tons of compost per acre, representing a significant investment to the grower. It is important to determine if almond growers can improve tree performance and/or yield enough to recover such a substantial input cost.

Two replicated field trials were established in 2015 to document the effects of composted green waste and manure on the performance of new almond orchards. Orchard A is planted in a Hanford sandy loam soil that has not been previously farmed. The variety is Nonpareil on Nemaguard rootstock and is irrigated with full coverage sprinklers. Orchard B is planted in a loamy sand in a replant site following an almond orchard removed four months prior and is irrigated with microsprinklers. The variety is Independence on Nemaguard rootstock. In both locations, 5.2 tons of compost per acre were applied in a concentrated band about four feet wide and incorporated into the soil at planting. An additional 0.5 tons / acre was applied to the soil surface at the base of the new trees after one month of growth. Each subsequent spring (2016 – 2019), approximately 10 tons of composted green waste or manure has been applied to the soil surface under the tree canopy in a band approximately eight feet wide. Trees are periodically monitored for stem water potential (water stress), and annually for leaf nutrients, nematodes, growth, and yield.

Discussion

Composted green waste and manure significantly affected July leaf levels of some elements. Nitrogen and chloride levels were sometimes higher in both compost treatments compared to the unamended controls ($P \geq 0.05$) while leaf calcium was significantly lower in the compost treatments every year. Potassium was increased in the composted manure treatment but not the green waste treatment. No other significant changes in leaf nutrients occurred.

After five years of study, the application of composted green waste or manure has not increased yield or tree growth as measured by trunk circumference or canopy size (photosynthetically active radiation - PAR). Stem water potential measurements with a pressure bomb indicated that compost did not improve tree water stress compared to trees growing in unamended soil. Compost treatments have not affected pathogenic nematode numbers.

Costs for purchase and application of composts at 10 tons / acre were approximately $265 annually, or $1,325 per acre over the five-year period. After five years of study, it does not appear that application of composted green waste or manure is an economically sound practice in conventionally farmed almond orchards. This was true whether orchards were grown in excellent, first generation orchard soil or very sandy, second generation orchard soil. Composts may be more beneficial in orchards with surface water penetration problems or if deprived of commercial fertilizers. It is also possible that benefits may be very long term and not observable in just five years.

Establishing a long-term phosphorus trial in almond.

Project Leaders: Franz Niederholzer[1]
[1]UCCE Colusa, 100 Sunrise Blvd, Suite E, Colusa, CA 95932 (530) 218-2359, fjniederholzer@ucanr.edu

Objectives

- Establish a replicated +/- phosphorus (P) trial in almonds.

Background

The goal of sustainable almond production is maximizing net annual income without risking decline in long-term productivity and negative environmental impact.

Phosphorus is an essential plant nutrient. Almonds remove phosphorus (P) from the orchard at a rate of 15-20 lbs P_2O_5 per 1000 lb kernel crop produced (hulls, shells and kernels).

Fertilizing almonds with P can increase yield, but evidence is limited. In a four year study at Nickels Soil Lab in the late 1990's comparing yield from multiple potassium (K) fertilizer treatments, adding a K+P source (Mono-potassium phosphate – MKP at the rate of 63 lbs P_2O_5/acre + 124 lb K2O/acre) increased yield in Year 4 by just under 800 lbs/acre over untreated control. This yield increase was not significantly different from 2 other treatments that added the same amount of K and no P. There was no yield increase in Years 1-3 from any K source. It is not clear from this work if the increase in yield from MKP was due to the P, alone.

Annual replacement P fertilizer application is not recommended in current UC almond production publications. However, many growers apply P in the range of 25-50 lbs P_2O_5/acre in the Sacramento Valley. Replacing P exported in a 3000 lb kernel/acre crop runs about $50/acre
(MAP at 60 lbs P_2O_5/acre and $0.8/lb P_2O_5.)

The question remains, does it pay to added P to bearing almonds?

We will establish a long-term P trial in bearing almonds at the Nickels Estate in Arbuckle (Colusa Co) in the fall, 2020. Fertilizer P will be applied in the amount removed in the previous crop year.

Prior to laying out the trial, the orchard will be surveyed assess potential differences in P availability (soil P), tree P status (leaf P) and yield.

If such survey work is not done prior to establishing a fertilizer/amendment trial, the results don't measure the impact of the input(s), but some confusing mix of natural productivity differences and input results.

Project Cooperators and Personnel:
Bruce Lampinen, UC Davis; Stan Cutter, Nickels Soil Lab, Arbuckle,

Drying Fresh Harvested Almonds

Project Leaders: Michael Coates[1] and Irwin Donis-Gonzalez[2]
[1]Plant & Food Res. Australia, Waite Campus, Urrbrae, SA 5064 + 61 4 3171 0647, michael.coates@plantandfood.com.au
[2]Dept. of Bio and Ag. Eng., UC Davis, One Shields Ave., Davis, CA 95616 +1 530 752-8986, irdonisgon@ucdavis.edu

Objectives

- Harvest fruit when kernel moisture is 10-15% with shaker, sweeper, conditioner and pickup
- Test effectiveness of dryers on in-hull fruit, maintaining RH below saturation. Optimise airflow and depth of product
- Compare stockpile and batch drying with control
- Test on multiple cultivars
- Evaluate for concealed damage, cavities, shell staining and pest damage

Background

Visible dust is a growing concern as both production increases and traditional growing regions become more urbanized.

Most dust is created after the fruit have been shaken from the tree. It is the act of sweeping the fruit into windrows and picking up the combination of fruit and soil (from the windrow) that billows dense dust into the surrounding air.

Off-ground harvesting is a potential solution but requires additional mechanical drying to prevent the fruit from spoiling, by maintaining temperature and RH low enough to prevent mold and bacterial growth. This project is establishing protocols surrounding the airflow delivery requirements to maintain that quality within a closed environment (batch) and within an open air, centrally aerated stockpile.

Discussion

This project makes use of both the Californian and Australian almond harvests to accelerate the research process. The 2020 Australian season has been cut short by Covid-19 restrictions, but what can be reported is as follows. Four factors affected stockpile drying: the shape of the stockpile; the velocity of the air moving through the stockpile; how that air was distributed within the stockpile; and the overall fruit moisture content (MC). Each of three 20-tonne 'Nonpareil' stockpiles of different moisture contents (14%, 12%, 10% kernel MC) tested using different tunnel aeration techniques (2 kW fan, solar fan, natural air). Each showed similar results. Fruit on the outside edges of the stockpile (Furthest from the air tunnel) didn't get adequate air flow and grew moldier. This indicates a need for a more structured stockpile with the outside boundaries equidistant from the air tunnel, or potentially additional air tunnels.

Fruit surrounding the tunnel dried inconsistently, with higher MC on the floor; this indicates a need for greater back pressure within the plenum to achieve better air distribution.

Air velocity, as well as air volume, played a large role in stockpile drying. Results demonstrated the importance of providing a large volume of air to keep the air saturation or relative humidity (RH) below 100% (reducing the potential for concealed damage). Additionally, the air needs to move through the almonds fast enough so that only a fraction of the moisture-carrying capacity is used. This velocity value varies with MC and quantity of product. If the air moves too slowly through the fruit, absorbing its true capacity, the resulting evaporative cooling effect will drop the core temperature of the stockpile enough to result in near-zero dehydration. At the same time, it would create a cool moist environment (>75% RH) ideal for mold to flourish. Ultimately, the MC of the stockpile (mainly the hull) will affect the size of a 'drying' stockpile. Unfortunately, the final test of the season, which combined all these factors to dry a 300-tonne stockpile of 'Monterey' with a 30-kW fan, was postponed by Covid-19.

Meanwhile, velocity and 'depth of product' values are being determined using the batch dryer developed during this project on both 'Nonpareil' and 'Carmel' cultivars.

Project Cooperators and Personnel: Almont Orchards (Fred and Mark Montgomery), Ismael Mayanja (MSc. Student), Nichols soil lab orchards (Dr Franz Neidermier), Dr Andrew Granger (PFR), Century Orchard (Scott McKenzie, Brendan Sidhu) and Cannonball Almonds (Luke and Mark Stoeckel)

Developing Effective Drying Methods for Minimizing Quality Defects for Off ground Harvest Almonds

Project Leader: Zhongli Pan[1]

[1]3018 Bainer Hall, Department of Biological and Agricultural Engineering University of California, Davis, One Shields Avenue, Davis, CA 95616 (530) 400-3400, zlpan@ucdavis.edu

Objectives

Develop engineering tool charts using the drying kinetics model to predict the drying times and energy consumptions under different drying temperature and air velocity conditions for almonds with different initial moisture contents and target final moisture contents.

Material and Methods

The Page model was used to predict the drying times and energy consumptions for almonds with different initial moisture contents and target final moisture contents. These data obtained from model simulation were used to create the engineering tool charts that can be used by the almond processors to determine the suitable drying conditions.

Results and discussions

The engineering tool charts were created using the Page model and could be used as prediction tools to determine the drying times and energy consumptions (heater and blower) of the column drying process under the range of tested operating conditions. The engineering tool charts are shown in the Figures U.1 to Figures U.4 in the appendix.

Project Cooperators and Personnel:

Co-Principle Investigator: Franz Niederholzer, Farm Advisor – Orchard systems, UC Cooperative Extension Colusa County, Colusa, CA 95932

Food Processing Lab at UCD: Dr. Ragab Khir, Chang Chen, Dr. Yi Shen, Zhaokun Ning, Rentang Zhang, Xiangyu Cao, Xingzhu Wu, Lizhen Deng

Almond Board of California: Guangwei Huang

Handling Fresh Harvested Almonds

Project Leaders: Michael Coates[1] and Reza Ehsani[2]
[1]Plant & Food Res. Australia, Waite Campus, Urrbrae, SA 5064 +61 4 3171 0647 michael.coates@plantandfood.com.au
[2]Dept. of Mech. and Aero. Eng., UC Merced, 5200 N Lake Rd, Merced, CA 95343 +1 863 396 9585, rehsani@ucmerced.edu

Objectives

- Continue development of a shaking guard to shield the tree line from fruit fall
- Evaluate fruit distribution
- Using current UC Merced research, continue development of a finger wheel pick-up and slow-arm sweeper
- Visually compare levels of dust creation to traditional sweep and pick-up methods

Background

Visible dust is a growing concern within the industry as both production increases and traditional growing regions become more urbanized. What was once a seasonal disturbance is becoming a more significant problem, with orchard dust moving into towns and across main traffic thoroughfares. Most of the dust is created after the fruit have been shaken from the tree. It's the act of sweeping the fruit into windrows that creates the problem by simultaneously agitating and blowing the soil along with the fruit. The soil billows up in clouds of dust, creating near-zero visibility. This ultimately creates a windrow that is equally filled with fruit and soil and is further exacerbated by picking up the combination of fruit and soil (within the windrow) and billowing a second round of dense dust into the surrounding air.

Solutions to the dust problem focus on removing the sweepers and potentially the 'pick-ups'. This ranges from modifying current equipment to completely changing the harvest model to an off-ground process that catches the fruit before they hit the ground. This project looks at keeping fruit off the tree line by deflecting the fruit during the shaking process and then adapting a 'finger wheel' harvester with a low-velocity sweeping arm to collect the fruit from the orchard floor without the need to windrow.

Discussion

This project makes use of both the Californian and Australian almond harvests to accelerate the research process. In the 2020 Australian season, screens were built to deflect fruit off the tree line. Initially two screens were attached to a cart that could be rolled up to the tree and extend 1.8 m either side of the tree, enough to shield the tree line vertically from falling fruit, but still provide line of sight with the boom of the shaker. Eighteen trees were shaken, 9 screened and 9 open. The quantity of fruit on the tree line was reduced by ~50% when using the screens. The follow-up to this was to attach the shields to the shaker along with the aeration system from the previous season, but Covid-19 restrictions have delayed that portion of the research. Hopefully restrictions will be eased to allow some testing to be done with the machine adaptations.

The 2019 Californian season showed success with the pinwheel pickup. There was essentially no visible dust, but it highlighted that the system worked best with a single layer of fruit. Large volumes of fruit were problematic as the pinwheels rolled over stacks of fruit. The pinwheel was capable of retrieving only the topmost layer of almonds. The UC Merced team has devised a new system to deal with larger volumes of fruit for the upcoming season but recognizing that this has an application for windfall and spill over. PFR has continued with a smaller pinwheel pickup that focuses on leftover fruit as a secondary collection method for orchard floor sanitation. Construction development has been hampered by Covid-19 restrictions. We will continue with tests once we can return to the orchards and manually distribute fruit under the trees to simulate fruit fall.

Project Cooperators and Personnel: Reza Serajian, Joseph Trotochaud, Arash Toudeshki, Hughson Nut (David Pohl), Walker Flat Almonds (Peter Cavallaro) and Century Orchard (Scott McKenzie, Brendan Sidhu)

Quantitative and qualitative impacts of windfall on almond yield and quality

Project Leaders: Patrick Brown[1], Christopher Simmons[2] and Sat Darshan Khalsa[1]
[1]Dept. of Plant Sciences, UC Davis, One Shields Ave., Davis, CA (530) 752-0929, phbrown@ucdavis.edu
[1]Dept. of Plant Sciences, UC Davis, One Shields Ave., Davis, CA (707) 205-7007, sdskhalsa@ucdavis.edu
[2]Dept. of Food Science and Technology, UC Davis, One Shields Ave., Davis, CA (530) 752-2109, cwsimmons@ucdavis.edu

Objectives

- Characterize the quantity of windfall and what environmental, biological and management factors impact windfall
- Assess the quality of the windfallen nuts collected from multiple regions

Background

By 2025, the California almond community commits to reduce dust during almond harvest by 50%. One option is transition to alternative off-ground harvest systems like catch-frames. Even though a transition to catch-frames would reduce harvest passes and subsequently dust, there is a potential unknown loss of marketable yield in the form of windfall that would not be picked up if ground harvest is abandoned. Windfall is the premature fall of almonds before harvest, usually due to variety characteristics or weather conditions but, also as a result of mechanical knocking by machine passes through the orchard. Windfall is relevant and a concern for alternative off-ground harvesting systems that mitigate dust because windfallen nuts may never be harvested. Additionally, windfall is a concern for current harvest systems since windfallen nuts that lie on the orchard floor for extended periods might disproportionately affect crop quality, food quality and food safety of the resultant harvested nuts. Research is therefore needed to 1) quantify the magnitude of windfall in relation to variety, location, and timing and 2) determine the impact of windfall on nut quality and food safety.

The distribution of windfall of almonds across the Central Valley is relatively unknown. Furthermore, the factors that impact windfall of almonds like wind speed, cultivar, temperature, harvest date, and relative humidity have not been quantified. Almond hull split begins in the top of the tree and progresses downward with harvest is generally scheduled at 95% hull split contributing to the potential for the early maturing nuts to fall to the floor prior to tree shaking. It is unknown if 1) windfall nuts are of equivalent maturity and quality as shaken nuts and; 2) if windfallen nuts are subject to a higher degree of damage from insects, moisture, mechanical damage from orchard machinery and contamination from orchard chemicals and/or pathogens.

Discussion

This project seeks to identify quantitative and qualitative impacts of windfall and to evaluate the opportunities and challenges of transitioning to off-ground harvest. The overall question is how to estimate both the quantity and quality of nuts that prematurely fall prior to harvest. In 2019, quantitative windfall assessments were conducted on 30 orchards in all major almond growing regions with data collection of two or more varieties at 20 of the orchards. Preliminary analysis shows windfall from zero to 1% of total yield with the majority of sites showing less than 0.4%. Windfall at 4 or more before weeks harvest showed very poor-quality characteristics. Quality and size of kernels was not compromised at 2 to 4 weeks before harvest. Qualitative and quantitative differences between windfall and nuts at harvest will influence the overall economic impact from the adoption of off ground harvest.

Project Cooperators and Personnel: Ricardo Camargo and Gustave Cirhigiri, Graduate Student Researchers, Dept. of Plant Sciences, UC Davis

Whole Tree ET Responses to Mind and Moderate Water Stress

Project Leader: Ken Shackel
Dept. of Plant Sciences, UC Davis, One Shields Ave., Davis, CA 95616 (530) 752-0928, kashackel@ucdavis.edu

Objectives

The overall objective of this study is to accurately measure almond evapotranspiration (ETc) using a weighing lysimeter (the ETc "gold standard"), and to determine how ETc is influenced by water stress. In order to account for weather conditions, measured ETc is always expressed as a 'crop coefficient' (Kc). The main sub-objectives of this study are to: 1) establish the relation between Kc and canopy shaded area from planting to maturity; 2) estimate the reductions in Kc (and water savings) that are associated with the practice of regulated deficit irrigation (RDI) for hull split and/or harvest; 3) utilize the lysimeter tree and other trees in the experimental plot to test alternative methods of measuring water stress and managing irrigation; and 4) in cooperation with other scientists, use the lysimeter data as the 'ground truth' for other indirect measures of Kc (e.g., remote sensing and micro-meteorological methods)

Background

This research project is based on determining almond tree water use (ETc) using a weighing lysimeter, which is the only direct ('gold standard') method for this measurement. In order to interpret water use values independent of local weather conditions, ETc is expressed as a crop coefficient (Kc) for each crop. The measured midseason Kc in this project has increased each year after planting, as expected based on the annual increase in tree size, but this increase in Kc has been much more rapid than reported in the literature, Effective tree size is measured by canopy shaded area, and the currently accepted midsummer Kc for a "mature" almond orchard (at least 60% shaded area) is about 1.2, which was reached in year 3 of this project (2017), even though the canopy shaded area was only about 40% at that time. Hence, one important result found in this project is either that young orchards may exhibit 'mature' water use much sooner than is normally assumed, or that fully mature almond orchards may use substantially more water than previously thought. Since 2017, shaded area has only increased to about 50%, with no measurable change in Kc, while Nonpareil yield has increased from 770 to 2070 kernel pounds/acre (2017-2019), indicating a substantial increase in 'crop per drop' from year 3 to 5.

Discussion

In 2019, deficit irrigation during hull split and harvest resulted in a substantial reduction in Kc, and a net savings of 4.3" in tree consumptive water use. A reduction in Kc of 0.7 was found to be linearly associated to a 10-bar decline in stem water potential (SWP), remarkably consistent with the same value that was reported by S. Johnson for peach, also using a weighing lysimeter. If these values are reproducible over years, then they will represent a documented savings of water that can be attributed to hull split and harvest deficit irrigation management in almonds. The lysimeter is also being used to 'ground truth' the more widely used, but indirect, micrometeorological (eddy co-variance) method for measuring Kc. 2019 was the first full year for this comparison, and the results indicated that the micrometeorological method may substantially (20%) underestimate Kc from July-August. The reasons for this discrepancy are not clear and are currently under study. The lysimeter tree and an adjacent tree were also used to test a recently developed sensor (microtensiometer) which allows continuous and automated SWP measurement. The sensors clearly showed that midday SWP could change from a value associated with fully irrigated conditions, to a value associated with a substantial level of stress, over a very short period (1-2 days). This was particularly evident when trees were deficit irrigated during hull split and harvest. These results underscore the practical benefits of using automated sensors, or daily pressure chamber SWP measurements, to fine tune the irrigation schedule, particularly under deficit irrigation conditions.

Project Cooperators and Personnel: Bruce Lampinen, UC Davis; Mae Culumber, UCCE - Fresno and Madera Counties; Andrew McElrone, USDA/ARS, UC Davis; Alireza Pourreza, UCCE; Reza Ehsani, UC Merced; Florent Trouillas, UCCE.

Evaluating the Effectiveness of Surface Renewal and other Technologies to Determine Almond Tree Water Use and Stress

Project Leaders: Andrew J. McElrone[1,2] and Nico E. Bambach[2,3]
[1]USDA-ARS, UC Davis, One Shields Ave., Davis, CA 95616; 530-754-9763; ajmcelrone@ucdavis.edu; andrew.mcelrone@usda.gov; [2]Dept. of Viticulture and Enology, UC Davis, One Shields Ave., Davis, CA 95616; nbambach@ucdavis.edu

Objectives
Evaluate the effectiveness of the new stand-alone SR method to accurately quantify almond orchard ET; Compare stress indices from SR with several other methods to determine how effective they are for almonds; Continue efforts to develop IRT based measurements of ET.

Background
Surface Renewal (SR) technology is now patented and being provided to growers as a commercial product, however, it has yet to be evaluated against weighing lysimetry and other stress indicators under field conditions for almonds. We continued to evaluate the following research question: How effective is SR as a cost-effective irrigation management tool that provides growers with information about both the amount and timing of irrigation events for almond orchards? We found that tree water use measured with SR correlates well with other measurements from a weighing lysimeter and eddy covariance methods, but the magnitude of measurements differs. A stress index determined from SR was also well correlated with other stress proxies like stem water potentials. We also successfully developed a new method to measure ET based on canopy temperature measurements.

Discussion
We continued our ongoing efforts with collaborators to validate the new SR technique for measuring almond ET, and compared our estimates with those from weighing lysimetry (with Ken Shackel) and eddy covariance. We evaluated how well SR works when radiation and ground heat flux are measured directly versus modelled. We also tested how effectively the SR technique works for detecting water stress compared to other methods like water potential measurements, leaf gas exchange and crop water stress indicators derived from measurements of canopy temperature (with Infrared Radiometers- IRT).

Our flux footprint analysis and station location and instrumentation was improved in the 2018 season, and we continued measurements to gather a full season of data in 2019. We now have adequate to make a sufficient evaluation of the effectiveness of SR for use in almond orchards. We also evaluated IRT sensors to estimate ET and will continue these efforts based on promising results. We began comparing our results with those of satellite-based ET approaches for all years of the study and established a new tower at Nickel's Ranch (with Tom Buckley) to compare output with sap flow output. We are preparing multiple peer-reviewed manuscripts to be submitted in 2020, which will include comparison with other techniques collected by collaborators.
Growers require information on how much, when, and where to irrigate. ET estimates can provide data on the quantity of water lost via ET from crop surfaces. Based on the results from the new approach presented here, IRT sensors provide an opportunity to determine ET at the single plant resolution, but also estimates of stress based on changes in canopy temperature with adequate resolution to account for infield variability. Such data would also be particularly useful for ground-truthing remotely sensing based estimates of ET from energy balance approaches.

Project Cooperators and Personnel: Ken Shackel UC Davis, Kyaw Tha Paw U UC Davis

Assessment of Almond Water Status Using Inexpensive Thermographic Imagery

Project Leader: Brian Bailey
Dept. of Plant Sciences, UC Davis, One Shields Ave., Davis, CA 95616 (530) 752-7478, bnbailey@ucdavis.edu

Objectives

- Quantify the sensitivity of various crop water stress indices (CWSIs) to environmental conditions and stomatal conductance
- Conduct field experiment to monitor the onset of stress during dry-down events based on measurements of temperature (leaf, canopy, trunk), stomatal conductance, photosynthesis, and leaf/stem water potential
- Evaluate the performance a number of methods based on thermal imaging in measuring the onset of water stress, and ultimately in their ability to inform irrigation decisions

Background

In order for almond growers to meet the future demands for reduced water consumption, the efficiency of irrigation practices must be improved to minimize waste. The traditional means for accomplishing this goal is to use some indication of tree water demand to guide irrigation scheduling. While various sensing techniques do indeed give improved indications of plant water demand than traditional crop coefficients, adoption of such technologies is often slow because of expense, both in terms of money and time. Sensing networks are expensive to maintain and rapidly become dilapidated if significant maintenance resources are not allocated.

Estimation of tree water status through measurement of leaf/canopy temperature has been used for decades, but its performance is still not well understood, and adoption is low due to challenges in applying these methods. Smartphone-based sensing platforms present a potentially feasible yet challenging means for measuring plant water status. Utilizing the computational power of smartphones can reduce the cost to purchase and maintain sensing systems but have the drawback that they only provide measurements at a single point (or limited number of points) in time and space. The overall goal of this project was to determine whether thermal-sensing-based approaches were useful for irrigation scheduling in almond orchards, and specifically evaluate the use of smartphone-based thermal cameras as the temperature sensing platform.

To perform this evaluation, a field experiment was conducted in which a saturating irrigation event was applied, and the water status of the trees were tracked for a long period after water was withheld. Thermal sensing techniques were applied periodically throughout the dry-down period, which were accompanied by several physiological measurements such as stomatal conductance, photosynthesis, and water potential.

Results & Discussion

Before applying the thermal sensing techniques in the field, a theoretical analysis was conducted to study the performance of several crop water stress indices (CWSIs), which are used to normalize leaf/canopy temperature measurements for ambient weather conditions. This study revealed that standard CWSIs are still quite sensitive to weather conditions, and that the CWSI was as sensitive to wind speed as it is to water status. This indicated the potential for severe accuracy issues when CWSIs are calculated in field.

The results of the dry-down experiments in the field provided a number of valuable insights regarding thermal-based water status measurement, and for irrigation scheduling in almond in general. Following the saturating irrigation event, water potentials and photosynthesis declined nearly linearly with time. However, stomata remained unaffected by declining soil water potentials for about 10 days following irrigation. By the time that stomata began to respond to ensuing drought, photosynthesis had already declined to about 30% of its maximum value. This suggested that photosynthesis limitation due to water availability was primarily due to non-stomatal factors. This meant that temperature-based approaches, which are based on the impacts of stomata on leaf temperature, did not respond during this decline in water status. CWSI did not begin to respond to the onset of drought until the trees were very stressed (about 3 weeks after irrigation).

We concluded that the CWSI has relatively low sensitivity to changes in tree water status when applied consecutively in time and is contaminated by a number of errors. Even if the CWSI was perfectly correlated with stomatal conductance, it is unlikely to be useful for scheduling irrigation in almond because photosynthesis was primarily limited by non-stomatal factors.

Project Cooperators and Personnel:
Magalie Poirier-Pocovi, Bruce Lampinen, Astrid Volder (UC Davis)

Variable Rate Irrigation Practices on Almond

Project Leaders: Khaled Bali[1] and Catherine Culumber[2]
[1]UCANR Kearney Agricultural Research & Extension Center, 9240 S. Riverbend Ave., Parlier, CA 93648. (559) 646.6541, kmbali@ucanr.edu
[2] UCCE - Fresno County, 550 E. Shaw Ave. Ste 210-B, Fresno, CA 93710, (559) 241-7515, cmculumber@ucanr.edu

Objectives

- Develop irrigation decision support system to determine irrigation requirements based on site specific conditions related to climatic conditions, soil type, growth stage, soil salinity, water quality, and other site-specific factors to improve water use efficiency.
- Evaluate the impact of variable rate irrigation on crop yield, water use efficiency, fertilizer use efficiency, reduction in energy use and reductions in greenhouse gas emissions and document the economic benefits of variable rate irrigation on almond.

Background

Almond growers in California are under continuous pressure to grow orchards with limited water supplies. Sustainable almond production in California requires careful management of limited water supplies and smart irrigation practices to stretch the available water and minimize the environmental issues associated with over-irrigation. New technologies related to variable irrigation and water status technologies such as Tule, in addition to other soil and plant-based methods could be used to conserve water and improve efficiency. A primary goal of this program is to determine an orchard's irrigation needs more precisely and then have irrigation schedules to meet crop water requirements, thereby reducing water and nitrogen losses, and energy use.

We retrofitted the irrigation system in a commercial almond field in Tulare county with a variable rate irrigation (VRI) system that allows the grower to manage irrigation zones as small as one acre. The variable rate irrigation practices were implemented in the 2019 growing season at the one-acre level and in the 2020 growing season on selected trees in some of the 1-acre zones.

Discussion

A 70 acre, 10-year old commercial almond orchard near Hanford was selected and 36 one-acre variable irrigation (VRI) zones were implemented on approximately 50% of the field and the other 50% were used as control. Netafim installed the variable rate irrigation system in 2018 and the 2018 growing season was used as a baseline for yield and other variables. The VRI practices were implemented with the start of the 2019 growing season using a VRI schedule on each of the 36 zones. The schedule was based on canopy coverage and NDVI index. The weekly irrigation schedule was based on current reference evapotranspiration, crop coefficient, and field measurements such as actual evapotranspiration from Tule stations, soil moisture sensors, and stem water potential from selected management zones. We implemented six different irrigation management strategies (A, B, C, D, E, and F) and each of the 36 zones received the required irrigation amount based on one of the management strategies. In the 2020 season the VRI practices were implemented on just six trees in each of the 36 zones. We determined soil moisture content, canopy light interception as midday canopy photosynthetically active radiation (PAR), NDVI, applied water, soil moisture content, and yield for each zone during the growing season. We had drone flights to determine tree size, height, and percent shaded area, and NDVI. Yield was determined from selected rows in all VRI plots and control in August 2018 and 2019 and will be determined again in August 2020.

Project Cooperators and Personnel:
Ashley Correia (Almond Board of California), Todd Rinkenberger (Netafim), James Nichols (Nichols Farms), Daniele Zaccaria and Bruce Lampinen (UCD), Alireza Pourreza (KARE), Dan Munk (UCCE-Fresno County), Blake Sanden (UCCE-Kern County), David Doll (UCCE- Merced County), Allan Fulton (UCCE-Tehama County)

Data-Driven Physiological Modeling of Canopy Photosynthesis for Precision Irrigation Management

Project Leaders: Tom Buckley[1]
[1]Dept. of Plant Sciences, UC Davis, One Shields Ave., Davis, CA 95616 (530) 752-0320, tnbuckley@ucdavis.edu

Objectives

- (1) Parameterize a canopy photosynthesis model in Nonpareil and Aldrich and drive it with continuous measurements of canopy conductance from sap flow.
- (2) To help scale the model from leaf to orchard level, quantify variation in PAR within tree crowns, and in intrinsic water use efficiency (iWUE, the ratio of photosynthesis to stomatal conductance) among trees.
- (3) Determine how changes in irrigation affect modeled canopy photosynthesis and measured SWP as environmental conditions vary across a growing season.
- (4) To allow canopy photosynthesis to be modeled without sap flow data, parameterize, and test a physiological model for canopy conductance in Nonpareil and Aldrich.
- (5) To determine guidelines for more effective irrigation scheduling, use the model to simulate the impact of a range of potential irrigation strategies.

Background

Irrigation scheduling in almond is usually based on target ET estimated from meteorological data (ETo) or midday stem water potential (SWP). Though practical and straightforward, these measures are distantly related to the purpose of irrigation, which is to enable trees to keep their stomata open to allow photosynthesis -- which provides all the energy and carbon that underpins yield. This project aims to understand how irrigation and climate (especially ETo and temperature) affect canopy photosynthesis, and to use that understanding to compare alternative irrigation strategies and identify optimal strategies, using physiological modeling.

We are using sap flow to obtain a continuous estimate of canopy conductance (stomatal opening), and using that to drive a physiological model of canopy photosynthesis and to parameterize a physiological model of canopy conductance. We will use the combined model to simulate alternative irrigation strategies, with the ultimate objective of identifying a strategy that makes the best possible use of limited water while also being practical for growers to apply.

In 2017-2018, we developed a new sap flow method and tested it at an orchard near Arbuckle. In 2019, we experimentally withheld irrigation for two 7-day periods in half of our instrumented trees, and measured leaf stomatal conductance, photosynthetic parameters and pre-dawn and mid-day leaf and stem water potentials, before and after each artificial drought. We are now (early October) processing these data to apply them to our canopy photosynthesis model.

Discussion

We found that (1) photosynthetic biochemistry was unaffected by soil drought sufficient to cause nearly total closure of leaf stomata; (2) variations in VPD, but not temperature, mildly decoupled photosynthesis from transpiration; (3) in this orchard, photosynthesis was nowhere near saturated with respect to water loss, such that (3a) yield decline under stress was proportional to the reduction in canopy photosynthesis caused by stomatal closure, and (3b) optimal redistribution of irrigation over the season would have little impact on total carbon gain; and (4) even mild water stress such as associated with 20% reduction in irrigation prior to harvest may cause substantial decline in water transport (hydraulic conductance), which has the potential to suppress photosynthesis even after resumption of full irrigation.

We conclude that irrigating to ETc during the main growing season is fairly efficient in terms of carbon gain, but that withholding water around harvest may substantially harm tree health and reduce current-year and future yield.

Project Cooperators and Personnel: The project is being carried out by the Buckley Lab (Tom Buckley & PhD students Heather Vice and Marshall Pierce)

Advanced Irrigation Management of Young and Mature Almond Orchards

Project Leader: Isaya Kisekka
Dept. of Land, Air, and Water Resources[1], UC Davis, One Shields Ave., Davis, CA 95616 ikisekka@ucdavis.edu
Dept. of Biological and Agricultural Engineering[2], UC Davis, One Shields Ave., Davis, CA 95616

Objectives

1. Establish an irrigation scheduling regime for implementing regulated deficit irrigation (RDI) during variety-specific hull-split using a remote valve control system and crop evapotranspiration (ET_c) estimates.
2. Evaluate RDI in Nonpareil, Butte, and Aldrich in the period from hull-split initiation to harvest and quantify effects on yield, nut quality, and the amount of water used to produce a pound of almonds.
3. Quantify crop water use (ET) of young almond orchards and determine crop coefficients (K_c).

Background

This project aims to understand the importance of site-specific factors, including tree variety and tree age, in achieving sustainable irrigation management. We are conducting this project in two independent field studies, the first one in Arbuckle, CA to study the effect of tree variety, and the second one in Corning, CA to study the effect of tree age on irrigation management.

At the Arbuckle site, we are investigating how to implement regulated deficit irrigation (RDI) during variety-specific hull-split using a drip irrigation system with remote valve control. Almond orchards typically have two or three different varieties within the same orchard to establish effective cross-pollination. Each almond tree variety experiences hull-split at different times in the year. RDI during hull-split can reduce water use while minimally affecting yield and possibly reducing hull rot. Without an irrigation system that allows a farmer to independently irrigate the varieties, a farmer would likely implement RDI in all varieties during the hull-split period of whichever variety profits the most.

At the Corning site, we are quantifying crop water use in young almond trees. An increase in new almond orchards has motivated us to determine crop water use and crop coefficients in young almond orchards (ages 1-5 years) using a variety of ET modeling and measurement methods.

Discussion

This project addresses the following research questions:

1. Does RDI during variety-specific hull-split improve production in terms of yield, quality, water use efficiency, and profitability?
2. How can RDI during variety-specific hull-split periods be practically implemented on a commercial orchard and how much would a retrofit cost on a per acre basis?
3. How much water do young almond orchards use and what are the crop coefficients (K_c) growers should be using for different ages of young almond orchards (e.g., 1st, 2nd, 3rd, 4th, and 5th leaf)?

During the 2020 season, we will conduct the second year of the study implementing RDI during variety-specific hull-split periods in a 4-acre plot in Arbuckle, CA. We will implement 50% ET_c and 75% ET_c treatments from hull-split initiation to harvest in all varieties in two different ways, (1) implementing RDI in all varieties according to Nonpareil hull-split initiation and harvest dates to simulate an orchard in which Nonpareil growth stages drive irrigation scheduling, and (2) implementing RDI according to variety specific hull-split initiation and harvest dates to simulate an orchard with the ability to irrigate by variety. At the end of the 2020 season, we will determine the effects of two consecutive years of RDI during variety-specific hull-split on yield, quality, and the amount of water used to produce a pound of almonds in each variety.
At the Corning site, we will quantify the water consumption and crop coefficients of young almond orchards in 1st, 2nd, 3rd, 4th, and 5th leaf almond orchards using eddy covariance (energy-budget method), surface renewal, soil water balance, and a two-source energy balance modeling scheme. We will determine how the different ET measurement and modeling methods compare in estimating crop water use in young almond trees. During the 2020 season, we will focus on quantifying crop water use and crop coefficients in the 3rd, 4th, and 5th leaf almond trees

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Project Cooperators and Personnel: Kelley Drechsler, UC Davis; Franz Niederholzer, UCCE – Colusa County; Allan Fulton, UCCE – Tehama County; Andre Daccache, UC Davis

Managing for Soil Health: Targets and Potential in Almond Orchards

Project leader: Amélie Gaudin[1]
[1]Department of Plant Sciences; UC Davis; 1 Shields Ave., Davis, CA 95616; (530) 752-1212 agaudin@ucdavis.edu

Objectives

- Investigate relationships between soil health and sustainability metrics
- Explore relationships between established metrics of soil health and novel biological indicators of soil health and function
- Develop benchmark values of physical, chemical, and biological indicators of soil health for the study region
- Create outreach materials for almond growers

Background

There is an increased focus on soil health-building practices to improve the overall sustainability and resilience of crop production whilst maintaining productivity. The functions provided by a healthy, living soil could also address almond-specific management challenges such as salinity, water use inefficiencies, poor infiltration, and soil-borne pests and diseases. However, much of the current research on the role of soil health building practices remains focused on annual cropping systems, leading to a knowledge gap about the functional potential of soil health in almond orchards. It is becoming increasingly important that we define the functional potential of soil health building practices because improvements in soil functions supports the ecosystem services that drive sustainability, resilience, and productivity outcomes.

We are combining multiple statistical approaches to understand the relationships between soil health metrics and soil functions of particular importance to almond growers as well as for overall agroecosystem sustainability and productivity. A comprehensive framework was developed to define soil health's potential in almond agroecosystems and identifies the seven key functions being used to evaluate the functional potential of a variety of management approaches for building soil health. These functions include 1) building and storing soil carbon, 2) regulating nitrogen cycling, 3) conserving and cycling water, 4) supporting provisioning, 5) improving soil structure, 6) improving resource use, and 7) supporting diverse & active microbial communities.

Discussion

During a May 2019 field season 13 almond orchards were sampled that represent a gradient of strategies for building soil health – from continuously grazed vegetative understories (*not a recommended practice by ABC for food safety reasons*) and winter cover crops to soil managed to maintain clean floors. Restricting the geographic study region (Yolo Co.) minimizes the variations in climate and soil type and let us create a management gradient using an ecological survey approach. Initial analyses suggest we were able to capture variation in soil health and soil functions between orchards. Principle component analyses were conducted as the first step and allowed us to explore relationships between measured indicators of soil health.

Our initial results suggest that applying organic amendments (i.e., compost, almond shells) alone did not significantly improve soil functions. Winter and continuous cover crops were instrumental in positively shifting soil physical properties such as wet aggregate stability and available water capacity. The living roots maintained in continuous cover crop systems were particularly effective at positively shifting these soil physical properties compared to just winter cover crops. Finally, orchards that integrated livestock for grazing of either winter cover crops or continuous pastures improved pools of soil carbon and nitrogen, soil micronutrients, and microbial activity. The trends exhibited in the initial analyses suggest that stacking soil health building practices have the greatest potential to achieve soil health targets necessary for addressing management challenges and production goals in almond agroecosystems. We are continuing with more sampling and will integrate production metrics (yields, inputs…) so explore linkages between soil health and sustainability.

Project Cooperators and Personnel: Katherine Jarvis-Shean (UCCE); Amanda Hodson (UCD); Jorge Rodriguez (UCD); Tim Bowles (UCB); Krista Marshall (PhD student)

Using SWP to Delay the Start of Irrigation in the Spring

Project Leader: Ken Shackel
Dept. of Plant Sciences, UC Davis, One Shields Ave., Davis, CA 95616 (530) 752-0928, kashackel@ucdavis.edu

Objectives

The objective for 2019 was to collect data on grower irrigation management, SWP, PAR, and yield at north (Tehama) and mid-south (Modesto) commercial almond sites. These values will be used as pre-treatment reference values for the irrigation delay treatments to be applied in 2020.

Background

It is widely believed that irrigation should be sufficient to match orchard evapotranspiration (ET) demand throughout the season, and further, that early season irrigation (i.e., starting soon after leaf out) will maintain a 'bank account' of deep soil water to insure against excessive water stress at harvest. Recent results in walnut (a crop documented to be highly sensitive to water stress) have demonstrated that delaying the start of irrigation in the spring, based on SWP, can substantially reduce overall seasonal water application (10 – 20 inches, depending on the year), with no long term effect on crop yield and a general improvement in tree appearance. These delays, which varied from 1 to 2 months after the grower normally started irrigation, were also associated with small (5-10%) but measurable increases in crop load and nut quality (edible yield). Soil measurements indicated that these trees used stored soil moisture to compensate for the irrigation deficit. However, counter to expectations, this use of soil moisture did not result in water stress at harvest, and in fact, trees in the delayed treatments were *less* stressed at harvest compared to control trees. In almond, Prichard (1990 – 1993 study) reported that a plant-based deficit irrigation regime gave higher yields (93% of control yield with 66% applied water) than an ET-based deficit irrigation applying the same water (77% yield with 66% water), and it has become widely recognized that SWP is valuable for deficit irrigation approaches. However, this recent data in walnut may also be the first example of standard ET-based irrigation scheduling resulting in over-irrigation.

Discussion

Three almond orchards (two in Tehama and one in Modesto) were identified and monitored in 2019, prior to imposing any delayed irrigation treatments. Initial measurements indicate that each site is relatively uniform in terms of tree water status (SWP), but there are clear SWP differences between sites as a result of soil types and irrigation practices. All orchards showed near baseline (fully irrigated) SWP levels through April, indicating a good possibility that the start of irrigation could be delayed compared to current grower practice at all sites. In both Tehama and Modesto, there was also a trend for higher yields to be associated with somewhat higher levels of water stress. The contrasting sites in Tehama Co. are of particular interest as they are on contrasting soils, both are 50% Nonpareil, but one site (field #28) is on a class 1 Columbia silt-loam soil, and the other (field #11) is on a class 2/3 loam/gravely loam soil. The grower closely matched ET with irrigation over the season on the shallow soil (field 11), whereas ET was not matched in the deep soil at any time (Field 28). Field 11 trees experienced substantial stress (-20 bars) when applied irrigation did not match the calculated ET, but Field 28 trees were able to tolerate a substantial period of no irrigation without reaching this level. Field #28 trees are high vigor and it is clear that soil water is readily available because the trees show minimal water stress (SWP), despite being irrigated at about 50% ET. On the other hand, field #11 trees are less vigorous and show mild to moderate water stress, even though they are irrigated at 100% ET. Interestingly, 2019 yields were lower in field #28 (1760 lbs/ac) than field #11 (2840 lbs/ac), possibly suggesting that the vigor in field #28 may be 'excessive.' These are preliminary background monitoring results, with irrigation delay treatments to be applied in 2020.

Project Cooperators and Personnel: Bruce Lampinen, UC Davis; Roger Duncan, UCCE, Allan Fulton (/Luke Milliron), UCCE

Updating information on evapotranspiration (ET) and crop coefficients (Kc) of micro-irrigated almond production orchards grown in California for use in water resource management and irrigation scheduling decisions

Project Leaders: Richard L Snyder[1] and Daniele Zaccaria[1]
[1]Department of Land, Air and Water Resources, UC Davis, One Shields Ave., Davis, CA 95616
R. Snyder: (530) 752-4628, rlsnyder@ucdavis.edu; D. Zaccaria: (530) 219-7502, dzaccaria@ucdavis.edu.

Objectives

- This project started on 1 May 2020, so we are at the beginning of this effort to update information on almond evapotranspiration and crop coefficients. This report will discuss the background for this effort and what we hope to accomplish during this first year of the project.

Accuracy of water demand estimations has become critically important for climate-adaptive water resource planning, for securing reliable water supply, managing water allocations and deliveries, and for on-farm irrigation scheduling. Efficient water management requires accurate data on evapotranspiration, i.e. orchard water loss to the atmosphere.

Well-watered crop evapotranspiration (ETc) is estimated as the product of standardized reference evapotranspiration (ETo) and a crop coefficient (Kc), using the well-known formula ETc = ETo x Kc. In recent years, significant advancements have made accurate historical, near-real-time, and forecast ETo data readily available, but crop coefficient (Kc) information needs updating to effectively use ETo data for water resource planning and irrigation management, because the ETo estimation methodology, as well as irrigation methods and crop management practices have significantly changed during the last decades. For example, the broad adoption and use of frequent micro-irrigation rather than infrequent surface irrigation has affected water management.

In recent years, researchers from the University of California Cooperative Extension (UCCE) and scientists from the California Department of Water Resources (DWR) and Land IQ have attempted to address the existing knowledge gaps by conducting field studies on water use of almond orchards using energy balance data. The research teams measured crop ETc from well-watered orchards grown with micro-irrigation in the main almond production areas of California. The ETc was determined by measuring net radiation, ground heat flux, and sensible heat flux and calculating latent heat flux as the residual of the energy balance. Converting the latent heat flux in energy units to mass of water vapor flux units gives an estimate of the ETc.

Unfortunately, findings from these research studies have not been published to date for a variety of reasons. This has prevented almond growers and water managers from using updated ET and Kc information to further improve on-farm irrigation and water resources planning. The main objective of this project is to standardize the analysis of these field studies to attain comparable ETc and Kc data and to evaluate data both on a regional and statewide scale to address micro-climate impacts on Kc values.

Discussion

We currently have 14 seasons of ETc data collected over mature, well-water almond orchards ranging from Tehama to Kern County. During this coming year, because the studies were done by independent groups, we will develop a standardized method to quality control data and determine orchard ETc values that are comparable. Using CIMIS and weather data from each site, we will analyze for crop coefficients trends at each of the research sites. Time permitting, we will investigate the possibility to correct Kc values for micro-climate effects to see if we can derive a universal method to estimate local Kc patterns and trends for all production areas.

Project Cooperators and Personnel: Allan Fulton and Blake Sanden, UCCE Farm Advisor-Emeritus; Dan Munk, Mae Culumber, UCCE Farm Advisor; Khaled M. Bali and Bruce Lampinen UCCE Specialist; Morteza Orang and Steve Ewert, California Department of Water Resources; Doug Parker, California Institute of Water Resources.

Almond Orchard Recycling

Project Leader: Brent A. Holtz
University of California Cooperative Extension in San Joaquin County, 2101 East Earhart Ave, Suite 200, Stockton, CA 95206 (209) 953-6124 baholtz@ucdavis.edu

Objectives

- The overall goal of this project is to comprehensively assess the implications of whole orchard recycling (WOR) with the standard practices of orchard removal, either burning for energy co-generation or open field.
- Quantify the effect of WOR on physical, chemical, and biological soil properties.
- Assess the impact of WOR on replanted tree growth, nutrition, yield, and water and nitrogen efficiency.

Background

WOR, as an alternative to co-generation or open field burning, could reduce net orchard greenhouse gas emissions by sequestering carbon from orchard biomass. The wood residue generated by WOR, estimated to be 45-70 tons per acre, appears to increase soil carbon, organic matter, fertility, water infiltration rates and water retention. The first orchard grinding trial at Kearney, established in 2008, compared WOR of stone fruit trees to burning and incorporating the ash. The orchard was replanted to almond and greater yields, significantly more soil nutrients, organic matter, and organic carbon were observed in the grind treatment when compared to the burn. Cumulative yield shows a 2,000 pound per acre increase in kernel weight when the grind treatment is compared to the burn. Tree circumference was significantly greater in the grind treatment when compared to the burn. Leaf petiole analysis also revealed higher nutrients levels in trees growing in the grind treatment. A deficit irrigation trial documented increased water use efficiency and stomatal conductance with WOR (2020 PLOS One Jahanzad et al.) In WOR trials established since 2015, we have observed increased soil organic carbon, total nitrogen, and gravimetric water content with WOR, while observing decreased soil compaction and bulk densities. We observed that large soil macro-aggregates accumulated more carbon in the WOR soils, demonstrating that woodchips have the potential to store and physically protect carbon from microbial breakdown.

Discussion

Initial data from our earlier WOR trials suggested that our nitrogen (N) recommendation for first year almond trees was too low. We initiated a series of nutritional studies from 2017-2019 and observed that the timing of nitrogen application is critical following WOR. From these studies we would recommend to growers that they apply at least 5-8 ounces of actual N per tree (45-75 lbs N/acre). And that early applications, starting at planting time, are more important than applications later in the season. N applications should be spread out so that no more than 1 oz of actual N is applied per tree per application in the 1st year of tree growth, in order to prevent N burn.

In three trials the effects of WOR and preplant fumigation are being tested. Preplant soil fumigation significantly stimulated tree growth, with or without WOR, indicating that WOR did not interfere with fumigation. We have observed no impact of WOR on the incidence of crown rot, root rot, or canker disease. A new experimental orchard was established at Kearney to examine both nitrogen and irrigation efficiency after WOR. We hypothesize that increased nitrogen and irrigation efficiency will be observed as wood chips bind and hold both applied N and moisture, ultimately releasing both in the rootzone. We estimate almond growers have implemented WOR on over 40,000 acres since 2015 (personal estimate in discussion with growers).

Project Cooperators and Personnel: Mae Culumber, UCCE - Fresno, Greg Browne, Suduan Gao, Amisha Poret-Peterson, USDA-ARS, Cameron Zuber, UCCE - Merced, Amélie CM Gaudin, Emad Jahanzad, UC Davis, Mohammad Yaghmour, UCCE - Kern, Franz Niederholzer, UCCE - Colusa, Sutter, Yuba, Phoebe Gordon, UCCE – Madera.

Effect of Partial Fertilizer Substitution with Organic Matter Amendments on Nutrient Cycling

Project Leaders: Sat Darshan S. Khalsa[1] and Patrick Brown[2]
Department of Plant Sciences, University of California, Davis, One Shields Ave., Davis, CA 95616
(707) 205-7007, [1]sdskhalsa@ucdavis.edu [2]phbrown@ucdavis.edu;

Objectives

- To verify the efficacy of composted manure and green waste compost as a partial substitute to fertilizer
- To determine of how increases in soil organic carbon (SOC) and total nitrogen (TN) impact nitrogen (N) availability
- To contrast OMA *supplementation* (OMA plus standard N fertilizer) and *substitution* (OMA plus reduced N fertilizer)
- To test the impact of composted manure on soil moisture at different depths

Background

Organic matter amendments (OMA) may offer a viable option to supplement or partially substitute synthetic fertilizers. We examined the effects of OMA sources compared to an unamended control along with supplementation versus substitution N fertilizer treatments. We applied 7 tons per acre of composted manure and green waste compost in fall 2018 and maintained an unamended control as main plot treatments. The same OMA plots received 7 tons per acre each year during the fall of 2016 and 2017. During the spring of 2019, these main plot treatments were divided into split plot treatments and followed with the recommended standard N fertilizer rate for the control for both OMA and unamended plots (OMA supplementation) compared to a 20% reduced N fertilizer application rate (OMA substitution).

Orchard measurements in in this study include effects on soil phosphorus (P) and exchangeable potassium (K), soil inorganic N availability, soil moisture, leaf nutrient status in July for N, P and K, and almond yield. Multiple results offer evidence that OMA use may act as a partial substitute for fertilizer while at the same time provided associated co-benefits of increasing soil organic carbon (SOC), total nitrogen (TN), soil P and exchangeable K. OMA use also continues to show a measurable impact on soil moisture at different depth within the tree profile. There was been no measurable effect on tree size measured by trunk circumference collected in January each year.

Discussion

During postharvest 2018, soil organic carbon (SOC) was greater for both the OMA sources compared to the control. The same trend was apparent for total nitrogen (TN). This build-up in SOC and TN in 2018 provided the basis for soil organic matter to supply plant essential N, P and K during the subsequent 2019 growing season. The increase in soil organic matter measured during postharvest 2018 was the results of OMA applications in 2016 and 2017.

During postharvest 2018, Olsen-P from composted manure was greater than both green waste compost and the control. OMA sources were greater in soil exchangeable K compared to the control during postharvest 2018. Increased P and K from OMA use provide an added co-benefit to OMA use in almond orchards. Fertilizer K can be one of the most expensive input investments for growers and the K inputs for different OMA sources offers an important benefit to OMA use.

In 2018, N availability from OMA continued to increase as evidenced by higher leaf N concentrations. In 2019, N availability did not different between supplementation and substitution treatments. This result affirms the build-up in TN from OMA use acts as a partial substitute to N fertilizer due to the subsequent release of N during the growing season.

Soil moisture measured using soil moisture sensors by depth was higher in the composted manure (CM) compared to the control during summer 2019 at 15 cm depth. However, VWC at 30 and 60 cm depth was higher for the unamended control. This result suggests irrigation water is retained in the upper soil layer from OMA use, but may more easily pass through the profile when soils are unamended. In 2019, 6th leaf almond yields did not differ at 1,848 lb per acre for the control and 1,937 lb per acre for the compost treatments. Yields also did not differ between supplementation and substitution treatments, which suggests OMA use acted as a viable partial nutrient source to fertilizer.

Project Cooperators and Personnel: Daniel Rivers and Brent Holtz, UCCE - San Joaquin; Jeffery A. McGarvey, USDA-ARS

Management and Benefits of Cover Crops in Almond Orchards

Project Leaders: Amélie Gaudin[1], Jeffrey Mitchell[2], Andreas Westphal[3]
[1]Department of Plant Sciences; UC Davis; One Shields Ave., Davis, CA 95616; (530) 752-1212; agaudin@ucdavis.edu;
[2]KARE; 9240 S. Riverbend Ave., Parlier, CA 93648; (559) 646-6564; jpmitchell@ucdavis.edu;
[3]Department of Nematology; University of California Riverside at KARE; (559) 646-6555; andreas.westphal@ucr.edu

Objectives

- Compile knowledge on the use, management, and barriers for adoption of cover crops in irrigated almond orchards.
- Quantify the benefits and tradeoffs of winter cover crops and resident vegetation compared to fallow on productivity and soil health, water balance, and weed pressure in three different geographical regions differing in average annual rainfall
- Determine the host status of various cover crops species and mixes to key plant-parasitic nematodes.

Background

Planted or natural vegetation cover crop during the fall/winter impact various aspects of soil quality: regulating N, water, and C dynamics which may be of benefit to the sustainability and resilience of the almond industry. Previous projects and farmers' experience show that this practice is compatible with large scale almond production and California growers recognize the theoretical benefits that might come from implementing cover cropping. However, we lack concrete information to guide cost-benefit analyses of implementing cover crops and to address concerns about potential increase in water usage. We are now finishing our third field season and aim to monitor benefits and trade-offs associated with two different winter cover crop mixes in terms of soil health, pest suppression and pollination across different rainfall zones.

Discussion

We have finalized our grower survey (~110 growers, both adopters and non-adopters) to better understand barriers to adoption of cover crops and data needs. The survey indicates that growers expect that cover cropping will provide multiple benefits, in addition to improvements in tree nutrition. The value of cover crop benefits is already recognized by most growers, with soil health and pollinator habitat being of highest importance.

We have conducted our final cover crop trials growing season (year 3) at our replicated trials across the Sacramento and San Joaquin Valleys with either (T1, T2) two winter planted cover crops (pollinator mix and soil mix), (T3) winter resident vegetation, and (T4) bare ground across four rainfall regions. We obtained excellent cover crop growth at all sites and are collecting data regarding soil health outcomes, weed suppression, cover crop phenology, support to pollinators, as well as interactions with winter sanitation (tackled in separate project at our sites). The first two years show that cover crops likely do not reduce almond yields and do not compete for water with the almond trees, have a minimal impact on water dynamics and no extra irrigation needs. Cover crops can rapidly improve aggregate stability compared to bare soils. The Brassica dominated mix has a large weed suppression potential. We have continued our field studies in nematode infected orchards, building on greenhouse studies, to evaluate parasitic nematode host status or suppression capability of various cover crop species and mixes, including the ones used in our trial.

Our results will help guide the design of locally adapted and practical orchard floor management strategies that do not interfere with imperatives of intensive almond production while maximizing benefits obtained from cover crops. This project will also assist the development of integrated pest and water/fertility management strategies necessary to meet the future needs of the California almond industry. We are planning to hold a series of roundtables to start developing best management practices guide. We have leveraged this project for Federal grant funding and our data has also been used to develop two manuscripts by another team related to costs-benefits and water footprint of this practice in almond and tomato systems.

Project Cooperators and Personnel: UCCE: M.Yaghmour, Cameron A Zuber; Project Apis M: B.Synk; UCD: A.Hodson, B.Hanson, and their respective personnel & PhD students: C.Creze (Gaudin/Horwath), S.Haring (Hanson); Growers: Sandridge (Bakersfield), Castle Farm (Merced), Bosque Verde (Corning)

Water12

Almond Variety Development

Project Leader: Thomas M. Gradziel
Dept. of Plant Sciences, UC Davis, One Shields Ave., Davis, CA 95616 tmgradziel@ucdavis.edu

Objectives

Develop and test a series of new California adapted varieties solving current and emerging needs, including self-fruitfulness, improved water-use efficiency, improved disease and pest resistance and adaptation to changing climates.

Background

While almond represents a diverse and highly adaptable species, most commercial production is dependent on the variety Nonpareil and its pollinizers, most of which have Nonpareil and Mission as direct parents. Consequently, the UCD Almond Variety Development program is pursuing the incorporation of new and diverse germplasm to provide new genetic solutions to current and emerging production challenges.

Discussion

Near optimal crossing conditions were experienced in spring, 2020 allowing over 100,000 crosses to be made among approximately 20 different parents. Very good seed-sets were achieved with initial seed harvesting anticipated by early August. Over 8000 seed from 2019 controlled hybridizations have been stratified and planted and are now entering the 1st stages of greenhouse preselection utilizing morphological and molecular screening tools. Both breeding parents and progeny are being selected towards high-quality Nonpareil-type kernels with consistent, high productivity facilitated by self-fruitfulness and improved water-use efficiency and resistance to important diseases and pests.

Self-fruitfulness is being achieved through selection for pollen self-compatibility and flower architectures ensuring a high degree of self-pollination with or without insect pollinators. Selection is through field screening followed by molecular marker validation. Additional selection targets tree architectures compatible with increasingly higher density orchard systems with reduced agrochemical inputs.

Advanced selections for these criteria are now in multiple Regional Variety Trials in the Sacramento and San Joaquin valleys. Self-fruitful selections such as UCD8-160 and UCD8-27 provide good overlap with the early Nonpareil bloom and so are effective cross-pollinizers for Nonpareil while their self-fruitfulness ensures high seed-set and productivity when they are the 1st to bloom in the orchard (i.e. no other cross-pollinizers available to them). Other advanced selections such as UCD18-20 and UCD1-271 have been selected for good bloom overlap with the early to late Nonpareil bloom while the final set of selections, including the recently released variety Kester and the self-fruitful selections UCD 1-232 and UCD 8-201, were selected for later bloom in order to better avoid damage from frosts and rain during the traditional bloom time.

The breeding program is currently evaluating over 22,000 bearing-trees for productivity and kernel quality, including post-harvest storage quality. The desirable blonde kernel-color of Nonpareil, Sonora, and advanced selections UCD 1-6 and UCD 1-16 have been found to be associated with longer post-harvest storage capacity (probably associated with lower tissue oxidation). Heritability studies have shown this market-preferred trait to be complex and we are currently developing molecular markers to improve future selection efficiency.

To fast-track regional testing of more promising advanced selections, an accelerated evaluation plot at the Nickels Research Station in Colusa County and a high-density test orchard in Kern County are being developed for planting in 2020/2. This will enable evaluation of an additional 30 promising 2nd-generation selections in addition to the 22 advanced selections currently being evaluated in current RVT and grower plots.

Project Cooperators: S. Marchand, B. Lampinen, UCCE-Davis; J. Adaskaveg, UCR; S. Metcalf, UCD; F. Niederholzer, UCCE-Colusa; R. Duncan, UCCE-Stanislaus; P. Gordon, L.K. Milliron, UCCE-Butte, M. Yaghmour UCCE-Kern and G. Brar, CSU-Fr.

Field Evaluation of Almond Varieties

Project Leader: Bruce Lampinen
Department of Plant Sciences, University of California, Davis, One Shields Ave., Davis, CA 95616
(530) 752-2588, bdlampinen@ucdavis.edu

Objectives

To evaluate the characteristics of the most promising almond varieties developed in the almond breeding programs at UC Davis, the USDA Agricultural Research Service as well as commercial introductions in an orchard setting.

- Continue collecting data on bloom, hullsplit, light interception, yield and quality in 2014 Regional Variety Trials (planted in the winter of 2014).

Background and Discussion

Regional almond variety trials provide both almond growers and researchers with a valuable information resource.

2019 was the fourth year for complete data collection at the next generation almond variety trials that were planted in the spring of 2014 in Butte, Stanislaus and Madera Counties. The Butte, Stanislaus and Madera trials were planted on Krymsk86, Nemaguard and Hansen 536 rootstocks, respectively. In the current generation trials, there are four replications of each of 30 pollinizers – an experimental improvement initiated with the completed McFarland trial. Nonpareil is planted in every other row in all three trials. Trees are planted at a density of 110, 130 and 173 trees per acre in the Butte, Stanislaus and Madera trials respectively. Fourteen of the pollinizer test varieties in the trials from the University of California, USDA Agricultural Research Service, and commercial nurseries are self-compatible. In 2015, missing trees were replanted.

In 2019, bloom and hullsplit data were collected at all 3 replicated trials. Light interception data was collected at the trials as well using the mobile platform lightbar. In addition, the trials were harvested for the fourth time in 2019. Yields at the Butte trial averaged from 870 to 3002 kernel pounds per acre in the fifth leaf. At the Stanislaus trial yield ranged from 964 to 2630 kernel pounds per acre in the fifth leaf. The yields at the Madera trial ranged from 462 to 3521 kernel pounds per acre.

Since the point of the variety trial is to identify varieties with issues that make them unsuitable for commercial production due to problems, in this early stage we are focusing on determining these factors that might make a variety or selection unsuitable for commercial production. So far, we have only eliminated selections that have been discarded by the nursery that developed it.

We are also working to evaluate yield and production efficiency by using data from the mobile platform lightbar that measures light intercepted by the tree canopies. We are also working with Ali Pourreza in Bio and Ag Engineering and Brian Bailey in Plant Sciences at UC Davis to further refine these relationships.

Project Cooperators and Personnel: Phoebe Gordon, UCCE- Madera County, Luke Milliron, UCCE-Butte/Glenn/Tehama Counties; Roger Duncan, UCCE - Stanislaus County; Tom Gradziel, Sam Metcalf, Ali Pourreza and Brian Bailey, UC Davis; Craig Ledbetter, USDA/ARS, SJVASC, Parlier

Field Evaluation of Almond Rootstocks

Project Leader: Roger Duncan
University of California Cooperative Extension, Stanislaus County, 3800 Cornucopia Way #A, Modesto, CA 95358
(209) 525-6800, raduncan@ucdavis.edu

Objectives

This project encompasses several long-term trials located in Butte, Yolo, Stanislaus and Fresno Counties. Each field trial separately evaluates the field performance of numerous rootstocks compared to long-time local standards. Each trial location presents different production challenges and includes the following objectives:

- Evaluation of alternative rootstocks irrigated with low quality (saline) irrigation water in alkaline, heavy soil (Stanislaus County).
- Evaluation of alternative rootstocks under very high boron conditions (Yolo County).
- Evaluation of rootstocks in unfumigated replant locations in sandy (Stanislaus) and heavy (Butte) soil.
- Performance comparison of conventional rootstocks with peach x almond hybrid rootstocks and dwarfing rootstocks in different EC zones (CSU Fresno).

Background and Discussion

Selecting the appropriate rootstock for specific soil conditions is critically important for the long-term success of an almond orchard. Rootstocks influence many aspects of tree performance, including vigor, anchorage, carbohydrate accumulation and storage and date of crop maturity. More importantly, rootstocks can guard against soil-borne pathogens like nematodes, crown gall, Phytophthora, Verticillium wilt and oak root fungus. They can also affect above ground pathogen susceptibility in the case of hull rot or band canker. Tolerant rootstocks enable plantings into areas with chemical soil challenges such as high pH, sodium, chloride or boron.

Nemaguard and Lovell, long-time industry standard rootstocks, have some significant flaws. Both often perform poorly in heavy, alkaline soils and are susceptible to Phytophthora, oak root fungus, crown gall, and other diseases. Nemaguard is also susceptible to ring nematode and bacterial canker while Lovell is highly susceptible to rootknot nematode and crown gall. This project involves several separate field trials evaluating over 25 different rootstocks from various breeding programs around the globe.

Some of the highlights documented in these field trials include:

- High chloride tolerance of most peach x almond hybrids, Rootpac R and Viking.
- Rootstocks least tolerant to chloride include Lovell, Krymsk 86, and Nemaguard.
- Most peach x almond hybrids and Viking accumulate significantly less hull boron than other rootstocks. However, no tested rootstock appears to be highly tolerant of excessive boron.
- Lovell, Krymsk 86, Atlas, Cadaman, and HBOK 50 accumulate the most hull B.
- Krymsk 86, PAC9908-02, Hansen, and Viking have exhibited very good anchorage while Hansen x Monegro (HM2) has unacceptably poor anchorage. Empyrean 1 is questionable.
- Atlas appears to be tolerant to Verticillium wilt disease while Lovell and Hansen appear highly susceptible.
- Hansen, Krymsk 86, & Brights 5 can host high levels of ring nematodes while Krymsk 86 can also host substantial numbers of root knot and root lesion nematodes. Viking, Lovell, Guardian and Empyrean 1 support fewer ring nematodes than Nemaguard.
- Krymsk 86 and Rootpac-R showed the highest potassium leaf levels in Butte County but not in other trials.

Project Cooperators and Personnel: Joseph Connell, UCCE – Butte County (Emeritus); Katherine Jarvis-Shean, UCCE – Yolo & Solano Counties; Gurreet Brar, CSU Fresno

Integration of Tree Spacing, Pruning, and Rootstock Selection for Efficient Almond Production

Project Leader: Roger Duncan
University of California Cooperative Extension, Stanislaus County, 3800 Cornucopia Way (#A), Modesto, CA 95358
(209) 525-6800, raduncan@ucdavis.edu

Objectives

- Evaluate the long-term effects of three key management factors: tree spacing (planting density), rootstock, and training/pruning techniques on orchard production and longevity.

Background

This project, initiated in 2000, has examined the long-term, integrated yield effects of various minimal training and pruning strategies with different tree spacings over a 20-year period. The Nonpareil and Carmel varieties are planted 22, 18, 14 or 10 feet apart down the row while the rows are 22 feet apart.

Discussion

Summary of Short & Long Term Results:

Effects of Pruning (or not)

- The pruning portion of this trial has been concluded. After twenty years, this trial has confirmed that pruning does not improve almond yield in the short term or long term. In many years, yields were slightly lower in annually pruned trees compared to trees that have been essentially unpruned for the duration of the experiment. This was true across all tree densities and rootstocks.
- Cumulatively, annually pruned Nonpareil trees yielded 508 – 1481 lb per acre less than unpruned trees during the duration of the pruning experiment, depending on rootstock.
- Pruning the Carmel variety reduced yields more than the Nonpareil variety. Depending on rootstock, annually pruned Carmel trees have cumulative yields 977 – 6075 lb per acre less than unpruned Carmel trees.
- Annually pruned trees have historically captured less photosynthetically active radiation (PAR) than unpruned trees. This indicates that pruned trees have a lower yield potential than unpruned trees.
- Based on the results of this experiment, annual pruning would have reduced cumulative gross income by over $13,000 per acre over a nineteen-year period, including yield reduction, but mostly due to the cost of pruning and brush disposal.

Effect of Tree Spacing

- Yield effects (positive and negative) have varied depending on the vigor and ultimate size of the trees. In general, the smaller Carmel variety on the moderate vigor Nemaguard rootstock has shown significant yield benefits to closer spacing. Through the 19th leaf, the closest spacing (10 feet between trees) accumulated 7564 lb per acre greater than the widest spacing (22 feet).

Cumulative Yield of Carmel on Nemaguard

- 10’ x 22’: 48,593 lb per acre
- 14’ x 22’: 45,595 lb per acre
- 18’ x 22’: 43,805 lb per acre
- 22’ x 22’: 41,029 lb per acre

- Conversely, very vigorous trees have had highest yields at more moderate spacings, although differences are modest over a 20-year period. Through the 20th leaf (including 2019 yield), Nonpareil trees on the very vigorous Hansen rootstock have the highest cumulative yields where planted 18 feet apart.

Cumulative Yield of Nonpareil on Hansen

- 10’ x 22’: 45,146 lb per acre
- 14’ x 22’: 46,029 lb per acre
- 18’ x 22’: 48,687 lb per acre
- 22’ x 22’: 44,776 lb per acre

Project Cooperators and Personnel: Bruce Lampinen, UC Davis

Rootstock Breeding

Project Leader: Thomas M. Gradziel
Dept. of Plant Sciences, UC Davis, One Shields Ave., Davis, CA 95616 tmgradziel@ucdavis.edu

Objective
Develop a series of multifunctional rootstocks combining required traits for continued production in the main California growing regions as well as expanded production into newer regions.

Background
Changes in field management practices have led to the need for a new generation of almond rootstocks. Most commercially successful almond rootstocks are derived from interspecies hybrids in order to achieve the largest possible selection of vigor and desirable horticultural and resistance traits. UCD breeding lines have now been developed combining almond, peach and plum as well as wild Prunus species including P. argentea, P. bleriana, P. bucharica, P. davidiana, P. fenzliana, P. domestica, P. mira, P. orthosepala, P. scoparia, P. tangutica and P. webbii. Working with collaborators we have identified likely sources of pest and disease resistance within related species and are currently working to bring together the most promising resistance from different sources into regionally adapted almond rootstocks.

Discussion
Our ability to generate these traditionally difficult interspecies crosses in large enough numbers will allow capturing a range of diverse traits required for commercial acceptance and to then propagate these inherently unbalanced genotypes in large enough numbers for accurate replicated testing. Currently, over 300 advanced interspecies selections are being evaluated by cooperators as well as in our established hotspots for nematode, oak-root fungus and crown gall.

Breeding lines demonstrating promising levels of resistance to nematodes (root-knot, dagger and/or ring) include P. davidiana, P. persica, P. cerasifera, and certain heirloom almond seedlings. Inter-species hybrids showing the most promise have been replicated and provided to the Westphal nematode screening program as well as the Sandhu program for screening for vigor and salt tolerance. Similarly, replicated clones of UCD rootstock selections (primarily P. cerasifera by peach and/or almond) demonstrating potential resistance to oak root fungus have been provided to the Browne disease screening program at UCD as well as cooperators at Clemson University. Interspecies hybrids combining P. persica and P. davidiana and/or P. bleriana appear to be segregating for tolerance to crown gall in our Davis test plot, while hybrids between P. persica and P. tangutica are segregating for susceptibility to botryosphaeria with no crown gall symptoms present at this time in either these hybrids or susceptible controls.

Molecular markers for promising resistance sources are being pursued through the generation of segregating populations from resistant by susceptible parents that are then evaluated for promising associations between resistance and any nearby, identifiable DNA markers. UCD populations segregating for resistance to botryosphaeria are currently being analyzed by cooperators at University of Florida while hybrid seed populations from 2019 and 2020 crosses are currently being analyzed by cooperators at Clemson for oak root fungus resistance. Population segregating for root knot and ring nematode resistance, including new crosses generated in 2020, are being analyzed for potential molecular markers by A, Westphal, KAC and Dr. Sideli who manages our genomics lab.

Project Cooperators: K. Gasic, J. Preece, M. Aradhya, G. Browne, D. Kluepfel, T. Michailides, A. Westphal, R. Duncan, J. Chaparro, G. Drakaki, A. Dandekar, P. Browne and California nurseries.

Development and Testing of a Mobile Platform for Measuring Canopy Light Interception

Project Leader: Bruce Lampinen
Department of Plant Sciences, University of California, Davis, One Shields Ave., Davis, CA 95616
(530) 752-2588, bdlampinen@ucdavis.edu

Objectives

- Utilize the mobile platform light bar to complete collection of light interception and yield data for long term studied orchards with the goal of determining what happens to this relationship as orchards age.
- Further refine the iPhone application to calculate canopy light interception and create a PC based version of image processing software to allow batch processing of images.

Background and Discussion

Data collected on tree canopy light interception has shown that it is a valuable indicator of an almond orchard's potential productivity. Results suggest that 50 kernel pounds of almond can be produced for each 1% of total incoming midday canopy photosynthetically active radiation (PAR) that is intercepted. These data are also valuable in evaluating new cultivars to assess whether higher yields can be attributed to higher efficiency or whether they simply grow faster.
Traditionally, obtaining the PAR data has been a slow and labor-intensive process based on use of a hand-held lightbar. Consequently, data gathering has often consisted of only limited and small-scale sampling and of collecting PAR data from only a portion of the row where yield data was collected.

Starting about 10 years ago, a mobile platform lightbar was developed on a Kawasaki Mule. It can span an entire row (up to 32 feet) and includes an advanced data logger and accurate GPS. With this setup, it is possible to gather data at a high rate of speed. Following the 2019 season, the mobile platform lightbar was redone on a Honda ATV which provides the advantage of more height adjustment and cruise control.
In 2019, we ran the mobile platform lightbar in a total of 32 almond trial sites. As well as collecting data for most other almond research projects, we research concentrated on collecting data on the orchards that had the longest term data sets with the goal of understanding what factors lead to yield declines as orchards age. We are also working with Ali Pourreza in Bio and Ag Engineering at UC Davis to use drone imagery as another means of assessing canopy size and with Brian Bailey in Plant Sciences at UC Davis to integrate our iPAR app with his modeling work.
A working version of the iPhone app for estimated midday canopy PAR interception was released in the Apple store under the iPAR name in the fall of 2016. This app gives growers a tool to estimate yield potential and estimated nitrogen needs of a given orchards based on canopy size.
Overall, this project has the potential to significantly improve orchard design and management by providing a basis for better managing canopyies and water, as well as providing quantitative estimates of production potential and crop nitrogen needs.

Project Cooperators and Personnel: Shrini Upadhyaya, Ken Shackel, Ali Pourreza, Brian Bailey, Tom Gradziel and Sam Metcalf UC Davis; Greg Browne, USDA-ARS, Davis; Mae Culumber, UCCE – Fresno County, David Doll, UCCE - Merced County; Roger Duncan, UCCE - Stanislaus County; Elizabeth Fichtner, UCCE- Tulare County; Allan Fulton, UCCE - Tehama County; Phoebe Gordon, UCCE- Madera County, Brent Holtz, UCCE - San Joaquin County; Dani Lightle and Luke Milliron, UCCE - Butte/Glenn/Tehama Counties; Franz Niederholzer, UCCE – Colusa/Sutter/Yuba Counties; Blake Sanden, UCCE – Kern County

Integrated Conventional & Genomic Approaches to Almond Rootstock Development

Project Leaders: Daniel Kluepfel[1], M. Aradhya[1], G. Browne[1] and A. Westphal[2]
[1]USDA-ARS Dept of Plant Pathology, UC Davis, One Shields Ave., Davis, CA 95616; (530) 219-8867, Daniel.kluepfel@usda.gov
[2]Kearney Agricultural Research & Extension Center 9240 S Riverbend Ave Parlier, CA 93648 559-646-6555
andreas.westphal@ucr.edu

Objectives
Disease resistance screening is central to this project. Reliable disease evaluation permits selection and development of improved rootstock with combined durable resistance to soil borne diseases. Highly reliable disease resistance data will be used to develop marker associations and genomic selection strategies to accelerate development of rootstocks in the future by avoiding expensive and time-consuming disease evaluations.
Specifically, this year we will focus on expanding field and greenhouse evaluations of our elite rootstock genotypes grafted onto common scions in comparison to commercially available clonal rootstocks. Supplemental pathogen inoculations will be performed in most of these field trials.

Background
We previously identified a large number of hybrids with putative disease resistance characteristics. In December 2019, 35 elite rootstock genotypes selected for positive growth characteristics and pathogen resistance were collected from our hybrid germplasm repository at UC Davis ("Armstrong"). Hardwood cuttings were made and were transferred to Sierra Gold Nurseries for propagation. During 2020, successfully propagated genotypes were grafted to scions to produce 25 finished trees each of 'Nonpareil' and of 'Monterey'. The complete, potted trees are now available for field testing at Kearney and UC Davis. These finished trees will be examined in 2020 under field, and in some cases, under greenhouse conditions. First-year data of these field evaluations will guide what genotypes will be used for subsequent grower-trial evaluations.

Discussion
This project has made a significant progress in the development of novel almond rootstock genotypes with putative resistance to soil borne diseases known to limit sustainable production of almond in California. We applied conventional breeding approaches to develop a wide range of experimental rootstock hybrids utilizing cultivated

and wild relative gene pools. This resulted in the generation of a wide range of novel interspecific hybrids. We maintain copies of these experimental hybrids under field conditions in our germplasm orchard at the UC Davis Armstrong Plant Pathology Field Facility to examine the vigor, growth habit and for further propagation and evaluation to confirm disease resistance. The ~400 hybrids represent a wide spectrum of genetic variability with diverse genetic background of peach and almond. We anticipate that this collection will provide us with the desirable genetic combinations required to select for improved almond rootstocks.

During the 2020 growing season we are focusing on the propagation of many of these hybrids for confirming resistance to nematodes, phytophthora, and crown gall, as well as for establishing field trials to assess for the horticultural traits. We anticipate that many of these novel genotypes will exhibit superior performance under competitive field conditions where they are experience elevated levels of disease pressure.

Project Cooperators Sierra Gold Nursery

Physiology and Management of Salinity Stress and Nitrate Leaching in Almond

Project Leader: Patrick Brown[1]
[1]Dept. of Plant Sciences, UC Davis, One Shields Ave., Davis, CA 95616 (530) 752-0929, phbrown@ucdavis.edu

Objectives

- Improve our understanding of plant response to spatial/temporal variation in ion distribution in the rootzone
- Determine which specific mineral element is responsible for heterogeneous root response
- Determine how water and ion uptake responds in different root ages and types
- Determine how root architecture parameters respond to heterogeneous nutrient/salinity stress
- Determine the influence of nitrification inhibitors on dynamics of N in the wetted root zone, the impact on root activity and growth and N leaching

Background

Heterogeneous ion distribution is a characteristic of micro-irrigation systems and yet our understanding of the biology and management of these systems is inadequate. Previous experiments have demonstrated that root response is proportional to salt concentration and that the response is quickly reversible when the salt distribution in the root zone changes. It has also been shown that the nutrient distribution determines the location within the root volume where roots will be active. Controlling the distribution of nutrients in the sub-zones of the whole root system may therefore be a useful tool to reduce root activity in saline zones, thereby minimizing salt uptake. Research is ongoing to determine which specific mineral element is responsible for root response and whether the response differs between different root ages and types. The findings from these greenhouse experiments are being contrasted with observations in an outdoor large lysimeter with 5 year old trees in which differential root zone heterogeneity is being produced in mature trees under drip irrigation. In this experiment, two different root zone ion distributions were created by employing two scenarios with different irrigation/fertigation treatment: irrigating every 4 days and fertigating every 8 days (low frequency scenario, LF) and irrigating and fertigating every day (high frequency scenario, HF). Root distribution, ion concentrations in the rootzone, and leaf ion concentrations in response to these scenarios are being measured.

Discussion

Pot trials conducted in 2019/20 are being analyzed. New pot trials are planned but have been delayed due to Covid. Irrigation and salinity treatments in outdoor lysimeters were maintained and monitored. Root distribution was measured in the lysimeters with a mini-rhizotron camera in horizontal access tubes at two different depths for a total of 6 trees (two in LF, two in HF). Images were taken bi-weekly from April to October 2019 and from July to October 2020. We are currently working on processing the images to derive root length distribution in the lysimeters as a consequence of irrigation/salinity treatments. Porewater samples were taken in March, May and July 2020 for nitrate and salinity. Results show an increase in porewater electrical conductivity with distance from the drip emitter indicating that differential salinity zones have been established however no difference in rootzone salinity distribution between the two scenarios has been observed yet. Leaf samples were taken in May, July, and October 2020 and will be analyzed for nutrient concentrations.

This experiment will be ‘mature’ and ready for primary analysis and interpretation in the 2021 season. Solute modeling to predict and describe ion distribution, measurement of above and below ground plant parameters, rhizotron analysis of root growth, tissue and soil ion accumulation and plant growth analysis will be conducted in 2021.

In a separate orchard location, an N stabilizer polymer designed to reduce nitrification of urea and ammonium-based fertilizers was tested. Potential N leaching measured using ion exchange resins was lower for the N stabilizer treatment compared to the grower control ($p = 0.10$). The largest effect was observed during the period from February to June when almond N demand and N fertilizer is greatest.

Project Cooperators and Personnel:
Francisco Valenzuela-Acevedo, Daniele Reineke, Sat Darshan Khalsa

Tree Architecture and Development of New Growing Systems

Project Leader: Grant Thorp
Plant & Food Research Australia Pty Ltd, 7 Bevan St, Albert Park, Melbourne, Victoria 3206, Australia.
+61 4 2261 0748 grant.thorp@plantandfood.com.au

Objectives

- Optimize pruning systems for current and future almond varieties and rootstocks planted at high density
- Accelerate identification, breeding and commercialization of almond varieties with tree architectures suitable for high density orchards.

Background and progress

Research is underway in California and Australia to redesign almond orchards using more intensive management systems and new varieties and rootstocks to increase orchard productivity and grower profits. Our key research hypothesis is that orchard yields and grower profits can be doubled by planting trees at high density and growing these trees with minimal pruning to produce a slender pyramid tree shape that optimizes orchard light interception, crop yield and quality.

As with many *Prunus* spp., almond trees generally exhibit a strong basitonic growth habit that produces a set of large scaffold branches near the base of the tree. With heavy cropping varieties, this can result in unstable fruiting canopies that require considerable pruning and training to maintain productivity. While this growth habit is suited to current large-tree growing systems, it is not suited to more intensive growing systems with smaller trees.

New varieties are required that have architectural attributes better suited to high density orchards, with high yields sustainable over the longer term.

Projects, including pruning responses and architectural studies, have been planted in California in collaboration with Burchell Nursery, UC Davis and CSU Fresno. These trials include several advanced genotypes from the UC Davis and Burchell Nursery breeding programs, planted alongside five commercial varieties — ‘Nonpareil’, ‘Monterey’, ‘Wood Colony’, ‘Winters’ and BA2 (Shasta®). Similar projects were also planted in Australia, utilizing genotypes from the University of Adelaide almond breeding program.

In these projects, we are characterizing desirable architectural traits in current and potential new almond varieties. All trees were planted as unpruned, central leader trees and then left in the field with no pruning for 2 years. Data on the growth habit of each genotype, including branching, flowering and fruiting patterns, are being collected. Methods and early data from this research have been shared with almond breeders in California, Australia and Spain to encourage tree architecture as a key selection criteria.

In related projects in California and Australia we are also comparing a range of tree training and minimal-pruning treatments to promote higher yields. New growing concepts are being tested, including working with central leader trees planted at high density and then narrow-pruned to eliminate any branches that, if left, would ultimately become large scaffold branches growing into the center of the rows. Data from our Australian trials have demonstrated that a single round of narrow pruning at the end of the second year’s growth was sufficient to maintain narrower canopies in subsequent years. This pruning did not reduce tree yields so that yield per canopy volume was higher. Data also showed that narrow-pruned trees intercepted less light than control trees. This means trees could be planted with narrow rows, potentially 16 ft wide, and narrow pruned, to increase orchard light interception and yields. The first yield data from Californian trials testing these systems, with rows 15 ft apart, will be collected in 2020.

This project is jointly funded by the Almond Board of California and by Hort Innovation in Australia.

Project Cooperators and Personnel: Gurreet Brar, CSU Fresno; Bruce Lampinen, Tom Gradziel, UC Davis; John Slaughter, Kaylan Roberts CA; María José Rubio Cabetas, CITA Spain; Michelle Wirthensohn, University of Adelaide Australia; and Ann Smith, Plant & Food Research Australia.

Gene prediction and genome functional annotation of 'Nonpareil'

Project Leader: Jonathan Fresnedo-Ramirez
Dept. of Horticulture and Crop Science, The Ohio State University, 1680 Madison Ave., Wooster, OH 44691
(330) 263-3822, fresnedoramirez.1@osu.edu

Objectives

- Develop a gene prediction model for the genome assembly of 'Nonpareil'.
- Develop a transcriptome with full length transcripts of genes in 'Nonpareil' using single-cell RNA sequencing technology.
- Develop a functional gene annotation of the 'Nonpareil' genome.
- Integrate optical mapping data to refine the 'Nonpareil' genome.

Background

The availability of well-developed genomic resources in almond are lacking despite the application of genomics in other fruit and nut trees. In this project, we developed the genome sequence of 'Nonpareil', the most important cultivar in California. This genome sequence was annotated to represent the predicted function of the 'Nonpareil' genes and the proteins these genes encode. This information represents a resource for researchers in the California almond community to enhance almond breeding to develop improved almond cultivars.
An interdisciplinary team including researchers from The Ohio State University and UC Davis are involved in this effort to develop the first complete genome for 'Nonpareil'.

Discussion

Using a combination of Illumina technology and high-throughput chromosome conformation capture (Hi-C), a high-quality, high-continuity draft assembly of the genome (specially the gene spaces) was produced.
Next, a gene prediction and functional annotation analysis was completed at the Ohio Supercomputer Center. This allows the mapping of specific genes, over half of which have predicted functions, to the assembled genome. Repeats and low complexity regions were identified and subsequently masked. As a result, 27% of the genome was soft-masked for repeat elements, a majority of which comprised of interspersed repeats (25%), suggestive of recurrent transposition or retro transposition events as seen in the 'Mission' genome.

Gene prediction analysis resulted in identification of 28,637 putative genes, with an average gene length of 2.82 kilobase pairs. Thus, ~48% of the 'Nonpareil' genome is made up of likely genes. For comparison, ~ 40% was reported for the genomes of 'Mission' and ~ 38% for peach. These numbers suggest that 'Nonpareil' is within the expected range in related trees.
The predicted gene models were supported by expression or transcript data generated from 'Nonpareil' tissues, specifically, leaf and fruit tissues (peel and mesocarp) collected from the same tree at UC Davis Plant Foundation Services.

The functional annotation of the protein sequences associated with the predicted models found ~68% were associated with at least one known biological function, while the rest (~32%) are of unknown function. This is a common outcome in annotation of plant genomes. Thus, 'Nonpareil' gene prediction exhibited ~90% completeness.

To improve completeness of genome and annotation, additional DNA sequencing and RNA-seq from flower and meristematic tissues were produced. Unfortunately, in the case of 'Nonpareil', it is almost impossible to obtain samples of true root tissues given that the original seedling no longer exists.

In addition, 0.5 TB of new PacBio genome sequence data was produced to improve the assembly (completeness ~98.9%). Currently, the integration of all genomic information is ongoing to produce a refined 'Nonpareil' genome sequence based on optical mapping which will be reannotated using previous and new transcriptomic information.

Project Cooperators: Wilbur Z. Ouma & Tea Meulia, MCIC – Ohio State University; Thomas Gradziel, UC Davis

Three-dimensional model-based analysis of the impact of variability in almond tree structure and configuration

Project Leaders: Brian N. Bailey[1]
[1]Dept. of Plant Sciences, UC Davis, One Shields Ave., Davis, CA 95616 (530) 752-7478, bnbailey@ucdavis.edu

Objectives

- Obj. 1: Collect phenotypic data for model parameterization:
  - Collect and process LiDAR structural data for three contrasting cultivars
- Obj. 2: Develop improved models to predict the 3D development of canopy structure:
  - Implement initial framework to describe 3D development of tree structure with time
  - Implement initial model for nut development

Background

Recent advances in genomic technologies have revolutionized plant breeding programs by providing an explicit linkage between traits of interest and the underlying genetic mechanisms. An extraordinary amount of effort has been allocated toward rapid development of genomic tools, and as a result, resources for mapping genomes are now widely available, rapid, and relatively inexpensive. However, deciphering underling genetic information is only one piece of the modern breeding pipeline. In order to make this genetic information useful, it must also be linked with variation in traits or phenotypes of interest, and critically how the particular environment that the plant experiences affects these traits. The rate of development of genomic technologies has vastly outpaced the development of technologies to quantify associated phenotypes and their interactions with the environment, thus creating a bottleneck in the modern breeding pipeline. This problem is exacerbated in perennial crops such as almond because of the long timeframe for development, the size of the plants, and higher variability in management practices. In this context, quantifying phenotypic variation across the wide range of possible configurations using traditional field trials with a factorial experiment design becomes impossible because the number of experiments required increases exponentially with every additional variable. Furthermore, it is often difficult to quantitatively understand how complex traits should be varied in order to achieve the ultimate breeding target of interest.

Overall Goals and Scope

The overall goal of this project is to develop a detailed, three-dimensional modeling framework that can inform breeding and selection practices by predicting how tree structure and growth/yield dynamics vary in response to variability in genotypes, management, and orchard configuration. The model will then be used to determine optimal tree structures that maximize yield over the lifespan of an orchard. This project fits within a wider vision of incorporating next-generation models within the modern breeding pipeline to accelerate the rate of new cultivar development and assessment. The work is also expected to produce near-term results directly relevant to almond orchard design and management. The project will result in a modeling framework that will be used to rapidly assess the impacts of varying orchard configuration and cultivar combinations in terms of growth behavior, spur dynamics, yield, etc.

Model development work will specifically focus on enhancing the Helios 3D modeling framework to be able to dynamically represent almond structural development. Structural evolution is described by providing the model with certain heuristic rules of growth such as branching angles, level of apical dominance, etc., from which 3D structure can be evolved in time. These rules provide the basic instructions for development, which then feed-back with environmental variables such as light to ultimately determine large-scale structure. The goal is to be able to represent how the structure, light availability, and photosynthetic production of various real or hypothetical cultivars interact to determine the evolution of spur populations and ultimately yield.

Project Cooperators and Personnel: Tom Gradziel, Ted DeJong, UC Davis Dept. Plant Sciences

The application of molecular tools and quantitative phenotyping for genomics-assisted breeding in almond.

Project Leaders: Gina M. Sideli[1] and Thomas M. Gradziel[2]
[1]Dept. of Plant Sciences, UC Davis, One Shields Ave., Davis, CA 95616 gsideli@ucdavis.edu
[2]Dept. of Plant Sciences, UC Davis, One Shields Ave., Davis, CA 95616 tmgradziel@ucdavis.edu

Objectives
To develop both large-scale phenotyping and genomic tools, and to apply these technologies to economically important traits for the selection of superior parents and progeny. This will involve

- Characterizing molecular genetic & phenotypic variation;
- Performing statistical and computational analysis to correlate molecular variation with phenotypes as the basis for developing breeding tools;
- Validating and employing these genomic tools in the UC Davis almond and rootstock breeding programs.

Background
The overall utilization of technologies such as molecular markers and quantitative phenotyping can increase the precision of selection for superior genotypes, thereby saving time and resources. The discovery of genetic variants can be used to assess relationships and the architecture of a desired trait. Accuracy in phenotyping is fundamental for obtaining a range of variation observed for a given trait where the quality of phenotypic data affects its application, in order to identify genetic variants associated with a phenotype or the ability to make predictions.

The following four traits exhibit economic importance and are the focus of this study: bloom, shell hardness, kernel lipids, nematode resistant rootstock. Bloom date is important to avoid adverse pollination conditions. Shell hardness affects kernel quality and susceptibility to navel orange worm (NOW). Understanding lipid profiles of the kernel is essential for the oxidative stability of almond oils under storage. Discovering natural genetic resistance to both root-knot and ring lesion nematodes is of great importance because infestations of these pests result in substantial crop loss.

Discussion
Thus far for 2020, phenotyping for bloom time has been collected. Utilizing traditional scoring, date of initial bloom and percentage of bloom as measured two-times per week during flowering, over 500 almond genotypes were phenotyped. In contrast, high-precision phenotyping was also collected on over 2000 almond genotypes utilizing large-scale, high-resolution aerial imagery phenotyping where camera and sensors were attached to a small autonomous aircraft (drone) flown by a specialized pilot.

For the shell-hardness trait, the shells of 170 genotypes varying from soft-shell, semi-hard and hard-shell have been measured using a standard texture-analyzer (model TA-XT2; Texture Technologies; Surry, England) machine and processed with Exponent software (Stable Micro System; London, UK) in order to obtain quantitative data such as initial rupture, maximum force, integral, and number of peaks (resistance to breakage) in the force applied.

For the evaluation of nematode resistance, 10 rootstock genotypes exhibiting promise for resistance to ring-lesion nematode were planted in Parlier, CA in April in order to perform a pre-liminary inoculation and screening.

The sequencing of founder trees will be underway which will then enable the genotyping of a large number of related breeding families.

Project Cooperators: Sabrina Marchand, Selina Wang, Andreas Westphal- UC Riverside, Jonathan Fresnedo-Ramirez- Ohio State, Michelle Wirthensohn and Diana Mather- University of Adelaide-Australia

Applied epigenomics towards measuring the risk of noninfectious bud failure in almond

Project Leader: Jonathan Fresnedo-Ramirez
Dept. of Horticulture and Crop Science, The Ohio State University, 1680 Madison Ave., Wooster, OH 44691
(330) 263-3822, fresnedoramirez.1@osu.edu

Objectives

- Sample clones of almond cultivars and clones exhibiting contrasting noninfectious bud failure grown in distinct production environments in California.
- Obtain samples from an almond pedigree derived from 'Nonpareil'.
- Begin profiling the methylome of Californian and Spanish samples.

Background

Noninfectious bud failure (NBF) has been recognized as threat to the almond industry since 1944 when the disorder was first systematically described and is still observed with the Carmel variety today. Even with the development of new cultivars and breeding germplasm, NBF is a risk to the productivity of the California almond industry.

Despite the threat posed by NBF, little is known about its origin and only recently have propagation practices in been described to reduce the risk of occurance. NBF is particularly concerning because it directly affects the performance of almond cultivars as vegetative buds fail to grow in the spring, limiting the availability of resources to sustain kernel yield in subsequent years. Further, once a tree exhibits NBF, the symptoms (i.e. canopy dieback) only continue to worsen each subsequent year.

NBF does not have a pattern that suggest it is pathogenic, but it can be transmitted through both vegetative propagations (e.g. grafting and budding) and in sexually-derived progenies (i.e. breeding), though manifestation in the latter case is not usually immediate. Finally, the severity of NBF tends to increase as trees age, classifying it as an age-related disorder. NBF is concerning to nurseries, orchards, and breeding programs.

Given the transmission patterns of NBF, the hypothesis that epigenetic mechanisms (i.e. mechanisms that modify gene expression without

modifications to the actual DNA sequence) may be involved in the onset and exhibition of NBF has gained acceptance. A particular epigenetic mechanism, known as DNA-methylation, was tested for association with NBF by our team. We found that the amount of DNA-methylation in the almond genome might serve as a measure of the potential of an almond cultivar to develop NBF.

Discussion

With the advent of sequencing technologies and the development of key genomic resources, we now possess the tools and knowledge to identify genomic aspects related to NBF. In the Fresnedo-Ramirez laboratory at the Ohio State University and with the support of the Almond Board of California, we developed a high-quality, annotated genome sequence of 'Nonpareil' (project HORT.35). This work enabled us to produce profiles of DNA-methylation in almond genotypes in the UC Davis almond breeding program. By comparing identical almond genotypes with contrasting NBF exhibition, we preliminarily identified regions in the almond genome containing genes, transcription factors, and regulatory sequences that may be involved in NBF exhibition.

This project will collect additional information from almond to improve our ability to 1) measure the risk that a given tree may develop NBF in the future and 2) implement remedial measures to avoid NBF exhibition in breeding programs and commercial orchards and reduce NBF potential in nursery stocks. Therefore, the aim of this project is to test and expand our current knowledge of the association of DNA-methylation and the exhibition of NBF in almond grown in distinct producing regions.

Project Cooperators: Katherine D'Amico-Willman & Tea Meulia, Ohio State University; Thomas Gradziel and Gina Sideli, UC Davis. Pedro J. Martinez-Garcia, CEBAS-CSIC, Spain.

Are Californian almond cultivars and rootstocks susceptible to PPV and can almonds be a host for the spread of Sharka in California?

Project Leader: Manuel Rubio[1]
[1]Dept. of Plant Breeding CEBAS-CSIC, Campus de Espinardo, 30100 Murcia, Spain+34 610742116, mrubio@cebas.csic.es

Objectives

- Selection of almond cultivars and rootstocks to be phenotyped against *Plum pox virus* (PPV, sharka). Plant material preparation, including rootstocks growth, inoculation and grafting of selected genotypes.

Background

Sharka, a disease caused by Plum pox virus (PPV), is one of the most serious limiting factors for Prunus production (apricot, plum, prune and peach) in those areas that are affected.
PPV was detected in the USA in 1999, and was declared eradicated by USDA in October 2019, after 20 years of fighting. Despite the official declaration of eradication, Sharka is still of great importance, mainly because of the huge quarantine efforts and millions of dollars spent on it, even though it has never been described in California.
Studies about Sharka on Californian almond cultivars and rootstocks are scarce, and the behavior of these cultivars and rootstocks to Sharka remains unknown. Fifteen years ago, our own results showed a limited potential role of almond as a virus source in Sharka epidemics, but mainly only when Type D isolates of PPV are present.
Based on the little information available, we consider that it is very interesting to know the level of susceptibility/resistance presented by almond varieties as well as the rootstocks used by the almond industry in California, clarifying the possible role almond trees could play in the permanent threat that Sharka presents for stone fruit production.

Discussion

As we mentioned before, the global objective of this project is to determine the susceptibility to PPV of the most important cultivars and rootstocks used in the almond industry in California, as well as the new promising Californian selections (UC Davis, USDA, private nurseries), to assess the role that almond could play in the spread of Sharka towards other stone fruit species in California.
This general objective should be divided into three secondary objectives:

1. Plant material selection and collection in California of almond cultivars, rootstocks, new releases and advanced selections from breeding programs. Introduction of these genotypes in Spain.
2. The PPV phenotyping process including the following: rootstock preparation (stratification, germination, growth in greenhouse, inoculation, grafting of all previously selected plant material); a rest period in a cold chamber and phenotyping (at least three phenotyping cycles).
3. Sharka susceptibility analysis, elimination of susceptible genotypes and re-grafting of genotypes that showed higher resistance to PPV in order to complete a second round of evaluation.

The PPV phenotyping process is a long procedure that we will carry out for four years. After the final analysis of the second phenotyping round, establishing the behavior of the genotypes tested, we will try to establish a conclusion about the risk almond represents for Sharka disease in California

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Project Cooperators and Personnel: Tom Gradziel, Ted DeJong, UC Davis.
Federico Dicenta, Pedro Martínez-Gómez. CEBAS-CSIC

Discovery of genetic variation in related self-fertile species of almond

Project Leaders: Martínez-García PJ
[1]Dept. of Plant Breeding, CEBAS-CSIC, Murcia, Spain +34 646522033, pjmartinez@cebas.csic.es

Objectives

- Discover candidate single nucleotide polymorphic markers (SNPs) on wild relatives of almond
- Annotate and select SNPs from millions of potential loci for a low-density array
- Organize genomic resources and final SNP selections into databases for community access.
- Develop a new tool for marker assisted selection of major S alleles in *Prunus*.
- Apply advanced technologies to California genotype breeding populations.
- Implement Marker-Assisted Selection in the almond breeding program at UC Davis.

Background

The almond breeding program at the University of California at Davis is pursuing the development of new California adapted varieties. These include varieties that are self-fruitful, have improved disease and pest resistance and adaptation to a changing climate. In the long term the program has developed advanced introgression lines transferring self-fruitfulness from multiple independent sources including *P. webbii*, cv. 'Tuono', *P. mira*, and *P. persica* (peach). Other relatives (species) such as *P. fenzliana* or *P. argentea* have been used as a source for improved tree architecture or other traits (e.g. amaretto flavor market.) rather than self-fruitfulness. Several factors affect the breeding efficiency for self-fruitfulness as the degree and stability of the trait and the tedious and time-consuming backcrossing required for successful trait introgression. Although, a few low-density methods for marker assisted breeding have been used, the number of improved self-fruitfulness cultivars om to the public almond breeding programs in California is low.

Advances in high-throughput sequencing technologies provide a great opportunity to bring molecular breeding to full application in screening of the large number of promising new germplasm is being developed at the UC Davis. Molecular breeding is based on the efficient selection of genomic variants known or hypothesized to be associated (tightly linked) with alleles with superior phenotypic effects. A robust breeding program requires knowledge of the many alleles at each genetic locus in the *Prunus* gene pool. Many allelic variants will have a different effect depending upon the desired phenotype, such self-fruitfulness. These allelic variants are largely caused by or tightly linked to single nucleotide polymorphisms (SNPs), the most abundant genetic variations within the genome.

Beyond the obvious value of detected SNPs as a resource for the development of genotyping tools, genomic variants can be annotated with respect to their potential phenotypic consequences, serving as a readily accessible source of candidates in gene-oriented breeding for self-fruitfulness. In addition, the development of a database of the genetic variation in wild relative species of almond, will support powerful annotations of genomic variation that are often the foundation of new avenues of research and breeding. The identified markers will be tested, validated, and fully integrated for the selection of desired parent and progeny possessing self-fruitfulness into the UCD almond breeding program.

Results & Discussion

This is a new project, beginning the summer of 2020.

Project Cooperators and Personnel: Thomas M Gradziel, Charles H. Langley, Gina Sideli, Kristian Stevens, UC Davis, Federico Dicenta, CEBAS-CSIC, Jonathan Fresnedo-Ramirez, OSU

Field Screening of Size Controlling Rootstocks for Higher Density, Off Ground-Harvested Almond Orchards

Project Leaders: Roger Duncan[1] and Giulia Marino[2]
[1]UC Cooperative Extension, Stanislaus County. 3800 Cornucopia Way, Suite A, Modesto CA, 95358. 209-525-6800, raduncan@ucdavis.edu
[2]Dept. of Plant Sciences, UC Kearney Ag Center, 9240 S Riverbend Ave, Parlier, CA 93648. 559-646-6500. giamarino@ucanr.edu

Objectives

- Establish a replicated field trial to evaluate the performance of several dwarfing rootstocks. The long-term objectives include determining the most suitable rootstocks for higher-density almond planting for off-ground harvest.

Background

Due to environmental and food safety concerns, the Almond Board of California is interested in exploring off-ground almond harvesting systems. Many current off-ground harvesting systems require smaller stature trees to accommodate machinery that extends over the treetops. For example, the current Tenias harvesting system allows for a maximum tree height of 18 feet and recommends a row spacing of 18 feet wide. Conventional peach and peach hybrid rootstocks likely have too much vigor and will require extensive annual or semi-annual pruning to maintain trees small enough. This will increase cultural costs, expose trees to wound pathogens and certainly reduce yield potential and the productive lifespan of the orchard.

Many stonefruit industries have turned to size controlling (dwarfing) rootstocks to maintain smaller tree size to accommodate cultural activities. Higher density almond orchard systems using dwarfing rootstocks has not been adequately explored. Many dwarfing rootstocks used in other stonefruit industries may not be compatible with almond, may exhibit different growth characteristics than other fruit tree species or may not be suitable for almond farming systems (minimal pruning, mechanical harvest, etc.). Current rootstock trials conducted by Duncan and others have shown significant growth retardation by many plum hybrid rootstocks compared to standard peach rootstocks. However, lower vigor rootstocks have been generally less productive than high vigor stocks under current, medium to low density almond farming systems. In addition, Duncan's long-term tree spacing trial has shown that tree planting density has a substantial effect on tree size. We need to test the suitability of many dwarfing rootstocks for high density, off ground harvesting almond systems.

A replicated field trial will be established at the UC Kearney Agricultural Research and Education Center in Parlier. Test trees are currently being grown at several commercial nurseries, with a target planting time of fall, 2020. Thirty trees each of Nonpareil and Monterey on all rootstocks will be planted. These varieties were chosen because of their importance to the California almond industry and because rootstock incompatibility has sometimes been a problem with these varieties. Dwarfing rootstocks will be compared against industry standard Nemaguard. Rows will be planted 18 feet apart to accommodate the Tenias off-ground harvester. The exact down the row spacing is yet to be decided and will be influenced by the dimensions of the field available at Kearney. The anticipated between-tree spacing will be in the range of seven to ten feet, for a total of 242 - 345 trees per acre. Prior to planting, the field will be deep ripped and fumigated (if needed). Trees will be irrigated with a double-line drip system.

Parameters including tree height, canopy width, trunk circumference, photosynthetically active radiation (PAR), tree anchorage, canopy architecture, and pathogenic nematodes will be measured annually, beginning in 2021. Trees will be examined for signs of incompatibility periodically throughout each season. Yield will be measured annually for both varieties once a commercially viable crop is produced (beginning at the end of the second or third leaf). Stem water potential will be measured periodically. The trial will persist for at least five years.

Project Cooperators and Personnel: Bruce Lampinen, UC Davis Dept. of Plant Sciences

Accelerated Assessment of Almond Variety Candidates

Project Leaders: Tom Gradziel[1] and Franz Niederholzer[2]
[1]Dept. of Plant Sciences, UC Davis, One Shields Ave., Davis, CA 95616 (530) 752-1575, tmgradziell@ucdavis.edu
[2]Franz Niederholzer, UCCE Farm Advisor, Colusa Counties. (530) 218-2359 fjniederholzer@ucanr.edu

Objectives

- Development multi-location sites for the interim assessment of promising UCD and international breeding selections.
- Establish plots at Arbuckle and Wolfskill, CA and pursue locations in the San Joaquin Valley.
- Collaborate with geneticist in Australia, Spain, France, the Middle East and Central Asia to identify and acquire for California testing, the most promising new varieties and germplasm.

Background

Ongoing changes in almond production are shifting industry needs in multiple areas including: new genetic approaches to pest and disease management, new and improved nut quality traits to allow a continued diversification of food and beverage products that use almonds, and an expansion in the geographic and environmental adaptability of almond production required by changes in the natural and regulatory climates. Achieving these goals while optimizing productivity and water use efficiency will require novel genetic solutions combined with similar advancements in orchard management practices. Regional Variety Trials (RVT) remain the only accurate and trustworthy method to compare yield potential and regional performance of new variety candidates because these long-term and multi-site trials provide growers and processors with unbiased information that helps them select varieties suitable to their needs. Of the 29 varieties currently in the RVTs, most come from UC-Davis and are a combination of recently released varieties and promising advanced selections. These advanced UCD selections represent the 1st generation of a dramatically enriched germplasm combining California quality and adaptability with novel traits for self-fruitfulness, disease and pest resistance and water use efficiency, while demonstrating the range of tree sizes, architectures and bearing habits required for developing more efficient orchard systems required in the future. Understanding how new varieties affect the overall orchard configuration (i.e. closed canopy orchards vs. hedgerow orchards) is the final layer of a crop improvement process because new trees architectures will require different management to reach full yield potential. Thus, it is important that characteristics including tree-size, bearing habit (spur versus dard-type shoot) and tree-architecture of advanced selections are well documented, particularly those which facilitate the ongoing industry shift toward future goals such as dust suppression and off-ground harvesting. Smaller interim evaluation trials for separating out variety candidates with inferior characteristics as well as flawed orchard management systems prior to intensive RVT assessment promise improved speed /efficiency.

Discussion

-A replicated Interim Assessment Field Plot has been established at Winters, CA with the assistance of cooperating CA nurseries where 8 advanced selections, 2 recently released varieties and 4 standard varieties were evaluated in 2020.

-A second Interim Assessment Field Plot is currently being prepared at the Nickels Soils Lab in Colusa County for initial planting in the fall of 2020. Twenty trees each of 30 advanced UCD varietal selections and 2 advanced UCD rootstock selections have been propagated with the assistance of cooperating CA nurseries for fall, 2020 planting. An additional Interim Assessment Plot is currently being negotiated with a grower in the southern San Joaquin Valley.

-Test agreements have been completed for three promising varieties from Spain and four promising varieties from Australia and clonal material has been acquired, successfully transferred through quarantine with the assistance of cooperating CA nurseries, and has been established and further virus tested at Foundation Plant Services, Davis CA. Wolfskill field plots are being prepared for planting in spring, 2021.

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Project Cooperators and Personnel:
Bruce Lampinen, Plant Sciences, UCD, bdlampinen@ucdavis.edu

Evaluating new breeding material for salinity tolerance in almond rootstocks and exploring novel sources of salinity tolerance in *Prunus*

Project Leader: Devinder Sandhu
USDA-ARS US Salinity Lab, 450 W Big Springs Rd., Riverside, CA 92507
(951) 369-4832, devinder.sandhu@usda.gov

Objectives

- Objective. Evaluate elite rootstocks from breeding populations for salinity tolerance.

Background and Discussion

Plants employ several mechanisms to cope with salinity stress and toxic ions. Understanding the roles of different component traits of plant salt tolerance mechanism is critical. In our previously ABC funded study, we showed that there is significant variation available for different components in almond rootstocks. We evaluated 16 commercial almond rootstocks under irrigation waters of 5 different salt compositions, including Atlas, BB106, Bright's 5, Cornerstone, Empyrean 1, Flordaguard x Alnem (F x A), Guardian, Hansen, Krymsk 86, Lovell, Nemaguard, Nickels, Rootpac 20, Rootpac 40, Rootpac R, and Viking. The treatments included a control, sodium sulfate-based solution, sodium chloride-based solution, sodium dominant water with mixed anions, and calcium and magnesium dominant water. Both survival rate and change in trunk diameter analyses suggested that mostly Na, and to a lesser extent, Cl concentration in irrigation water are the most critical ion toxicities for almond rootstocks. Top performing rootstocks under salinity were Rootpac 40, Empyrean 1, Cornerstone and BB 106. These rootstocks also had least amount of tissue accumulation of Na and Cl, suggesting that ion exclusion may be the main component trait of the salt tolerance mechanism in almond. Furthermore, the expression analysis of 24 genes that play important roles in salt tolerance revealed that treatments where Na and Cl were the main ions in irrigation water led to induction of most genes, suggesting the importance of both chloride and sodium toxicities during salt stress in almonds. The study on commercial rootstocks was valuable, however, screening breeding material and germplasm for salinity tolerance will be highly effective in combining vigor, biotic stress resistance and abiotic stress tolerance while developing new almond rootstocks.

Prunus species are generally sensitive to salinity. All the rootstocks previously evaluated at low salinity levels with irrigation water electrical conductivity (EC_{iw}) of 3 dS m^{-1} displayed poor survival rates. Our results suggest that gene pool for salinity tolerance is very narrow in commercial rootstocks. Targeted studies are needed to introduce new gene combinations from *Prunus* germplasm into breeding material.

Our previous studies on almond rootstocks provided detailed analysis on importance of different component traits in different rootstocks. However, coordinated effort is needed to combine multiple component traits into a single rootstock to enhance its salinity tolerance. The best way to tackle this issue will be screening of elite breeding lines for salinity tolerance in addition to other traits. Using this approach new rootstocks can be developed that are vigorous, resistant to diseases and insects and are tolerant to salinity.

Our approach involves working with breeders to identify elite hybrids (based on vigor and biotic stress resistance) and screen those for salinity tolerance. Several elite lines have been developed by Dr. Tom Gradziel (UC Davis) and Dr. Malli Aradhya (USDA-ARS, Davis, CA) in their breeding programs. In 2020-21, we will evaluate up to 38 rootstocks for salinity tolerance. We will focus on screening genotypes at moderate salinity levels (EC = 3 dS/m) as almonds are sensitive to salinity, at high-salinity levels other mechanisms such as osmotic shock play a dominating role that will likely dilute the effect of genes involved in salt tolerance and because this is the upper limit of salinity levels that would likely be used for future almond irrigation. Tissue samples will be analyzed for major and minor elements. Plants will be assessed for survival rate and trunk diameter before and after treatment and these parameters will be related to their Na and Cl tissue accumulation to establish salinity tolerance.

This comprehensive approach will lead to development of some elite rootstocks with multiple desirable traits, including salinity-tolerance, in short period of time.

Project Cooperators and Personnel:
Jorge Ferreira and Donald Suarez, USDA-ARS US Salinity Lab, Riverside, CA, Thomas Gradziel, UC Davis, Malli Aradhya, USDA-ARS, Davis

Three-Dimensional Modeling of Water Use and Photosynthesis in Almond Orchards

Project Leader: Brian Bailey[1]
[1]Dept. of Plant Sciences, UC Davis, One Shields Ave., Davis, CA 95616 (530) 752-7478, bnbailey@ucdavis.edu

Objectives

- Perform sub-leaf model validation against lysimeter data
- Simulate seasonal water use for virtual orchards with different designs and analyze trade-offs in water-use efficiency

Background

Field experimentation, along with intuition, has been the traditional approach used by agronomists and growers to better understand crops, and ultimately improve efficiency in production. Making robust conclusions that lead to the adoption of new practices is typically slow, as very large data sets are required in order to separate physiological effects from the high natural variability in the system of interest. Inability to rapidly vary environmental, geographical, and architectural conditions also limits the utility of field experimentation.

This project seeks to develop a robust modeling system that can be used to evaluate proposed management strategies in a virtual environment prior to field experimentation. This will accelerate the speed of innovation and add an additional layer of information to supplement data collected in the field.

Crop models have been utilized for many decades as a tool to better understand cropping systems. While models have been relatively successful in representing field/annual crops, modeling perennial cropping systems presents many considerable difficulties such as storage of carbohydrates over winter, and their complex and seasonally-varying architectures.

Our approach is to represent the full three-dimensional geometry of the canopy and associated physical processes at the leaf scale in order to study how management, environment, and cultivar interact at the field scale. The model outputs will be absorbed sunlight, evapotranspiration fluxes, and photosynthetic rates for every leaf and surface in the canopy, which can be easily aggregated to give whole-plant and whole-field values.

Our overall goal is to determine optimal management practices that minimize plant water requirements without sacrificing yields. We have been working to develop the modeling system, and to rigorously validate it against detailed field measurements.

Results & Discussion

One key finding of the past year's work is the importance of performing simulations at the sub-leaf scale rather than at the leaf scale. There can be considerable variability in light interception across an individual leaf and taking this into account rather than averaging over each leaf can decrease modeled canopy water fluxes considerably, with largest differences occurring for more dense canopies. Another lesson learned was the importance of using the appropriate within-canopy environmental conditions as input for simulations. Air temperature, humidity, and especially wind speed measurements collected within the canopy were significantly different from above-canopy conditions and conditions at the nearest CIMIS station. We have incorporated the ability of reading in multi-layer meteorological data to drive the model. Another factor that we are investigating is the reliability of our tree geometry measurements derived from LiDAR.

There is some indication that leaf area density may be overestimated using our current method, which was originally validated on a canopy with larger leaves (grapevine). We are generating simulated LiDAR to perform virtual measurements on a model canopy with known leaf area to test this. Once we are confident in reconstruction of the lysimeter tree, we will perform additional validation against the lysimeter measurements. Then we will use the validated model to construct virtual orchards of different densities and analyze trade-offs in water-use efficiency, and how particular orchard designs impact production versus water use.

Project Cooperators and Personnel:
Eric Kent, Ken Shackel, Bruce Lampinen, Ted DeJong, Matthew Gilbert, UC Davis

Development of Tree Carbohydrate Budget of Almonds Under Changing Central Valley Climatic Conditions

Project Leader: Maciej Zwieniecki
Dept. of Plant Sciences, UC Davis, One Shields Ave., Davis, CA 95616 (530) 752-1843, mzwienie@ucdavis.edu

Objectives
- Maintain and expand state-wide database on almond carbohydrate dynamics that provides information on non-structural carbohydrates (sugars) NSC content in respect to region, variety, age, and season
- Analyze seasonal dynamics of NSC content and determine NSC role in driving almond phenology
- Use available NSC content data base to investigate and document the impact of management practices on NSC and yield
- Research role of carbohydrates in almond phenology. Determine impact of dormancy temperature on bloom time and synchrony

Background
California's Central Valley is affected by a climatic shift that is reducing fog cover. The net result is an increased incidence of variable thermal conditions during winter including higher average temperatures, severe frost nights, and hot days that can affect tree phenology.

These factors combined with the increasing use of saline ground water supplies have produced an unprecedented set of new abiotic stresses that affect horticultural production. Almond life can be described as a continuous struggle to acquire, transfer, and store energy that is necessary to grow, reproduce, and protect from abiotic and biotic stresses and allow for the survival of dormancy.

Carbohydrates (sugars and starch) are responsible for the majority long-term storage of energy in plants. They are the currency that the plant uses to interact with the environment. The understanding of carbohydrate physiology is of key importance for predicting phenology, yields, analysis of stress, and a plant's ability to mediate salinity, drought, or winter survival.

Discussion
We are currently developing and maintaining the largest data base on carbohydrate content in orchard trees in California (and the world). Information is available online: http://zlab-carb-observatory.herokuapp.com/.

Information collected over multiple years provides insight on the impact of biological and climatic conditions on NSC content variation and allows for a deterministic link of NSC content to yield capacity and bloom. NSC content in almond trees during November and in February is positively correlated with the following year's yield. High yield results in low content of NSC in August. This suggest that NSC content recovery in fall (post-harvest management) is crucial for maintaining high orchard productivity. Specific management practices leading to NSC recovery (especially post-harvest irrigation) is a goal for future research.

Changes in NSC content during dormancy can be mechanistically linked to bloom time and synchrony. This suggest that NSC content is part of a dormancy 'clock' in almonds. A bloom time model developed for nonpareil is available online. This model suggest that both too high and too low NSC content in the fall can lead to asynchronous and delayed bloom in almond. The model allows for real time analysis of dormancy progression in an average orchard and a comparative analysis of the current year's winter to the previous for each available CIMIS station: https://zlab-chill-heat-model.herokuapp.com

Current research has expanded to analyze the role of NSC in spring phenology, and to determine bloom cost in terms of carbohydrates. This includes the respiration during dormancy, flower biomass and respiration during flower development. This research will provide information on what the minimum NSC storage requirement is in trees during fall to assure bloom potential. In addition, we are analyzing RNAseq of plants exposed to high and low chill to determine enzymatic pathways involved in plants' response to climatic variability. Preliminary results suggest that metabolic pathways of soluble sugars are key components of almond response to chill.

Project Cooperators and Personnel: Anna Davidson, Adele Amico Roxas, Kathelyn Cooper UC Davis,

Root data summary and publication

Project Leaders: Astrid Volder and Patrick Brown
[1]Dept. of Plant Sciences, UC Davis, One Shields Ave., Davis, CA 95616 .avolder@ucdavis.edu; phbrown@ucdavis.edu

Objectives

- Summarize and write up root data from past projects, including impacts of winter groundwater recharge and effects of pruning/irrigation (Volder lab) and fertilizer/water use efficiency (Brown lab) projects

Background

Root, shoot, and vascular traits are tightly linked to expected survival and growth rate under drought conditions. The supply of water to and within plants is determined by soil water availability (water content and soil type), plant architectural traits (e.g., root:shoot ratio, root depth, root surface area, leaf area, tissue density), as well as axial and radial hydraulic conductance of the root system. Generally, there are tradeoffs between characteristics that confer stress resistance and those that allow a high physiological activity. We aim to study variation in root morphological, anatomical and physiological traits in response to multiple irrigation and fertilization scenarios. We have considerable information about root growth patterns in response to winter recharge practices, as well as root responses to irrigation and aboveground management.

Discussion

In the past, preliminary data for these projects has been presented at the Almond Production short course (2016, 2019), Principles of Fruit and Nut production short course (2016 – 2019), various field days etc. to both growers, PCA's and farm advisors. However, converting preliminary data into a publishable manuscript takes a substantial amount of time and perseverance. A post-doctoral scholar (Max Ma) started in January 2020 and has taken the lead on (or contributed) to the following achievements so far:

The root dataset for root responses to winter recharge has been double checked for errors and summarized into an analyzable database.
A manuscript on the impact of winter groundwater recharge in mature almond orchards has been submitted for review to California Agriculture in August 2020 (Ma et al.).

Data analysis for another manuscript on root demographics in two California orchards subjected to winter recharge is being performed (to be submitted to Tree Physiology, Ma et al.). This manuscript will have detailed data on periods of high root production, root death and a root lifespan analysis for orchards at Delhi (sandy soil) and Modesto (sandy loam).
The root chapter for the almond production manual (Volder and Doll) is being updated with the newest information and insights from these projects.

A draft manuscript by Olivos et al. on the impact of nitrogen fertilizer and irrigation practices is being circulated with co-authors.

Project Cooperators and Personnel: Xiaochi (Max) Ma, Helen Dahlke, Andres Olivos, Roger Duncan, David Doll, Bruce Lampinen, Paul Martinez

Efficacy of AF36 Prevail® after Commercial Application

Project Leaders: Themis J. Michailides
[1] Kearney Agricultural Research & Extension Center9240, S. Riverbend Ave, Parlier, CA 93648 (559) 646-6546, tjmichailides@ucanr.edu

Objectives

Aflatoxins are toxic and carcinogenic metabolites produced by the fungi Aspergillus flavus and A. parasiticus, which contaminate crops, including almonds. The almond industry supports research to reduce aflatoxin contamination risks and meet compliance with aflatoxin regulations. A successful control of aflatoxin will benefit a) the growers by avoiding the loss of revenues due to lower risk of crop rejections and b) the public in general by consuming almonds free of aflatoxins. The overall objective of this project is to optimize the use of the atoxigenic A. flavus strain technology to reduce aflatoxin contamination in California almonds. This includes improving the sporulation efficiency of the AF36 Prevail®, changing the population structure of the aflatoxin producing fungi by implementing area-wide, long-term management programs to reduce the overall aflatoxin-producing potential of the population. Specific objectives are: 1) develop a qPCR protocol to evaluate the ratio AF36 / A. flavus & A. parasiticus, 2) study the risk of infection during the period that the almond fruit are drying on the ground, 3) determine the optimal period of application and improved sporulation of AF36 Prevail in almond, 4) determine the effect of almond cultivars on aflatoxin accumulation, and 5) monitor the atoxigenic A. flavus AF36 in commercial almond where AF36 is applied and evaluate its effect on area-wide long-term reduction of aflatoxin in almonds.

Background

Crops susceptible to aflatoxin contamination, including almond and pistachio, frequently are growing in the same area, implementing area-wide aflatoxin control programs might be the best strategy to lower the risk of aflatoxin contamination in tree nut crops. However, research to monitor the efficacy of area-wide programs requires the increase of sampling and Real Time PCR (SNP-qPCR) to quantify the proportions of both AF36 and A. flavus/A. parasiticus can expedite the analysis and reduce its costs, and increase the certainty of the study. We developed a qPCR system that differentiates AF36 from toxigenic Aspergillus in samples with known rates of AF36 and other Aspergillus fungi, which is being validated with field samples. Also, experiments on the risk of infection during drying on the ground determined that the risk of infection of almond by Aspergillus during this time is null. Experiments on the time of AF36 biocontrol application indicate that applications during the 2nd to 3rd week of July were optimal for product sporulation and displacement of toxigenic strains. Earlier applications (late June) even though have lower sporulation, they have similar displacement than application in July, indicating that better sporulation on earlier applications might improve displacement. Experiments on sporulation of biocontrol products indicate that Afla-Guard had better sporulation under lower temperatures and lower soil moisture than AF36 Prevail, which could improve sporulation in early applications. Experiments evaluating area-wide programs where in one area both pistachio and almond were treated and in another only pistachio was treated indicate that pistachio in the treated area had the highest percentage of strain L (94%) and the lowest percentage of toxigenic strains (6%), while almond orchards in the not treated area had the lowest percentage of strain L (69%) and higher percentage of toxigenic strains (22%), indicating that an area-wide program (treating both pistachio and almond) can improve efficacy of atoxigenic strain biocontrol in reducing aflatoxin contamination.

Discussion

Real Time PCR (SNP-qPCR) to quantify the proportions of both the applied AF36 and the resident A. flavus/A. parasiticus can expedite the analysis, reduce costs, and increase certainty of toxigenic strain displacement. AF36 Prevail® has better sporulation and displacement on applications during July. Earlier applications had lower sporulation, but similar displacement. Afla-Guard had better sporulation under lower temperatures and soil moistures. Biocontrol products with better sporulation on earlier applications might increase displacement and improve aflatoxin control. Area-wide program can improve efficacy of atoxigenic strain biocontrol for aflatoxin contamination.

Project Cooperators and Personnel: Ramon Jaimee

Altered Lipid Metabolism-Bifenthrin-Resistant Navel Orangeworm (NOW) Potential Cross-Resistance to Pesticides

Project Leader: May Berenbaum Researchers: Esther Ngumbi, Daniel Bush, Mark Demkovich
Dept. of Entomology, University of Illinois, 505 S. Goodwin Ave., Urbana, IL 61801 (217)333-7784 maybe@illinois.edu

Objectives

- **1:** Determine and compare cuticular hydrocarbon profiles between insecticide-resistant and susceptible strains of navel orangeworm (NOW)
- **2:** Determine LC_{50} contact toxicity of chlorantraniliprole and methoxyfenozide to larval NOW from a range of populations, with bifenthrin as a positive control
- **3:** Determine whether kaolin clay can prevent egg hatch and/or cause neonate mortality

Background, Results, and Discussion

Objective 1: The insect wax layer comprises cuticular hydrocarbons (CHCs) that both prevent desiccation and provide intraspecific communication signals. CHCs can also contribute to insecticide resistance via reduced penetrance. We assessed the role of CHCs in NOW insecticide resistance. We found differences in cuticular hydrocarbon profiles between adults of pyrethroid-resistant (R347) and susceptible strains. Hydrocarbon profiles consisted of 47 compounds of lengths ranging from C_{17} to C_{43}. Generally, R347 adults had greater total CHC quantities, but relative quantities of individual components were similar. We also found that CHC quantities increased with age. Our results indicate that CHCs vary by strain and age and could potentially be useful biomarkers to differentiate between insecticide-resistant and susceptible strains. A manuscript from this work has been published in *Journal of Chemical Ecology* (Ngumbi et al. 2020).

Objective 2: We developed a bioassay to assess insecticide toxicity through direct

exposure to eggs and contact exposure across a treated surface. Bifenthrin concentrations of 5, 10, 20, and 40 ppm were sprayed on eggs resting on filter paper placed on the surface of artificial diet in Petri dishes. Neonates thus experienced contact exposure to treated filter paper before reaching their food underneath the treated surface. The resistant strain experienced significantly lower egg mortality relative to the susceptible strain. More larvae survived the contact exposure in the resistant strain up to three weeks after the sprays. Also with this method using bifenthrin as a positive control, we have preliminary data on contact toxicity of chlorantraniliprole and methoxyfenozide.

Objective 3: Kaolin clay hampers feeding and survival in some insect pests; one suggested mechanism is abrasion or disruption of CHCs. We treated almond kernels with kaolin, chlorantraniliprole, or both together. In two sets of trials, we used kaolin concentrations based on field applications of both ~40 lb per 100 gallons and ~15 lb. In both versions of the experiment, kaolin and chlorantraniliprole reduced penetration of NOW larvae into the almonds and the percentage of kernels infested after one week. In addition, kaolin and chlorantraniliprole interacted synergistically, although at a reduced level in the 15 lb treatment. R347 was more resistant to all treatments (including chlorantraniliprole, which was unexpected) but was still significantly affected. These results indicate that kaolin can improve insecticide efficacy, possibly by disrupting CHCs. Dr. Joel Siegel has conducted field trials at ~15 lb per 100 gallons, and we have preliminary trials in the laboratory with bifenthrin and kaolin.

Reference: Ngumbi EN, LM Hanks, AV Suarez, JG Millar, MR Berenbaum, 2020. Factors associated with variation in cuticular hydrocarbon profiles in the navel orangeworm, *Amyelois transitella* (Lepidoptera: Pyralidae). Journal of Chemical Ecology 46: 40-47.

Project Cooperators and Personnel: Joel Siegel, USDA/ARS, Parlier, CA

Comparison of Navel Orangeworm Attractants

Project Leader: Chuck Burks
[1]USDA, Agricultural Research Service, San Joaquin Valley Agricultural Sciences Center, Parlier CA 93654
(559) 596-2757, charles.burks@usda.gov

Objectives

- Test of commercial prototype trap with a new attractant in the presence or absence of an additional pheromone lure

Background

NOW is the most economically important insect pest of almonds, and for California tree nut industries in general. It is important to have best practices for monitoring for NOW to inform management decisions as part of an IPM plan. Currently the greatest challenges for monitoring are that the monitoring system needs to detect reliably under low as well as high population, and to work in both the absence and the presence of mating disruption. It is also desirable that the trap counts in a NOW detection system should predict damage. Present traps and attractants do not predict damage well, although there is evidence that traps attracting females are more correlated with damage than those attracting males. A variety of systems for automation and remote monitoring of traps are starting to emerge, and this trend seems likely to continue.

Two attractants have shown promise for meeting these criteria; phenyl propionate (PPO); and ovibait traps (more widely known as Peterson traps, based on the predominant commercial provider). PPO, when emitted at high concentration, captures both sexes and works well in the presence of mating disruption as well as in its absence. Previous research in this product found that PPO attracted NOW in mating disruption more consistently than a kairomone blend developed for this purpose. When presented along with a pheromone lure, PPO capture similar numbers of NOW in both the presence and the absence of mating disruption. Trap type is, however, important to this effect and more open traps, like wing traps, work much better than the more closed delta traps that are more popular with pest control advisors.

Discussion

Based on these observations, experiments in the later 2019 and early 2020 crop years compared NOW captured in ovibait traps vs traps baited with PPO and pheromone. In June-September 2019, a trial in almonds under mating disruption compared, among other treatments, NOW captured in wing traps baited with PPO and pheromone, Peterson traps, and egg traps. The delta traps baited with PPO and pheromone captured >10x as many NOW as the Peterson traps, and fewer eggs were captured on egg traps compared to females on Peterson traps.

Experiments under mating disruption in early 2020 compared wing traps baited with PPO in almond and pistachio. In the early season (e.g., March), Peterson traps captured more NOW than PPO. This trend partially reversed itself as the season progress. In addition, a commercial prototype wing trap was tested in an attempt to make the wing trap more acceptable to users. It was determined that further refinement was needed to provide a viable alternative to the delta trap.

The superior performance of Peterson traps in early season might have been due to poor volatilization of PPO at low temperature, or it could have been a behavioral difference in recently overwintered NOW. Future work will examine whether ovibait or PPO is more correlated with subsequent NOW damage.

Project Cooperators and Personnel: Brad Higbee, Trécé Inc., John Beck, USDA-ARS Gainesville FL, Mike Strmiska, Advanced Nut Crop Science, Fresno CA. Jhalendra Rijal, UCCE, Stanislaus County

Arthropod Pest Management in the Lower San Joaquin Valley

Project Leader: David Haviland
UC Cooperative Extension, 1031 South Mount Vernon, Bakersfield, CA 93307, (661)868-6215, dhaviland@ucdavis.edu\

Objectives

- Evaluate novel approaches for mating disruption of navel orangeworm
- Improve management programs for spider mites
- Maintain a UC almond research orchard in the south Valley

Research Activities

Navel orangeworm and Pacific spider mite are two of the most important pests in the lower San Joaquin Valley, and successful management of both species requires an integrated approach.

Navel orangeworm

For NOW our research focused on the use of mating disruption. In past years we documented that the use of aerosol and meso emitters provided more than 90% shutdown of pheromone traps and an approximate 50% reduction in damage at harvest. Economic assessments showed that adding mating disruption systems resulted in a positive return on investment.

During the past year we expanded this research to include two new types of dispenser systems. This included the use of two microencapsulated (sprayable) formulations of NOW pheromone, a foam-like dispensing system that is applied by drone, and compared those to traditional aerosol and meso-based dispensing systems.

During the period from the initiation of hull split through the end of harvest, aerosol and meso-based systems reduced male captures by 93 to 97%. Four monthly applications of a sprayable pheromone product reduced captures by 78% while two applications of sprayable and foam-based products (hull split plus one month later) reduced captures by 40 to 46%.

At harvest there were no significant differences in Nonpareil damage. In Monterey, aerosol and meso-based products reduced damage by 36-45%, four applications of sprayable pheromone reduced damage by 25% (not significant), and two applications of sprayable or foam-based products reduced damage by <15% (not significant).

Ongoing trials in 2020 continue to evaluate mating disruption products. This includes a new and standard aerosol products, a meso product, and new approaches to the use of two sprayable products, one of which has undergone formulation changes since previous research was conducted. Results of these trials will be available for reporting at the 2020 Almond Industry Conference.

Spider mites

For the past few years our research team focused on the role of sixspotted thrips in spider mite control programs. We evaluated sticky card traps as an improved method for monitoring this natural enemy and used those traps to gain general information about thrips biology and phenology throughout the season. Correlations between trap captures and mite densities were used to develop a tentative predictive model that could be used to aid the decision-making process regarding the use of miticides.

All of this information is currently being distilled and prepared for formal publication during a sabbatical leave by PI Haviland during the summer of 2020. Once the monitoring and treatment threshold guidelines are published, efforts will be made to generate outreach materials that target almond growers and pest control advisors.

Research orchard

We continue to maintain a 7-acre almond orchard in Shafter, CA with partial support from the Almond Board of California. Between the years of 2010 and 2019 this orchard has been used for 73 different field trials by multiple public and private investigators that include pomologists, entomologists, weed scientists and pathologists.

Project Cooperators and Personnel: Stephanie Rill, Chelsea Gordon and Minerva Gonzalez from UCCE Kern. Pacific Biocontrol, Trece, Suterra, an ISCA Technologies

Another Look at Pheromonal or Related Attractants for Leaffooted Bugs

Project Leaders: Jocelyn Millar[1], Houston Wilson[1], and Kent Daane[2]
[1]Dept. of Entomology, UC Riverside, 900 University Ave., Riverside CA 92521 (951) 827-5821, millar@ucr.edu; (559) 646-6519, Houston.wilson@ucr.edu
[2]Environmental Sciences, Policy, and Management, UC Berkeley, 212 Wellman Hall, Berkeley, CA 94720 (510)-643-4019, kmdaane@berkeley.edu

Objectives

- Finish identification and synthesis of final pheromone candidate for *Leptoglossus zonatus* and *L. clypealis*.
- Test cuticular hydrocarbon mediated winter aggregation responses.
- Optimize traps for *Leptoglossus* spp.
- Test *Leptoglossus* responses to infrared radiation cues.

Background

Leaffooted bugs are a major problem in California nut crops, and there are currently no cost-effective methods of monitoring them. The problem is compounded by the tendency for large numbers of bugs to move into a crop rapidly, so that infestations can go from negligible to substantial within a few days. Previous research has shown that these bugs use pheromones in different contexts, from alarm to aggregation and mating. We hope to identify pheromones for two species, *Leptoglossus zonatus* and *L. clypealis*, to be developed for detection, monitoring, and possibly control of these major pests of nut crops.

In parallel, we are optimizing trapping/ monitoring methods, using traps with and without chemical attractants. In addition, Takacs et al. (2009) showed that *Leptoglossus occidentalis,* a congeneric pest in conifer seed orchards, used infrared (IR) cues to locate cones, which are warmer than the rest of the tree due to the high metabolic activity in the cones. We are testing *L. zonatus* to determine whether they also may be responsive to IR cues associated with developing nuts.

Discussion

Pheromone Identification and Synthesis:

1. We have completed an entirely new synthesis of one of the main pheromone components, alpha-bergamotene, producing several grams in preparation for field bioassays.
2. Through 2019, we collected volatiles from ~80 cohorts of male summerform *L. zonatus*, to get enough of the final pheromone compound to identify. The compound was purified, and NMR spectra were collected over 2 weeks in December. From those spectra, we think that we have finally identified the basic carbon skeleton as an 8,4-bicyclic sesquiterpene. We are literature researching routes to synthesize the compound as soon as labs reopen.
3. The cuticular hydrocarbon bioassays are on hold because we had to kill our insect colonies during COVID-19 lockdown. Colonies will be restarted as soon as lab research can resume.

Trap Development:

1. A field experiment to compare different trap types determined that a hanging cross-vane panel trap can be used to monitor *L. zonatus*.
2. Subsequent studies to refine the use of this trap demonstrated that trap catch can be improved through (i) the addition of fluon (a surface lubricant) and (ii) use of a yellow, blue or green trap.
3. Additional studies evaluated the utility of an unbaited trap in orchards and found that it was very low – highlighting the necessity of an attractant lure to pair with this trap.

Response to infrared cues:

1. A custom experimental arena was constructed in fall 2019 to evaluate *L. zonatus* response to infrared cues.
2. Initial tests produced mixed results, but this may be due to the use of biologically irrelevant infrared cues, which are not clearly understood for this species.
3. Studies in 2020 will focus on refining the experimental arena for this study and evaluating a wider range of infrared cues.

Project Cooperators and Personnel: Dr. Sean Halloran

Influence of Different Cover Crop Systems on Navel Orangeworm (NOW) and Its Natural Enemies

Project Leaders: Houston Wilson[1], Kent Daane[2], Amelie Gaudin[3]

[1]Dept. Entomology, UC Riverside, Kearney Ag. Center, 9240 S. Riverbend Ave., Parlier, CA 93648, (559) 646-6519, houston.wilson@ucr.edu; [2]Dept. Environmental Science, Policy and Management, UC Berkeley, 212 Wellman Hall, Berkeley, CA 94720, kdaane@ucanr.edu; [3]Dept. of Plant Sciences, UC Davis, One Shields Ave., Davis, CA 95616, (530) 752-1212, agaudin@ucdavis.edu

Objectives

Quantify the influence of ground covers on sanitation efficacy

- Determine the influence of ground covers on mummy mortality

Background

A team of UC researchers (A. Gaudin, J. Mitchell, N. Williams, B. Hanson, A. Westphal, H. Wilson, K. Daane) are investigating the potential of winter-spring cover crops to help growers improve the overall health of their orchards. The group is evaluating the use of (1) a pollinator mix and (2) a soil builder mix, the performance of which will be compared to plots with (3) bare soil with minimal ground cover.

Traditionally the use of cover crops in IPM has focused on their ability to either (a) increase biological control by supporting natural enemies or (b) reduce pest densities by (i) acting as a trap crop or by (ii) inducing changes in host-plant quality that make it less suitable for pest development. Unfortunately, none of these processes are likely to be enhanced enough by the addition of a cover crop to increase regulation of navel orangeworm (NOW) populations, much less below the extremely low damage thresholds (<2%) required for this commodity. These low thresholds are primarily due to the association between NOW and aflatoxin, which is heavily regulated in key export markets.

Alternately, it may be that the addition of winter cover crops influences NOW populations via impacts on sanitation and NOW mortality in mummy nuts. Sanitation of mummy nuts is the foundation of NOW management, and here the hypothesis is that the addition of ground covers could have both positive and negative effects. The potential negative effect is that ground covers could harbor mummy nuts and generally interfere with sanitation efforts (i.e. reduce sanitation efficacy). The potential positive effect is that ground covers are thought to create an inhospitable environment for overwintering NOW that ultimately increases mortality, as well as deters egg deposition on mummy nuts in the spring. Our goal is to better characterize and quantify these effects as a net positive or negative outcome.

Discussion

Cover crops were sown fall of 2019 in replicated experimental plots at the UC Westside Research and Extension Center (Five Points, CA) and on commercial almond acreage near Arvin, CA. Data collection is now coming to an end at the Westside REC while the field plots in Arvin did not successfully establish.

Cover Crop Height and Biomass – Few differences were observed early on as the cover crops developed, but by February both total stand height and biomass of the cover crop treatments was higher than resident weedy vegetation (RWV) that had developed in the bare soil plots. While height of the two cover crops was similar, the soil builder mix generated a greater total biomass.

NOW Egg Deposition – Overall egg deposition was highest on traps placed in the tree canopy while much lower in the burm and ground cover. Within the stands of cover crop egg deposition was generally lower compared to RWV in the control plots, but alternately was higher on the burms in cover crop plots. There were no consistent differences in egg deposition in the tree canopy across the different plots.

Data on NOW mortality in mummy nuts is still being gathered, and additional data points will be collected for ground cover biomass and egg deposition through June 1.

Project Cooperators and Personnel:

Mohammed Yaghmour, UC Cooperative Extension, Kern County; David Haviland, UC Cooperative Extension, Kern County; Jessica Maccaro, Kearney Ag. Center / UC Riverside.

Biology, Monitoring and Management of Brown Marmorated Stink Bug in Almond Orchard

Project Leaders: Jhalendra Rijal
UC Cooperative Extension, Stanislaus County, 3800 Cornucopia Way, Ste. A, Modesto, CA 95358, (209) 525-6800, jrijal@ucdavis.edu

Objectives

- Conduct BMSB detection and seasonal monitoring in almond orchards
- Characterize the temporal effect of BMSB feeding on developing almonds using cage studies
- Evaluate insecticides against BMSB

Background

Brown marmorated stink bug (BMSB) is an invasive stink bug species from Asia and has been spread to over 43 U.S. states causing a significant economic burden to growers. In California, we reported the first find of the BMSB population in a commercial almond orchard in Stanislaus county in 2017. Since then, the reproducing populations of BMSB have been in several almond orchards in Stanislaus and Merced counties with substantial crop damage in some cases. BMSB has a 'straw-like' mouthpart to pierce into the fruit, release digestive enzymes, and suck the liquified fruit content. BMSB adults overwinter in human-made structures such as houses, barns, shops, and even in woodpiles, and migrate to crops for an extended period beginning in Spring (March-June).

Discussion

Our study in the past two years suggested that BMSB can do damage to all stages of the fruit development in almonds and that they are present in the orchard throughout the season. Early season feeding causes nut abortion, resulting in substantial nut drop. Mid-to-late season infestations result in gummy, darken-spots, or dimpled kernels. Although there are other true bug pests such as leaffooted bug, native stink bugs can be present in almond orchards as well. The presence of BMSB throughout the season is often in high numbers which makes the BMSB a risky pest. Some varieties are more susceptible to the damage than others. For example, Monterey, Fritz showed a higher level of damage compared to Padre, Wood colony varieties; Nonpareil is in between, but no variety is immune to BMSB damage. We found that the sticky panel trap with BMSB lure is effective in detecting BMSB adults, and is currently in use by several pest control advisers in the northern San Joaquin Valley. Our study also found that BMSB infestation was much higher in the edge of the orchard compared to the internal part. We recommend that growers and pest control advisers (PCAs) pay close attention to BMSB activities in orchards and monitor the BMSB population at least in orchards in the area with high risks (i.e., area with a known infestation, near to highways; near to other hosts (e.g., tree of heaven). We evaluated insecticides against BMSB using laboratory bioassays, and found that bifenthrin and lambda-cyhalothrin are effective.

Future studies include the continuation of monitoring of BMSB activity in San Joaquin Valley and evaluate the BMSB feeding damage in almonds by addressing questions related to external factors such as the presence of other hosts near to the orchard, varietal difference, edge vs. internal damage, use of the potential of attract-and-kill, and insecticide evaluations.

Project Cooperators and Personnel: Frank Zalom, UC Davis

Survey of Navel Orangeworm Control Strategies

Project Leaders: Phoebe Gordon[1] and Houston Wilson[2]
[1]UC Cooperative Extension, 328 S Madera Avenue, Madera CA 93637 (559) 675-7879 ext. 7209, pegordon@ucanr.edu
[2]Dept. of Entomology, UCR, Kearney REC, 9240 S Riverbend Ave, Parlier, CA 93648 559-646-6519 hwilson@ucanr.edu

Objectives

Deploy the survey at UCCE regional meetings across the state: four almond meetings, three walnut meetings, and two pistachio meetings. The almond meetings were to be held in Yuba, Colusa, San Joaquin, and Kern Counties; the walnut were held in Tehema, San Joaquin, and Tulare Counties, and the pistachio meetings were to be held in Tulare and Madera Counties.

- Identity barriers to understanding or adoption to improve outreach and use of effective IPM tools for NOW.

Background

Navel orangeworm (NOW) (*Amyelois transitella*) is the most significant insect pest of almonds and pistachios and can be a pest of walnuts. This insect can damage nuts directly, and NOW damage has been positively correlated with aflatoxin contamination of nuts. Aflatoxin is a human carcinogen that is heavily regulated in key export markets for these commodities. Researchers have developed multiple tools for managing NOW, including monitoring and well-timed insecticide sprays, mating disruption, timely harvest and sanitation (i.e. removal of leftover 'mummy' nuts after harvest). However, anecdotal evidence suggests that adoption of the various strategies has been unequal. Thus, understanding industry-wide adoption of the various 'tools' in the IPM toolbox, as well as barriers to adoption, is of critical importance in order to develop more targeted research and/or education in order to improve adoption of all tools for effective NOW control.

We deployed this survey at several UCCE county extension meetings during the winter of 2020.

Initial Results

This project was jointly funded by the ABC, CPRB, and the CWB. The reported results are only for attendees at almond-focused meetings; the full results will be made available in the future.

Due to the COVID 19 pandemic, we were unable to deploy the survey at the Kern County almond meeting and the Madera County pistachio meeting. This survey is likely the beginning stage of our attempts to understand industry-wide control of NOW, and we will reevaluate future steps once the results have been fully analyzed.

Orchard owners and PCA/consultants were the most common respondents. More than 50% of respondents reported evaluating mummy nuts for infestation, using egg traps and pheromone traps every year, and more than 70% of respondents reported using biofix and degree day calculations every year. Less than 10% never evaluated mummy nuts for infestation, and approximately 30% of respondents reported never using egg traps or pheromone traps. Twenty percent reported never using biofix and degree day calculations. Only 20% of almond respondents reported using mating disruption regularly, and more than 60% have never used it. Approximately 80% reported sanitizing their orchards every year, and 90% use pesticides in their NOW control program. Orchard access was the most cited barrier for not doing winter sanitation, with over 50% of respondents selecting this as a barrier. Thirty percent reported cost of labor/equipment as a concern. Respondents could select more than one barrier to adoption. Forty percent of respondents reported that the cost of mating disruption products was a barrier to adoption, while approximately 35% reported that the efficacy of mating disruption was unclear. As a comparison, less than 20% cited cost as a barrier to adopting pesticide use, and less than 10% cited unclear efficacy. Over 90% of respondents at almond meetings reported frequently relying on PCAs and consultants as sources of NOW management information.

Project Cooperators: Jessica Maccaro, UC Riverside

Herbicide Performance and Crop Safety Evaluations in Conventional Almond Production Systems: Field Research and Extension Support

Project Leader: Bradley D. Hanson
Department of Plant Sciences, University of California, Davis, Robbins Hall, Davis, CA 95616
(530) 752-8115, bhanson@ucdavis.edu

Objectives

- Evaluation and comparison of registered preemergence and postemergence herbicides in commercial almond orchards
- Evaluate effective sequential herbicide programs for control of glyphosate-resistant summer annual grasses.
- Determine emergence timing and biology of threespike goosegrass, a growing challenge in several almond production regions, to inform better management practices.

Background

The overarching goal of our research and extension program (http://hanson.ucdavis.edu/) is to contribute to the economic and environmental sustainability of perennial cropping systems through effective weed management. Real world weed management and herbicide performance data form the backbone of an extension effort aimed at providing information to growers, Pest Control Advisors, Farm Advisors, the crop protection industry, and the regulatory community. This type of research is ongoing and is relevant to the greater than 99% of almond growers that utilize herbicides for weed management.

Discussion

The first objective focuses on the control of winter annual weeds with preemergence and postemergence herbicides in research station and commercial almond orchard locations in the Central Valley. Data and experience from this ongoing objective is shared via cooperative extension meetings, consultations, and guides industry and regulatory discussions.

The second objective addresses the need for season-long weed management in almond production systems. With the widespread presence of herbicide-resistant summer weed biotypes in California almond orchards, postemergence herbicide efficacy is reduced. Experiments were conducted to address the underlying hypotheses related to sequential application performance. Data have been presented in extension formats and are being analyzed for scientific reporting. Field-scale evaluations have been put on hold due to travel restrictions related to global health crisis.

The third objective addresses the need for up-to-date information on the biology and control of tough summer weed species, particularly threespike goosegrass, which is suspected to be resistant to glyphosate and is a rapidly expanding problem in tree nuts in California. As this species is a relatively new problem in California orchards and is not common elsewhere in the US, basic information on the biology of this species and effective control strategies are needed. This research has been completed and data are being analyzed and prepared for scientific reports and extension presentations.

Project Cooperators and Personnel: Seth Watkins, Katie Martin, Steven Haring, Drew Wolter, Matt Fatino, Adewale Osipitan, Caio Brunharo, and Gale Perez, UC Davis

Evaluation of the Effects of PHI on Detection of Glyphosate or Glufosinate in Harvested Almonds

Project Leader: Bradley D. Hanson
Department of Plant Sciences, University of California, Davis, Robbins Hall, Davis, CA 95616
(530) 752-8115, bhanson@ucdavis.edu

Objectives

- Determine to what extent do early-falling almonds and relatively short preharvest intervals affect the potential for detection of the postemergence herbicides glyphosate and glufosinate in nuts after harvest
- Determine whether glyphosate transfer from treated soil to harvested almonds can occur through mimicked physical contact

Background

There are occasionally residues of the postemergence herbicides glyphosate and glufosinate detected in almonds in the market. These both represent risks to the industry due to public concerns about pesticide residues, especially glyphosate, as well as potential challenges related to import tolerances in the EU and other markets.

However, potentially relevant to pesticide detections in harvested almonds, both herbicides can be used during the “preharvest burndown” application when the grower is preparing the orchard floor for the subsequent almond harvest. In this herbicide application window, growers typically treat the entire orchard floor to kill any vegetation and then closely mow the orchard after the weeds are desiccated. The goal of this practice is to remove or destroy plant material that could interfere with harvest operation, especially sweeping and pickup of nuts.

Most glyphosate herbicides have a preharvest interval (PHI) of 3 days while glufosinate herbicides have at PHI of 14 days. While most almonds are mechanically shaken from the tree during harvest operations, some nuts natural fall due to wind, orchard machinery traffic, or other factors.

The route of glyphosate or glufosinate onto (or into) almonds is not well understood. The research in this proposal will examine the impacts of longer PHI on analytical detections of the two herbicides harvest almond samples at the field scale and directly assess the transfer of glyphosate from treated soil to almond hulls, shells, and kernels at the lab scale.

Discussion

Pilot studies for the laboratory components of this research (using radio-labeled glyphosate) were initiated in late 2019 but were stopped by COVID-19 related campus closures. This is anticipated to restart in summer 2020.

The field component of this research, which is the core of the project, is scheduled to begin in summer 2020. This portion of the work is expected to be accomplished under university and governmental pandemic safety guidelines.

Project Cooperators and Personnel: Katie Martin, Seth Watkins, UC Davis; Franz Neiderholzer UCCE

Evaluation of Potential Pest Pressure Reduction for Early Off-ground Harvest

Project Leaders: Patrick Brown[1] and Sat Darshan S. Khalsa[2]
Department of Plant Sciences, University of California, Davis, One Shields Ave., Davis, CA 95616
(530) 752-0929, [1]phbrown@ucdavis.edu [2]sdskhalsa@ucdavis.edu;

Objectives

- Understand the impacts of early almond harvest on navel orange worm incidence
- Assess the effects of early harvest on hull rot incidence
- Evaluate the impacts of early harvest on shaker efficacy

Background

The California almond industry aims to reduce dust during almond harvest by 50%. One option is the transition to off-ground harvest up to 3 weeks earlier than standard on-ground harvest. Transition to early off-ground harvest would reduce harvest passes and dust. Additional benefits may include reduced pest pressure from hull rot (HR) and navel orangeworm (NOW) leading to lower dependence on pesticides, and increased marketable yield.

In the summer of 2020, we examined the effects on NOW and HR incidence of drying down and harvesting almonds approximately three weeks earlier (EH) than standard harvest compared to standard harvest (SH) timing. This research was carried out at field sites in Davis and Denair, CA. Both trials locations were randomized complete block designs. During 2-3 months leading up to harvest, we took weekly or biweekly midday stem water potential (SWP) measurements on three trees per plot, totaling 12 trees per treatment (24 trees total). We used SWP data to determine an optimal irrigation schedule for tree dry down and harvest for the EH and SH treatments.

Data collected around and after both EH and SH included hull split at harvest and HR incidence. NOW incidence, yield, and shaker efficacy data collection is ongoing. Preliminary results show that early harvest may be a strong control method for HR in almond orchards.

Discussion

During EH at the Denair site, there was no incidence of HR on any of the trees. During SH, the number of HR strikes was greater for the SH trees, averaging 7 strikes per tree with a range of 2 to 12 compared to an average of 4 strikes per tree ranging from 1 to 6 for EH trees.

Each treatment tree was rated for hull split prior to harvest, with an average of 85.6% hull split and a range of 69-99% on EH before the early harvest, and an average of 99.3% and range of 96-100% hull split on SH harvest trees before the standard harvest. Hull split suture size on EH trees at harvest appeared to be much smaller than those on SH trees at harvest. This factor may contribute to HR control during EH, as smaller hull split suture size is correlated with lower HR incidence.

The research team is currently cracking open subsamples of nuts from EH and SH trees and inspecting for NOW presence and damage. The number of almond kernels damaged by NOW will give us an estimate of the percentage of marketable almonds. The percent NOW damage from our EH and SH sampling will be applied to grower collected yield for those orchards. Data will be reported as marketable and cull yields in lb kernel per acre.

A second shake of EH and SH trees will be conducted at the Denair site in October 2020. The total dry weight of the nuts left in the tree will be determined. As NOW overwinters on nuts left on the tree, this information about possible differences in shaker efficacy between EH and SH treatments will provide information about potential additional implications of early almond harvest on NOW control.

Project Cooperators and Personnel: Evelyn Smith and Ken Shackel, UC Davis; Brent Holtz, UCCE - San Joaquin

Non-Fumigant Approaches and Diagnostics for Orchard Replacement and Soilborne Disease Management

Project Leader: Greg Browne
USDA-ARS Crops Pathology and Genetics Research Unit, Department of Plant Pathology, UC Davis, One Shields Ave., Davis, CA 95616; (530) 754-9351, gregory.browne@usda.gov

Objectives

- Optimize preplant soil remediation strategies based on almond byproducts and anaerobic soil disinfestation (ASD) for replant problems.
- Relate benefits of ASD and soil fumigation to physicochemical and microbial variables.
- Determine causal species and manageable factors associated with incidence of *Phytophthora* in almond orchards.
- Improve diagnostics for prescriptive management of almond replant problems.

Background

Development of young, replanted almond orchards can be compromised by soilborne diseases, including: Prunus replant disease (PRD) (growth suppression mediated by a soilborne complex in successive plantings of *Prunus*); plant parasitic nematodes (root damage caused by several species), and Phytophthora crown and root rots (caused by >10 species of *Phytophthora*). Soil fumigation is used to manage the former two issues, whereas integrated cultural, chemical, and genetic approaches are most effective for the latter. Due to increasing restrictions on soil fumigation, we are working with ASD as an alternative. We previously found that ASD can be as effective as optimal soil fumigation for management of PRD but continue work to optimize ASD for affordability. For Phytophthora problems we are focused on the causal species, which seem to change over time and with orchard practices; identification of the specific pathogens is essential for effective fungicide and rootstock testing. Our work with replant diagnostics is designed to improve decision-making about whether an orchard being replaced would benefit from preplant soil fumigation or other remediation strategies.

Progress and Discussion

ASD. In 2020 we established a new ASD trial at Kearney Agricultural Research and Extension Center (KARE, Parlier), and we continued monitoring orchard performance and yields in ASD trials initiated in 2017 and 2018. The 2020 trial tests ASD treatments driven by rice bran (RB) and ground almond hull and shell mixture (GAhs); after soil incorporation, each substrate was used alone as a soil amendment and in combination with the full complement of ASD components (drip irrigation under tarp, 5 wks.). Additional fertilization treatments are included in KARE trials and are being established with and without preplant soil fumigation to examine soil nutrient interactions with PRD. Growth and yield data from the previous trials indicated that full ASD with RB is the most dependable fumigation alternative, but ASD based on GAhs matched the performance of RB in some trials. The best ASD treatments approached, but did not always match, the benefit of shank fumigation with 1,3-dichloropropene plus chloropicrin.

Phytophthora. In 2020, we diagnosed serious Phytophthora disease losses in 8 orchards among 3 counties, obtaining 45 isolates representing 5 different *Phytophthora* species. The species were identified by diagnostic DNA sequencing, and selected isolates are being used for the determination of baseline fungicide sensitivities (Adaskaveg Lab) and for field evaluations of rootstock resistance at KARE (Browne Lab). Observations suggested: 1) that dual line drip may be facilitating infection by *Phytophthora* when left too long by tree trunks, and 2) a resurgence of canker disease in Kern Co. associated with intense late-spring rain(s).

Replant Diagnostics. Our replant diagnostics efforts were hampered by pandemic restrictions. Soils from three orchards slated for replacement were sampled and the orchards were replanted, including fumigated and non-fumigated areas. Also, two large operations, one in Tulare and Fresno Cos., and another in Kern Co., agreed to soil sampling in support of the research. The latter operation agreed to host fumigated and non-fumigated plots. Also, we submitted a manuscript that documented use of our microbial and physicochemical data from 25 replant soils in a machine learning approach (Random Forests modeling) to discriminate between the soils that induced PRD (19 of them) those that did not (6), suggesting viability of the diagnostics objective.

Project Cooperators and Personnel: USDA-ARS: N. Ott, A. Khan, A. Poret-Peterson, K. Sanchez, M. Gillis; UCR: J. Adaskaveg, H. Forster; UCD: A. Hodson; UCCE: M. Yaghmour, B. Holtz, P. Gordon, M. Culumber, F. Niederholzer, and F. Trouillas

Biology and Management of Almond Scab and Alternaria Leaf Spot

Project Leader: J. E. Adaskaveg
Dept. of Microbiology and Plant Pathology, UC Riverside, CA 92521 (951) 827-7577 jim.adaskaveg@ucr.edu

Objectives

- Determine population composition of the scab pathogen *Fusicladium carpophilum* and occurrence of sexual reproduction Alternaria alternata and the scab pathogen.
- Evaluate new and registered fungicides for their efficacy against scab and Alternaria.
- For scab management, evaluate the effect of dormant and in-season applications.
- Establish baseline sensitivities, monitor for fungicide sensitivity shifts in the pathogens, and characterize mechanisms for resistance against SDHI and DMI fungicides.

Background and Discussion

Scab caused by *Fusicladium carpophilum* and Alternaria leaf spot caused by *Alternaria alternata* and *A. arborescens* are economically important summer diseases of almond, especially in locations with high humidity and poor air circulation. Severity of Alternaria leaf spot is expected to be higher but lower for scab in 2020. For scab this is because of the relatively dry spring during bloom and early stages of fruit development (petal fall period). In contrast, the forecasted warm environments, cool nights, and scheduled irrigations to keep up with evapotranspiration should provide favorable conditions for dew formation and consequently for Alternaria leaf spot. *Alternaria* spp. are ubiquitous and typically occur in high-production orchards that are planted densely and have high inputs (e.g., water, nitrogen). The result is low air movement and consistent wetness periods from dew. Many of the current pollinators are highly susceptible to Alternaria leaf spot (e.g., Monterey, Carmel).

We plan to continue our evaluations of the pathogens from different areas in the state. *Fusicladium* and *Alternaria* spp. appear to only reproduce asexually by conidia in California. Based on molecular analyses, however, two species of *Fusicladium* may exist sympatrically and occupy the same niche on almond tissues and may even be present in the same lesions. Additional sampling is planned in the summer of 2020. Effects of wetness and drying on spore germination on isolates of *F. carpophilum* are planned in laboratory studies.

In dormant fungicide trials on almond, the FRAC codes (FC) M3 (ziram), M4 (captan), and M5 (chlorothalonil) were applied in 3% oil mixtures without any phytotoxicity. Dormant treatments delay scab inoculum production on overwintering twig lesions and are an anti-resistance strategy (a smaller population is exposed to selection processes) that align in-season, springtime treatments for scab, Alternaria leaf spot, and rust. Disease incidence will be evaluated later this summer.

Field trials to evaluate in-season applications of organic and conventional fungicides were established in early May for Alternaria leaf spot and scab. Emphasis was placed on mixtures of different FCs to prevent selection of resistance in target populations. Boscalid, fluopyram, fluxapyroxad, isofetamid, and penthiopyrad are registered, and pydiflumetofen, pyraziflumid, and an experimental are in development. We will collect diseased almond leaves and we plan to continue our genetic studies in *Alternaria* spp. in SDH subunits B, C, and D that correspond with resistance to selected SDHI fungicides (FC 7). The highest incidence of resistance occurs against boscalid, fluxapyroxad, penthiopyrad, and pyraziflumid. Only moderate resistance (EC_{50} <0.5 ppm) has been detected to fluopyram and low resistance to the new pydiflumetofen. We identified cross resistance patterns among SDHI fungicides that correlated with mutations SDHB-H277Y, SDHB-277L, or SDHC-H134R (most common). Resistance to newer SDHI sub-groups is detected before commercial introduction. Thus, variants most likely pre-exist, and non-detrimental mutations occur without significant fitness penalties to the pathogen.

DMI fungicides (FC 3) including the new Cevya are highly active against *Alternaria* isolates and against isolates molecularly identified as *F. carpophilum*. A relatively narrow range of sensitivity was observed in baseline studies with DMI fungicides. Outliers were detected in laboratory studies, however, these fungicides remain highly effective in managing both diseases in the field.

In our two 2020 trials each on Alternaria leaf spot and scab, Regev, Cevya, the experimentals V-10424 and 10570, as well as the pre-mixtures Miravis Top, Miravis Prime, and UC-2 are being evaluated and compared to other mixtures (e.g., Fervent, Fontelis + Teb, Luna Experience, and Quadris Top).

The four trials also include organic compounds including natural products (e.g., plant extracts, SARs), food grade treatments exempt from tolerance (e.g., potassium sorbate/sodium lauryl sulfate), and experimental biocontrols (e.g., *Bacillus amyloliquifaciens*).

Project Cooperators and Personnel: H. Förster, D. Thompson, Y. Luo, D. Cary (UC Riverside); L. Milliron and F. Niederholzer, (UCCE Butte Co. and UCCE - Colusa Co., respectively), and L. Wade (Grow West).

Biology and Management of Almond Brown Rot, Jacket Rot, Shot Hole, Rust, and Hull Rot

Project Leader: J. E. Adaskaveg
Dept. of Microbiology and Plant Pathology, UC Riverside, CA 92521 (951) 827-7577 jim.adaskaveg@ucr.edu

Objectives

- Evaluate new conventional and organic compounds for their spectrum of activity, systemic action, and persistence in managing brown rot, jacket rot, shot hole, gray mold, rust, and hull rot.
  - Pre- and post-infection - brown rot
  - Natural incidence – all disease listed
  - Cultural and nutritional strategies for hull rot management
- Establish baseline sensitivities of fungal pathogens against new fungicides and determine shifts in fungicide sensitivity.
- Evaluate almond genotype susceptibility to foliar diseases that develop naturally in an almond variety trial at UC Davis.

Background and Discussion

In 2020, we set up trials to evaluate new treatments against major foliar and fruit diseases of almond in California in the laboratory and field. We included the newly registered Cevya (FRAC Code - FC 3) and Fervent (FC 3/7), as well as the experimentals pyraziflumid (FC 7), Miravis Prime (FC 7/12), and Miravis Top (FC 3/7). Numbered products with FCs partially or not disclosed included UC-2 (FC 3/?), F4406-3 (FC 7/?), EXP-19A, V-10424, and GWN-10570. Among biologicals, we tested Dart, Cr-7, GWN-10474, Ecoswing, Double Nickel, and LifeGard. These latter compounds are natural products, biological agents, or are naturally derived systemic acquired resistance or SAR compounds. These were compared to registered pre-mixture compounds. Under California conditions, the availability and use of fungicides with different modes of action in rotation or mixture programs will prevent the selection and build-up of resistant pathogen populations. Furthermore, the use of pre-mixtures and tank mixtures expands the spectrum of activity allowing management of several diseases with a single treatment.

Incidence of brown rot, shot hole, and Botrytis gray mold was generally low in the spring of 2020 due to low rainfall and cool to warm temperatures during bloom. For brown rot management on cvs. Drake and Wood Colony, all conventional fungicides were highly effective, reducing the incidence compared to the untreated controls of each cultivar. For shot hole on cv. Drake, evaluations are ongoing.

In evaluation of natural host resistance to diseases in our variety block, data were obtained for brown rot incidence to date, and other diseases such as rust and shot hole (incidence and severity) are pending for 23 varieties or genotypes in 2020. Other diseases will be recorded if they develop as the season progresses.

For hull rot, we will continue to evaluate orchards for the presence of *R. stolonifer, Monilinia* spp., and *Aspergillus niger.* In previous years, hull rot was predominantly caused by *R. stolonifer* in Butte, Colusa, Sutter, San Joaquin, and Stanislaus Co., *M. fructicola* was found mostly in Stanislaus Co., whereas *A. niger* was much less commonly present in these central to northern regions of the state.

Studies on the management of hull rot are planned in orchards where *Rhizopus stolonifer* is the main pathogen. Fungicides containing FC 3, 7, 11, 19, or undisclosed experimentals, as well as Cinetis and the alkalizing foliar fertilizer dipotassium-phosphate (di-KPO_4) will be continued. The goal of the nutrients like Di-KPO_4 and Cinetis are to possibly neutralize fumaric acid that is produced by hull rot pathogens and is responsible in part for dieback symptoms of branches. Additionally, they are to balance the overuse of nitrogen with the two other macronutrients potassium and phosphate. Phytotoxicity was not observed in any treatments in previous years.

Hull rot management should always include cultural methods including proper nitrogen fertilization and irrigation practices. A two-spray program is suggested for hull rot at pre-hull split in early/mid-June (targeting *Monilinia* pathogens) and at early hull split (targeting the *Rhizopus* and possibly the *Aspergillus* pathogen). Soil applied treatments to reduce inoculum and stimulate SAR effects will be continued with Serenade ASO and LifeGuard. In previous years there was sometimes a trend for improving hull rot control.

In baseline sensitivity studies, isolates of *M. laxa* were highly sensitive to the new SDHIs pyraziflumid, pydiflumetofen, and isofetamid. Other new SDHIs (e.g., GWN-10570) will be evaluated in 2020. A much wider range in sensitivities was determined for *B. cinerea,* and sensitivity characteristics of isolates were similar for pydiflumetofen and pyraziflumid. This indicates that cross resistance is present among SDHI sub-groups and that SDHI fungicides should always be rotated with different FRAC codes. Other new modes of action (EXP-19A) will also be included in our baseline studies in 2020.

Project Cooperators and Personnel: D. Thompson, H. Förster, D. Cary, UC Riverside; R. Duncan, UCCE - Stanislaus Co.; B. Holtz, UCCE - San Joaquin Co.; M. Yaghmour, UCCE Kern Co.; and L. Wade, GrowWest.

Biology and Management of Bacterial Spot of Almond in California

Project Leader: J. E. Adaskaveg
Dept. of Microbiology and Plant Pathology, UC Riverside, CA 92521 (951) 827-7577 jim.adaskaveg@ucr.edu

Objectives

- Disease epidemiology
  - Pathogen identification and characterization of populations - genetic variability
  - Minimum temperature for growth in selected wetness periods
  - Field evaluation of temperature threshold under different wetness conditions and fruit stages
- In vitro sensitivity of *Xap* against new food grade biobactericides (e.g., nisin, poly-L-lysine)
- Develop field management programs
  - Dormant applications in winter
  - Spring-time applications with kasugamycin, nisin, Ɛ-poly-L-lysine, Serenade ASO, Blossom Protect, and others

Background and Discussion

Bacterial spot caused by *Xanthomonas arboricola* pv. *pruni* (*Xap*) continues to be a problem and has been reported in Butte, Colusa, Kern, San Joaquin, Merced, Madera, and Stanislaus, Co. mostly on cv. Fritz, but also on Nonpareil, Aldrich, Butte, Carmel, NePlus Ultra, and Price. Wet springs are highly favorable for the disease, but it can develop throughout the growing season in orchards where foliage is frequently wet from dew, rain, or high-angle sprinklers. With high rainfall in May, outbreaks occurred in the spring of 2019. We detected no copper resistance in the pathogen populations during surveys in 2015 to 2019. There was little genetic diversity among strains which may indicate a recent introduction of *Xap*.

In the spring of 2019, the pathogen was again isolated from overwintering symptomatic fruit mummies and fruit peduncles, but also from healthy flower buds, emerging leaves, and spurs close to infected mummies in the tree. This confirmed that mummies and peduncles remaining on the tree are the major primary inoculum source. In the spring of 2020, field inoculations were done on cv. Fritz, and flowers and developing fruit were bagged for selected times to evaluate the effect of different wetness periods on disease development. Data are pending, but disease after flower inoculation was first observed 8 weeks after inoculation. Still, it may be possible that flower inoculations may not directly result in an infection, but surviving inoculum later infects fruit. It has been suspected that the *Xap* pathogen is able to produce cankers, but these have not been observed to date in California. Twig inoculations using different methods were performed in early spring, and these twigs are being observed for canker development.

The efficacy of dormant and in-season applications with bactericides on the management of bacterial spot was evaluated by us over several seasons. Additionally, we have established that among in-season treatments, full bloom and petal fall treatments are most effective in reducing the disease. In the spring of 2020, small-scale field studies were conducted at UC Davis on cv. Fritz. Furthermore, two field trials were done with three applications that started at full bloom and continued through petal fall. Treatments applied include copper, Kasumin, Mycoshield/ FireLine, the food preservatives nisin and poly-L-lysine, Serenade, and several new bactericides. Selected additives including zinc nitrate and capric/caprylic acids were used for some treatments. These trials will be evaluated in June. Our goal is to find effective copper alternatives in addition to Kasumin, as well as treatments that could be used in organic production. Some of the new treatments are not highly effective by themselves, and therefore we are testing different additives. In previous trials, the most effective and consistent treatments included copper alone or mixed with mancozeb; kasugamycin mixed with mancozeb or copper; and oxytetracycline. Among organic products, Blossom Protect/buffer and Serenade ASO also significantly reduced the disease.

Based on our results from several years of field studies, in wet winter/spring seasons, a delayed dormant bactericide application to reduce inoculum should be followed by bloom and petal fall treatments around rainfall events and rising temperatures to prevent new infections. Bloom applications with copper cause minimal phytotoxicity. In drier spring seasons, only a dormant treatment or bloom/petal fall applications may be necessary for effective disease management. Kasugamycin is pending registration on almond. Integration of different compounds should reduce the potential of resistance to any one mode of action and overuse of copper that may cause phytotoxicity.

Project Cooperators and Personnel: H. Förster; R. Duncan, UCCE - Stanislaus Co.; B. Holtz, UCCE - San Joaquin Co.; L. Wade, Grow West.

Control and management of the newly introduced destructive wood decay pathogen, *Ganoderma adspersum*.

Project Leaders: Dave Rizzo[1] and Bob Johnson[2]
[1]Dept. of Plant Pathology, UC Davis, One Shields Ave., Davis, CA 95616 (530) 754-9894, dmrizzo@ucdavis.edu
[2]Dept. of Plant Pathology, UC Davis, One Shields Ave., Davis, CA 95616 bobjohnson@ucdavis.edu

Objectives
1. Confirm infection pathway and process
   a. Develop spore-based inoculation protocols for *G. adspersum*
   b. Determine type of wound conducive to *Ganoderma* spore infection
2. Access rootstocks for susceptibility to infection and decay
3. Increase capacity of private diagnostic labs to identify *G. adspersum*
4. Provide outreach and extension

Background
Ganoderma adspersum was first identified in Kings County, in 2016, as the cause of butt rot of almond resulting in extensive tree failure from loss of structure and breakage at the ground level. *G. adspersum* is more aggressive and destructive than endemic California *Ganoderma* species previously identified in almond. More than 75 orchards infected with *G. adspersum* have been confirmed across Kern, Tulare, Kings, Fresno and Madera counties. Based on discussions with PCAs, growers, and UCCE personnel, these orchards likely represent only a small fraction of total infected acreage and distribution likely extends further north into Merced county.

Impacts of *G. adspersum* vary between orchards but are typically quite severe and have led to the removal of orchards as young as 9 years. Substantial tree losses in orchards as young as 4 years have been observed. *Ganoderma* spores are airborne and the site of initial infection appears to be wounds near, or below, the soil line that are consistent with potential shaker damage. All confirmed reports have been on Nemaguard rootstock. However, studies in wood blocks and on young, potted trees found that Nemaguard is no more susceptible to decay than other available rootstocks. The reason for the apparent susceptibility of Nemaguard is yet to be determined. It is possible that physical properties of the wood and bark might make it more easily wounded than other rootstocks.

Currently, we are limited to inoculating trees with *Ganoderma*-colonized wood dowels. Since spores are most likely the primary inoculum source in the field, development of protocols for spore inoculation of trees is required. *Ganoderma* spores are produced on the underside of the fruiting body. Fruiting bodies may be produced on trees from 0.5 to 5 years after infection, although specific timing and conducive conditions are largely unknown. Laboratory grown spores are necessary for understanding inoculum dispersal, movement, and possible control strategies.

Discussion
Studies are underway to examine the propensity of spores to percolate down into the soil matrix and contact potential below ground wounds. Preliminary results show observable spores at least 8 to 12 inches below the soil surface in core samples collected from infected orchards.

Ganoderma fruiting bodies of several species have been successfully produced in the laboratory. However, the conditions necessary for *G. adspersum* sporulation have not yet been achieved. Studies altering environment, light, and growth media are ongoing. It is possible that *G. adspersum* only sporulates on live trees, so 15 potted 5-year old almond trees were inoculated in December 2019. As of April 2020, fruiting bodies have yet to form.

Potted trees of six almond rootstocks are ready for spore inoculations once laboratory-produced spores are obtained. Selective growth media that distinguish between *G. adspersum* and other wood decay fungi found in almond are underway. Problematic *Ganoderma* species show clear growth differences on media amended with gallic acid. To ensure accurate diagnostics, trials with larger amounts and range of fungi are ongoing.

Project Cooperators and Personnel: Daisy Hernandez, Neil McRoberts and Tom Gradziel, UC Davis; Franz Niederholzer, UCCE-Yuba/Sutter/Colusa Counties; Roger Duncan, UCCE- Stanislaus County

Management of Trunk and Scaffold Canker Diseases of Almond

Project Leaders: Florent P. Trouillas[1, 2] and Leslie A. Holland[1,2]
[1]Dept. of Plant Pathology, UC Davis, One Shields Ave., Davis, CA 95616 (559) 646-6566, flotrouillas@ucanr.edu
[2]UC Kearney Agricultural REC, 9240 S. Riverbend Ave, Parlier, CA 93648 (559) 646-6570, lholland@ucdavis.edu

Objective

- Provide effective control strategies for the management of almond canker diseases

Background

Trunk and scaffold canker diseases (TSCD) constitute the major cause of tree death in almond orchards in California. The broad cultivation of almond combined with intensive production practices including mechanical harvest and repeated pruning have contributed to a recent increase in canker disease occurrence. Sampling of orchards from 2015-2018 revealed five major canker diseases of almond. The main diseases are Botryosphaeriaceae and band cankers, and Ceratocystis canker. Three newly identified almond canker diseases include Cytospora, Eutypa and Collophora cankers.

Pruning wounds serve as the main entry sites for infection by canker pathogens, and protection of pruning wounds following primary and secondary scaffold selections is recommended to prevent early infection and ensure almond tree longevity. Pruning wound protection and susceptibility trials were established to determine the best fungicidal products as well as best pruning timing to lower risks of infection. From 2016-2018, eight independent field trials were conducted to test the efficacy of 26 fungicidal products including fungicides, paint, and biocontrols to protect pruning wounds from infection by canker pathogens. During two consecutive dormant seasons (2017/18 and 2018/19), four independent field trials were conducted to investigate the seasonal susceptibility of pruning wounds to infection, as well as the duration of pruning wound susceptibility in order to determine pruning periods with reduced risk of infection.

Discussion

Results revealed that several products provided great efficacy to protect pruning wounds. Topsin M (FRAC 1) and Vintec (*Trichoderma atroviride* SC1) provided the best protection (70 to 100% disease control) against all canker pathogens. Merivon (FRAC 7/11) also was highly efficient. Paint applied for pruning wound protection provided limited efficacy. Pruning wound susceptibility field trials were conducted in the field to determine the month (Sept, Oct, Nov, Dec, Jan) and duration (0, 1, 2, 3, 5, 8 weeks) when pruning wounds are most susceptible to infection by canker pathogens. Both the 2017/18 and 2018/19 trials indicated that fresh pruning wounds (inoculated immediately after pruning) in September through January were the most susceptible to pathogen infection in comparison to wounds inoculated after 1 to 8 weeks after pruning. Overall pruning wound susceptibility declined substantially after 2 weeks following pruning and continue to decrease overtime.

September had the highest infection rate across all wound ages in all trials. January had the lowest infection rate overall and the shortest duration of wound susceptibility. Fall and early winter (Sept through Dec) pruning appeared more conducive to infection by canker pathogens than the month of January. This work suggests that one application of a pruning wound protectant such as Topsin M or Vintec following pruning in January should significantly reduce risks of infection of pruning wounds by canker pathogens. Topsin M has received a 2(ee) recommendation for use as pruning wound protectant on almonds in California. Vintec is currently in the registration phase for California almond.

Project Cooperators and Personnel: Mohamed Nouri, and Brent Holtz UCCE – San Joaquin County; Franz Niederholzer, UCCE – Sutter-Yuba Counties; David Haviland and Mohammad Yaghmour – UCCE – Kern County.

Epidemiology and Management of Phytophthora Root and Crown Rot of Almond in California

Project Leaders: James E. Adaskaveg[1] and Greg Browne[2]
[1]Dept. of Microbiology and Plant Pathology, UC Riverside, Riverside, CA 92521 (951) 827-7577, jim.adaskaveg@ucr.edu
[2] USDA, UC Davis, 347 Hutchison Hall, Davis, CA 95616 (530) 754-9351, gtbrowne@ucdavis.edu

Objectives

- Evaluate the in vitro toxicity of new fungicides against *Phytophthora* species from almond
- Evaluate new fungicides for the management of root rot and crown rot of almond and compare to the registered fungicides.
- Evaluate for phytotoxicity and tree growth

Background and Discussion

Phytophthora root rot and crown rot of almond can be caused by several species of *Phytophthora* including *P. cactorum, P. cambivora, P. cryptogea*, *P. megasperma*, and *P. niederhauseri*. Additionally, the newly described, taxon *P.* sp. ax. was identified to be highly virulent on almond, causing a rapidly progressing crown rot and tree death. We determined that *P. niederhauseri* and *P.* sp. ax. have a high temperature optimum of 30°C and therefore, will be most damaging during the warmer times of the year. In our inoculation studies with five species, *P. niederhauseri* and *P.* sp. ax caused the largest and *P. syringae* the smallest stem lesions. Phytophthora diseases are widely distributed and may cause high losses in newly planted orchards. Disease development depends in part on the rootstock and the species of *Phytophthora* involved, but the disease is most severe in soils with poor drainage that are waterlogged. The pathogens survive as oospores, chlamydospores, and/or hyphae in plant debris in the soil, depending on the species. Under favorable conditions, hyphae start growing or chlamydospores and oospores germinate to produce sporangia that contain zoospores - the main infective propagules. Zoospores are motile in water, are attracted by plant exudates, and infect feeder roots and the crown.

Management of Phytophthora diseases is by use of tolerant rootstocks, proper irrigation, and use of mefenoxam or phosphonate (e.g., potassium phosphite, fosetyl-Al) fungicides. New fungicides including mandipropamid (Revus), fluopicolide (Presidio), ethaboxam (Intego), and oxathiapiprolin (Orondis) with high activity against *Phytophthora* species have become available for evaluation. They have different modes of action (belong to different FRAC Codes) and will possibly allow for better disease control and fungicide resistance management in almond. Registrants support their registration on almond.

The in vitro activity of the new fungicides against 11 *Phytophthora* species was generally higher than for mefenoxam and potassium phosphite. Oxathiapiprolin had the highest activity with EC_{50} values for mycelial growth inhibition of <0.001 µg/ml. A rather narrow range of EC_{50} values (0.001 to 0.01 µg/ml) was also found for mandipropamid, but for ethaboxam and fluopicolide higher rates were generally needed and the range was wider.

We are evaluating the effectiveness of the fungicides on almond in two field studies at UC Davis. In the first trial planted in 2016 with Nonpareil on Hansen or Nemaguard rootstocks and inoculated with *Phytophthora* spp., two years after the second of two soil applications, 30% of control trees showed gumming cankers on the lower trunk, and many trees were dead. All except one tree treated with Orondis 200OD at 2.4 to 9.6 fl oz/A were healthy and thus, Orondis showed long-lasting, high efficacy. The other treatments were less or not effective and may have to be applied more frequently.

In the second orchard (planted and inoculated in 2017 and treated three times), 34.4% of control trees had trunk cankers, and no disease was observed for Orondis, Revus, and ProPhyt/Ridomil Gold. Disease incidence was 6.3% for Presidio and Presidio-Intego. Incidence for Intego and Ridomil Gold by themselves was not significantly different from the control. Treatments and evaluations will be continued in this orchard.

We also initiated three large studies in commercial orchards in 2019 and 2020. In the first two plots in Yuba Co., new bareroot trees were planted and inoculated at a site not previously cultivated with almonds; whereas in the third plot in Kern Co., trees were replanted in an orchard with a history of Phytophthora crown rot. Results from these studies are expected in late 2020 or 2021.

Thus, new effective fungicides for managing Phytophthora diseases of are being developed for the almond industry. This is important because phosphonate and mefenoxam resistance in *Phytophthora* species has been found in other crops. The new fungicides will have international MRLs, and Orondis and Presidio are now commercially available on other tree crops.

Project Cooperators and Personnel: H. Förster, D. Thompson, W. Hao, and R. Belisle, Department of Microbiology and Plant Pathology, University of California, Riverside.

Detection of Band Canker Pathogens in Young Almond Trees in Nurseries and Orchards and Disease Management

Project Leader: Themis J. Michailides
University of California Kearney Agricultural Research and Extension Center, 9240 South Riverbend Ave., Parlier, CA 93648
(559) 646-6546, tjmichailides@ucanr.edu

Objectives

Continue to evaluate the effects of fungicide treatment on trunks of young almond trees to reduce canker development.

Background

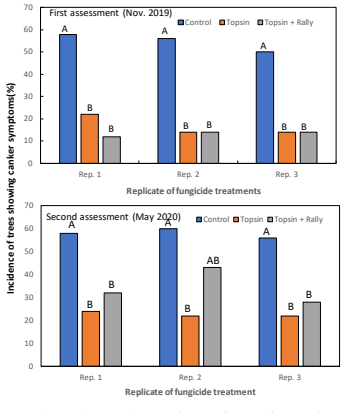
As an important almond disease, band canker has become very severe in the last several years, significantly affecting almond production in California. The causal pathogens of this disease are classified into different fungi species in the family of Botryosphaeriaceae. Through our multi-year research, we determined that these pathogens can exist in plant tissues for long period of time in a latent infection phase until plant and environmental conditions favor disease development and symptom appearance. We also found that in orchards with severe disease, the pathogen can produce huge amounts of pycnidial and ascosporic spores, which may cause new infections under favorable conditions. Both latent infections and new infections could serve as sources to initiate disease epidemics in young plantings.

Since it is difficult to determine latent infection in shoots conventionally, we developed a sensitive quantitative real-time PCR (*q*PCR) approach to accurately quantify six canker-causing pathogen groups in shoots. Thus, we tracked the level of changes of latent infection in shoots of almond trees of different ages. Interestingly, we found an accumulation of latent infections from old to young shoots. We also found that the three predominant populations, i.e. *Lasiodiplodia* spp., *Neofusicoccum* spp. and *Cytospora* spp. varied significantly among sampled almond orchards. We started to investigate the possibility of young trees carrying the pathogens from nurseries to new-planted orchards. From shoots of potted and bare-root young trees collected from different nurseries, we found various levels of latent infection of some pathogens using our *q*PCR approach. Consistently, we also found the latent infection in shoots from budwood trees in few nurseries. The results demonstrated that risk of young trees carrying pathogens to new-planted orchards could exist in California, despite nursery efforts to clean the plant material.

Thus, disease control should focus on both young orchards and in nurseries. Nurseries are doing a great job in protecting cuttings, but still latent infection can occur. In multi-year trials in a prune orchard, we found that the fungicide Topsin M70 could significantly delay the canker development after spraying young trees. In the spring 2019, trees of a 1st - and a 2nd -leaf almond orchard were sprayed with Topsin and disease assessments was done in November of 2019 and May of 2020.

Discussion

The disease was not assessed in Nov. 2019 in the 1st-leaf orchard since the trunk protectors of young trees covered the trunk of treated trees. While in May of 2020, the grower sprayed Topsin in the entire orchard as we had suggested.



In the 2nd -leaf orchard, Topsin resulted in a significant reduction of incidence of trees showing gumming and canker symptoms. In the first assessment, the incidence of untreated control was 50 to 58%, while those treated with Topsin + Rally and Topsin only were 12 to 14% and 14 to 22%, respectively. In the second assessment, these three values were 56-60%, 28 to 43% and 22 to 24%, respectively. The results indicated that, in addition to treatments in the nursery, young trees as soon as they are planted in the orchard need to be protected by spraying Topsin M.

Project Cooperators and Personnel:
Yong Luo and Dan Felts, UC Kearney Agric. Res. & Ext. Center; Franz Niederholzer, UCCE Yuba/Sutter/Colusa; Dani Lightle, UCCE Glenn/Butte; and Chris Taylor, almond grower and PCA in Glenn Co.

Investigation of *Aspergillus niger* causing Hull Rot and Conditions Conductive to Disease Development in Kern County

Project Leaders: Mohammad Yaghmour[1] and Themis J. Michailides[2]
[1]UCCE – Kern County, 1031 S Mt. Vernon Ave., Bakersfield, CA 93312 (661) 868-6211, mayaghmour@ucanr.edu
[2]UC Davis/Kearney Agricultural Research and Extension Center (KARE) (559) 646-6546, tjmichailides@ucanr.edu

Objectives

- To complete *Aspergillus niger* pathogenicity tests and study almond fruit susceptibility.
- To assess disease incidence and monitor inoculum dispersal in the orchard.
- Effect of tree water and nitrogen status on disease development.
- Establish cultural and chemical control strategies of hull rot caused by *A. niger*.

Background and Discussion

Hull rot in California almond orchards is primarily caused by *Rhizopus stolonifer* and *Monilinia fructicola.* Infections by these fungi result in killing of leaves, spurs, and parts of the shoot bearing the infected fruits. In Kern County, and the Southern San Joaquin Valley, *R. stolonifer* is more prevalent, and this fungus produces a toxin (fumaric acid) which moves from the infected fruit into the surrounding tissues killing the vascular tissues. Thus, hull rot affects future yields by killing fruiting spurs and wood. In the past years, orchards affected with hull rot in Kern County and other counties in the central valley showed the presence of *Aspergillus niger* growing mainly between the hull and shell in fruit with hull rot. Many samples were processed at Kearney Agricultural Research and Extension Center in Dr. Michailides' lab showed that hull rot samples from the San Joaquin and Sacramento Valleys were also infected with *A. niger* alone and/or *R. stolonifer*.

In July of 2019, we repeated the pathogenicity tests in field inoculations of Nonpareil variety. A large number of fruits were inoculated with *A. niger*. While inoculated fruits reproduced the typical symptoms of hull rot with leaves shriveling and developing necrotic peduncles and tissue. Many fruit that were sprayed with water (uninoculated control) also developed hull rot, but it was mainly caused by *R. stolonifer.* Furthermore, some of the inoculated spurs had mixed infections (both *A. niger* and *R. stolonifer*). We will repeat this experiment again in 2020. Looking at the most susceptible developmental stage, inoculated fruit at the three developmental stages [unsplit, deep V (b2 stage)], and split less than 1 cm (stage c) showed that Inoculated fruit at stage (c) had the highest percentage of spurs developing hull rot symptoms. In addition, results showed that populations of *A. niger* on the surface of the fruit were the highest later in July and through early August and corresponded with fruit that already split with less than 1 cm hull opening.

July leaf nitrogen analyses were not significantly different between the two experimental plots, which showed differences in hull rot incidence for the second year in a row. The levels in nitrogen were within the optimal range and did not explain the difference in disease incidence in the two plots in this orchard.

In 2019, preliminary work looking at sensitivity of *A. niger* to different groups of fungicides was tested *in vitro* (lab tests) and in the field. *In vitro* tests showed that *A. niger* was sensitive to fungicides in FRAC groups 3, 7+11, and 7. An experiment using three fungicides belonging in the FRAC groups 3, 7+11, and 11, were conducted in a commercial orchard and all fungicides reduced the number of symptomatic spurs by approximately 39-54% as compared to non-treated control fruit. We are repeating this work in 2020.

Project Cooperators and Personnel: Brent Holtz, UCCE-San Joaquin County; Mae Culumber, UCCE-Fresno County; Florent Trouillas, UC Davis-KARE

Continued Assessment of Almond Orchard Performance and Soil Health Following Biosolarization Using Almond Residue Amendments

Project Leaders: Christopher Simmons[1] and Amanda Hodson[2]
[1]Dept. of Food Science and Technology, UC Davis, One Shields Ave., Davis, CA 95616 (530) 752-2109, cwsimmons@ucdavis.edu
[2]Dept. of Entomology and Nematology, UC Davis, One Shields Ave., Davis, CA 95616 (530) 752-2592, akhodson@ucdavis.edu

Objectives

- Measure nematode community composition, including phytoparasitic species, in biosolarized and control soils.
- Measure major plant macronutrients (total and mineral nitrogen, extractable phosphorus, and potassium) in biosolarized and control soils.
- Measure soil physical and chemical properties (organic matter content, cation exchange capacity, aggregation) in biosolarized and control soils.
- Measure trunk diameter and several multispectral imaging indices for various almond varieties grown in biosolarized and control soils.

Background

Biosolarization(aka anaerobic soil disinfestation) is an integrated soil pest management technique that uses passive solar heating and organic matter amendments to create anaerobic soil conditions that are antagonistic to pests. and beneficial to overall soil health. In the summer of 2017, a biosolarization field trial was conducted at a commercial orchard in Chico, CA. Biosolarization was implemented using almond hulls and shells from different varieties as soil amendments to help drive pesticidal conditions in the soil. The treatment occurred during the preplant period and the orchard was planted in January of 2018. Soil properties, including nutrient and nematode profiles, were monitored since the onset of biosolarization. Tree growth and multispectral imaging measurements were incorporated after orchard planting. The current project continues to monitor these metrics following orchard planting in order to understand the long terms effects of biosolarization on soil health and soil interactions with almond trees.

Discussion

Current data show that there have been persistent benefits to soil nutrient content in the roughly 2.5 years following introduction of almond hulls and shells to the orchard soil during biosolarization. Specifically, organic matter, total nitrogen, total carbon, and potassium remain significantly elevated in biosolarized plots compared to untreated control soils regardless of whether hulls and shells from Nonpareil or pollinator varieties were used as amendment. Additionally, soils biosolarized using Nonpareil biomass continue to exhibit significantly higher nitrate and phosphorus levels compared to untreated soils. Early measurements of trunk diameter for trees grown in biosolarized and untreated soils indicated trees required adaptation to the biosolarized soil, as indicated by slower growth. However, by 24 months post-planting, the growth rate uniquely accelerated for trees in biosolarized soils, indicating successful adaptation. Over the following 6 months, Nonpareil and Bennett-Hickman trees have maintained significantly greater growth rates in soils biosolarized with hulls and shells from Nonpareil and pollinator varieties, respectively. Furthermore, imaging data show that Bennett-Hickman trees in soils biosolarized with Nonpareil biomass exhibit canopy reflectance and color properties associated with improved nutrition. These results indicate that benefits to almond trees may depend on interaction effects between biosolarization conditions and tree variety.

Project Cooperators and Personnel:
Emily A. Shea, Stephanie Chen, PhD students, Janina Milkereit, Postdoctoral scholar UC-Davis; Rory P. Crowley, Nicolaus Nut Company, Chico, CA

Development of Spray Backstop: A Low-maintenance System to Reduce Spray Drift without Limiting the Spray and Air Delivery

Project Leaders: Alireza Pourreza[1]
[1]Dept. of Biological and Agricultural Engineering, UC Davis, One Shields Ave., Davis, CA 95616
Tel: (530) 752-9290, apourreza@ucdavis.edu

Objectives

- Development of the Spray Backstop mechanism
- Evaluate and quantify the effectiveness of spray backstop on reducing drift and improving deposition

Background

In this project, we aim to evaluate a solution that can potentially address two major issues in almond spray application: off-target drift and on-target deposition. It is difficult to obtain a uniform and full spray coverage on almond trees mainly because of their dense and tall canopy profile. Using nozzles that generate fine droplets and increasing air may offer a better coverage especially on treetops; however, it will increase the chance for off-target drift of spray droplets.

Off-target drift of chemicals is possibly dangerous to human health, ecosystems and wildlife around the agricultural fields. California has the severest chemical spray application laws and regulations in the United States. Unmanaged off-target movement of chemicals may as well contribute to tighten regulations for pest control activities. Consequently, managing drift becomes a big priority.

Discussion

We developed and tested the first prototype of Spray Backstop, a foldable structure that can be mounted on the sprayer and covers trees from top. The prototype included a screen assembly covering trees from top and foldable mast powered by the tractor hydraulic system. The test was conducted in an almond orchard planted in 2009 (11th leaf) at the Nickels Soil Lab. A method adopted from a continuous loop sampling technique was used to quantify drift. We stretched a cotton ribbon with 1in width on top and both sides of the row that was sprayed. Leaf samples were also collected from the top third portion of the trees, where deposition efficiency is usually low. A mix of water and Pyranine (fluorescent dye) was used for spraying. The test was conducted with and without the spray backstop system. The ribbon samples were cut and tested for dye deposition.

A significant reduction of 78% in droplets drifting from top was observed when the spray backstop was used. Drifting from the sides was also reduced by an average of 47% when the spray backstop was used. Analysis of dye deposition of leaf samples on the top third portion of the trees showed a slight average improvement of 9%. Aerial videos of the spray cloud movement were captured during the experiment. The videos visualized the effectiveness of the backstop system in blocking the droplets scaping from the treetops.

Using the spray backstop system as an attachment to the sprayer can substantially reduce airborne drift. Use of such a system could potentially enable growers to adjust their sprayer fan for more air and use nozzles that generate finer droplets to obtain an improved overall coverage, particularly a better deposition in hard-to-reach portions of trees, without being concerned about drifting. Next steps are to test the robustness of the system under a variety of orchard conditions. The research to date shows that the system can reduce spray drift while improving spray coverage.

Project Cooperators and Personnel: Ali Moghimi (postdoc), German Zuniga-Ramirez (SRA), Farzaneh Khorsandi (Specialist), Ken Giles (Professor), UC-Davis; Franz Niederholzer (Advisor), Peter Larbi (Specialist), UCCE

Integrating Almond Data into ongoing Development of Airblast Spray Deposition and Drift

Project Leader: Peter Ako Larbi
Kearney Agricultural Research and Extension Center, UC Agriculture and Natural Resources, 9240 S Riverbend Ave., Parlier, CA 93648 (559) 646-6577, palarbi@ucanr.edu

Objectives

- Conduct airblast spray trials to generate spray drift data for validating a mechanistic airblast spray drift risk assessment model being developed to estimate exposure values with several crops.

Background

The U.S. Environmental Protection Agency (EPA) has been using a 20-year old AgDRIFT® model to assess human and environmental exposure to pesticides applied on all crops. This model was developed with data generated from young dormant apple trees, a much different structure from foliated almond canopies. Thus, the dormant tree scenario can significantly overestimate drift that occur in almond orchard airblast spray applications, leading to regulations that are potentially stricter than needed. The development of a new mechanistic model that considers actual application parameters used in real almond orchard situations is therefore needed.

The development of such a model is underway, an effort which started in Washington State in 2016. With intent to validate the model using a subset of the same series of tree crops used by AgDRIFT® to develop the three composite groups, aggregated by orchard characteristics into Normal [grapes, apples], Dense [almonds, oranges], and Sparse [dormant apples], validation data has been generated for Washington apples. Washington-California collaboration in 2019 led to a vision to generate validation data for almond, citrus, and grape in California, with all the commodity groups agreeing to jointly fund shared equipment/instruments for efforts in all three commodities to minimize cost. This project contributes to part of the effort in almond.

Discussion

We recruited a project staff research associate in December 2019 to start work in February 2020. Currently, most field/lab supplies for sampling have been acquired and prepared, with the remaining ongoing. Later-added funding for shared resources has aided the ongoing building of sampling structures. Although funds from other commodity groups for purchase of all shared resources is pending due to different funding cycles, we have identified a site for the experiment that suits the EPA-approved protocol and are preparing for data collection once resource acquisition is completed.

Meanwhile, the following have been achieved:

- *Fluorometer Calibration* – We conducted a lab-based study in February 2020 to calibrate the fluorometer system to be used for analyzing samples from the field experiment. The calibration was done for Pyranine dyes obtained from two different sources, AA (Alta Aesar Pyranine 5g) and AB (AmBeed sodium-hydroxypyrene-1,3,6-trisulfonate 5g 85% purity). Different standard solutions of known concentration were made and analyzed to create calibration curves that will be used to convert the instrument reading to concentration. Both dyes showed a strong linear correlation ($R2 \approx 1$), but AB values were consistently ~0.71 of AA values from 5 to 100K ppb, and both were detectable within the same range. The results were used to identify the preferred dye for the planned spray drift experiment.
- *Sprayer Air Velocity Study* – We conducted another lab-based study to evaluate the air velocity characteristics of an airblast sprayer. Air velocity was measured at seven heights and four distances away from the sprayer outlet on both sides. Percent fan input opening was varied in different runs. Generally, air velocity profile varied across height and the air reduced gradually with distance from the sprayer outlet and with decreasing opening. A first draft report has been developed.

Project Cooperators and Personnel: Mae Culumber UCCE Fresno, Christian Basulto, Kearney Ag REC, Mark McKean, Mark McKean Farms

Technical Transfer Teams Serving Commercial Beekeepers in Almonds

Project Leaders: Dr. Dennis vanEngelsdorp and Dr. Annette Meredith
Bee Informed Partnership, 4112 Plant Sciences Building, College Park, MD 20742, 240.484.0664
Annette.meredith@beeinformed.org

Objectives

The Almond Board has co-funded the Bee Informed Partnership's (BIP's) Technical Transfer Teams (TTTs) since 2011. This funding helps sustain, in part, the objectives for this ongoing program:

- Provide on-site colony health and disease assessments for U.S. beekeepers. BIP's highly trained Field Specialists work with commercial beekeepers who provide pollination services to California almond growers.
- Perform hygienic testing for queen producers who sell queens to beekeepers across the country.
- Deliver Field Specialist rapid response to beekeeper – reported colony health incidents and any resulting diagnostic testing needed to make rapid, data-driven management decisions to minimize colony harm.

Background

Bee Informed Partnership is a nonprofit organization dedicated to working with beekeepers to improve the health and sustainability of U.S. honey bees. To accomplish this goal, BIP:

1) Conducts an annual survey of U.S. beekeepers to monitor colony mortality rates and beekeeper management practices.

2) Runs the Sentinel Apiary Program, a citizen science program engaging with U.S. beekeepers to increase awareness of major threats facing honey bees and to encourage monitoring and timely colony treatments. This program is also meant to serve as an early warning system, alerting other beekeepers to colony health issues in their region.

3) Provides field and laboratory honey bee health support services to commercial beekeepers who manage the vast majority of the nation's honey bee colonies.

Discussion

One of BIP's primary goals is to help beekeepers monitor colony health and identify disease pressures by conducting frequent monitoring of major colony risk factors and rapidly returning actionable data to beekeepers. This is critical to ensure that a sufficient number of colonies are alive and able to provide almond pollination services.

By funding BIP, funds are sent directly to all teams that service commercial operations and improve the health of colonies during almond pollination.

Commercial beekeepers face a growing set of challenges to keep honey bee colonies healthy. BIP Field Specialists serve commercial beekeepers much like Crop Protection Agents serve commercial growers. Each team, trained extensively and consistently on our standard sampling and diagnostic protocols, acts independently to serve participating beekeepers in their specific regions. Regions vary considerably by management practices, migratory routes and main commodities.

Nationwide, the Field Specialists service 110 beekeepers who collectively manage over 580,000 colonies (~21% of the managed colonies in the US), an estimated 80% of whom are used for California almond pollination, representing ~23% of the colonies used by California almond growers.

Between 1 January and 30 April, 2020, Field Specialists have already conducted 2,772 colony inspections, performing over 2,097 field *Varroa* tests, 1346 *Nosema* lab exams and 509 hygienic tests and several samples were taken to test for pesticides in response to a number of bee-kill incidents reported in California this spring.

Project Cooperators: Dr. Marla Spivak, University of Minnesota, Dr. Ramesh Sagili, Oregon State University, Dr. Elina L. Niño, University of California Davis, Dr. Meghan Milbrath, Michigan State University, Dr. Juliana Rangel, Texas A&M

Evaluating cover crop benefits to pollinators and pollination in almond orchards

Project Leader: Neal M. Williams[2]
[1]Entomology and Nematology, UC Davis, One Shields Ave., Davis, CA 95616 (530) 752-9358, nmwilliams@ucdavis.edu

Objectives

- Quantify flowering density and phenology of different cover crops across growing regions.
- Quantify the use of cover crop by honeybees and native pollinators before, during and after bloom of the almond orchard.
- Compare the rate of honeybee visitation to almond trees in rows planted with cover crop to the rate of visitation to trees without cover to determine if the cover crop impacts bee visitation to almond flowers.

We are coordinating with researchers working on soil health to integrate results from multiple projects and optimize cover crop mixes to increase multi-functionality.

Background

Almond pollination relies on healthy honey bees and honey bee pollination quality can increase through synergistic interactions with wild bees (Brittain et al. 2013). Although almond pollen and nectar are important forage resources, alternative pollen and nectar sources immediately preceding and following almond bloom would benefit bee health through increased nutritional diversity.

Planting flowering cover crops in orchard understories is a potential strategy to augment bee forage immediately preceding and following orchard bloom (DeGrandi-Hoffman et al. 2016, PAm 2018). Cover crops also offer potential agronomic benefits in orchards (Ramos et al. 2010) through reduced compaction, improved soil nutrient dynamics and water infiltration. Informed choice of plant species in orchard cover crop mixes might promote dual goals of supporting bees and soil health. We collaborated with the UC research and extension team exploring soil health to examine the ability of cover crop mixes targeting soil health versus supporting pollinators to benefit bees and bolster almond yield.

Discussion

Cover crop plantings produced higher densities of flowers on orchard floors compared to similar areas lacking a cover crop. During the study period, mixes targeting soil health and pollinators established robustly in the northern and southern regions and

provided resources to bees late in almond bloom and after it. In both regions, flowering of the cover crops peaked after almond bloom. Cover crops in the central region site established poorly, grew more slowly, and were mowed by the farmer immediately following orchard bloom, preventing a full assessment of performance. Lack of rainfall during winter during the study period and limited irrigation likely explains the limited bloom and late flowering at the central and northern sites, respectively. Careful irrigation management will be needed if consistent early bloom is desired.

Both soil and pollinator mixes supported bees; however, relative to bloom density, and bees preferred the pollinator mix four-fold over to the soil mix. Bees' use of the mixes increased sharply following almond bloom. This change corresponded to increasing flowering of the cover plantings, but also to the shift from almond to these alternate resources. Thus, cover crops can extend resources for bees.

Specific flower species in each cover mix were most used by the bees. Clovers and vetch generally bloomed too late to support honey bees because hives are removed within 1-2 weeks after orchard bloom. Instead, Brassicaceae were most important. Relative to bloom density, honey bees preferred Bracco white mustard, Canola and secondarily Daikon radish.

Consistent with the shift of bees to cover crop mostly after almond bloom, we saw no consistent evidence of competition for pollinators between almond and the cover crops. Visitation to almond flowers did not differ between control rows and those with a cover crop. We note that the spatial scale of the trials is not well matched to conclusively address this question. We recommend additional whole orchard comparisons.

Project Cooperators and Personnel: Anna Britzman, Amelie Gaudin UC Davis, Cameron Zuber, David Haviland, Cynthia Montes, Mohammad Yagmour, UCC

Effect of Almond Insecticides, Fungicides, and Phytochemicals on Honey Bees

Project Leaders: Reed Johnson[1] and Chia-Hua Lin[2]
[1]Dept. of Entomology, Ohio State Univ., 1680 Madison Ave., Wooster, OH 44691 (330) 202-3523, johnson.5005@osu.edu
[2]Dept. of Entomology, Ohio State Univ., 2021 Coffey Road, Columbus, OH 43210 (614) 292-4624, lin.724@osu.edu

Objectives

- Test the potential for spray adjuvants to kill adult and larval honey bees alone and in combination with insecticides and fungicides in field relevant tank-mix combinations.
- Determine the potential for phytochemicals to reduce the toxicity of insecticide-fungicide-adjuvant combinations to bees
- Using a queen rearing test, determine whether adjuvants causing developmental problems in workers can also affect queen development, survival and success.

Background

Beekeepers providing honey bees for almonds continue to experience unacceptable colony losses related to pollination that may be related to insecticide-fungicide-adjuvant mixtures applied during bloom. Some beekeepers observe death of adult bees, larvae, or pupae while others report having trouble rearing queens or experience lingering problems in colonies weeks following almond pollination. Data from the California Department of Pesticide Regulation shows that in 2017 individual insecticide, fungicide and spray adjuvant formulations were applied to 55,000, 1.6 million and 3.8 million acres of almonds, respectively, during the blooming period (Feb. 15 – Mar. 15). Nearly all pesticides are applied as a tank-mix including multiple adjuvants. Research is needed to identify the particular combinations of pesticides that are capable of producing effects observed by beekeepers. Findings will allow for more focused recommendations regarding pesticide application to protect bees and pollination. Additional work will seek to ameliorate the harm caused by combinatorial pesticide exposure through feeding bees phytochemicals that can naturally be found in the hive.

Discussion

Previous work identified the combination of the insecticide Altacor (chlorantraniliprole) and the fungicide Tilt (propiconazole) as potentially toxic to adult and larval honey bee workers. We found that the addition of the organosilicone-containing spray adjuvant Dyne-Amic to this combination, applied in a spray to bees at the maximum label rate, caused substantially increased mortality in adult bees.

A similar effect was observed in honey bee queens developing in queen-rearing boxes provisioned with pollen contaminated with this combination of pesticides. Queen larvae exposed the Altacor-Tilt-Dyne-Amic mixture showed reduced survival to capping and adult emergence relative to treatments not receiving the spray adjuvant. Worker larvae that were artificially reared with diet contaminated with the active ingredients in these pesticides also showed reduced survival.

Spray adjuvants have the potential to further increase the toxicity of insecticides and combinations and may demonstrate toxicity against larvae and adults in their own right. Work to date has only looked at the potential of a single spray adjuvant to harm bees. Adjuvants are exceptionally diverse, with 236 different products used in almonds in 2017. [i]Work in 2020 will focus on identifying the spray adjuvant constituents with the greatest potential to harm bees in tank mixes.

Project Cooperators and Personnel: Michael Chapman, Colin Kurkul, Will Passifiume, Adrianna Pollee, Dylan Ricke, Nicole Sammons, Sreelakshmi Suresh and Emily Walker, Ohio State University; Wyatt Foss, College of Wooster; Jessica Lyons, Kent State University

Mitigating adverse effects of pesticides on honey bees through dietary phytochemicals

Project Leaders: May R. Berenbaum Researchers: Ling-Hsiu Liao, Wen Yen Wu
Dept. Entomology, University of Illinois, Urbana, IL 61801-3795 217 333 7784 maybe@illinois.edu

Objectives

- Determine effects of pesticide-adjuvant-phytochemical interactions on honey bees.
- Evaluate impacts of almond-characteristic phytochemicals.

Background

Many fungicides applied to almond orchards during bloom are considered to bee-safe. However, beekeepers have reported sporadic occurrences of significant bee losses, particularly during larval and pupal stages, after fungicide applications. Factors other than the fungicides might be involved in this intermittent bee mortality.

Bees often ingest pesticides, including fungicides, tank-mixed insecticides, and adjuvants, along with phytochemical-rich foods. These phytochemicals can detoxify the pesticides impacts. Our previous work showed that consuming *p*-coumaric acid and quercetin, ubiquitous dietary phytochemicals in honey and pollen induces forager CYP450 gene expression and enhances detoxification of co-occurring tau-fluvalinate, bifenthrin, β-cyfluthrin and imidacloprid.

For colony viability, healthy queen-rearing is essential. Nurses feed queen larvae in their queen cells and tend them throughout development, feeding them royal jelly. Nurse bees that consume pesticide-contaminated diets may experience changes in behavior and physiology that compromise queen care. Pesticides in bee food might quantitatively and qualitatively alter royal jelly, by affecting mandibular and hypopharyngeal gland function, which in turn may compromise the quality of the next generation of queens.

Discussion

We found that when foragers consume the fungicide boscalid, their wingbeat frequency declines, possibly impairing foraging ability. Consuming quercetin, however, boosts ATP levels in flight muscles; when bees consumed quercetin with boscalid, wingbeat frequencies were restored to normal levels. Thus, natural dietary phytochemicals may protect bees against fungicide toxicity (Liao et al. 2019).

Quercetin and p-coumaric acid at natural levels also enhance forager longevity. We determined that the synergistic impacts of fungicide (Tilt,propiconazole)-insecticide (Altacor, chlorantraniliprole) ingestion, frequently encountered as a tank mix by foragers during almond bloom, can be ameliorated by the consumption of these two phytochemicals, albeit only at low pesticide concentrations (Liao et al. submitted). Moreover, Tilt and Altacor have synergistic effects not only on worker longevity but also nursing behavior. Mixed Tilt and Altacor reduced the duration of visiting and nursing behaviors of nurse bees consuming phytochemical-free sugar water. Consuming quercetin-supplemented sugar water, in contrast, neutralized all impaired nursing behaviors with all pesticide treatments.

Additionally, we conducted assays to assess pesticide-induced altered olfactory responsiveness to brood pheromone as a possible mechanism underlying nursing behavior changes. We fed workers treated pollen for one week and then measured electroantennogram responses to synthetic non-volatile brood pheromone and a volatile brood pheromone (ocimene) to detect signs of olfactory impairment. Neither pesticide altered nurse olfactory responses in our preliminary assays; however, the adjuvant Dyne-Amic altered olfactory response of nurse bees to brood pheromones and to the alarm pheromone, 2-heptanone.

With respect to the second objective, we obtained samples of almond honey from a California producer and characterized major phytochemical constituents. In a pilot study, amygdalin, a cyanogenic glycoside in almonds, did not affect forager survival in the presence of 0.4ppm chlorantraniliprole but did reduce survival on diets with 0.9ppm fungicide propiconazole. Surprisingly, amygdalin neutralized the impaired survival caused by the mixed propiconazole+chlorantraniliprole diet. How other almond phytochemicals interact with pesticides to affect forager and queen health remain an important knowledge gap for almond pollination.

Reference

Liao L-H, W-Y Wu, A Dad, MR Berenbaum, 2019. Fungicide suppression of flight performance in the honey bee (*Apis mellifera*) and its amelioration by quercetin. Proc. Roy. Soc. B 286: https://doi.org/10.1098/rspb.2019.2041

Project Cooperators: Reed Johnson, Chia-Hua Lin, Ohio State University

Using cold storage to stabilize the supply of honey bees for pollination

Project Leaders: Brandon Hopkins[1]
[1]Dept. of Entomology, Washington State University, FSHN 166, Pullman, WA 99164 (509) 335-0881, bhopkins@wsu.edu

Objectives
Complete design and construction phase for controlled atmosphere chambers.

- Manipulate CO_2 in chambers with full size colonies to increase mite mortality during winter months
- Move colonies into chambers during summer months to stop brood production to increase varroa control efficacy

Background
Honey bees provide critical pollination services for California's almond crop. However, the commercial beekeeping industry has been struggling in recent years to maintain reliable/consistent colony numbers, leading to concerns about the supply of commercial colonies and increased pollination costs. Well documented factors have contributed to the unstable supply of commercial colonies: poor nutrition, pest and diseases, queen bee genetics & quality, and pesticide exposure. Commercial beekeeping management practices also play a critical role in colony losses and, surprisingly, have been largely understudied. Varroa mites continue to plague the industry even with a number of chemical control options. Varroa and the secondary issues that come with Varroa infestation and treatment, such as bee viruses, build up of chemical residues in wax from treatment, sublethal effects of treatments on developing bees, and increased labor costs contributes to concerns about the adequacy and cost of honey bees for almond pollination. Varroa and the associated issues are exacerbated by the current beekeeping industry practice of staging bees in California (holding yards) during the winter months prior to almond bloom. In these high colony density situations with continuous brood is produced (and therefore, Varroa production) under warmer California conditions, together with robbing from one hive to another, mite dispersal, diseases, poor nutrition, and vandalism all contribute to heavy losses when bees are stacked into holding yards. An alternative approach that has shown promise is the storage of bees indoors during the winter months. However, there is a lack of research and the potential benefit of this strategy is largely untapped.

Discussion
Almond Board funding was leveraged to successfully secure a $500,000 USDA-NIFA grant. Co-funding of this research is also provided by Project Apis m. We have accomplished Objectives 1 and 2 listed above. Field work related to Objective 3 is currently underway. Results and related work supported by this funding has been summarized into an online publication on Project Apis m website and presented on Almond Board webinar on March 26th, 2020.

Project Cooperators and Personnel: Walter S Sheppard – WSU; Bud Wilhelm – 2B Apiarie

A tough nut to crack: Understanding and improving honeybee pollination input in self-sterile and self-fertile almond varieties

Project Leaders: Elina L. Niño[1]
[1]Dept. of Entomology and nematology, UC Davis, One Shields Ave., Davis, CA 95616, (530) 500-2747, elnino@ucdavis.edu

Objectives

- Meta-analysis of available data to glean preliminary insights about the effect of hive stocking rates on nut yield.
- Develop methods to measure the stocking rates on nut yield.
- Extend information to growers, beekeepers, researchers.

Background

Almond production is highly dependent on successful pollination by honey bees. A standard recommended stocking rate for a mature, nut-bearing orchard is 2-2.5 hives per acre, with average of 6-8 frames per hive. Honey bee colony losses have led to continued concern over the sufficiency of the bee supply to fulfill almond pollination needs. Together with the adoption of self-fertile almond varieties, there is a need to update hive stocking rate recommendations for growers. In addition, anecdotal evidence suggests that growers could get by on 1.5 or even 1 hive per acre as long as the colonies are strong 8-frame colonies, for conventional varieties, and possibly 0.5-1 colony/acre for self-fertile varieties. Preliminary data, however, indicates that Independence trees in absence of bees had approximately 50% lower nut yield.

Understanding and updating pollination requirements of both self-compatible and conventional varieties has the potential to improve costs for almond growers without negatively affecting production. Secondarily, if lower stocking rates are effective at supporting high yields, this should also alleviate pressure on the bee industry. *Thus, our ultimate goal is to test the effects of diverse stocking rates and colony strengths on nut yield of traditional and self-compatible varieties in order to improve pollination management strategies and increase both grower and beekeeper satisfaction.*

Discussion

In 2020, our research focused on nut-bearing trees in the south San Joaquin Valley (Olam Farms) and Sacramento Valley (with F. Niederholzer), determining the effect of the number of bee visits on nut set, nut yield and quality (for Independence and Nonpareil), and calculating bee flight hours necessary for nut set (Independence) for both trees where bees were excluded from the flowers and control trees. We will also conduct a meta-analysis of available information to determine what can be gleaned about hive stocking rates' impact on nut yield.

We have accomplished the exclusion of honey bees from almond flowers of Independence almonds at both sites by using the standard pollination research method of bagging the flowers in mesh bags. At both sites we used 5 bags per tree for minimum of 20 trees per site. At the Northern site we implemented this technique for Nonpareil variety as a control comparison, and we also established a protocol for observing honey bee visitation rates on nut set.

At the Southern site, in addition to bagging the flowers we completely caged trees of Independence variety thereby excluding the bees from pollinating. We also noticed that Independence seems to have an extraordinary number of double flowers so we counted the number of single and double nuts set in Independence variety as well as three additional self-sterile varieties for minimum of 20 trees and three branches per tree, to determine if this might lead to lower nut set and yield in self-fertile varieties. At the time of this report we have completed the post-June drop counts for nut set at both locations, and have arranged to harvest mature nuts later in the summer to determine the effect of bee visitation on nut yield, size and quality. We also collected fertilized and unfertilized flowers to determine the mechanism of self-fertility.

Lastly, the meta-analysis will be underway starting in August due to COVID-19 impacting the staff availability.

Project Cooperators and Personnel: Franz Niederholzer, UCCE; Zac Ellis, Olam Farms.

Influence of Whole Orchard Recycling on GHG Emissions and Soil Health in a Newly Established Almond Orchard

Project Leaders: Mae Culumber[1] and Suduan Gao[2]
[1]UCCE-Fresno County, 550 E Shaw Ave. Suite 210-B, Fresno, CA 93710 (559) 241-7526, cmculumber@ucdavis.edu
[2]USDA-ARS 9611 S. Riverbend Ave. Parlier, CA 93648 (559) 596-2870, suduan.gao@ars.usda.gov

Objectives

- Monitor field level variability of nitrous oxide (N_2O) and carbon dioxide (CO_2) emissions, after a one-time WOR mulching rate of 45 T/ac dry wt at a commercial almond farm in Parlier, Ca. three years after recycling
- Describe the effect of WOR on soil carbon and nitrogen cycling

Background

Loss of co-generation facilities disposal methods have coincided with an increase in the volume of orchard removal waste across the Central Valley. Some growers have reverted to burning, which emits regulated air quality pollutants. Whole orchard recycling (WOR) incorporates orchard biomass on-site, without burning or moving the debris to another location, preventing the release of air pollutants into the atmosphere. When mulched into the soil, high carbon (C) containing amendments like wood chips increase soil organic matter (SOM). Agricultural systems with high SOM that receive frequent applications of water, and various rates and types of fertilizer, have shown both decreases and increases in carbon dioxide (CO_2) and nitrous oxide (N_2O) greenhouse gas (GHG) emissions. A study was initiated in April 2018 to find what impact a high rate (45 T/ac dry wt) of recycled wood chips will have on N_2O and CO_2 emissions, soil edaphic factors, and tree establishment for three years after planting. Gas flux chambers were installed in wood chip mulched and control plots to measure differences in GHG flux and soil N pools in the tree rootzone and inter-tree spaces where applied nutrients are not taken up. Flux sampling is conducted weekly at a minimum, and more frequently during management events like fertigation, irrigation, and tillage. Net changes in soil N and labile dissolved organic C pools from 0-15 cm are measured monthly one day following fertigation.

Discussion

Wood chip treatments had higher N_2O and CO_2 emissions compared to conventional in the fertigated drip line during the first through third year after orchard recycling in a replanted orchard. Similar to the control, woodchip treatment N_2O fluxes were consistently low except for the one to four-day period after fertigation. N_2O and CO_2 fluxes appeared to decline after mid-to-late summer in 2018 and 2019. The wood chip N_2O emission factor (EF) 1.1% was greater than the control (0.6%) in 2018 but dropped significantly (0.5% and 0.2%) in 2019. Data collection and analysis of the impacts of WOR and orchard management on cumulative GHG emissions is ongoing. Preliminary soil analyses suggest consistently higher ammonium levels in wood chip soil prior to fertigation events. Wood biomass in the alleyway remained largely the same from December 2017 to October 2018, suggesting potential for long term C storage in the unirrigated, unfertilized portions of the orchard. Rapid degradation of wood chips in the fertigated berm, and improved nutrition and growth in wood chip trees in 2019 indicate growers may lower fertilization to standard rates by the second growing season. Initial low vigor and insufficient leaf N points to the need for early spring fertilization to mitigate stunting problems after planting. Further research is needed to pinpoint the optimal timing and necessary season long fertility rates in the first two growing season after recycling.

Project Cooperators and Personnel: Brent Holtz UCCE-San Joaquin County; Greg Browne, USDA-ARS, Davis; Amelie Gaudin, UC-Davis; Elias Marvinney UC-Davis, Amisha Poret-Petersen USDA-ARS Davis, Emad Jahanzad UC-Davis; Cameron Zuber UCCE-Merced; Luis Toledo UCCE-Fresno; Dan Rivers UCCE-San Joaquin

Geophysical Imaging of Sediment Texture

Project Leader: Rosemary Knight
Department of Geophysics, Stanford University, Mitchell Building, 397 Panama Mall, Stanford, CA 94305
(650) 736-1487, rknight@stanford.edu

Objectives

- Improve ability to assess water infiltration rates for ground water recharge.
- Obtain water content profiles from nuclear magnetic resonance (NMR) borehole logs to support the interpretation of previously acquired tow-TEM, DroneTEM, and cone penetrometer testing (CPT) data in an almond orchard outside Tulare, CA.
- Use computer simulations to assess best practices for designing rock physics transforms linking geophysical data and sediment texture.
- Densely map sediment texture in 3D using combinations of local well logs, CPT, tow-TEM, Drone-TEM and borehole NMR data to show added value of each dataset.
- Develop 3D recharge pathway heatmaps from sediment texture models.
- Collate findings into a final report and video that documents our recommended workflow for assessing recharge pathways.

Background

On-farm flood-managed aquifer recharge is being explored as a means of replenishing aquifers in the California Central Valley. Optimizing recharge site selection can increase recharge efficiency, but optimal site selection requires accurate, 3D maps of the spatial distribution of sediment textures that control the recharge pathways to the aquifer. Our goal is to map the recharge pathways at a 32-hectare almond orchard in the Tulare Irrigation District to great depth (~60 m) with high lateral resolution (~10 m) and meter-scale vertical resolution.

To map the spatial distribution of sediment texture, we can directly sample the sediments down to ~30 m depth using cone penetrometer testing (CPT). However, mapping the sediments over the entire almond orchard using CPT is impractical. To achieve the desired resolution and coverage, we instead use a geophysical method called tow-TEM, developed by Aarhus University in Denmark. The tow-TEM instrument is pulled behind an all-terrain vehicle to densely map the subsurface electrical resistivity at our desired resolution and depth.

Electrical resistivity is controlled by the sediment texture and water content, so we can use the tow-TEM measurements to map the sediments in the subsurface. We use rock physics transforms to translate electrical resistivity to sediment texture and calibrate the models using CPT data. However, variable sediment saturation above the water table can complicate interpretation of the tow-TEM signal. We can employ another geophysical method called nuclear magnetic resonance (NMR) logging, to directly image water content and account for its effect on our rock physics transforms. By applying this transform to the entire tow-TEM dataset, we produce dense, 3D maps of sediment texture.

In 2017, we acquired an outstanding set of tow-TEM data at the almond orchard. In 2019, we drilled five CPT holes to calibrate rock physics models and, thus, sediment texture models. We also contracted a Japanese company, Neo Science, and OYO Corporation, to test the DroneTEM method, where a drone tows he geophysical instrument. This may allow us to monitor the subsurface during a recharge event.

In 2020, we will use computer simulations to assess different methods for building rock physics transforms in variably saturated sediments. In the fall, we will contract the consulting firm Vista Clara to acquire NMR logs at the almond orchard in the fall. We can then build rock physics transforms informed by our computer simulations that incorporate tow-TEM, NMR and CPT data to develop 3D maps of sediment texture. Finally, we can use pathfinding algorithms to build heatmaps of the recharge pathways to inform best practices for on-farm managed aquifer recharge.

Project Cooperators and Personnel:
Meredith Goebel and Gordon Osterman, Stanford University; Hiro Shima, OYO Corporation; Akira Jomori, Neo Sciences; Aaron Fukuda, Tulare Irrigation District

Using Rice Fields as Managed Floodplains to Help Recover Fish Populations

Project Leaders: Andrew L. Rypel[1]
[1]Center for Watershed Sciences and Dept. of Wildlife, Fish & Conservation Biology, UC Davis, One Shields Ave., Davis, CA 95616, (530) 752-9567, rypel@ucdavis.edu

Objectives

- Receive, grow, and implant 450 juvenile Chinook salmon with JSAT tags in a rice field. Subsequently track outmigration routes and survival (2019 and 2020).
- Bench test methods for growing, marking, and volitionally releasing salmon in rice fields (2019-2021).
- Develop partnerships and technical advisory groups with fish managements agencies, agricultural interests, NGOs, and other resource management groups (2019-2021).
- Train a graduate student at UC Davis on this work.

Background

Water availability for fishes and farms continues to be a central challenge in California. The number of fish species listed under the US Endangered Species Act is growing and more stringent water policies are linked with declining populations of native fishes. Diminished salmon populations are particularly problematic because of exceedingly complicated life cycles involving access to habitats at critical times. Furthermore, the importance of salmon as a cultural, indigenous, commercial and recreational fishery resource mandates improved conservation science to aid in recovering populations.

There is growing recognition that availability of floodplain habitats during crucial periods might be key for juvenile salmon in California. The Central Valley was in fact once a vast floodplain wetland teeming with diverse fishes and wildlife. Salmon were born in upstream gravel beds and transported to fertile floodplain habitats where juveniles reared. After growth and acquisition of large energy reserves, fish returned to the river healthy and prepared for out-migration.

Discussion

We developed a study aimed at understanding how preparation of rice fields by California growers could contribute to survival and local production of juvenile salmon. 9000 juvenile salmon were stocked into eight replicate 0.5-acre fields prepared in the following ways: installation of ditches; installation of wood; installation of ditches + wood; and control plots. We monitored survival of salmon over a 3-4 week rearing period using mark-recapture techniques involving PIT tags. Fields were drained in mid-March and PIT tags used to examine volitional egress of salmon through rice boxes. In addition, we performed surgeries on 450 rice-reared salmon using JSATS tags and released these fish into the Sacramento River. An additional 450 lab-reared fish were tagged and released as controls in June upon reaching taggable size.

Final results from this year's work are pending; however preliminary analysis revealed survival of salmon in rice fields was quite high (>80%) and that survival did not vary substantially by treatment type. Salmon reared in rice fields grew exceptionally fast, reaching taggable size in only 3-4 weeks. Preliminary tracking results are also encouraging with ~5% of tagged rice fish reaching ocean entry; expectation for survival in water years like 2020 (i.e., drought years) is 0-3%. This study posted high survival numbers compared to more than10 other concurrent telemetry projects in the region. Additional tracking analytics available here: https://bit.ly/30O1Aw3

This work is being conducted in close collaboration with the California Rice Commission as part of a grant from USDA-NRCS. Our project will be used to develop a practice standard for growers to help raise salmon in agricultural fields in the Central Valley. Growers are directly involved in our project, specifically to test and refine field management and provide salmon the ability to exit fields naturally.

Project Cooperators and Personnel: Paul Buttner - California Rice Commission; Jacob Katz, Jacob Montgomery - California Trout; Rachelle Tallman, Dennis Cocherell, Wilson Xiong, Nann Fangue - UC Davis. Roger Cornwell and Dominic Bruno – River Garden Farms

Diet And Lifestyle Intervention Strategies To Treat B-Cell Dysfunction And Insulin Resistance In Asian Indians

Principle Investigator: Jeannie Tay, Singapore Institute of Clinical Sciences (SICS), Agency for Science, Technology & Research (A*STAR), Singapore
Co-PIs: Johan Eriksson, Human Development, SICS, A*STAR, Singapore
Sendhil Velan, Magnetic Resonance Spectroscopy & Metabolic Imaging Group, Singapore Bioimaging Consortium (SBIC), A*STAR, Singapore

Background – Asian Indians have a 2-4-fold greater Type 2 Diabetes (T2D) risk and develop diabetes 5-10 years younger than Caucasians. In Singapore, Indians have the highest prevalence of T2D and exhibit the greatest insulin resistance compared to Malays and Chinese. Abnormalities in glucose homeostasis, in the absence of obesity and advanced age, are prevalent among Indians, indicating a possible genetic predisposition to greater insulin resistance and reduced β-cell function. Accompanying this insulin resistant metabolic phenotype is a body composition characterized by preferential accumulation of abdominal and ectopic adiposity and deficit of peripheral fat.

Objectives – The study will examine the etiology underlying the greater insulin resistance in Indians by studying potential molecular mechanisms that differentiate from obesity-induced insulin resistance

Methods and Results – This study is a 24-week, parallel arm, randomized controlled trial (RCT); 60 participants matched for age, gender and BMI will be randomized to one of two groups in 1: 1 ratio by computer-generated assignment: (1) Low glycemic (LG) diet or (2) Control diet (compatible with Singapore Health Promotion Board recommendations).

Specific Aim 1: To compare differences in the change in ectopic fat (pancreas, liver, visceral and intramyocellular) assessed with magnetic resonance imaging (MRI) and magnetic resonance spectroscopy (MRS); first- phase insulin secretion and insulin sensitivity assessed with the C-peptide model and oral minimal model, respectively, during an oral glucose tolerance test (OGTT).

Specific Aim 2: To understand the mechanisms underlying the genetic predisposition to increased insulin resistance in Indians by validating a 53-SNP genetic risk score associated with insulin resistance. To substantiate these findings by examining the association between these genetic variants and insulin sensitivity.

Current Status – The study is underway.

Effect Of Almond Supplementation In Overweight Indian Adults On Insulin Resistance, Glycemic Markers, Inflammation, And Satiety.

Principle Investigator: Sudha Vasudevan, Madras Diabetes Research Foundation (MDRF), Chennai, India
Co-PIs: R.M. Anjana, MDRF, Chennai, India; Unnikrishnan R, MDRF, Chennai, India

Background - South Asia is one of the epicenters of the diabetes epidemic. India alone has nearly 70 million with diabetes and 80 million with pre-diabetes. The higher prevalence of diabetes and insulin resistance among Indians is partly due to the Asian Indian phenotype and thrifty genotype characterized by metabolic abnormalities such as insulin resistance, abdominal obesity, glucose intolerance, lower adiponectin and increased levels of high sensitivity C-reactive proteins. These combined with altered lifestyles (poor dietary practices and physical inactivity) are one of the key drivers of escalating prevalence of obesity, further fueling the diabetes epidemic in India. Poor dietary practices include higher intake of refined carbohydrates such as white rice, lower dietary fiber and monounsaturated fats (MUFAs) in both urban and rural Indian diets. Such a diet has shown a strong association with insulin resistance, low HDL levels, metabolic syndrome and type 2 diabetes. Consumption of nuts, a rich source of MUFA, has historically been low in India.

Objectives - The proposed trial will evaluate the efficacy of providing nearly half of the MUFA calories (≈7%calories) almond supplementation (≈ 43g/day) in Indian diets on insulin resistance in overweight and obese subjects. This will address the current gaps in understanding the healthier role of nuts like almond in the high carbohydrate Indian diets.

Methods and Results - This randomized, controlled, two-arm study aims to determine the effects of almond supplementation on 1) insulin resistance (HOMA IR) and beta cell dysfunction, 2) biomarkers of glucose metabolism (i.e. fasting glucose and HbA1c), lipid profile (triglycerides, total cholesterol, LDL-C, and HDL-C), plasma fatty acid profile, satiety related gut hormones and selected inflammatory markers (hs-CRP, TNF alpha, MCP-1). A 1-week run-in period will - 2 - Diabetes & Metabolic Syndrome be conducted to evaluate participant motivation and compliance and to obtain baseline data. Diet and physical activity pattern will be assessed at regular intervals. Blood samples will be collected at the baseline, mid and end of the study.

Current Status - The assessments of lipid profile (i.e., triglycerides, total cholesterol, LDL- cholesterol, and HDL-cholesterol), apo A and apo B and adiponectin are completed. Analysis will be carried out once other pending assessments are completed. They are currently working on the assessment of satiety related gut hormones (GLP- 1, GIP, CCK, PYY and ghrelin) and selected inflammatory markers (i.e., TNF-α, MCP-1).Due to COVID 19 lockdown they nearly lost almost 6 months and as a result there is a delay in completing the assessments of secondary outcomes including- plasma fatty acid profile, inflammatory markers and gut hormones.

Evaluation of pre-meal load of raw almonds on postprandial hyperglycemia and other metabolic responses in Asian Indians with prediabetes

Principal Investigator: Anoop Misra, Fortis-C-DOC Centre of Excellence for Diabetes, Metabolic Diseases and Endocrinology
Co-Investigator: Dr. Seema Gulati, Head, Nutrition Research Group

Background - Type 2 diabetes is rapidly increasing in India due to multiple factors; predominant among them are dietary and lifestyle transitions. Not only is the prevalence of type 2 diabetes increasing in South Asians, but they are diagnosed a decade earlier than white Europeans. Post prandial hyperglycemia (PPHG) is the earliest defect in the pathophysiology of diabetes, occurring 4-7 years before fasting blood glucose begins to rise. Magnitude of post prandial hyperglycemia in Asian Indians is higher than other ethnic groups. A typical Indian major meal largely comprises of starchy foods, consisting of high amounts of carbohydrates, which is a possible reason for PPHG. Dietary strategies to curb PPHG are warranted especially in Asian In Indians, who consume high carbohydrate meals and have higher PPHG.

Objectives - This study is designed to investigate acute as well as chronic effect of pre-load of almond ingestion (60g per day; 20g) before 3 main meals (breakfast, lunch and dinner) on postprandial hyperglycemia.

The secondary objectives include effect of pre-load of almonds on serum insulin, pro-insulin, glucagon, glycosylated hemoglobin, c-peptide, TNF- α, hs-CRP, HOMA-IR, free fatty acids.

Methods and Results - Asian Indian subjects with prediabetes, in the age range 18-60 years without any end organ disease or allergy to nuts are being recruited in the study. Eligible subjects are randomized to one of the two study arms: control or almond. The study has 3 phases:

1) acute phase (single preload almond study evaluated with OGTT, n=60, cross over) : Subjects randomized to almond arm are given a pre load of 20 g of almonds 30 minutes prior to ingestion of 75 g of glucose, while those in control arm do not have any food/almonds before OGTT. Blood samples are collected at (minutes):- 30, 0, 30, 60, 90 and 120. Cross over of these subjects is done OGTT repeated in similar manner.

2) Sub-acute Phase (three- day almond preload study monitored with CGMS, n=60, cross over): All individuals undergoing OGTT are further studied with CGMS for three days each and then after crossed over. Those in almond arm continue to receive preload of almonds for three days and then studied without almond preload after cross over. A washout period of 7 days separates cross over period;

3) Chronic Phase (Ninety days almond preload study monitored with self-monitoring of blood glucose and glycosylated Hemoglobin, parallel arm, n, 60): This is a randomized controlled parallel arm study to evaluate the long term effect of preload of almonds on hyperglycemia. Subjects fulfilling the inclusion/exclusion criteria are randomized to either the control arm or the almond arm.

Composition of Diets

I. Composition of diets: Diets incorporating almonds as preload: Carbohydrates, 49%; fat, 32%; protein, 19%.
II. Diets for subjects in the control arm: carbohydrates, 50%; fat, 35%; protein,15% (Figure 9)
III. Subjects will be advised to consume one standard meal a day, which will be consisting of 250 Kcal and 50 g of carbohydrates in the days CGMS will be affixed.

Current Status - This study is currently underway.

Effect of Almond Consumption on Blood Glucose Regulation and Cardio-Metabolic Risk Factors in Adolescents and Young Adults (16-25years) with Prediabetes

Principle Investigator: Jagmeet Madan, Dept. of Food Nutrition and Dietetics, Sir Vithaldas Thackersey College of Home Science (Autonomous) SNDTWU, Juhu, Mumbai
Co-PIs: Rama Vaidya, Division of Endocrine and Metabolic Research, Medical Research Center of Kasturba Research Health Society, Vile Parle (west) Mumbai
Shobha Udipi, Integrative Nutrition and Ayurceuticals Medical Research Center of Kasturba Research Health Society, Vile Parle (west) Mumbai

Background - Type 2 diabetes is rapidly increasing in India due to multiple factors; predominant among them are dietary and lifestyle transitions. Not only is the prevalence of type 2 diabetes increasing in South Asians, but they are diagnosed a decade earlier than white Europeans. Post prandial hyperglycemia (PPHG) is the earliest defect in the pathophysiology of diabetes, occurring 4-7 years before fasting blood glucose begins to rise. Magnitude of post prandial hyperglycemia in Asian Indians is higher than other ethnic groups. A typical Indian major meal largely comprises of starchy foods, consisting of high amounts of carbohydrates, which is a possible reason for PPHG. Dietary strategies to curb PPHG are warranted especially in Asian In Indians, who consume high carbohydrate meals and have higher PPHG.

Objectives - This study is designed to investigate acute as well as chronic effect of pre-load of almond ingestion (60g per day; 20g) before 3 main meals (breakfast, lunch and dinner) on postprandial hyperglycemia.

The secondary objectives include effect of pre-load of almonds on serum insulin, pro-insulin, glucagon, glycosylated hemoglobin, c-peptide, TNF- α, hs-CRP, HOMA-IR, free fatty acids.

Methods and Results - This is a randomized, controlled, parallel arm study design with a 3-month intervention. . . The study was designed to provide 20 percent of the total caloric intake as snacks (almonds or control snack) in line with local snacking habits. Participants followed their usual diet patterns otherwise. The following study outcomes have been assessed: Primary outcome - changes in prediabetic state, in fasting blood glucose or 2 hourly blood glucose level. Secondary outcome - changes in insulin sensitivity, lipid profile, and Quality of Life & in oxidized LDL and inflammatory markers

Current Status - The study intervention is complete. A manuscript draft is underway.

Understanding the mechanism of long-term almond consumption on chronic glucose regulation and its translation into improved vascular function and cognitive performance: The AL-INCLUSIVE trial

Principle Investigator Jogchum Plat, Maastricht University, The Netherlands
Co-PI: Prof. Dr. R. P. Mensink, Maastricht University, The Netherlands

Background - Globally, the number of subjects with type 2 diabetes (T2DM) is rapidly rising, largely driven by the obesity epidemic. The well-known PREDIMED study in subjects at high cardiovascular risk showed that regular nut consumption was associated with a 50 % reduction in incident diabetes, a 30 % reduction in CVD and a 52% reduction in stroke. Almond consumption has been shown to be associated with ameliorations in various factors associated with CVD risk such as hyperglycemia, hyperlipidemia, and hypertension.

Objectives - The main aim for the current project is to investigate the impact of long-term almond consumption on chronic glucose regulation in subjects with impaired glucose tolerance (IGT).

Primary objective:

- To examine and understand the impact of long-term almond consumption on chronic glucose metabolism in subjects with IGT

Secondary objectives:

- To investigate if improved chronic glucose metabolism in subjects with IGT after long-term almond consumption translates into improved peripheral and brain vascular function and enhanced cognitive performance.
- To examine to what extent improved chronic glucose metabolism in subjects with IGT after long-term almond consumption can be explained by (combined) effects of lowered hepatic lipid accumulation and inflammation, skeletal muscle characteristics, visceral and subcutaneous fat accumulation, pancreatic function or fecal microbiota composition.

Methods and Results - This is a 12-month long-term randomized well-controlled intervention trial with a cross-over design, including 34 overweight and obese (BMI: 25-35 kg/m^2) men and women with IGT. During the intervention period of 5 months, subjects will receive daily 50 gr almonds but not in the 2 months wash out and 5 months control periods.

During the control period, subjects will not receive any other foods to balance the amount of energy provided by the almond. Both groups will receive instructions about recommended dietary guidelines in terms of macronutrient compositions. In this way, effects of almonds will be evaluated on top of an advised healthy background diet. This approach has deliberately been chosen, as it closely resembles the real-life setting, maximizing the applicability of the results. The primary outcome parameter is whole body insulin sensitivity measured as glucose infusion rate during a hyper-insulinemic-euglycemic clamp, the current gold standard for assessing insulin action *in vivo*.

Current Status - The study is ongoing.

The Effects of Long-Term Almond Consumption in Adults With Different Body Fat Distributions On Carbohydrate And Lipid Metabolism

Principle Investigator: Richard D. Mattes Nutrition Science, Purdue University, W. Lafayette, IN
Co-PIs: Robert Considine, Indiana University School of Medicine, Indianapolis, IN

Background - Almond consumption can improve some risk factors for type 2 diabetes mellitus (T2DM), including body weight and postprandial glycemia. However, the effect of consuming almonds on long-term measures of glycemic control is unclear. Different body fat distributions (BFD) carry different risks for T2DM and may be a reason for mixed evidence. High truncal visceral fat (VAT) is associated with insulin resistance, high truncal subcutaneous fat (SAT) carries some metabolic risk but likely not as problematic as VAT, while high gluteo-femoral fat is not associated with insulin resistance.

Objectives - The purpose of this study was to determine the effects of long-term almond consumption on body composition, glycemia and lipemia in adults with different BFD associated with different risks of insulin resistance and onset of T2DM.

Methods and Results - A 6-month RCT in 134 adults was conducted. Subjects were randomly assigned to the almond or control treatment based on their BFD. Those in the almond group consumed 1.5 oz. of almonds with their breakfast and as their afternoon snack every day and were instructed not to consume any other nuts. Those in the control group continued their habitual breakfast and afternoon snack routines but were instructed not to consume any nuts. Body composition was measured, and blood samples were collected for determination of HbA1c, glycemia and lipemia at 0 and 6 months. Appetite and dietary intake were collected at 0, 2, 4 and 6 months as was a blood sample for compliance testing. Body weight was measured every 2 weeks. An ITT linear mixed model analysis with Bonferroni correction for pairwise comparisons on energy intake and body composition and HbA1c change values was performed.

The almond group consumed more energy compared to the control group (A:2010±60, C:1816±63; p=0.028). However, there were no differences in BMI, total fat mass %, total lean mass %, or HbA1c between groups. Subjects with high SAT on the almond treatment had a greater decrease in truncal fat mass % (A:-0.94±0.6, C:1.06±0.6), preserved truncal lean mass % (A:0.90±0.6, C:-1.03±0.6), and trended towards a decrease in truncal VAT mass (A:-7.8±52.9 g, C:129.8±52.9 g) compared to those with high SAT on the control treatment (p=0.02, 0.02 and 0.09, respectively).

Current Status - The clinical trial is complete. The team is currently analyzing blood samples to determine glucose, insulin, and lipid results, while also preparing a manuscript for publication.

Effects of Almond Consumption on the Human Gastrointestinal Microbiota and Metabolic Health

Priniciple Investigator: Hannah Holscher, University of Illinois at Urbana-Champaign, Urbana, IL
Co-PIs: N. A. Burd, Ph.D., Naiman A. Khan, Ph.D., R.D., Jason M. Ridlon, Ph.D.

Background -The multifactorial causes of obesity complicate the implementation of efficacious interventions. Dietary modifications, including caloric restriction, increased dietary fiber, and modulation of dietary fat intake are important first lines of defense against weight gain and obesity. Further, a growing body of evidence suggests that there are associations between gastrointestinal microbiota and obesity in humans.

Objectives -The overall objective of this proposal is to utilize a whole foods approach to elucidate the microbial and molecular underpinnings of the beneficial metabolic effects of almond consumption in adults with overweight and obesity.

Methods and Results -This study is a randomized, controlled, parallel arm clinical trial with participants being assigned to either the treatment group (almonds), or the isocaloric control (pretzels). Each participant will provide stool samples, blood samples, and anthropometric measurements at the beginning and the end of the 12-week intervention. From the stool samples, DNA is extracted and the V4 region of the 16S rRNA gene is amplified and sequenced to asses the changes in microbiota composition between groups. Additionally, microbial metabolites and bile acids are measured from these fecal samples. Blood samples are collected as part of mixed-meal tolerance tests and are used to measure metabolic and inflammatory markers.

Current Status -The study is actively recruiting participants.

Almond Snacks and Satiety: A Randomized Intervention Trial Examining Acute and Chronic Effects.

Principle Investigator: **Nancy L. Keim,** USDA ARS Western Human Nutrition Research Center, Davis, CA
Co-PIs: Sridevi Krishnan, John Newman, Brian Bennett, Lindsay Allen

Background – In the U.S. there is a widespread "permissive" food environment that may be an important contributing factor to the high prevalence of obesity and its related comorbidities. It is plausible to posit that the habit of consuming unhealthy snacks may contribute to overeating and energy surplus.
Current models depicting appetite regulation involve meshing of physiological signals and psychological/cognitive influences. Fat-free mass and fat mass are thought to provide tonic signals to the brain to stimulate the drive to eat or inhibit hunger, respectively. Episodic signals for food intake also arise from the GI tract in response to meals. Overlaying, and possibly overriding, this long-established control of food intake is the neuroendocrine circuitry established through attitudes and perceptions toward foods. Finally, the 'executive brain' (residing in frontal region) may allow for top down control of decisions about whether and how much to eat. Satiety signals, consisting of physical and psychological components, are primary drivers of food intake regulation. Human intervention trials often deal with large variability in response to dietary interventions, and study conclusions and interpretations suffer from inability to account for this natural variance. This study will evaluate satiety response to almond snacks versus control snacks, investigating causes for variability, which is the new approach to precision nutrition.

Objectives
Determine if regular almond consumption, as a snack, increases satiety compared to the control snack using an acute meal challenge protocol in the laboratory and also monitoring food intake and satiety in a free-living, natural setting.
Hypothesis 1a: A multivariate model of satiety that predicts ad libitum food intake based on acute responses of satiety hormones to a meal challenge will differ between almond and control intervention.
Hypothesis 1b: Under free-living conditions, self-selected food intake will show appropriate energy compensation for the added calories of the almond snack, whereas under the condition of the control snack, less than adequate energy compensation will occur and total daily energy intake will exceed that needed for energy balance.

Methods and Results
A randomized, parallel arm design is used, and qualified participants (healthy pre-menopausal women, 18-45y, BMI 25 to 39.9 kg/m^2) are assigned to either the almond snack intervention, 56 g/day (divided into morning and afternoon snacks) or the control group snacks that are a mixture of dry cereal, pretzels, and bread sticks of equal calorie content to almond snacks. The intervention period lasts 4 weeks, during which time both a pre- and post-intervention laboratory test day is conducted to determine the response to short-term satiety signals in response to the snacks, assessing self-reported perception of hunger and satiety, and measuring subsequent food intake at a provided dinner meal. To gauge the effect of snacks on free-living food choice, participants will keep diet records.
The study assesses; circulating hormones, analytes, and vitamin E status, Feelings of hunger, fullness, desire to eat, and prospective consumption, Energy and macronutrient intake in the test day protocol, Eating behaviors, preferences, and craving, Food records, Body composition, Metabolic rate, Single nucleotide polypeptides related to food intake

Current Status
The study is currently suspended due to COVID closures.

The Effects of Consuming Almonds on Body Weight Management and Satiety

Principle Investigator: Rachel Brown, University of Otago, Dunedin, New Zealand
Co PIs: Lara Ware, Andrew Gray, Alexandra Chisholm, Siew Ling Tey

Background - Although nuts are high in fat and energy dense, several epidemiologic studies have found that regular nut consumers are leaner than non-consumers. In support of this finding, interventions designed to examine the effect of regular nut consumption on body weight report either no weight gain or less weight gain than predicted based on energy content alone. However, many of these studies have been of relatively short duration or have had a crossover design, which is problematic when the outcome is body weight and carryover effects are difficult to avoid. Therefore, longer-term studies using a parallel design are needed to confirm previous findings.

Objectives - The objective of this study was to examine the long-term effects of consuming almonds on body weight and satiety over 12 months among healthy people with a BMI<30kg/m2 who regularly snack. The primary aim of this study was to compare the effects of daily consumption of whole almonds or biscuits (as a comparison snack providing the same energy content) on body weight and composition, and satiety. Secondary outcomes included risk factors of chronic disease and consumer acceptance.

Methods and Results - This was a 12-month parallel, randomized controlled trial, with two arms: 1. Almonds (providing either 42.5 g/d or 10% of total energy requirement (whichever is the highest value)); 2. control arm of biscuits (providing the same energy as the almonds). We recruited 136 participants who reported they regularly snack on discretionary foods. Body weight, body composition (by dual-energy x-ray absorptiometry (DXA)), satiety, blood lipids and lipoproteins, inflammation, endothelial function, dietary intake, physical activity, resting metabolic rate (RMR), blood pressure, arterial stiffness; inflammation; endothelial function, glycated hemoglobin, vitamin E, and sleep were measured at baseline and 12 months. All measures except for DXA, RMR and arterial stiffness were also measured 3 and 6 months. Consumer acceptance of almonds and biscuits were assessed weekly. It is estimated that 109 will be included in the intention-to-treat-analysis.

Current Status - In total 79 participants have completed the study. A further 30 participants are due to complete the final measures by the end of October 2020. Biochemical analysis will begin in November 2020.

Does inclusion of almonds in an energy restricted diet enhance weight loss and protect against weight gain?

Principle Investigator: Alison Coates, University of South Australia, Adelaide, Australia
Co PIs: Jonathan Buckley, Alliance for Research in Exercise, Nutrition and Activity (ARENA); Alison Hill, Alliance for Research in Exercise, Nutrition and Activity (ARENA); Sze Yen Tan, Institute for Physical Activity and Nutrition (IPAN); Geraint Rogers, Microbiome Research, South Australian Health and Medical Research Institute (SAHMRI)

Background – Previous studies have demonstrated the effectiveness of adding almonds to an energy restricted diet for weight loss. Currently, data are lacking on the role of almonds in weight control diets to limit weight regain. The profile of almonds, which are rich in monounsaturated fats, protein and fiber, may assist with weight maintenance through increased satiety.

Objectives – to perform a randomized, controlled trial using a parallel study design to evaluate whether inclusion of 1.5 serves of almonds (42.5g) in an energy restricted diet (almond enriched diet; AED) compared with a nut free energy restricted diet (NFD) for 3 months to induce weight loss, followed by 6 months of weight maintenance

1) improves weight loss during energy restriction and limits weight regain during weight maintenance;
2) improves anthropometric measures and body composition after weight loss and weight maintenance;
3) improves subjective and objective measures of satiety after weight loss and weight maintenance;
4) improves body fat distribution and reduces body fat depots;
5) results in potentially beneficial changes in the composition of the gut microbiome;
6) reduces fat accumulation in the liver and improves liver enzyme profiles;
7) improves blood lipid profiles, blood glucose and inflammatory biomarkers.

Methods and Results - A 9 month randomized, controlled parallel-arm dietary intervention comprising a 3 month energy restriction phase (approximately ~30% energy deficit to induce weight loss) followed by a 6 month weight maintenance phase with maintenance of physical activity and lifestyle habits.

Individuals are randomly allocated to an almond enriched diet (AED) or a nut free diet (NFD) with groups matched on age, gender and baseline body mass index (BMI). All participants will be supported by a dietitian for 3 months to lose weight (~30% energy restriction). To achieve weight maintenance, both groups will be instructed to consume a healthy diet based on Australian dietary guidelines. The AED group will incorporate 42.5g (1.5 oz) of unsalted, whole, natural almonds with skins 6 days/week during both weight loss and weight maintenance while the NFD group will consume a nut free diet. During weight maintenance all participants will be asked to consume an energy intake appropriate to maintain weight, allowing for nuts to be incorporated into the diet 6 days per week in the AED group. Participants will be assessed at baseline, after 3 months of weight loss, and after 6 months weight maintenance. The full protocol has been published Carter et al 2020 BMJ open DOI: 10.1136/bmjopen-2019-036542.

Current Status - The study is underway.

Almonds and Their Impact on Gastrointestinal Physiology, Microbiology, And Function.

Principle Investigator: Kevin Whelan, King's College London, London, UK
Co PIs: Alice Creedon, King's College London; Eirini Dimidi; King's College London, Mark Scott, Queen Mary University of London; Chris Probert, University of Liverpool

Background – Almonds contain fibre, unsaturated fatty acids and polyphenols that may impact the composition of the gut microbiota and overall gut health. Early clinical trials reported a prebiotic effect of almond consumption on faecal *bifidobacteria*. Results of subsequent randomised controlled trials (RCTs) are conflicting. Few studies have investigated the impact of almond consumption on gut microbiology, physiology or gut symptoms. Additionally, potential mechanisms of action are unclear.

Objectives –The primary objective was to conduct a randomised controlled trial to investigate the effect almonds on fecal *Bifidobacteria* concentrations in healthy adults. The secondary objective was to investigate the role of almonds lipid bioavailability in the observed prebiotic effect by comparing the impact of consumption of whole almonds (low bioaccesible lipid) or ground almonds (high bioccessible lipid) on gut physiology, microbiology and symptoms in healthy adults.

Methods and Results - We conducted a parallel design RCT with three treatment arms in 87 habitual snackers with low fiber intake (<22 g/d). Participants were randomised to receive whole almonds (56 g/d), ground almonds (56 g/d) or a control snack muffin (2/day) for 4 weeks and instructed to consume products in place of their usual snack foods. Outcome measures were fecal microbiota composition and diversity (16S RNA sequencing), microbial metabolites (SCFA – gas liquid chromatography; VOCs gas-chromatography mass-spectrometry), gut transit time (Smartpill motility capsule), stool frequency (stool diary) and stool consistency (Bristol stool form scale and lyophilisation).

The trial was completed in September 2019. To date, analysis of gut microbiota composition and diversity, faecal VOCs and stool frequency and consistency have been completed. Almond consumption modulates the relative abundance of several gut bacteria at the genus level, including increased relative abundance of *Lachnospiraceae_UCG-003* ($p=0.004$) and decreased abundance of *Corynebacterium* ($p<0.001$) and *CHKCI002* ($p=0.03$) but had no effect on fecal *Bifidobacteria*. There was no effect of almond consumption on bacterial phyla or diversity. Fecal VOCs were increased by almond consumption in comparison to control (2-methylbutanoic acid, $p=0.002$; 3-methylbutanoic acid, $p=0.003$; butanoic acid, $p=0.004$). Almond consumption had no effect on stool frequency or stool consistency. There were no differences between either whole or ground almonds and control for any outcome measure.

Current Status- Analysis of the impact of almond consumption on fecal SCFA, gut transit time and objective measures of stool consistency are ongoing.

Roles of Almonds in Physical Performance

Principle Investigator: Mark Kern, San Diego State University
Co PIs: Shirin Hooshmand, San Diego State University; Changqi Liu, San Diego State University; Mee Young Hong, San Diego State University; Oliver Witard, Exercise Metabolism and Nutrition, King's College London, UK

Background - The profile of nutrients found in almonds likely confers numerous advantages over more commonly consumed snack foods. Based on their composition alone, almonds should improve subjective feelings and promote recovery following strenuous exercise of active individuals.

Objectives - Our primary objectives are to determine the impacts of almonds for promotion of recovery from vigorous exercise, exercise performance, physical activity and subjective assessments of vigor. Recovery assessments will include delayed onset muscle soreness outcomes following eccentric exercise (downhill running), creatine kinase as a marker of muscle damage, and exercise performance recovery (quadricep and hamstring muscle strength testing using an isokinetic dynamometer as well as vertical jump). Secondary objectives include assessing metabolic and health outcomes including body weight regulation, body composition, food cravings, total antioxidant capacity, inflammatory responses, blood lipid concentrations, and blood glucose and insulin regulation.

Methods and Results - To test our hypotheses, sixty men (n=30) and women (n=30) aged 18-45 years will be recruited to participate. A cross-over design performed in random order will be utilized in which participants will complete 2 trials separated by at least 4 weeks at two testing sites, one in the United States (San Diego, CA) and one in Europe (London, UK). At each testing site, 30 active (at least 2 hours of structured exercise per week) men (n=15/site) and women (n=15/site) will be assessed. Participants will consume 1.5 oz of almonds or an isocaloric amount of unsalted pretzels daily for 8 weeks during 2 separate trials performed in a randomized, cross-over fashion. Prior to and at the end of each 8-week period, subjects will report to the laboratory following an overnight fast and after abstaining from strenuous exercise for at least 24 hours for collection of blood and assessments of height, body weight, and body composition using dual-energy x-ray absorptiometry. Blood will be analyzed for total antioxidant capacity, creatine kinase, TNF-a, blood lipid concentrations, glucose, and insulin. Throughout each period, activity will be monitored during 4 random days using Actigraph meters. Additionally, the Profile of Mood States for assessing vigor/activity will be administered. During each period, food cravings will be monitored. During the testing session that occurs at the end of each 8-week period, participants will complete a 40-min downhill run at -10% grade. A recovery serving of the test food will be provided upon completion of the downhill run. Antecubital blood will be collected before the bout of exercise and 24, 48, and 72 hours afterward. Muscle soreness will be assessed at each timepoint. Quadricep and hamstring strength as well as vertical jump will be assessed prior to the exercise bout and at each recovery timepoint. Daily consumption of the almonds will continue during the 72-hour recovery period.

Current Status - The study is underway.

The Effects of Almond Consumption on Functional Performance, Aerobic Capacity, and Physical Activity in Overweight and Obese Active Older Adults

Principle Investigator: Bahram Arjmandi, Center for Advancing Exercise and Nutrition Research on Aging, Florida State University
Co PIs: Lynn Panton; Claire Berryman, Robert Hickner, Neda Akhavan, Nutrition, Food and Exercise Sciences, Florida State University Scientist

Background - The aging process is strongly associated with reduced aerobic capacity, functional performance, and resting metabolic rate, which can promote alterations in body composition as well as increases in inflammation and oxidative stress. In addition to the increased number of older adults in the U.S. contributing the demographic changes, eating patterns have also changed for Americans within the past 30 years, with a majority of Americans consuming at least one snack/day; with the types of snacks consumed being processed foods that are high in refined carbohydrates, saturated fats, and low in fiber. Therefore, healthy, nutrient-dense foods are of primary importance for this growing population. Almonds (Prunus dulcis) are a nutrient-dense food that can be consumed as a snack and is an excellent source of α-tocopherol and a good source of monounsaturated fat, magnesium, fiber, and protein. Although the health benefits of almond consumption on indices of cardiovascular health are well established, the benefits of almond consumption on functional performance, aerobic capacity, physical activity, sleep quality and energy expenditure in active, overweight, older adults, to our knowledge, has not been examined.

Objectives - The objectives of this randomized, crossover study is to determine the effects of roasted almonds for 12 weeks as a snack, on functional performance, aerobic capacity, physical activity, sleep quality, mood status, body composition, vascular function, and inflammation/oxidative stress in active, overweight, older adults compared to an isocaloric commercially available snack.

Methods and Results - Fifty men and postmenopausal women, will follow a 12-week randomized-controlled crossover design, with a 4- week washout period. After the screening visit, physical activity, sleep patterns, and dietary intake will be assessed for the next seven days, prior to participants Baseline Study Visit #1. During the Baseline Study Visit #1 anthropometric, metabolic, hemodynamics, blood draw, and mood/sleep assessments will be completed. The following day, participants' will return for their Baseline Physical Performance Visit #1 to complete performance and functional tests and will be provided with almonds or the isocaloric snack. Participants will come in after 6 weeks to monitor compliance and be given the remaining snack regimen. During week 11, participants will have their physical activity, sleep patterns, and dietary intake assessed for the next seven days prior to the Post-Test Study Visit #1. After 12 weeks, participants will return to complete their Post-Test Study Visit #1, which will involve all the same assessments from the baseline study visit, as well as their Post-Test Physical Performance Visit #1, the next day. After the 4-week washout period, participants will similarly have their physical activity, sleep patterns, and dietary intake assessed for the next seven days prior to their Baseline Study Visit #2, where participants will crossover into receiving the corresponding dietary regimen and will follow the same procedures for the following 12 weeks.

There are no results to share at this time as recruitment has not started at this moment.

Current Status - This study has recently been approved from Florida State University's IRB and will soon begin recruitment of participants.

Influence of Almond Supplementation on Energy, Performance, and Inflammation Resolution from Exercise-Induced Muscle Damage

Principle Investigator: David Nieman, Dept. of Biology, College of Arts and Sciences, at Appalachian State University

Background – Eccentric muscle movement occurs when the muscle lengthens as it contracts. Common eccentric exercises include going downstairs, running downhill, lowering weights, and the downward motion of squats, push-ups, or pull-ups. These types of exercises often cause some muscle damage and soreness. Muscle soreness from this type of exercise can last several days. Nutrition supplements may help reduce muscle damage and soreness during recovery from eccentric exercise. Almonds are high in protein, healthy types of fats, and important vitamins and minerals. Eating almonds may reduce post-exercise muscle damage, soreness, and inflammation (swelling and pain).

Objectives – This study will determine if eating almonds for four weeks reduces muscle damage, soreness, and inflammation during several days of recovery from eccentric exercise.

Methods - An acute 90-minute bout of eccentric exercise will be used to induce muscle damage and inflammation in a group of 60 relatively untrained male and female adults (ages 30-65 years). Using a randomized, parallel group design, almond supplementation (57 g/d) for a 4-week period prior to the eccentric exercise challenge will be investigated as a countermeasure strategy to exercise-induced muscle damage and inflammation. The control group will ingest a calorie-matched common snack (cereal bars). To improve the potential for capturing an intervention effect, advanced mass spectrometry procedures will be utilized to measure changes in 70 different oxylipins and lipid mediators that are involved in regulating inflammation. C-reactive protein (CRP), six inflammatory cytokines, and muscle damage biomarkers will also be measured. Blood samples (7 total) will be collected before and after the 4-week supplementation period, and then daily during 5 days of recovery from the eccentric exercise bout. The Profile of Mood States (POMS) will be used to measure mood state and specific domains including vigor or activity, and fatigue or inertia. Exercise performance will be measured each time a blood sample is collected using a comprehensive battery that includes vertical jump, leg/back muscle strength, bench press muscle endurance, and anaerobic power (30-second Wingate test).

Current Status - The study is currently suspended due to COVID restrictions.

Almond Consumption Increased UVB Resistance in Healthy Asian Women

Principle Investigator: Zhaoping Li, Dept. of Medicine, Division of Clinical Nutrition, University of California, Los Angles
Co PIs: Jenny Kim. Dept. of Medicine, Division of Dermatology, University of California, Los Angles

Background – Almonds are a rich dietary source of phenolic and polyphenolic compounds, known for their antioxidant capacity. In vitro and in vivo studies have demonstrated that topical application of almond oil and almond skin extract reduces UVB-induced photoaging.

Objectives – Investigated whether oral almond consumption can increase resistance to UVB radiation and reduce skin aging in healthy Asian women.

Methods and Results - Thirty-nine female participants (18-45 years) with Fitzpatrick skin type II-IV were randomly assigned to consume either 1.5 oz of almonds or 1.8 oz of pretzels, for calorie control, daily for 12 weeks. Minimal erythema dose (MED) was determined by standardized protocol at inner arm. Facial skin texture was evaluated by two dermatologists using the Clinician's Erythema scale and Allergan Roughness scale. Facial melanin index, hydration, sebum and erythema were determined using a cutometer. The MED was significantly increased in the almond group compared to the pretzel group. We did not observe any change in facial rubor, Allergan roughness, melanin, hydration and sebum. Our findings suggest that daily oral almond consumption may lead to enhanced protection from UV photodamage.

Current Status - Manuscript is submitted and review.

The Impact of Almond Nut Consumption on Emerging Markers of Cardiovascular and Metabolic Disease

Principle Investigator: Wendy Hall and Sarah Berry, Diabetes and Nutrition Sciences Division, Faculty of Life Sciences & Medicine, King's College London, UK.
Co PIs: Peter Ellis; Geoff Charles-Edwards; Phil Chowienczyk; Gerda Pot

Background – There is convincing evidence that daily whole almond consumption lowers blood LDL-cholesterol concentrations, but effects on other cardiometabolic risk factors such as endothelial function and liver fat are still to be determined. Very little is known about population-level intakes of almonds, but prevalence of consumption is low according to US estimates, although almond consumption was associated with lower adiposity, better diet quality and greater nutrient adequacy.

Objectives – The program of work included a randomized controlled trial and an observational study. The Almonds Trial Targeting Dietary Intervention with Snacks (ATTIS) study aimed to investigate whether substituting almonds for typical snacks (high in available carbohydrates and saturated fats, and low in fibre) influenced cardiometabolic risk factors. We hypothesized that almonds would increase endothelial function and decrease liver fat. This project also aimed to estimate whole tree nut and specifically almond consumption in a nationally representative UK survey population and examine associations with diet quality and cardiovascular disease (CVD) risk.

Methods and Results - The ATTIS Study: Healthy adults aged 30-70 y who were habitual snackers and at moderate risk of developing CVD were recruited. Following a 2-wk run-in period consuming control snacks, a 6-wk parallel arm trial was conducted where participants were randomized to isoenergetic treatments: 1) control snacks (mini-muffins) replicating an average snack nutrient profile, calculated from snack foods identified in 4-d food diaries from a national dietary intake database; or 2) dry-roasted whole almonds, providing 20% estimated energy requirement. Endothelial function; abdominal subcutaneous and visceral fat, and liver, muscle and pancreatic fat (via MRI/ MRS); day- and night-time heart rate variability (HRV) and 24 h ambulatory blood pressure (ABP); blood biomarkers of insulin sensitivity, lipid profile, plasma fatty acid composition and metabolomics; and fecal short chain fatty acids (SCFA) were assessed at baseline and endpoint. Five-minute periods of supine heart rate (HR) and HRV were also measured at resting and during mental stress (Stroop color-word test). 107 participants (75 F, 32 M; mean age 56.2 y, SD 10.4) were randomized and 105 subjects completed. Almonds significantly increased FMD (mean difference 4.1%, 95% CI 2.2, 5.9), the HRV index, night-time very-low frequency (VLF) power (mean difference 337 ms^2, 95% CI 12, 661) and plasma oleic acid levels (mean difference 228 µmol, 95% CI 7, 449) relative to control adjusted for baseline BMI and baseline dependent outcome values, but there were no treatment differences in ABP and subcutaneous, visceral, liver, muscle and pancreatic fat. Plasma LDL cholesterol concentrations were significantly decreased by almonds (mean difference -0.25 mmol/L, 95% CI -0.45, -0.04), but no differences were found in other blood lipids, insulin sensitivity, metabolomic measures, nor fecal SCFA levels. High frequency (HF) power, which reflects parasympathetic regulation of HR, was significantly increased following almonds during the mental stress task relative to control (mean difference between groups 124 ms^2; 95% CI 11, 237); other HRV indices were not significantly affected. These novel findings suggest almonds may be cardioprotective by increasing availability of nitric oxide and improving cardiac autonomic function during sleep and acute mental stress, in addition to their well-established LDL cholesterol-lowering properties.

The observational studies: Four-day food record data from the National Diet and Nutrition Survey (NDNS) were analyzed to investigate associations between tree nut snack (TNS) consumption (NDNS 2008-2014, n=4,738, age ≥19y) and whole almond consumption (NDNS 2008-2017, n=6,802, age ≥19y) and diet quality, measured by the modified Mediterranean Diet Score (MDS) and modified Healthy Diet Score (HDS), and CVD risk markers, using survey-adjusted multivariable linear regression. Whole almond consumption was reported in 7.6% of the population, with a median intake of 5 g/d (IQR 9.3) in consumers. TNS and almond consumers had higher modified MDS and HDS relative to non-consumers. TNS and almond consumption was also associated with higher intake of total fat, unsaturated fatty acids, fiber, and a number of vitamins and minerals; and lower intake of trans fatty acids, total carbohydrate, sugar, and sodium. TNS and almond consumers also had lower BMI (almond consumers 25.5 kg/m^2 (95% CI 24.9, 26.2) vs non-consumers 26.3 kg/m^2 (25.9, 26.7), and waist circumference (almond consumers 88.0 cm (86.2, 89.8) vs non-consumers 90.1 cm (89.1, 91.2)). There was a dose-related fully adjusted significant association between increasing TNS intake (g per 1000 kcal energy intake) and lower SBP. Consumers of whole almonds and tree nuts in general report better dietary quality and have lower CVD risk factors. Encouraging replacement of less healthy snacks with nuts such as almonds should be encouraged as part of general dietary guidelines.

Current Status - This project is complete, and all the results have been published (1-4) with a further cross-sectional analysis of baseline measures underway (5).

Nutrition Research Publications 2020

The following is a list of scientific papers published with the research partially or wholly funded by the Almond Board of California. Copies of the abstracts may be obtained by contacting the Almond Board of California.

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Conversion of Almond Hulls to Protein-Rich Animal Feed Supplements

Principle Investigator: Zhiliang Fan, Dept. of Biological and Agricultural Engineering, University of California, Davis

Background and Objectives- Almond hulls are a byproduct of the almond industry and are typically used as a dairy feed supplement. Due to the dairy industry's recent downsizing, there are fewer needs for almond hulls as the dairy feed. This new development drives down the almond hull price and leaves an excessive amount of almond hulls, which urgently need to find an alternative use. Meanwhile, poultry and aquatic industries are pursuing new materials for protein-rich feed supplements. Almond hulls contain free sugars (21-25%), cellulose (9-15%), hemicellulose (7-10%), pectin 4-6%, lignin (4-15%), protein (4-6%), and fat (1-2%) on a dry weight basis. They are too low in protein content and too high in fiber content for direct use as poultry or aquatic feed supplements. This project aims to test the feasibility of the conversion of almond hulls to a protein-enriched animal feed. The conversion process will use almonds hulls as a substrate to produce fungal biomass through a solid-state fermentation process using thermophilic fungus. By supplementing nutrients and fungal spores, fungi can grow on almond hulls. They will consume most of the free sugars and the carbohydrate fibers (cellulose, hemicellulose, and pectin) in the almond hulls to produce more fungal biomass, which contains 40-50% crude protein. This process can lower the hull fermentation residue's fiber content and increase the protein content, both of which are more suitable for poultry feed supplements.

The overall objective is to conduct a preliminary study to evaluate the feasibility of converting the hulls to protein-rich fungal biomass and the fermentation residue's suitability as poultry feed supplements. The first objective is to pretreat the hulls for fungal growth. The second objective is to screen various fungal strains for biomass production using the pretreated hull as the substrate.

Methods and Results- Microwave pretreatment has been conducted to improve the digestibility of the almond hulls. The milled almond hulls were added different amounts of water and were treated under different microwave intensity and time. The resulting pretreated almond hulls have been tested the amenability to enzymatic hydrolysis. Cellulase enzymes were added to the pretreated almond hulls, and the amounts of sugar released were measured. Our results show that the microwave pretreatment did not improve the almond hulls' digestibility under the tested conditions.

Three different thermophilic fungus strains were obtained from ATCC or DSMZ. We have tested them in both the submerged fermentation and solid-state fermentation using almond hull as the substrate in Vogel's media at 45°C-50°C. All strains were able to grow on the milled almond hull and produced fungal biomass. All fungi could increase the fermentation residue's protein content to above 20% in the submerged fermentation and above 15% in solid-state fermentation in 4 days. The optimal fermentation growth media for all fungi was Vogel's media. We are now working on optimizing the solid-state fermentation conditions to improve the fungal biomass production, including the culturing temperature, moisture content, and media composition.

Conclusions- The fermentation process can convert almond hulls to a fermentation residue containing a protein level appropriate for use as a poultry feed supplement.

Utilizing Almond Hulls as a Novel Feed Ingredient for Poultry

Principle Investigator: **Woo Kyun Kim and Fanbing, Kong**, Dept. of Poultry Science, University of Georgia

Background and Objectives- Because almond hulls contain digestible fibers and antioxidants, it could be a functional feed ingredient for poultry (Broilers and Laying hens). Since the public demand for antibiotic free chicken meat and eggs is growing, almond hulls could be a potential feed ingredient in antibiotic free systems. The objective of the projects was to evaluate effects of different inclusion levels of almond hulls on growth performance and egg production in broilers and laying hens

Methods and Results- In trial 1 (broiler trial), a total of 560 one-day-old Cobb male chicks were randomly placed to 7 experimental treatments with 8 replicates of 10 birds each. Seven treatments consisted of a corn-soybean meal control diet and diets containing prime hulls or California type hulls at 3, 6, and 9 %. The nitrogen corrected true metabolizable energy, crude protein, and crude fiber from prime hulls and California type hulls were 1,624 and 1,514 kcal/kg, 4.8 and 5.0 %, and 13.1 and 26.45 %, respectively. During 0-18 d of age, the inclusion of the prime hulls at three levels had no significant effects on growth performance, but the California type hulls at 9 % increased feed intake ($P < 0.05$) and feed conversion ratio ($P < 0.05$), compared to control. The prime hulls at 9 % decreased ($P < 0.05$) ileal dry matter and ileal nitrogen digestibility, and the California type hulls at 9 % only decreased ileal dry matter digestibility, but both prime hulls and California type hulls at 6 % had no effects on ileal dry matter digestibility and nitrogen corrected apparent metabolizable energy compared to control. There were no significant differences in cecal microbiota diversity at a phylum or genus level among treatments, but 9 % inclusion rate of the California type hulls increased ($P < 0.05$) the population of certain bacteria in the genus *Clostridium* and *Oscillospira* compared to control. In conclusion, as a dietary energy and fiber source, the prime hulls can be used at up to 9 % without a negative effect on body weight gain, while the California type hulls can be used up to 6 %.

In trial 2 (Laying hen trial), The objective of this study was to evaluate two varieties of almond hulls (Nonpareil, **NP**; Carmel, **CA**) as an alternative feed ingredient on the performance, egg quality, nutrient digestibility, and body composition using 100 Hy-Line W36 hens from 24 to 32 wk of age. Treatments consisted of a control diet based on corn and soybean meal; T2 and T3 were formulated to contain 7.5 and 15% of NP; and T4 and T5 contained 7.5 and 15% of CA. Inclusion of NP and CA had no effects on feed intake, egg laying rate, and feed conversion ratio, but CA at 7.5% decreased ($P < 0.001$) body weight gain compared to the control. NP at 7.5% and CA at two doses improved ($P \leq 0.022$) AMEn and N retention. Both NP and CA had no effects on egg size, specific gravity, Haugh unit, and percentages of yolk, albumen and shell, but yolk color appeared green and less yellow ($P \leq 0.009$) by NP and less yellow ($P = 0.001$) by CA. For body composition, NP and CA at two doses lowered ($P \leq 0.017$) body fat and CA at 7.5% decreased ($P = 0.001$) lean weight. In summary, inclusion of NP or CA hulls up to 15% had no negative effect on egg production and egg quality while reduced the body fat percentage and mass.

Conclusions- We found that up to 9% of NP or 6% of CA can be supplemented into broiler diets without any negative effects, while up to 15% of both almond hulls can be supplemented into laying hen diets. Furthermore, we found that dietary fibers from Almond hulls could enhance intestinal fermentation and stimulate beneficial bacteria in the gut of poultry.

Publication-

1. Effect of almond hulls as an alternative ingredient on broiler performance, nutrient digestibility and cecal microbiota diversity (Submitted to Poultry Science)
2. Effect of almond hulls as an alternative ingredient on the performance, egg quality, nutrient digestibility, and body composition of laying hens (In progress)

Effects of Almond Hulls on Coccidiosis in Broilers

Principle Investigator, **Woo Kyun Kim**, Dept. of Poultry Science, University of Georgia

Background and Objectives- Coccidiosis caused by intestinal parasites such as Eimeria spp. (Eimeria Maxima, Eimeria Tenella, and Eimeria acervulina) is important enteric disorders in broilers, causing tremendous economic loss for the poultry industry. Thus, coccidiostats (anti-coccidiosis drugs) are commonly supplemented into poultry diets. However, frequently the poultry develops resistance to these drugs. Therefore, the poultry industry is looking for alternatives to such drugs and feed additives to alleviate symptoms of coccidiosis. The typical symptoms of coccidiosis in broilers are growth retardation, poor feed conversion, diarrhea, intestinal damage, and mortality. Coccidiosis induces severe oxidative stress which induces inflammation and aggravates these symptoms. One of the potential strategies to reduce severity of the symptoms in broilers is supplementing strong antioxidants. *Since almond hulls contains high levels of phytochemicals which acts as antioxidants, we would like to evaluate effects of almond hulls containing high phytochemicals on coccidiosis symptoms in broilers challenged with Eimeria spp..* We expect that almond hulls can improve growth performance and oxidative related enzyme activity in Eimeria challenged broilers. **Specific Aim:** To evaluate the effects of almond hulls on growth performance, immunity, intestinal damage/recovery, and gut health in broilers challenged with Eimeria spp. It is *hypothesized* that almond hulls containing fibers and antioxidants will promote intestinal fermentation and reduce inflammation and oxidative stress, promoting growth performance, gut health, and protection in broilers.

Methods and Results A total of 360 one-day Cobb 500 birds will be used in the study. There will be 12 birds per replicated battery cage and 6 replicate cages per treatment. Broilers will be given feed and water ad libitum. Broilers in challenged group (T2 to T5) will be inoculated with 25,000 oocyst of *E. maxima, E tenella and 125,000 oocysts of E. acervulina* on d14, and birds in non- challenged group will be inoculated with same amount of PBS.

Growth performance: As we expected, challenge with the middle dose of the coccidiosis compromised the body weight gain and feed conversion ratio. Supplementation of the almond hulls did not further reduce the growth performance compared to challenged control. This may due to that there are very limited amount of protein in the almond hulls. It is reported some high fibrous by product exacerbated the cocci challenge due to the undigested crude protein. Aside from the growth performance, gut lesion score, gut permeability, oocysts counting in fecal samples and oxidative stress in liver are all important parameters to evaluate the cocci infection and recovery.

Gut health and oxidative stress: As shown in Table 2 below, challenged with coccidiosis increased the gut permeability and reduced SOD dismutase compared to the non-challenge positive control. When supplementation at 3 and 9% level, the SOD dismutase activity is improved compared to the challenged negative control. When supplementation at 3 and 9% level, the SOD dismutase activity is improved compared to the challenged negative control. However, supplementation of the almond hulls at 9% increased the ceca lesion score compared to the challenged control group, which is not as our expected.

Conclusions: Supplementation of the almond hulls were able to improve the SOD dismutase activity in coccidiosis challenged group and 3% seems an optimum dose based on lesion score and SOD dismutase activity to ABC.

Determination of Fatty Acids and Tocopherols in Egg Yolks from Laying Hens Fed Almond Hulls

Principle Investigator: **Woo Kyun Kim and Ron Pegg.** Dept. of Poultry Science, University of Georgia

Background and Objectives- Many studies have demonstrated that dietary fibers and phytochemicals changes blood fatty acid profiles and body antioxidant properties including Vitamin E levels in humans and rats. Since almond hulls contain high levels of dietary fibers and phytochemicals, feeding almond hulls to poultry has potential to modify lipid profiles and vitamin E contents in meat and eggs. Thus, we hypothesize that almond hulls containing fermentable fibers and phytochemicals will modify fatty acids and tocopherols in egg yolks.

Specific Aim: To evaluate the effects of almond hulls on fatty acids and tocopherols in egg yolks.

Methods and Results

I. Treatments

1) Corn and SBM diet meet the Hyline recommendations.
2) 7.5% Non-pareil included to the diet.
3) 15% Non-pareil included to the diet.
4) 7.5% Carmel included to the diet.
5) 15% Carmel included to the diet.

II. Egg collection

There are 5 treatments in total with 10 eggs in each treatment. Egg yolks from a treatment will be randomly pooled into 3 lots comprising 3, 4, and 3 yolks, respectively. The 3 lots for a treatment will be frozen and then lyophilized. Extraction of lipids will be performed from each freeze-dried lot for fatty acid (via GC-FID) and tocopherol (HPLC-fluorescence) analyses. Samples from each lot (NB, derivatization required for GC-FID FAME analysis) will be injected in duplicate.

III. Extraction of Lipid Constituents.

There are several approaches that can be used here: (1) the yolks can be lyophilized before the onset of extraction to base the calculations on a dry weight basis; or (2) lipids can be extract from portions of yolks (fresh weight), and a moisture analysis (AOAC Method 922.06) should be performed. The best, as noted above, is the lyophilization of an egg yolk lot using a FreeZone 2.5 L freeze dryer (Labconco Corporation, Kansas City, MO, USA).

After lyophilization, ~0.5 g of ground freeze-dried yolk powder will be transferred to a glass tube and mixed with 5.0 mL of $CHCl_3/CH_3OH$ (2:1, *v/v*) as described by Folch et al. (1957). The tubes will be capped and vortexed for 30 s, and then placed in a shaker at ambient temperature overnight for optimal extraction of lipid constituents. The tubes will be centrifuged to obtain a clean layer. Roughly 2 mL of the supernatant will be filtered through a 0.45 µm PTFE filter with a glass syringe. One milliliter of the filtrate will be transferred to a preweighed glass tube and solvent evaporated using an N-EVAP (Organomation Associates, Inc., Berlin, MA, USA) system. The tube will be reweighed to determine the lipid mass. This lipid sample can be used either for FAME or tocopherol analyses.

IV. Yolk Fatty Acid Composition

Extracted yolk lipids will be derivatized to their fatty acid methyl esters (FAMEs) according to established procedures in Dr. Pegg's lab. An Agilent 6890N GC-FID system equipped with electronic pneumatic control (EPC) split/splitless injector and a 7683 autosampler module will be employed for FAMEs analysis. A Supelco SP-2560 highly polar biscyanopropyl column (0.25 mm × 100 m, 0.20 µm film thickness, Bellefonte, PA, USA) will be used. The analysis will be operated in the split mode at a split ratio of 50:1 with UHP-grade helium as the carrier gas. The column head pressure will be set at ~60 psi. UHP-grade hydrogen and scientific-grade air will be the fuel gases for the FID at 450 and 40 mL/min, respectively. The

initial oven temperature will be set at 140 °C and held for 5 min before ramping up to a final temperature of 240 °C at a rate of 4 °C/min. This final temperature will be maintained for 15 min.

V. FAME Identifications

FAME identifications will be made by retention time mapping against GLC-463 FAME standard mixture (Nu-Chek Prep., Inc., Elsyian, MN, USA). A relative response factor will be calculated for each FAME using methyl heptadecanoate as the internal standard (IS). Each FAME has a different response to the FID depending on its chain length, saturation, and *cis/trans* configuration, as shown in the following equation:

$$R_i = (Ps_i \times Ws_{17:0}) / (Ps_{17:0} \times Ws_{is})$$

where, R_i = relative response factor for fatty acid *i*; Ps_i = peak area of individual FAME *i* in the FAMEs standard solution; $Ws_{17:0}$ = mg of C17:0 FAME in injected FAMEs standard solution; $Ps_{17:0}$ = peak area of C17:0 FAME in FAMEs standard solution; and Ws_{is} = mg of individual FAMEs *i* in injected FAMEs standard solution.

VI. Tocopherol (T) Analysis

Lipid Sample Preparation. The lipid extract will be passed through a 0.45 µm nylon membrane filter. A 1.0-mL aliquot of the extract will be evaporated under a stream of nitrogen using the N-EVAP system. The lipids will be redissolved in 1 mL of the mobile phase of 0.85% (v/v) isopropanol in hexanes + 0.01% (v/w) butylated hydroxytoluene (BHT) prior to manual injection in the HPLC. All extraction steps will be carried out under yellow light to prevent oxidation of the Ts.

HPLC Quantitation. The prepared samples or T standard solutions will be injected directly into a 20-µL Rheodyne loop of an HPLC system comprising a Shimadzu LC-10AT (Tokyo, Japan) controller/ pump, CBM-20A Prominence communications bus module, DG-14A degasser, RF-10AXL fluorescence detector and EZStart chromatography software (Shimadzu Corporation, Columbia, MD, USA). A LiChrosorb® Si 60 column (4 mm × 250 mm, 5 µm particle size; Hibar Fertigsäule RT, Merck, Darmstadt, Germany) will be connected to a LiChroCART® 4-4 guard column packed with LiChrospher® Si 60 (5 µm), and an isocratic mobile phase comprising 0.85% (v/v) isopropanol in hexanes + 0.01% (v/w) BHT at a flow rate of 0.8 mL/min will be employed. Before use, the mobile phase will be vacuum filtered through a 0.45 µm nylon membrane filter (MSI, Westboro, MA, USA). The excitation and emission wavelengths for fluorescence determination of the T isomers will be 290 and 330 nm, respectively. Concentrations of the Ts will be calculated from each peak area determined by the integrator based on the fluorescence response of each T in a 20-µL injection of the working standard stock solution.

Conclusions- Because of COVID-19 pandemic, we could not conduct the study. Now, UGA reopened for research. We are working on generating data.

Effects of Almond Hulls on Salmonella Infection in Broilers and Laying Hens

Principle Investigator: **Woo Kyun Kim,** Dept. of Poultry Science, University of Georgia

Background and Objectives- Food-borne illness caused by *Salmonella* is of both public health and economic concerns. *Salmonella* is one of the most prevalent pathogen causing egg and chicken meat contamination. Thus, Limiting *Salmonella* contamination in eggs and meats is a critical issue for the poultry industry. Moreover, it is challenging to prevent *Salmonella* infection in poultry under antibiotics-free conditions. Thus, feed amendments and interventions have received increased interests. Some of these amendments include feeding prebiotics and probiotics and have been successfully used in broilers or laying hens to reduce the *Salmonella* infection in their internal organs. Prebiotics or probiotics promote gut fermentation, beneficial bacteria growth (Lactobacillus and Bifidobacteria), and short-chain fatty acid (SCFA) production such as acetic, propionic, and butyric acids and reduce gut pH, eliminating pathogens in the intestine. Since almond hulls contain dietary fibers, we hypothesize that almond hulls will stimulate gut fermentation and SCFA production and reduce gut pH in order to limit Salmonella colonization and promote immune response and gut health in poultry.

Specific Aim: To evaluate the effects of almond hulls on Salmonella infection, Salmonella colonization in organs and immune response in broilers and laying hens challenged with Salmonella.

Methods and Results

Study1 (Broiler trial)

	Control	6% almond hulls (AH)	9% almond hulls
Salmonella challenge	T1 (PC)	T2 (6% AH)	T3 (9% AH)

Birds and *Salmonella.* infection

A total of 300 one-day Cobb 500 birds will be used in the study. There will be 10 birds per replicated battery cage and 10 replicate cages per treatment. Broilers will be given feed and water ad libitum.

I. **Challenge:** Chicks will be challenged orally with Nalidixic acid-resistant *Salmonella typhimurium* (NAL^R ST). The NAL^R ST cultures used in our challenge protocol will be provided by USDA Athens, GA. A single colony of NAL^R ST. A night before, NAL^R ST will be streaked out into a BGS-NAL plate and will be grown overnight at 37°C and 42°C, respectively.

II. **Dose: Chicks will be individually challenged with 10^7 cfu/ml NAL^R ST on Day 2**

Study2 (Laying hen trial)

	Control	7.5% almond hulls (AH)	15% almond hulls
Salmonella challenge	T1 (PC)	T2 (7.5% AH)	T3 (15% AH)

Birds and *Salmonella.* infection

A total of 60 laying hens (Hyline W26 = 34 weeks old) will be used for the study. Two laying hens will be housed together per cage (30 total cages) in M house at the Poultry Science Research Farm at the University of Georgia. The layers will be fed ad libitum feed and water. Feed will be provided in mash form. There will be three treatments.

I. **Challenge:** Hens will be challenged orally with Nalidixic acid-resistant *Salmonella Enteritidis* (NAL^R SE). The NAL^R SE cultures used in our challenge protocol will be provided by USDA Athens, GA. A single colony of NAL^R SE. A night before, NAL^R SE will be streaked out into a BGS-NAL plate and will be grown overnight at 37°C and 42°C, respectively.

II. **Dose: Laying hens will be individually challenged with 10^6 cfu/ml NAL^R ST on Day 7**

Conclusions- Because of COVID-19 pandemic, we could not conduct the studies. Now, UGA reopened for research. We are planning to conduct trial 1 in November 2020, and trial 2 in February or March 2021.

Evaluation of Biochar for On-Farm Soil Management in California

Principle Investigator: Sanjai Parikh, Dept. of Land, Air and Water Resources, University of California Davis
Co PIs: Danielle Gelardi, William Horwath, Daniel Geissler, Milt McGiffen, Michelle Leinfelder-Miles, Toby O'Geen, Kate Scow

Background and Objectives- Farmers, researchers, and policymakers are increasingly interested in the use of biochar as a soil amendment, as it may offer solutions to pressing agricultural issues such as nitrate leaching, low nutrient use efficiency, vulnerability of soils to drought conditions, and depleted soil carbon stocks. Previous research shows inconsistent results, due to differences in biochar feedstock, production methods, soil properties, climate, and cropping systems. Furthermore, biochar studies are dominated by short-term laboratory experiments that are difficult to extrapolate to field-scale. The overarching objective of this study is to provide field-scale data specific to CA regarding the potential for biochar to provide benefits for agriculture in the Central Valley. Specific objectives are:

1. Characterize biochars produced from local CA biomass, with an emphasis on almond shell
2. Evaluate the impact of biochar amendments on soil-water dynamics, nutrient use efficiency (including leaching), carbon (C) stocks, and crop productivity
3. Evaluate soil conditions and biochar parameters, including biochar and nitrogen (N) fertilizer application rates, which are most likely to lead to beneficial outcomes

Methods and Results- In year 3 of this ABC-funded project, significant progress was made in each of the proposed tasks. **Task 1: Produce and characterize biochar** was completed, with analysis of total hydrogen and particle size distribution added to list of biochar properties measured. Analysis of trends in physical and chemical properties by feedstock and temperature is currently underway. For **Task 2: Field trials in Yolo and Fresno Counties,** two seasons of processing tomato trials were completed, with the third and final year currently planted and awaiting harvest in late August 2021. In years 1 and 2, no significant differences in yield or nitrogen use efficiency were observed in soils with any biochar at any rate. There was a slight and significant increase in pH in soils amended with almond shell pyrolyzed at 800°C (AS800), though the effect was not substantial enough to influence yield. More extensive analysis on yield, nitrogen use efficiency, pH, and other soil health indicators such as microbial community structure and soil C respiration are currently underway and will be completed by early 2021. For **Task 3: Lab Trials,** batch sorption and column experiments were completed. Batch sorption experiments showed that almond shell biochar pyrolyzed at 500°C (AS500) best retained nitrate, where AS800 best retained ammonium. These two biochars, as well as a third biochar produced from softwood at 500°C were chosen for further experimentation. Columns of Hanford Sandy Loam (HSL) and Yolo Silt Loam (YSiL) (chosen to match soils in field trials) were amended with 0 and 2% of each of the three biochars. Each biochar significantly decreased saturated hydraulic conductivity (K_{sat}) in each soil, with the exception of AS800, which increased K_{sat} in the HSL. Further experimentation included nitrate and ammonium at 50ppm, gravity-fed through HSL columns to measure the quantity and timing of N leaching. Biochar significantly influenced both the timing and the quantity of ammonium, delaying its movement through the soil profile, and reducing the mass quantity leached, especially as a result of AS800 addition. There was a similar but much smaller effect for nitrate. Micro-CT scans of soils with and without biochar have been taken, and image processing to quantify porosity and pore structure is currently underway. **Task 4: Life cycle assessment of biochar amendments in CA** will ramp up in early 2021, to be completed by the end of the year.

Conclusions- While biochar did not increase processing tomato yield in years 1 and 2, evidence from the literature suggests that the effect of biochar on yield may be delayed as biochar weathers. Year 3 trials will may still show an effect on yield. The increase in pH from AS800 may also suggest that this biochar could have a greater effect in more acidic soils than those chosen for this study. Results from lab trials reveal that almond shell biochars best retained nitrate and ammonium at multiple pyrolysis temperatures when compared to coconut shell and softwood feedstocks. This suggests that almond shell biochar may be an appropriate material for use as a soil amendment to reduce nitrogen loss, an amendment in composting to retain biomass/compost nutrients, and in the treatment of wastewater.

Valorization of Almond Hull: Physiochemical Properties, Functional Application and Prospective Products - Non-thermal Processes for Making Clean Label Food and Nutraceutical Ingredients from Almond Hull

Principle Investigator: Roger Ruan, Center for Biorefining, Dept. of Bioproducts and Biosystems Engr., Dept. of Food Science and Nutrition, University of Minnesota
Co PIs: Juer Liu, Jun An, Yiwei Ma, Yanling Cheng, Peng, Min Addy, Chi Chen, Guangwei Huang, and Paul Chen

Conclusions – Various potential product development ideas have been derived from the preliminary evaluation of the characteristics of almond hull samples through different processing, which provide the industry with the new opportunities to valorize this low-cost ingredient. From our previous findings, powder A was chosen for its excellent antioxidant capacity and physiochemical properties to proceed to further research. The effect of different temperature of steeping water and non-thermal process including ultra-sonication and high-pressure homogenization (HPH) on the antioxidant capacity of the almond hull beverages was studied. In general, the samples dissolved at 60 ˚C assisted with non-thermal process achieved highest total polyphenol content while the most flavonoid contents were released when samples were dissolved at 100 ˚C followed up with non-thermal process. Moreover, other eight top-shelfed commercial beverages were selected for comparing their antioxidant capacity and they were performed poorer antioxidant capacity, the beverage with 5% hard-shell powder dissolved in 100 ˚C water assisted with non-thermal process scored the highest DPPH scavenging capacity as 1.56 mg ± 0.56 TE/ mL sample, which was more than 40 times than one of the most popular antioxidant beverages in market.

Then, further extraction and enrichment of phenolic compounds from almond hull (PEAH) were done and prepared to study the bioavailability of these compounds by using an in-vitro digestion/Caco-2 cell culture model. The total phenolic content (TPC) in PEAH was 481.52 ± 3.02 mg GAE/g dw, among which the contents of vanillic acid and chlorogenic acid are 119.47 mg GAE/g dw, 14.35 mg GAE/g dw respectively. Two in vitro methods, DPPH radical scavenging activity and FRAP, were used to evaluate the antioxidant activities of the PEAH, and both showed significantly higher antioxidant activities in both assay systems, with a TE value of 2697.73 ± 113.08 μmol TE/g dw and a AAE value of 2654.55 ± 64.28 μmol AAE/g dw. Tert-Butyl hydroperoxide (t-BOOH) is an organic peroxide widely used in a variety of oxidation process. It was shown that the PEAH had a positive protect effect Caco-2 cells against t-BOOH with higher cell viability and lower LDH leakage percentage. Pre-treatment with PEAH protected Caco-2 cells against the increase of intracellular ROS induced by t-BOOH, especially 5 μg/mL of PEAH prevented the increase by almost 73% compared to that of oxidative stressed cells. Additionally, PEAH was exhibiting a protective effect by recovery GSH content against t-BOOH oxidative insult alongside with lower carbonyl content resulted in less protein damage in Caco-2 cells.

Carbon Sequestration and Soil Heath Improvement in Almond Orchards Using Dairy Manure and Woody Biomass Compost

Principle Investigator: Ruihong Zhang, Dept. of Biological and Agricultural Engineering, University of California Davis
Co-PIs: Patrick Brown, Sat Darshan S Khalsa, Frank Mitloehner, Pramod Pandey, and Hamed El Mashad
Research Team: Hossein Edalati, Yike Chen, Tyler Barzee, and Allan Chio

Background and Objectives - The application of organic amendments to almond orchards can add organic carbon and nutrients to the soil, reduce greenhouse gas emissions and contribute to California's goals of building healthy soils. The objectives of this project are to: 1) Produce and characterize the physical and chemical properties of dairy manure and almond woody biomass compost products with and without pelletizing; 2) Investigate and demonstrate the effectiveness of the compost products in sequestering carbon and improving soil health in a commercial almond orchard and determine the impact of compost application on the greenhouse gas emissions; and 3) conduct outreach activities and disseminate project results to stakeholders and the public.

Methods and Results - In the first year (July to October 2019), we successfully produced loose and pelletized compost products, using dairy manure and almond sticks and trigs as raw materials. Windrow-composting was undertaken for 11 weeks. The pellets were produced using a pilot-scale pelletizer (Buskirk PM605). In the second year (August-October 2020), composting lasted 10 weeks and pelletizing is about to begin.

The produced compost products were characterized for bulk density and the contents of moisture, volatile solids, and pathogens (*E. coli O157 and Salmonella*). Pelletized and loose compost products were applied at a rate of 4 dry tons per acre to the almond orchard in March 2020. Second year application is planned to take place in November 2020. Prior to compost application, baseline tree circumference measurements and soil samples were taken for baseline physicochemical characteristics and pathogen contents of the demonstration plot. Leaf samples were collected in July 2020. GHG emissions from the orchard floor were measured using a static chamber technique were also measured in July 200. Gas samples were collected on the day before irrigation, one day after irrigation, and four days after irrigation. Gas chromatography was used to measure CO_2, CH_4, and N_2O contents in air samples collected from the flux chambers. Almonds were harvested in August 2020. A week prior to harvesting, soil and almond samples were collected for pathogen analysis. Almonds were mechanically shaken and almonds and field debris from 1 tree per microplot were hand harvested and weighed. Samples were collected and brought back to lab for analysis. The resulting yields of nuts and debris were determined to understand any changes caused by the compost amendments.

During 58 days of composting, the moisture content of manure and wood mixture dropped from 77.4% to 39.9%. Wet, total solids, volatile solids mass loss were 76.0%, 36.2%, 62.1%, respectively. Compost lost 9.1% in wet mass during pelletization. Data are being analyzed for determining the emissions (CO_2, CH_4, and N_2O), carbon and nutrient contents of compost and pellets, pathogens contents of compost and almond, and almond yields and will be reported as soon as the results are available.

Conclusions - Successful production and application of compost products to almond orchard soils were carried out in 2019-2020. Project progress was presented on posters at the 2020 ASABE California Nevada Section Annual Meeting; the 2020 CA Dairy Sustainability Summit; and the 2020 ASABE Annual International Meeting.

Chemical Characterization of New Almond Varieties

Principle Investigator: Alyson Mitchell, Dept. of Food Science and Technology, University of California, Davis
PIs Co: Haruka Tomishima, Dept. of Food Science and Technology, University of California, Davis

Background and Objectives- The objective of this proposal is to measure a range of chemical and physical markers of almond quality in 63 varieties of almonds in support of the 2019 “crack out” event held by the Almond Board of California. The overarching goal of this project is to create a comprehensive guide for analyzing almond quality and apply this approach to identify varieties of almonds that have the greatest potential for commercial production. During the “crack-out” event, 63 varieties of almonds were identified with potential for commercialization. The primary growing regions of these almonds include California, Spain, and Australia. This research will quantify chemical and physical markers of quality to help determine which varieties look promising for further field evaluation. Physical parameters being measured include almond color, moisture, and almond texture. The chemical markers being evaluated include volatile aroma compounds, including the primary compounds associated with raw almond aroma (amygdalin and benzaldehyde), vitamin E levels (i.e. tocopherols) and fatty acid profiles.

Methods and Results- The 63 almond varieties identified in the “crack-out” were collected and stored in a cold storage room at 0ºC in the dark. Humidity was not controlled in the cooler. The samples were double bagged in the Ziploc bags to slow down the loss of volatile compounds.

Color: Almond skin color was evaluated in unblanched whole almonds using L* a* b* color space values on a LabScan XE spectrophotometer (HunterLab). All samples were observed under D65/10 light source. Two replicates were taken for each variety and were averaged. The average values for L, a*, and b* are as follows: 41.03, 13.93, and 26.53; individual values will be available in table format.

Moisture: Moisture was determined in ground almonds that were passed through a size 20 sieve for uniform size. A Mark3 Moisture Analyzer (Sartorius Inc.,) was used to analyze moisture content. The average moisture of all varieties is 4.64%; individual values will be available in table format.

Texture: Texture will be analyzed in single kernels (10 per variety) using a TA.XT2 texture analyzer (Texture Technology Corp). Results will be presented as a graph with force in grams or Newtons on the Y axis and time/distance on the X axis. The total work done, maximum force, and force at the will be measured.

Amygdalin: Amygladin is analyzed in ground almond samples (replicates) after solid phase extraction (SPE) using ultra-high-performance liquid chromatography–electrospray ionization triple quadrupole–tandem mass spectrometry (Agilent Technologies). The calibration curves have been made, and data collection is in progress.

Benzaldehyde and Volatiles: Volatile aroma compounds will be measured in the headspace of ground almond samples (replicates) using a GC system coupled with a mass selective detector to identify and quantify the volatile samples (Agilent Technologies). Solid phase microextraction or SPME fiber will be used to extract the headspace volatiles. The results will be quantified using the standard curve and analyzed by analysis of variance. The graduate student has started training on the GC.

Fatty acid Profiles- Fatty acid profiles will be measured in almonds oil by trans esterifying the fatty acids into fatty acid methyl esters (FAMEs) and run by gas chromatography mass spectrometry (GC/MS).

Tocopherol (Vitamin E): Tocopherols will be measured in almond oil using an HPLC with a reverse phase C30 column (Agilent Technologies).

Conclusions- Research has been significantly delayed due to Covis-19, and data is in the collection stage.

Influence of Pasteurization on Oxidation in Raw Almonds over Storage

Principle Investigator: Alyson Mitchell, Dept. of Food Science and Technology, University of California, Davis
Co PIs: Kathleen Luo, Dawn Chapman, Dept. of Food Science and Technology, University of California, Davis

Background and Objectives- Since 2007, raw almonds in produced in California are required by law to undergo pasteurization to reduce the potential for foodborne illness. The goal of pasteurization is to kill surface bacteria on almonds using the least invasive process needed to preserve the nutritional and sensory characteristics of almonds. Thermal moist air pasteurization (TMA) involves exposing almonds to hot moist air for a short time, followed by a cooling/drying step. This type of pasteurization is widely employed in the industry, using patented technology that achieves TMA pasteurization with different parameters. Propylene oxide (PPO) is a fumigant used on raw agricultural products since the 1970s for pasteurization. In almonds, the approved process involves exposing kernels to PPO agents for 4 hours in a heated chamber (47-51 °C), followed by ventilation at 38-43 °C for 2 days or 15 °C for 5 days. Almonds are susceptible to lipid oxidation during storage as they are 50-60 % lipid by weight, in which 95 % are unsaturated fatty acids. Lipid oxidation can be initiated and accelerated by heat, moisture, enzyme activation, and UV light. During the pasteurization process, almonds are exposed to heat and moisture. The heat may increase enzyme activity or deactivate enzymes related to lipid oxidation. Moisture exposure can increase enzyme activity and fatty acid hydrolysis and accelerate lipid oxidation. Fumigation agents such as PPO, have the potential to deactivate enzyme through protein alkylation. Many studies have evaluated the effectiveness of different pasteurization methods on reducing foodborne pathogens, but the effect of pasteurization on lipid oxidation and nut quality is not known. The objective of this study was to understand the effect of PPO and TMA pasteurization on lipid oxidation in raw almond through shelf-life by monitoring chemical markers of lipid oxidation and the sensory characteristics of raw almonds.

Methods and Results- Nonpareil almonds from the same lot were separated into three subsamples; control (not pasteurized), PPO pasteurized, and TMA pasteurized. Pasteurization was carried out by Blue Diamond Growers (Sacramento, CA). Raw almonds were stored under accelerated conditions (32˚C, 60% relative humidity) to promote rancidity development over 12 months. Conjugated dienes levels (CD), free fatty acid levels (FFA), peroxide values (PV), and headspace volatile profiles were measured monthly. Sensory descriptive analysis was performed on samples stored for 0, 2, 4, 6, 8, and 10 months. Descriptive analysis was performed at The National Food Lab Inc. (Livermore, CA).

PV levels remained below 1.6 mEq/Kg oil in all groups tested over storage. PV levels in the TMA almonds were lower than control and PPO almonds for 10 months. PV levels were similar in PPO and control almonds until 7 months then increased. FFAs were significantly higher in control samples relative to TMA and PPO almonds over storage. CD levels were significantly lower in control samples relative to TMA and PPO almonds over 12-months of storage. A principle component analysis (PCA) was performed on the 60 headspace volatiles that were identified as significantly different between treatments using ANOVA. The PCA result indicated that after 6 months of accelerated storage, control samples have higher levels of lipid oxidation related volatiles (e.g. heptanol, nonanol, 1-octen-3-ol, and heptanoic acid) as compared to PPO and TMA almonds. Sensory evaluation indicated that total oxidized flavor and painty/solvent flavors are significantly lower in both TMA and PPO pasteurized almonds relative to the control. Clean nutty flavor decreased over storage time in all almonds, yet TMA pasteurized almonds maintained significant higher levels relative to the PPO and control almonds.

Conclusions- Pasteurization not only decreases foodborne pathogen, but also protects almonds by decreasing lipid oxidation during storage. Chemical measurements demonstrate lower amounts of FFAs, and lower levels of headspace volatiles related to lipid oxidation in pasteurized almonds as compared to controls. Descriptive analysis revealed that PPO and TMA pasteurized samples have significantly lower total oxidized flavor and painty/solvent flavor over storage relative to controls. These results indicate that pasteurization protects raw almonds against rancidity development during storage.

Developing Effective Drying Methods for Minimizing Quality Defects for Off-Ground Harvested Almonds

Principle Investigator: Zhongli Pan, Dept. of Biological and Agricultural Engineering, University of California Davis

Background and Objectives:
This research was conducted using a pilot-scale hot air column dryer of 6-feet height and a benchtop dryer. The main objective of this research was to study the hot air-drying performance and quality characteristics of off-ground harvested almonds under different drying conditions. The off ground harvested almonds with varieties of Nonpareil, Monterey, and Fritz were collected from Nickels Soil Lab, Arbuckle, CA and used for conducting the drying tests.

Methods and Results:
The almonds were dried at four temperatures of 45°C, 50°C, 55°C, and 60°C and air velocities of 1 m/s and 2 m/s with both the benchtop dryer as single-layer drying and the pilot-scale column dryer as deep-bed drying. Initial characteristics of freshly harvested almonds, including average moisture content (MC), moisture distribution in the almond components (hull, shell, and kernel), bulk density, weight ratio of major fractions (loose hulls, in-shell almonds, and in-hull almonds), insect damage, were measured. Drying characteristics, including drying time, throughput, hot air temperature/velocity/relative humidity (RH), energy consumption and energy cost, were determined. Quality attributes of the dried almonds, including finial MC distribution, cavity, concealed damage, kernel color, and oil quality, were also measured. The quality results of the almonds dried with hot air were compared with those of dried almonds from conventional harvesting.

Key findings and recommendations from this research include:

- Off-ground harvested almonds were cleaner and had less insect damage.
- The initial MC of in-hull almonds was much higher than the in-shell almonds.
- The drying time from their initial MC (17.7-20.9%) to kernels moisture content of about 6% ranged from 2.5 to 6 h for the column drying and from 1.0 to 4.3 h for the benchtop drying.
- Hot air drying did not show any adverse effects on the quality at even up to 60°C drying because of no significant differences in cavity, kernel color change, concealed damage, and oil quality compared to those of conventional harvested almonds.
- Drying at high temperature and air velocity increased throughput, reduced the drying time but generally increased the specific energy consumption and energy cost. For example, for Nonpareil variety, when the temperature increased from 45 to 60°C at 1.0 m/s velocity, the drying time was reduced by 21%, but the specific energy consumption and energy cost increased by 39%.
- The energy cost for the column drying of almonds ranged from 1.2 to 3.7 cents/lb of dried whole almonds when electricity was used as heating source with the rate of 14.08 cents/kWh.
- The energy consumption models, and engineering tool charts were developed based on different drying conditions and MCs.
- Up to 60% of the total energy was used for drying the hulls. While, only about 20% of energy was used to dry almond kernels.
- The research results suggested that presorting the harvested almonds into in-shell and in-hull almonds and hulls, followed by dehulling, and then drying inshell almonds should significantly reduce drying time and energy use, and improve moisture uniformity of almonds.

Performance Evaluation of Commercial Dryers for Drying of In-Hull Almonds

Principle Investigator: Zhongli Pan, Dept. of Biological and Agricultural Engineering, University of California Davis

Background and Objectives - This research was conducted using three different commercial dryers, including tunnel dryers at Campos Brother Farms, Caruthers, CA; stadium dryer at Emerald Farm, Maxwell, CA; and trailer dryer at West Valley Hulling Company, Firebaugh, CA. The main objective of this research was to evaluate the drying performance of different commercial dryers for drying off-ground harvested almonds. The off-ground harvested almonds with varieties of Independence, Monterey, and Fritz were used for conducting the drying tests.

Methods and Results - For the tunnel drying, Independence almonds were dried using six tunnels under three different drying conditions, including ambient air drying started at the daytime, ambient air drying started at the nighttime, and hot air drying at 46°C (115°F) with the air velocity of 1 m/s. For the stadium drying, Monterey and Fritz almonds were dried by using four bins with heated air at 35°C (95°F) and air velocity of 0.7 m/s. For the trailer drying, the Monterey almonds were dried by using four trailers under two different drying conditions of 43°C (110°F) and 54°C (130°F) with the air velocity of 1.2 m/s.

Initial characteristics of freshly harvested almonds, including average moisture content, moisture content distribution, bulk density, weight ratio of major fractions (hulls, in-shell almonds, and in-hull almonds), insect damage, and kernel color, were measured. Drying characteristics, such as drying time, throughput, ambient temperature/relative humidity (RH), air temperature/velocity, and utility use, were determined. Quality attributes of the dried almonds, including finial moisture content distribution, cavity, concealed damage, kernel color, and oil quality, were also determined. The quality results of the dried almonds from the drying testes were compared with those of dried almonds from the conventional harvest.

The key findings and recommendations from this research project are summarized as below:

- Off-ground harvested almonds were much cleaner and reduced insect damage by 57-67% compared to the conventional harvested almonds (2.0 to 10%).
- The harvest moisture (20.0-37.6%) had significant effect on hot air-drying time that ranged from 5.8 to 16.9 hours to 3.9-5.8% kernel moisture for Monterey.
- The drying temperature had a significant effect on the drying time and cost. For the tunnel drying, the drying at 115°F reduced the drying time by 74% and had higher energy cost by 15% compared to the ambient air drying. For trailer drying, the hot air drying at 130°F had 11% shorter drying time but 109% higher energy cost than the drying at 110°F.
- All dried products had no cavity, concealed damage, and change of kernel color after drying. Only almonds from trailer drying had peroxide values (PV) (1.33-1.83 meq/kg) and free fatty acid (AC) levels (0.26-0.31%) significantly higher than the conventional harvested almonds but far below the industrial standards (5 meq/kg PV and 1.5% AC). It is likely that the high numbers were due to the low harvest MC.
- The energy costs ranged from 0.07 to 1.91 cents/lb of whole dried almonds or 0.21 to 6.5 cents/lb of dried almond kernels that were calculated using the peak and off-peak rates of electricity at 0.15 and 0.25 $/kWh, 90 cents/therm for natural gas and 0.584 $/gallon for propane. The energy costs depended up on utility rate, initial moisture, dryer type and drying condition. It appeared that trailer drying was the most efficient method among the dryers.
- The available commercial dryers can be used for drying the off ground harvested almonds without quality concerns.

Optimization of Drying Conditions for Off-Ground Harvested Almonds Using Trailer Dryer

Principle Investigator: Zhongli Pan, Dept. of Biological and Agricultural Engineering, University of California Davis

Background and Objectives- California almonds are conventionally harvested by shaking the crop to the ground, where it dries naturally for extended periods before being swept and collected. The off-ground/shake-catch harvesting method is considered as a better alternative to the conventional harvesting method to reduce dust during harvesting, insect damage, and microbial contamination of almonds. With the support from ABC, the effort in toward implementing the off-ground harvest method has been made by testing existing three different types of commercial dryers, including tunnel dryers, stadium dryers, and trailer dryers, in the last harvesting season. Among the three types of dryers, trailer dryers had an efficient drying performance and low energy cost without compromising the quality of dried almonds. Due to the limited time and at the late harvest season, the trailer drying tests were conducted with only two drying temperatures of 43ºC and 54ºC and one harvest moisture of 20%. Therefore, the goal of this research project was to optimize the drying conditions with extended temperature range of trailer dryers for drying off-ground harvested almonds with high moisture. Specific objectives include: 1) Determine the initial characteristics and moisture content (MC) of off-ground harvested almonds; 2) Evaluate the drying time, drying rate, and throughput capacity of trailer dryers using different air temperatures and almond varieties; 3) Determine the effect of drying temperatures of trailer dryers on quality characteristics of dried almonds, energy use and cost of the drying process; 4) Compare the qualities of almonds dried using trailer dryers with those of almonds dried with the conventional harvest method; 5) Determine the optimum drying conditions of trailer dryer to achieve high drying rate and throughput, low energy use and cost, and improved quality for dried almonds.

Methods and Results- The trailer dryers at West Valley Hulling Company, Firebaugh, CA were used to conduct the drying experiments. Three almond varieties of Nonpareil, Wood colony, and Monterey obtained from off-ground harvesting were dried at different conditions with targeted hot air temperatures of 50ºC, and 60ºC at air velocity of 1.2 m/s. Two trailers were tested at each drying condition. Due to the variation of temperature control, the actual average temperatures of the two trailers were 61ºC and 72ºC, plus ambient air, for Nonpareil, 56ºC and 65ºC for Wood Colony, and 50ºC and 59ºC for Monterey almonds. The initial characteristics, insect damage, drying performance, product qualities, energy consumption and cost of the dried almonds were determined and compared with those of almonds harvested with the conventional method. Drying characteristics, including drying time, throughput, ambient temperature and relative humidity of air, air temperature and velocity, and energy use were recorded.

The results showed that trailers had wet loading weight from 32520 to 45920 lb per trailer, depending on the varieties and moisture contents. Nonpareil used 6.8, 9.4, and 74.4 h for drying from moisture 34.4% to 9.7%, 13.9% and 7.5% at 72ºC, 61ºC and ambient temperature, respectively. Wood Colony took 25.3 and 40.9 h for drying from moisture 57.4% to 6.0% and 4.7%, at temperatures of 65ºC and 56ºC, respectively. Monterey used 9.6 and 17.8 h for drying from 24.3% and 32.4% to 8.1% and 8.5% at temperatures of 59ºC and 50ºC, respectively. The specific energy consumption and utility cost were in the range of 0.67 to 5.37 MJ/kg and 0.31 to 3.20 ¢/lb of dried whole almonds under all tested conditions. In general, high drying temperature reduced drying time, but increased energy consumption. The high initial moisture also resulted in high drying cost. Based on all obtained almond quality data, all of the tested drying conditions did not show any negative effect on the product quality. We are continuing quality measurement and data analysis.

Conclusions- The tested conditions with even up to 72ºC did not show significant negative effect on product quality. The high moisture of almonds, e.g. 57.4% for Wood colony, resulted in a long drying time and increased energy consumption and cost.

Developing an Efficient Drying Technology for Simultaneous Disinfestation and Disinfection of Off-Ground Harvested Almonds

Principle Investigator: Zhongli Pan, Dept. of Biological and Agricultural Engineering, University of California Davis
Co PIs: Franz Niederholzer, Cooperative Extension Colusa County, UC Agriculture and Natural Resources Luxin Wang, Dept. of Food Science and Technology, University of California, Davis

Background and Objectives- The current harvest and on-ground drying methods have three major problems, including insect infestation, microbial infection, and dust generation. The ongoing efforts of ABC and our research may provide the needed solutions to the problems by developing new off-ground harvest and drying technologies. With ABC's support, we successfully completed two research projects on the hot air drying of off-ground harvested almonds in 2019. We tested column air drying in the temperature range from ambient to 60ºC and found that all of the drying tests had no adverse effect on the quality characteristics of dried almonds, including cavity, kernel color, concealed damage, and oil quality. It is believed that a higher drying temperature might be used to further improve drying rate and throughput, at the same time possibility achieve for disinfection and disinfestation since almond quality showed high heat tolerance. Therefore, the goal of this research project was to develop drying technology for simultaneous disinfestation and disinfection of off-ground harvested almonds. Specific objectives include: 1) Determine the initial characteristics and moisture content (MC) of off-ground harvested almonds; 2) Assess the insect development and microbial risk/load of the almonds harvested with conventional methods and naturally dried on orchard floor and on tarp to identify the cause of insect damage and microbial risk; 3) Compare the insect and microbial risk results with that from off-ground harvested almonds; 4) Determine the influence of new constant temperature drying and stepwise temperature drying on insect disinfestation, microbial disinfection, drying and quality characteristics, energy use and drying cost of off-ground harvested almonds; 5) Compare the insect damage, microbial safety and quality characteristics of off-ground harvested almonds dried with the new drying method with those of almonds dried with the conventional method; and 6) Determine the optimum conditions to achieve effective insect disinfection, microbial disinfection, high drying rate, low energy use and cost, and maintained quality of dried almonds.

Methods and Results- The insect damage and natural prevalence of Salmonella in the off-ground and conventionally harvested almonds were determined and evaluated. The changes of total bacterial and fungal amounts in the freshly harvested almonds during temporary outdoor storage were also studied. A pilot-scale hot air column dryer of 2-ft height was used to conduct the drying experiments. Three almond varieties of Nonpareil (NP), Wood colony (WC) and Monterey (MT) obtained from off-ground harvesting were dried at constant temperatures of ambient, 40ºC, 50ºC and 60ºC, and at the stepwise temperature drying with starting temperatures of 80ºC and 90ºC followed by holding and finishing at 60ºC. The initial characteristics, insect damage, drying performance, product qualities, energy consumption and microbial safety of the dried almonds were determined and compared with those of almonds harvested with the conventional method. Most of proposed work has been completed and the results need to be statistically analyzed.

The results showed that off-ground harvested almonds were cleaner and had less insect damage, less natural Salmonella prevalence and coliform levels compared to conventionally harvested and on-ground dried almonds. Three days of temporary storage of the wet off-ground harvested almonds caused significant increase in the total bacterial and fungal counts and microbial safety risk. The stepwise drying of even up to 90ºC at the starting stage did not show any adverse effect on the product quality compared to conventional harvest and drying. The stepwise drying, and 50ºC and 60ºC constant drying effectively killed all insects in the almonds and achieved up to 0.8 log total bacterial reduction. The stepwise drying had similar drying times (7 to 10.6 h), specific energy consumptions (14.3 to 22.0 MJ/kg almonds) and energy costs (11.3 to 17.9 ¢/lb of dried whole almonds) compared to 60ºC constant temperatures. It was also found that drying the in-shell almonds through dehulling before drying resulted in 77% lower energy cost and 75% less drying time compared to drying the in-hull almonds harvested from the same batch.

Conclusions- Off-ground harvested almonds had less insects and food safety risk than the conventionally harvested and dried almonds. Stepwise high temperature drying could improve the drying throughput and achieve effective disinfestation and reduce the microbial risk. It is suggested to use presorting followed by dehulling, and then only dry in-shell almonds to reduce the drying cost, improve the drying efficiency and throughput.

Assessing the Impact of Inshell Storage on the Shelf Stability of Almonds

Principle Investigator: Ronald Pegg, Dept. of Food Science and Technology, College of Agriculture and Environmental Science
Co PIs: William Kerr, George Cavender, Dept. of Food Science and Technology, College of Agriculture and Environmental

Background and Objectives- The impact of storing raw almonds inshell, relative to shelled almond storage, on kernel shelf-stability is currently being investigated. In February and March of 2019, raw 'Nonpareil' and 'Butte' almonds were stored both inshell and as shelled kernels with brown skins into woven polypropylene bags (10 x 10 weave, 1000 denier). These samples were stored at selected environmental conditions, which varied in temperature (T) and percent relative humidity (%RH) to help determine/assess optimal and possibly abusive storage conditions. For this study, the following storage treatments were used: 25°C/70 %RH; 25°C/55 %RH; 15°C/70 %RH; 15°C/55 %RH; 10°C/65% RH; 4°C/No %RH control; ambient T in GA/No %RH control; and ambient T in CA/No %RH control.

Methods and Results- Baseline data for the chemical (*i.e.*, peroxide value, percent free fatty acids, headspace volatiles composition), textural (*i.e.*, 'crunchiness' as measured through the area under force displacement curves), and sensory (*i.e.*, consumer acceptance) attributes of all almond types were collected upon receipt of samples to be used for comparative purposes throughout the study.

The almonds that currently remain in this study are those, which have not been rejected by a consumer acceptance sensory analysis. Almond samples that achieved a PV > 2.0 meq. active O_2/kg oil or a 'crunchiness' texture analysis value that deviated > 15% from its baseline value, were triggered to be evaluated by a consumer acceptance panel (n ≥ 100). All almond types of the 4°C/No %RH control and 25°C/70 %RH conditions were rejected after 6 and 10 months of storage, respectively, thereby highlighting these conditions as being the most abusive to almond quality and shelf-life. Following the onset of the COVID-19 pandemic, sensory testing had to shift to an "in-house" panel consisting of 4 researchers and graduate students. Using this panel, and the same triggers, the shelled 'Butte' almonds of the 15°C/70 %RH and 25°C/55 %RH conditions as well as the shelled 'Nonpareil' almonds of the ambient T in GA/No %RH control condition were rejected after 16 months of storage.

Certain trends have been witnessed in the chemical attributes of these stored almonds. Currently, and throughout the study, inshell almond samples have reported lower %FFAs than their shelled counterparts. Yet with regard to PVs, this trend has only consistently been observed for almonds of the 'Butte' cultivar, as some inshell 'Nonpareil' almonds have reported similar values to shelled 'Nonpareil' almonds from the same condition.

Conclusions- The variation in PV results between the 'Nonpareil' and 'Butte' cultivars is believed to be a result of the differences in shell properties/composition between almonds of the 'Nonpareil' and 'Butte' cultivars. The shell of 'Butte' almonds is a semi-hard shell with a low suture opening, while that of 'Nonpareil' almonds is a soft shell with a high suture opening. It is predicted that the 'Nonpareil' shell is more penetrable by molecular oxygen, which drives the mechanism of lipid autoxidation, therefore resulting in higher PVs. This study will continue to investigate the impact of inshell almond storage until March 2021 (24 months of storage) or until all samples have been rejected from the study.

Survival of inoculated generic *E. coli* on developing almonds between fruit set and harvest

Principle Investigator: Linda Harris, Dept. of Food Science and Technology, University of California, Davis
Co PIs: Chris Theofel, Western Center for Food Safety, University of California, Davis

Background and Objectives- Agricultural water is recognized as an important potential vehicle for contamination of crops with foodborne pathogens. Developing almonds are more likely to contact agricultural water through crop protection sprays than other routes. In-orchard studies designed to mimic application of a contaminated crop protection spray showed that inoculated generic *Escherichia coli* (non-pathogenic *Salmonella* surrogate) can survive for up to 5 weeks, and in some cases multiply, on developing almonds. While these studies demonstrated an ability for a surrogate organism to survive in the orchard canopy on developing fruit, it is unknown how the crop protection chemicals may impact the survival or growth of a potential pathogen. The objective of this study is to determine the ability of potential pathogens to survive or grow in a crop protection spray mixture. Crop protection chemicals likely to be used near (≤ 4weeks) harvest were considered in these studies.

Methods and Results- In consultation with UCDavis crop specialists and registered Pest Control Advisors (PCA) three products that could be applied to almonds within 4 weeks of harvest were selected – the insecticides Bifen 2 Ag Gold (Winfield United) and Intrepid 2F (Dow), and the fungicide PH-D (UPL). Label strength chemicals were inoculated at approximately 5 or 2 log CFU/ml with a cocktail of tree-nut relevant *Salmonella* serovars (Enteritidis PT30 [almonds], Montevideo [pistachios], Senftenberg [pistachios], Saintpaul [walnuts]) and the same generic *E. coli* cocktail used in the in-orchard studies. Inoculated samples were held at room temperature (24 ± 2°C) in sealed tubes and microbial populations were enumerated on Tryptic Soy Agar, CHROMagar Salmonella (*Salmonella*) and CHROMagar ECC (*E. coli*) at 0 and 24 h.

At an inoculum concentration of ~5 log CFU/ml, *Salmonella* and *E. coli* cocktails populations fell below the lower limit of detection in PH-D over 24 h (a decline of >4.30 log CFU/ml; n=3). In contrast, a decline of < 0.50 log CFU/ml was observed for both cocktails after 24 h in Intrepid 2F and Bifen 2 Ag Gold (n=9). Similar results (declines of <0.1 log CFU/ml) were observed in Intrepid 2F when inoculated at initial populations of ~2 log CFU/ml (n=3). However, declines of 0.49 ± 0.02 and 0.89 ± 0.08 log CFU/ml, respectively of *Salmonella* and *E. coli* of were observed in Bifen 2 Ag Gold inoculated at ~2 log CFU/ml) after 24 h (n = 3).

Conclusions- *Salmonella* and *E.* coli can survive for at least 24 h in the pesticides Bifen 2 Ag Gold and Intrepid 2F at label strength, but not the fungicide PH-D. Growers should take extra care to ensure water used in crop protection sprays during this period is of high microbial quality.

Characterization of *Salmonella* isolated from almonds by whole genome sequencing

Principle Investigator: Linda Harris, Dept. of Food Science and Technology, University of California, Davis

Co PIs: Anne-laure Moyne, Western Center for Food Safety, University of California, Davis

Background and Objectives- From 2001 to 2013, almonds were collected from seven almond handlers representing processing facilities located throughout the almond-growing regions of California. *Salmonella* isolates were retrieved from 1% of ~15,000 raw almond samples. In recent years, whole genome sequencing has become the standard method for characterization of foodborne pathogens. Many of the isolates that have been sequenced are clinical isolates with far fewer food and environmental isolates generally available. Sequencing of the almond survey isolates contributes to understanding environmentally sourced *Salmonella* and their genetic stability and variability. Ultimately it should also serve to assist the industry in interpretation of Food and Drug Administration (FDA) and Centers for Disease Control and Prevention (CDC)-derived sequence analysis.

Methods and Results- The genome sequences of 171 unique *Salmonella* isolates recovered from an almond survey conducted over 9 years were processed using standard methods (sequences were aligned with Parnsp followed by single nucleotide polymorphisms (SNPs) detection with the FDA Center for Food Safety and Applied Nutrition (CFSAN) SNP pipeline. All isolates belonged to the *Salmonella* species *enterica*, with a majority of the serovars (165) belonging to the subspecies *enterica*. Three isolates were classified as subspecies *arizonae* and three as *diarizonae*. Serovars identified by classical serotyping matched the serovars predicted by whole genome sequencing (WGS) in 92% of isolates. Fifteen serovars were represented only by one isolate, and 22 serovars were isolated multiple times. Genetic distance between multiple isolates within an individual serovar was calculated with the CFSAN SNP pipeline. Isolates with less than 10 SNPs differences between their genomes were considered genetically similar. *Salmonella* Enteritidis clustered in three groups that corresponded with Phage type: PT8, PT9, and PT30. Genomes of *Salmonella* Enteritidis, with less than 10 SNPs differences with the 2001 outbreak associated-strain, were retrieved in 2005 and 2006. Closely related genomes for several isolates of serovars Anatum, Infantis, Montevideo, Muenchen, Newport, Thompson, and Typhimurium, were identified 2, 8, 12, 10, 10, 1 and 3 years apart, respectively. Identification of genetically similar isolates of multiple serovars suggesting that some of these organisms may persist in the production environment.

A total of 18 antibiotic resistance genes (AMR) were identified with the ResFinder database that classified into seven different antimicrobial protein groups: aminoglycosides, β-lactams, fosfomycin, phenicol, sulfonamide, tetracycline, and trimethoprim. One gene, *aaa (6)-Iaa*, that conferred resistance to aminoglycosides, was detected in all *Salmonella* subspecies *diarizonae* (3) and *enterica* (165) but not in *arizonae* (3). The other 17 AMR genes were detected in 35 isolates, with *fosA7* being the most common gene. Virulence genes present in all isolates encoded proteins involved in type III secretion system (T3SS), magnesium uptake and some adhesins.

Conclusions- Multiple serovars of *Salmonella* were retrieved from the almond survey indicating that the contamination of almonds is likely a random environmental event that likely occurs during harvest or hulling and shelling. However, genetically similar isolates of multiple serovars were identified with WGS suggesting that some of these organisms may persist in the production environment.

Almond Quality and Food Safety Research Publications 2020

The following is a list of scientific papers published with the research partially or wholly funded by the Almond Board of California. Copies of the abstracts may be obtained by contacting the Almond Board of California.

An, J., J. Liu, Y. Liang, Y. Ma, C. Chen, Y. Cheng, P. Peng, N. Zhou, R. Zhang, M. Addy, P. Chen, Y. Liu, G. Huang, D. Ren, R. Ruan. 2020.
Characterization, bioavailability, and protective effects of phenolic-rich extracts from almond hulls against pro-oxidant induced toxicity in Caco-2 cells. *Food Chem.*
https://doi.org/10.1016/j.foodchem.2020.126742

Luo, K.K., D.M. Chapman, L.A. Lerno, G. Huang, A.E. Mitchell. 2020.
Influence of post-harvest moisture on roasted almond shelf life and consumer acceptance *J. Sci. Food Ag*. DOI 10.1002/jsfa.10624

Palma, L., J. Fernandez-Bayo, F. Putri, J.S. VanderGheynst. 2020.
Almond by-product composition impacts the rearing of black soldier fly larvae and quality of the spent substrate as a soil amendment *J. Sci. Food and Agric*. DOI 10.1002/jsfa.10522.

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Almond Board of California
1150 Ninth Street, Suite 1500
Modesto, CA 95354
T: 209.549.8262 | F: 209.549.8267
Almonds.com

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