Executive Summaries
2021

California Pistachio Research Board
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2021 Manager's Report

Bob Klein

The challenges to growing, harvesting, processing, and marketing each year's pistachio crop are constantly changing. What doesn't change is that each successive year seems to set a record. The 2021 US pistachio crop fits into this trend with a total crop of 1.167 billion pounds of which 98.9% (1.154 billion pounds) were produced in California. The industry has had record total crops in 4 of the last 6 years and the other two years were record off-year crops.

Growers had several challenges going into the 2021 growing season. Winter chill accumulation was relatively low, water supplies were limited, and use of an important pesticide for mealybug control was discouraged due to concerns over potential residues at harvest. Nut release at harvest was erratic, especially in cv. Golden Hills. Processing plants kept up with harvest but with the specter of machine malfunctions, there was little extra capacity across the industry. The harvested nuts are smaller than normal, tending to the smaller end of 21-25 ounce counts rather than the larger end as was seen in 2020. Split percentage was high at 84.7%, the highest since 1999. While high split percentages are generally positive, they do create some problems in efficiently meeting the expanding market for kernels.

As UCCE Kings County Farm Advisor Emeritus Bob Beede has noted, the pattern of winter chill accumulation has shifted. In the past, significant chill was accumulated in November and early December and virtually none after late February. Now, early winter chill accumulation has dramatically reduced but late winter chill accumulation has increased. We have been fortunate for the last few years that the late winter chill has occurred and helped with bloom synchrony. However, the shift creates problems for growers in timing oil applications and in the use of potential rest-breaking agents like hydrogen cyanamide. Because hydrogen cyanamide is newly registered for pistachios, there is a lack of experience with its use and effects in pistachio. We will certainly know more about it after 2022.

Gill's mealybug first appeared in pistachio orchards in the mid-1990s in a single location in Kings County before expanding across the industry in the early 2000s. Research by UCCE IPM Advisor David Haviland elucidated the life cycle and its control with a single well-timed pesticide application. In recent years, control has been inconsistent and sometimes requires more than one application. This is one aspect of a collision between increased post-harvest residues and decreased limits in large export markets which caused processors to discourage use of imidacloprid in 2021. Fortunately, there was another pesticide available, sulfoxaflor, to use for mealybug control. As we go into the 2022 season, the issues with imidacloprid have not been completely resolved and growers should expect some restrictions on its use. To complicate matters, a California Superior Court judge ruled on December 3 that the California Department of Pesticide Regulation erred in registering sulfoxaflor and banned its use. This might be resolved in time for 2022 use.

Growers have been successfully meeting one of our ongoing challenges, navel orangeworm (NOW) damage and the resulting aflatoxin contamination. Since the 2018 crop, there has been 4 years of very low (0.5% to 1%) NOW damage and very low aflatoxin levels in each crop. This has allowed the US pistachio industry to lower the number of inspections in the EU from 20% to about 2-3% of the lots shipped to the European Union. Growers – do not relax! The EU is very quick to increase mandatory testing and very slow to reduce it.

It is difficult to say how conditions are setting up for the 2022 crop. At the end of December, both precipitation and chill looked very promising but not so much at the end of January. Individual orchards continue to alternate bear but the weather conditions and the large acreages that have come into bearing in the last 5 years have suppressed the industry-wide total crop alternate bearing. As discussed at Pistachio Day, it is hard to envision conditions that would drive the average yield below 2200 pounds/acre and, conversely, that would drive it above 2900 pounds per acre. With about 427,000 bearing acres in 2022, the crop should be between 940 million and 1.24 billion pounds. Another new record crop is not unlikely.

Please read through the Executive Summaries printed in this booklet to better understand the research that will help drive and maintain a vibrant, profitable, and sustainable pistachio industry. The outcome of research is never certain, but it can help us all in our pistachio endeavors.

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Authors: **Julia Stover**, Academic Coordinator, Department of Plant Sciences, UC Davis; **Kevin Taniguchi**, GIS Programmer, Department of Plant Sciences, UC Davis

Introduction

The Fruit and Nut Research & Information Center (FNRIC) has provided support for the California agricultural community since its establishment by the University of California in 1995. The center core website (http://fruitsandnuts.ucdavis.edu) provides information and relevant links for specific crops. In addition to the website, FNRIC works to coordinate UC and industry communication through conferences, meetings, and courses.

Between the California Pistachio Research Board and the UC ANR Pistachio Workgroup there are 4 annual pistachio research events as well as other events that occur less frequently. Until 2019, the organization of these events has been handled separately, by the ANR PSU and the California Pistachio Research Board.

Results and Discussion

In 2019, FNRIC began providing support for upcoming Pistachio events, working with ANR PSU to develop a social media presence for Pistachio Day 2020 and facilitating the review process for the 2020 CPRB proposals.

Despite COVID-19 related cancellations and changes, in 2021 FNRIC continued and expanded these activities, assisting with abstract review for the 2022 Pistachio and Almond ISHS Meeting, and generally supporting ANR PSU. We have once again supported the review of the 2021 CPRB proposals and helped coordinate a restructuring of the review process for the 2022 proposals.

Conclusion

Overall feedback has suggested that our support has proven especially valuable for the proposal review process and the promotion of virtual events. We believe that we have continued to provide a valuable service to the California Pistachio Research Board and the UC ANR Pistachio Workgroup.
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California Pistachio Workgroup Website Development

Authors: Julia Stover, Academic Coordinator, Department of Plant Sciences, UC Davis; **Kevin Taniguchi**, GIS Programmer, Department of Plant Sciences, UC Davis

Introduction

The Fruit and Nut Research & Information Center (FNRIC) has provided support for the California agricultural community since its establishment by the University of California in 1995. The center core website (http://fruitsandnuts.ucdavis.edu) provides information and relevant links for specific crops. Content also includes interactive weather-related models, general management information, links to Cooperative Extension newsletters, and links to associated websites developed by the FNRIC to focus on extending current research to the agricultural community.

The Pistachio Workgroup is responsible for Statewide Pistachio Day, as well as the Pistachio Short Course, and the development and revision of the Pistachio Production Manual. In addition to these events, the workgroup meets annually to discuss new and ongoing research. The members of this workgroup are spread across the state from the UC Davis campus to Kern county.

Results and Discussion

Funding was requested to develop a site for California pistachio research and supporting the Pistachio Workgroup, in association with the main FNRIC site. This site will be used to create a central location for workgroup members, researchers, and growers to find information about upcoming events and will be used for timely media pushes for upcoming meetings and other events, such as the Pistachio Short Course. The site will also be used to provide an organized list of links to related research and other useful links, host grower tools for decision making, and facilitate communication between pistachio researchers with interactive boards.

Conclusion

The site is live as of Spring 2021 (https://ucanr.edu/sites/CaliforniaPistachioResearch/) and was presented to the Pistachio Workgroup at this year's meeting. We have not had the anticipated level of engagement with the site to date, we will be continuing to work with the Pistachio Workgroup to ensure that we are meeting their needs and that the site is kept up-to-date.

Raman Spectroscopy to Detect and Measure NOW Pheromones

Authors: **Tiziana Bond**, PhD, Senior Staff Engineer, **Sarah Sahota,** junior staff, and **Allan Chang**, PhD, staff, Lawrence Livermore National Laboratory; **Houston Wilson,** PhD, Asst. Coop. Extension Specialist, Kearney Agricultural Research and Extension Center, Dept. Entomology, UC Riverside; **Chuck Burks**, PhD, Research Entomologist, USDA Agricultural Research Service.

Introduction

Insect sex pheromones are chemical compounds that insects release to attract mates over distances of hundreds of meters or even kilometers, in complete darkness and without any audible signals. Use of synthetic forms of key compounds have in some cases become an essential component of monitoring and/or managing key pests of agricultural crops, including navel orangeworm (*Amyelois transitella*) (NOW) in California tree nuts. There are currently multiple commercially available mating disruption products available for NOW and recent studies have demonstrated that they can effectively help reducing crop damage. How mating disruption works is not fully established and likely varies across products and target species. For instance, it is not well understood how synthetic pheromones compete with natural ones and, in the case of monitoring, how efficaciously the insect follows the diffusing plumes and how those evolve from emission points, especially across large blocks and at plot borders.

We proposed to evaluate the use of enhanced Raman Spectroscopy (RS) for the detection of synthetic and natural NOW pheromones and, were that successful, use it in the future to measure pheromone diffusion in orchards. This year we continued Year 1 work, targeting two main objectives: (1) *Use Raman to Detect Pheromones and (2) Use Raman to Measure Pheromone Diffusion*. In Year 1 we were able to generate the Raman signatures for the main chemicals in synthetic pheromones, and now in Year 2 we have been able to provide a clear distinction of such signatures in mixed samples via Principal Component Analysis (PCA), validating RS for detecting emissions in more realistic conditions. In Year 2, we also continued our preparative efforts in measuring any release from dispensers or lures and setup some of our first "orchard in a box" experiments for diffusion studies. Given the low concentrations of pheromone release, we invested in boosting detection capacity by implementing two approaches: (1) preconcentrate samples with Solid Phase MicroExtraction (SPME) fibers[3] following GC-MS approaches applied to Raman and (2) pursue Surface Enhanced RS (SERS) to enhance the overall optical signals.

Funding from the CPRB was only approved within LLNL in May 2021, causing delays that were further accentuated by the lingering COVID-19 crisis, since laboratories had limited access until July 2021. During this downtime we requested and received DoE and State approval to work with live NOW moths for future experiments in collaboration with Dr. Wilson at UC Riverside. Despite all these constraints, we generated new and promising data on Raman selectivity and on pathways to enhancing its sensitivity for concentration-dependent diffusion studies of synthetic vs. natural pheromones.

Results

Since we had demonstrated the ability to detect Raman signals for various concentrations of what are accepted as most critical components in pheromone, i.e. ((Z,Z)-11,13-Hexadecadienal, (Z,Z,Z,Z,Z)-(3,6,9,12,15)-Tricosapentene, and (E,Z)-7,9-Deocedadien-1-yl Acetate`, this year we extended our Raman exploration to mixtures diluted in hexane or acetone. Our Raman system works in reflection, and comprises a 785nm, 30mW power source with a built-in spectrometer with ~10cm^{-1} resolution. The portable gun aims at the core of a built-in vial holder where the vials with the chemicals, or lures/dispensers/moths, can be set. This configuration offers the best focusing of the laser beam on the samples and thus best Signal-to-Noise ratio. The mixtures clearly show variations in the spectrum from a single component (Figure 1A) which were also analyzed by PCA (Figure 1B) that can help isolate the response of each mixture. A database of such mixtures will be built for reference.

Our preliminary test with various lures contained in vials were not conclusive, likely because of the extremely low concentration they release and leakages through the vials. We then moved on to mating disruption dispensers, since they have 10-100x higher emission rates and we are currently testing them.

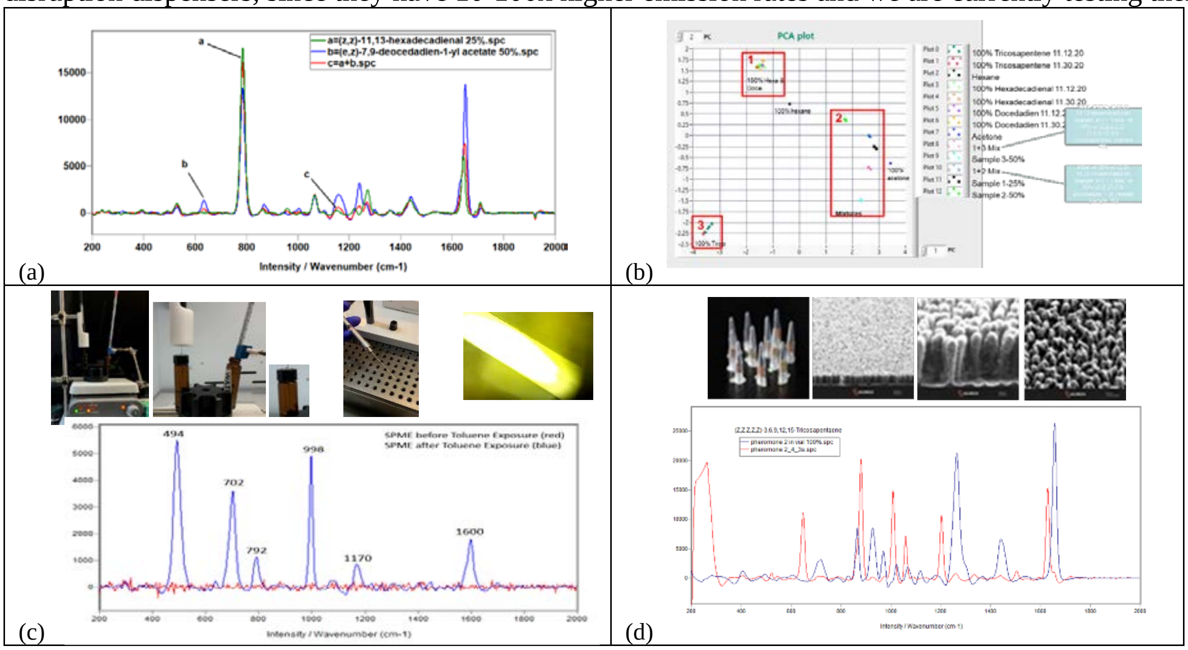

Figure 1. (a) Raman of single samples and their mixture: (mix #1) 150uL of 25% of (Z,Z)-11,13-Hexadecadienal & 150uL of 50% of (E,Z)-7,9-Deocedadien-1-yl Acetate; (b) PCA main components (p1,p2) plots showing isolation of single components and mixtures mix #1 and mix #2 (150uL of 25% of (Z,Z)-11,13-Hexadecadienal and 150uL of 50% of (Z,Z,Z,Z,Z)-(3,6,9,12,15)-Tricosapentene); (c) *top:* SPME test setup: fibers exposed just to the headspace of the vial on hotplate, partially filled with toluene, *bottom*: Raman of SPME before (blue plot) and after (red plot) exposure to toluene for 5 minutes at 130C indicating successful uptake; (c) SERS (red) vs Raman (blue) of (Z,Z,Z,Z,Z)-3,6,9,12,15-Tricosapentaene on Ag nanosubstrate.

We have also increased sensitivity by using SPME to preconcentrate compounds. The SPME 50/30um DVB/CAR/PDMS, Stableflex 24Ga (Sigma-Aldrich) was first tested with toluene uptake and proved successful (Fig. 1C). The second test with the release of adsorbed toluene in another vial headspace did not provide any meaningful results, likely due to inadequate heating (<200C) to enable the release. We are now verifying headspace release and semiochemical uptake at >200C. We are also exploring SERS for increasing sensitivity using nanopatterned leaning pillars, which introduce many hotspots that augment light shining onto and scattering back from the sample (preliminary results, Fig. 1D). The SERS peaks are shifted from bulk Raman since vibrational modes are likely affected by how molecules orientate on the substrate. Finally, we are improving setups for diffusion experiments, starting with emulating diffusion conditions (i.e. test for concentrations mapping). This will be verified by preparing lab samples or positioning lures, and exploiting SPME, SERS if possible.

Conclusion and Practical Applications

We are on the path to demonstrate RS to detect synthetic pheromone compounds emitted by trap lures, mating disruption products, and natural pheromone from NOW adults. This detection capability will be tested in an experiment (i.e. “orchard in a box”) to quantify pheromone diffusion in a very stable environment. Preconcentrating via SPME or SERS might be key elements. Preliminary data on pheromone detection will be used to attract additional funding from Federal agencies to fully mature the technology. Ultimately, the goal of this work is to characterize the nature of pheromone diffusion through the orchard environment, which will contribute to improved use of mating disruption and monitoring NOW with pheromone lures, as well as provide new information on the chemical ecology of NOW pheromones.

This work was performed under the auspices of the U.S. Department of Energy by Lawrence Livermore National Laboratory under Contract DE-AC52-07NA27344. Lawrence Livermore National Security, LLC; LLNL-SR-829414

Use of Pistachio Mummy Volatiles as Lures for Navel Orangeworm

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Introduction

The navel orangeworm has been the most persistent and economically damaging insect pest of pistachios. Monitoring for the navel orangeworm is an important part of management of this pest, but methods used for this purpose have changed in recent years and monitoring has been complicated by the increasing use of navel orangeworm mating disruption. Attractants used for navel orangeworm monitoring include: sex pheromone lures; ovipositional attractants such as egg traps or bait bags used with sticky traps for females (ovibaits); and natural products such as commercially sold phenyl propionate (PPO) lures or a kairomone blend previously patented and tested.

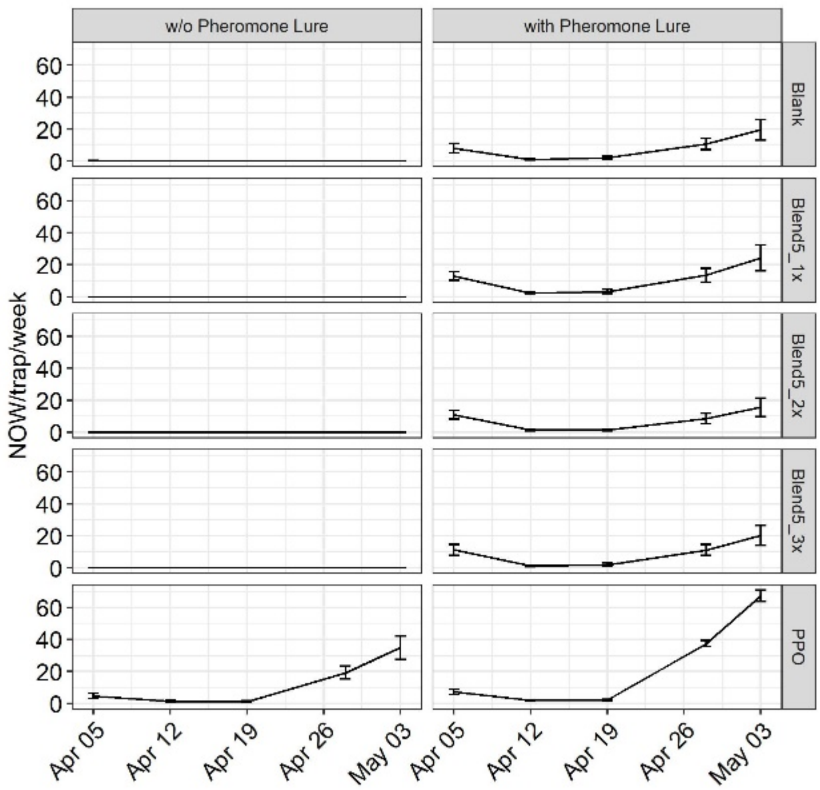
These products each have advantages and disadvantages. Pheromone lures provide sensitive detection of navel orangeworm males under favorable conditions but they are suppressed by mating disruption, even in neighboring orchards. Ovibaits are logically more immediately relevant to damage and they work well in spring (e.g., for biofix for first flight), but after that few moths or eggs are captured in these traps as they are out-competed in pistachio orchards. The previous natural products attractants (kairomone blend and phenyl propionate) capture both sexes, are apparently not affected by mating disruption, and capture more females than bait traps under the same conditions. Phenyl propionate works more effectively than kairomone blends under mating disruption and is synergized if presented in a trap along with a pheromone lure. Phenyl propionate captures variable ratios of both sexes; typically more males than females but also more females than the number captured in ovibait traps under similar circumstances. Phenyl propionate lures also have strong odors and capture large number of non-target insects, which can make collecting data from monitoring traps more difficult.

An ongoing goal of this project has been to identify improved attractants based on volatiles associated with pistachios, which navel orangeworm are known to prefer over almonds. In 2021 we examined a blend of three pistachio volatiles which were identified in experiments in 2020 as having potential as improved navel orangeworm attractants (identified in this and the 2020 report as Blend 5). Randomized complete block experiments examined a 2 × 5 factorial combination of treatments. Five candidate treatments (a blank trap, Blend 5 presented a 20%, 40%, and 60% neat material in a 3 x 0.3 cm glass tube, and an experimental PPO pouch lure) were presented in a standard wing trap one of two conditions—alone, or along with a monitoring lure (NOW Biolure, Suterra LLC, Bend OR). Treatments were arranged in seven replicate blocks (orchard rows), with 50 meters between treatments within the replicate and 100 meters between the rows serving as replicate blocks. Traps were serviced and data were collected weekly, and trap liners were returned to the laboratory to examine sex. This experiment was conducted in two pistachio orchards with a history of high navel orangeworm abundance. One, southeast of Huron, was not under mating disruption. The other, near Five Points, was under treatment with aerosol mating disruption (Pacific Biocontrol, Vancouver WA). The experiment in the absence of mating disruption was conducted from April 1 to April 20, 2021, and the experiment in the presence of mating disruption was conducted from April 30 to May 26, 2021.

Results and Discussion

In the orchard under mating disruption, no navel orangeworm were captured in the blank trap or in any of the traps baited with Blend 5 (see figure below). In contrast, significantly more males were captured with phenyl propionate (Kruskal-Wallis non-parametric ANOVA and Dunn post-test with Benjamini-Hochberg adjustment for multiple comparisons, chi-square = 30.55, df = 4, $P < 0.001$).

When synergized with a pheromone lure, the number of navel orangeworm captured was not significantly different (cumulative means per trap from 30 to 50 moths) between the blank (a pheromone synergized blank trap is of course a standard pheromone trap) and the traps baited with pheromone-synergized Blend 5. However, the trap baited with PPO captured significantly more males (100 per trap, chi-square = 15.28, df = 4, $P = 0.004$). Capturing more navel orangeworm in a PPO trap with pheromone than in a trap with pheromone alone is not expected far from mating disruption, but other fields in the region of our non-mating disruption test site were under mating disruption for navel orangeworm. Over 95% of the moths in all traps were male, except in the PPO-baited traps where females were 25-33% of the total navel orangeworm captured. This suggests that the pheromone lure was the primary attractant in traps in which it was presented with Blend 5.



Weekly capture of navel orangeworm in wing traps with various lures (mean and SE, n = 7) presented alone (left column), or in combination with a commercial pheromone monitoring lure (right column). Traps with PPO captured significantly more navel orangeworm than other traps

In the presence of mating disruption, only the PPO traps captured NOW. Traps with PPO alone captured a total of 16 moths (all reps), and traps with PPO synergized with pheromone captured a total of 55 moths.

Conclusion

The attractants tested in the present test do not appear promising for purposes of practical monitoring. In fact, examples of defined chemical attractant compounds or compound blends that attract only female moths are quite rare in the literature. Short- to intermediate-term research on improved navel orangeworm attractants should concentrate on identifying aspects of complex natural materials that optimize female attraction and developing trapping systems that use artificial intelligence to distinguish species and sex.

Attraction and suitability of trap crops for large bug pests in pistachio

Authors: **Judith M. Stahl**, Postdoctoral Researcher, Department of Environmental Science, Policy and Management, UC Berkeley; **Kent M. Daane**, Cooperative Extension Specialist, Department of Environmental Science, Policy and Management, UC Berkeley; **Houston Wilson**, Assistant Cooperative Extension Specialist, Department of Entomology, UC Riverside

Introduction

Large bugs, comprised of Pentatomidae and Coreidae, most notably a redshouldered stink bug (*Thyanta pallidovirens*), Uhler's and Say's stink bugs (*Chlorochroa uhleri* and *C. sayi*), the flat green stink bug (*Chinavia hilaris*), and leaffooted bugs (*Leptoglossus zonatus* and *L. clypealis*), can be a damaging group requiring annual treatments in pistachio. Here, we report on three aspects of large bug biology and management.

The first part investigated the potential of controlling large bug pests in pistachio with annual trap crops. The basic requirement for a trap crop system to work is that plant bugs are attracted and migrate to the cover crop in order to reduce population in the pistachio canopy. The second part determined the reproductive status of leaffooted bugs going into and out of overwintering sites, which tells us which cues can be exploited for monitoring and (yet to be developed) mass trapping in early spring before migration into orchards. The third part investigated the potential involvement of large bug feeding in the internal kernel damage that has been reported increasingly in Golden Hills pistachios.

Results and Discussion

Previously, a large bug preference for feeding on plants in the reproductive stage (flowering or seeding) could be reported. Buckwheat and mustard were the two cover crop species where more than ten cage study replicates could be completed with plants in the reproductive stage. When offered four different plant species, large bugs chose buckwheat as the first plant to contact in 35% of the cases and mustard only in 7%, a significant difference. However, while the 'first contact' and 'feeding' values are very similar for buckwheat, large bugs chose mustard as the plant to feed on in more than 20% of the cases – which is not significantly different from buckwheat. This could indicate that buckwheat is better at attracting large bugs, but once the insects are in close proximity, mustard is just as popular and could retain large bugs in the groundcover to a similar extent.

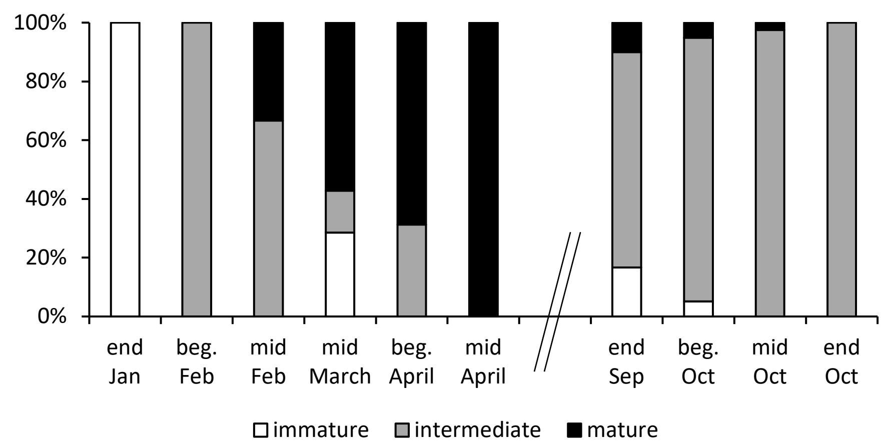


Fig. 1 Ovarian development over time in field collected *L. zonatus*.

For the second part, determining leaffooted bug (*L. zonatus*) reproductive status going into and out of overwintering sites, we could confirm and complete last year's data. Field collected leaffooted bug females show a mix of immature, intermediate and mature ovaries at the end of September, but until the end of October only females with intermediately developed ovaries could be found (Fig. 1). Towards the end of the overwintering period, end of January, all collected and dissected females showed immature ovaries, indicating that egg resorption is taking place over winter to regain nutrients. Mid-February the first mature ovaries are present, which suggest leaffooted bug females are ready to lay the first eggs. In addition to dissection of field-collected individuals, a subset of females from the field was reared in the laboratory in individual containers to obtain information about their mating status. The first females from the field to successfully produce offspring – indicating they have mated – were collected beginning of February. This corroborates the results obtained by dissection. Since the reproducing females were collected in their overwintering aggregates in citrus, it seems likely that mating occurred before or during overwintering as opposed to after leaving the overwintering sites as previously assumed.

For the third part, investigating whether large bugs could be responsible for the newly emerged internal kernel damage particularly in Golden Hill pistachios, a cage study in two orchards was conducted over the course of the season. Flat green stink bugs (GSB) and leaffooted bugs (LFB) were caged on separate pistachio clusters for two days in April, June, and August. Simulating large bug feeding without direct transmission of pathogens, nuts of other clusters were pierced with a sterilized needle, and control clusters were caged without insects to exclude feeding. All treatments including the control clusters, apart from the needle treatment in April, showed internal kernel damage (Fig. 2). There were no significant differences between the treatments (Fig. 2).

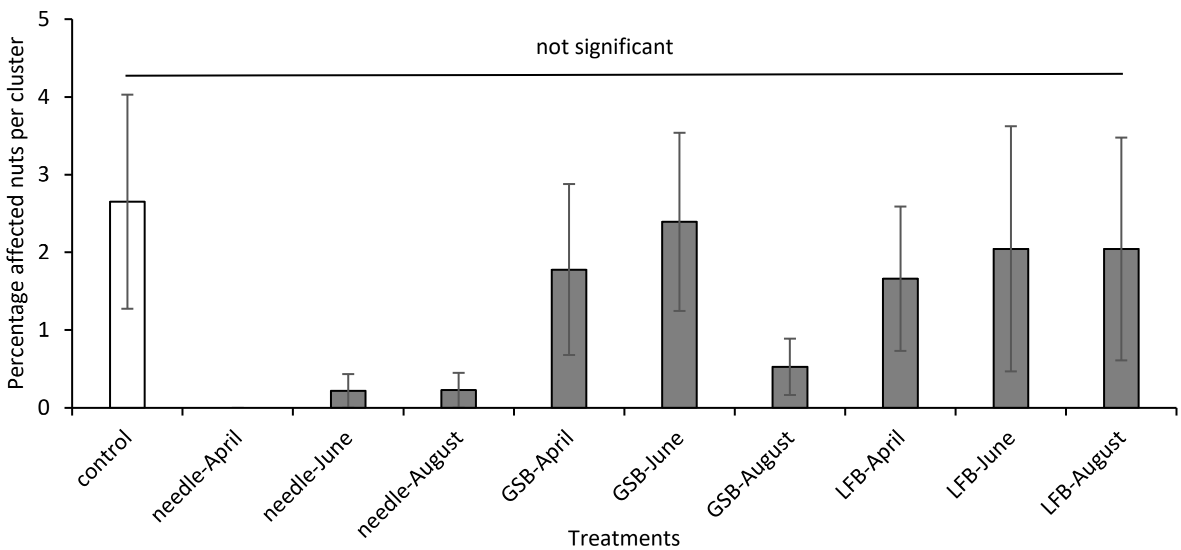


Fig. 2 Nuts with internal kernel damage in Golden Hills pistachios.

Conclusion

(1) Buckwheat had previously been reported as being the most attractive tested groundcover for large bugs. Here we add that mustard could have the potential to keep large bugs away from the pistachio canopy to a similar extent, but more plant species in their reproductive stages should be tested; (2) The fact that *L. zonatus* seems to mate at least to a certain extent during their overwintering indicates that plant-based volatiles could add to the attractive cocktail of species-specific pheromones in early spring since already mated females will likely be looking for food; (3) We could find no evidence that large bugs cause the internal kernel damage in the Golden Hills cultivar.

Seasonal biology and controls of the Gill's mealybug in pistachio

Authors: Nathan Mercer, Postdoctoral Researcher, Department of Environmental Science, Policy and Management, UC Berkeley; **Judith M. Stahl**, Postdoctoral Researcher, Department of Environmental Science, Policy and Management, UC Berkeley; **Kent M. Daane**, Cooperative Extension Specialist, Department of Environmental Science, Policy and Management, UC Berkeley; **Houston Wilson**, Assistant Cooperative Extension Specialist, Department of Entomology, UC Riverside

Introduction

Gill's mealybug, *Ferrisia gilli* Gullan, attacks pistachios in California's San Joaquin Valley and can cause significant damage in untreated orchards, and increasingly, in treated orchards as well. Mealybugs are phloem feeders that use needle-like mouthparts to pierce the plant, suck out plant sap and then excrete the excess fluids and nutrients as honeydew, a sugar-rich fluid. Gill's mealybug is a newcomer in a growing group of mealybug species that attack pistachios as well as almonds and grapes. For control, previous research showed that a well-timed application of an insect growth regulator (IGR) or neonicotinoid to 'crawlers' of the first generation (typically late May to early June) provided adequate to excellent control.

The increasing problems over the last few years, as well as 2019-2020 insecticide studies showing poorer performance (D. R. Haviland, presentations in November 2020), suggest that this program is losing efficacy. Two possible explanations are insecticide resistance and asynchronous population development. Insecticide resistance is unlikely as both the IGRs and the neonicotinoids have seen a reduction in their performance, and it would be surprising for resistance to develop concurrently to materials with different modes of action. The more likely culprit is asynchronous population development, such that there is a prolonged period of crawler emergence, which thereby reduces the performance of IGRs and neonicotinoids, both of which tend to better kill the smaller mealybug stages. For this reason, we will repeat some of the seasonal phenology work to determine if asynchronous populations have developed, and if insecticide programs should be altered accordingly.

We also note that natural enemies, especially parasitoids, have been important in Gill's mealybug control in pistachios, almonds, and vineyards, but are now rarely found in pistachios. To assess the natural enemy community of Gill's mealybug in pistachios and the potential impact of insecticidal treatments on natural enemy populations, conventional and organic blocks will be included in our population dynamic surveys.

Our objectives were to (1) study the seasonal phenology of Gill's mealybug to determine if asynchronous populations are reducing the effectiveness of current spray programs; (2) categorize the natural enemies of Gill's mealybug in pistachio orchards under different management programs; and (3) evaluate insecticide controls on Gill's mealybug and its natural enemies.

Results and Discussion

The seasonal phenology of Gill's mealybug was evaluated biweekly in eight sites throughout the San Joaquin Valley from April 2021 onwards. Branches with visible infestation on either cluster, leaves or petioles were cut around fifteen inches from the tip and mealybugs and natural enemies were counted in the laboratory. Three distinct crawler peaks could be identified, with overlapping larger nymphs and adult mealybugs present in high numbers during the second and third (Fig. 1). This confirmed asynchronous population development in mid- and late season could decrease insecticide efficacy if control of the early peak in May is missed. Further, there are consistently low numbers of larger nymphs and adults present in early season (Fig. 1) that could produce population surges later even if the first crawler peak is successfully controlled. Gill's mealybug phenology was largely synchronous between sites, indicating that treatments could be scheduled for the region rather than individual sites.

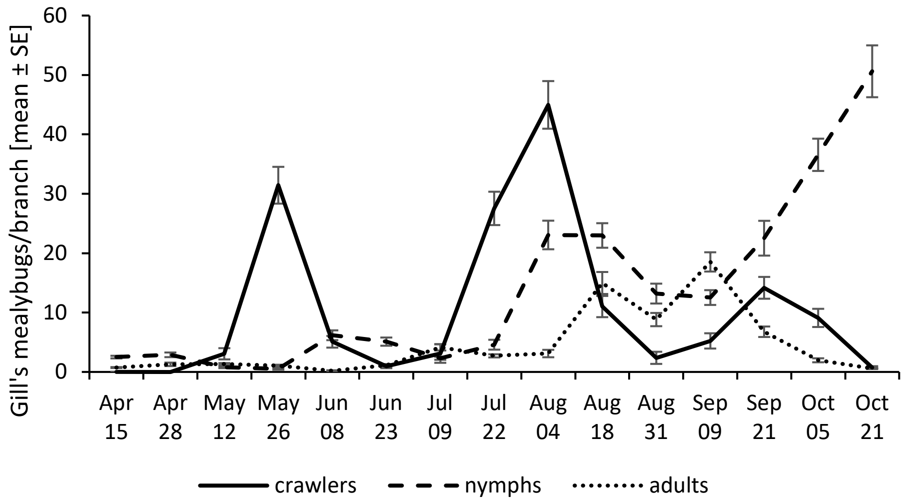


Fig. 1 Gill's mealybug phenology in eight sites in the SJV. Crawlers were counted up to 100 per branch.

In fall, larger nymphs were the most prevalent phenological stage on both remaining clusters after harvest as well as additional trunk evaluations. Both will be continued throughout the winter to understand overwintering dynamics and early season developments.

The most common natural enemy was the lacewing other occasionally recovered predators were the lady beetle larvae in the subfamily Scymninae, assassin bugs, minute pirate bugs, spiders, and predatory thrips. Initial results suggest that insecticide treatments did not lower natural enemy abundance in the sampled sites. Of the organic insecticides tested on natural enemies, pyrethrin residue 1 or 24 hr old had a negative impact on *Cryptolaemus montrouzieri* (mealybug destroyer) adults, and to a lesser extent *Chrysoperla rufilabris* (green lacewings) larvae. Insecticidal soap residue after 1 hr caused mortality in *C. montrouzieri*, but not 24, likely because some liquid soap remained in containers. Other organic insecticides tested in the lab (oil, diatomaceous earth, neem oil and azadirachtin) did not significantly impact either natural enemy. No parasitoids were found from any of the collected Gill's mealybugs. Surveys of historically infested vineyards for Gill's mealybug yielded few individuals and no parasitoids emerged from those collected.

Conclusion

All life stages of Gill's mealybug were present from April to October. Three distinctive peaks of crawlers occurred, much like was found in the original Gill's mealybug work. However, larger nymphs and adults were found during those peaks and insecticides targeted at crawlers would be ineffective on these other life stages. This problem would be exacerbated in the 2nd and 3rd crawler peaks. This finding agrees with our hypothesis of asynchronous generations of Gill's mealybug in pistachios and could be leading to the reduction in insecticide control. Natural enemies tested were largely unaffected by organic insecticides. The lack of parasitoids, an important natural control of Gill's mealybug, that likely contribute to pest suppression in vineyards and almonds could be further explanation for increased mealybug populations in pistachios. In the coming year, this survey will be repeated, more emphasis will be placed on collecting Gill's mealybug outside of pistachio orchards where the chance of retrieving parasitoids will be higher. With parasitoid colonies, insecticide applications can be further evaluated as a potential cause of the absence of parasitoids.

Optimizing Chemical Control Programs for Gill's Mealybug in Pistachio

Authors: **David Haviland**, Entomology and Pest Management Advisor, UC Cooperative Extension, Kern Co; **Stephanie M. Rill**, Staff Research Associate, UC Cooperative Extension, Kern Co.

Introduction

Gill's mealybug is an exotic pest that has become widely established within the California pistachio industry. Mealybug feeding within the pistachio cluster can intercept nutrients required for kernel development, resulting in reduced kernel size and lower percentages of split in-shell nuts. Additionally, honeydew accumulation on the surface of hulls can cause nuts within harvest bins to become sticky and gummy to the extent that they do not fall freely from hoppers used to transport raw products to the processor.

For nearly two decades, insecticides have provided growers with their primary defense against Gill's mealybug. However, chemical control options continue to change as new products become available, resistance and other factors make existing products less effective over time, as regulatory changes limit how certain products can be used, and as growers seek organic solutions.

The purpose of this project was to establish two research trials to evaluate insecticide options available to pistachio growers. It is a two-year project that is halfway through completion. During year 1 we evaluated insecticides at the traditional spray timing in late May, at an alternate timing in late July, and as two-spray programs including both timings. The second trial will be completed in year 2 of the project (2022), and consists of evaluations of sprays made post-harvest, in late March, in late May, and in combinations thereof.

Results and Discussion

Data on the number of mealybugs per cluster during seven evaluation dates, including average mealybug densities during the first and second mealybug generations, can be found in the full report of this project. That report analyzes each set of data as part of one of three separate analyses, 1) comparisons of application in May, 2) comparisons of May vs. July applications and combinations thereof, and 3) evalautions of organic products. Figure 1 shows the average numbers of mealybug per cluster during the second mealybug generation, after combining the results of all three analyses into one figure for purposes of brevity.

The most effective mealybug products were those containing spirotetramat (Movento and Senstar) applied on 26 May. These results are highly consistent with the result of previous work. The efficacy of spirotetramat in May was also reflected in the combination treatments of Movento in May followed by either Assail or Sequoia during second-generation crawler hatch on 22 July. Those two combination treatments resulted in the lowest overall mealybug densities across all treatments.

Assail and Sequoia provided significant reductions in mealybug densities when applied in either May or July. Densities of mealybugs in plots treated with these two products were typically numerically higher than, but statistically equivalent to, the best treatment. These results are consistent with the results of previous trials that evaluted the effects of these products when applied in May.

Sivanto and Imidan applied in May also resulted in significant reductions in mealybug density compared to the untreated check that was numerically higher than, but statistically equivalent to, the best treaments on most evaluation dates. When the results for Sivanto are considered along with the results of other trials, the conclusion is that it has potential to provide mealybug control, but has been inconsistent across trials in the level of control provided. This is the first trial we have conducted to evaluate Imidan, such that it would be premature to make predictions regarding consistency of control.

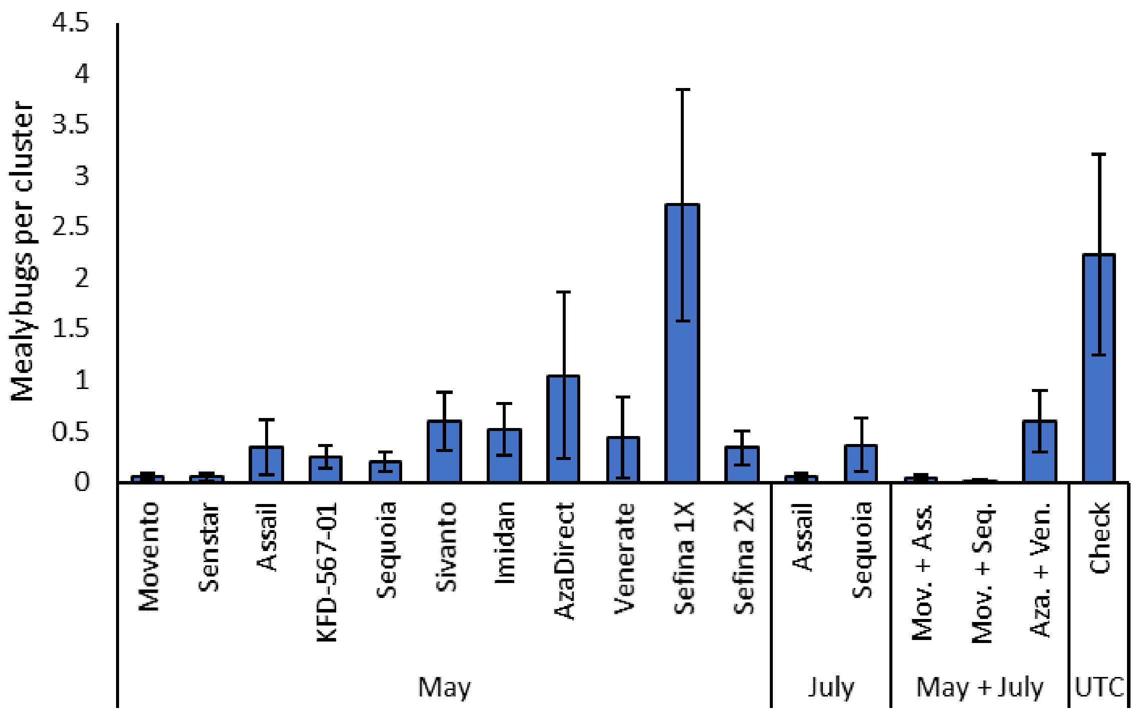
Sefina, when used at the current labeled rate of 6 fl oz per acre (for aphid control in tree crops), did not provide any mealybug control. However, when double the label rate was used (12 fl oz), Sefina reduced mealybug densities to levels comparable to middle-tier conventional treatments in this trial. Manufacturers of Sefina are currently determining whether or not they want to pursue changes to the label for tree nuts that would allow commerical applications for mealybugs at the higher rate.

Since the early 2000s, Centaur has been an industry standard insecticide for Gill's mealybug when applied in May. However, due to changes in maximum residue limits that are allowed on nuts being exported to the European Union, it was not included in this trial. Nevertheless, it continues to be a standard in pistachio orchards producing fruit that will not go to the E.U., while evaluations of alternate timings of this product, especially in March, are conducted during year 2 of this project.

The organic products Aza-Direct and Venerate reduced the density of Gill's mealybug compared to the untreated check on the majority of individual evaluation dates, and when data were evaluated by generations. Evaluations of single applications of the two products in May, compared to a two-spray program containing Aza-Direct in May and Venerate in July, did not result in any increases in mealybug control with the July application.

<u>Conclusion</u>

This trial provided insights into the relative efficacy of chemical control programs for Gill's mealybug. Details regarding each product and the relative fit that each can provide within a mealybug managemement program can be found within the full report of this project. Additionally, a second trial evaluating post-harvest, March, and May application timings, and combinations thereof, has already begun as part of the second half (year 2 of 2) of this multi-year project that will be evauated in 2022.



Attracttants for Leaffooted Bugs in Pistachios

Authors: **Andrea Joyce,** Associate Professor, Sierra Nevada Research Institute (SNRI), University of California Merced

Introduction

The leaffooted bug species, *Leptoglossus zonatus*, is a large insect found on over sixty plants including pistachio, and bug feeding can result in crop loss. There is a need to develop traps or monitoring devices to detect the presence of this insect in orchards prior to observing bug feeding damage. There are few insecticides known to be effective to control these large bugs in conventional or organic orchards. Chlorpyrifos is now eliminated as a control option in California, and pyrethroids are regulated to prevent water contamination. The overall goal of this proposed work is to further our understanding of potential attractants for leaffooted bugs in order to aid in monitoring of this pest. *L. zonatus* which moves into pistachio orchards could be attracted either to food sources or mates. Previous studies of potential attractants for *L. zonatus* include investigating host plant associated products such as pistachio or almond meal, while others have investigated insect-produced odors such as pheromones. We investigated adult *L. zonatus* flight behavior to attractants in a wind tunnel and found that mating pairs of *L. zonatus* were attractive to both *L. zonatus* males and females. Panel traps were investigated by Wilson et al. (2020) who found they could be used for monitoring leaffooted bugs. A commercial attractant is not yet available for leaffooted bugs.

Another factor which could influence monitoring or control of *L. zonatus* is that there are two genetically distinct strains of this species in California. *Leptoglossus zonatus* in California has been collected from Butte County to the Bakersfield area; two genetically distinct populations exist (Joyce et al. 2017). One strain is primarily found in California, while the second strain is found from California all the way to Brazil; this strain will be called the 'widespread' strain. We found that the widespread strain is the dominant strain found from California to Brazil. Nearly all individuals of the widespread strain are clones, the exact same genotype, and it is possible the genotype may be invasive (Joyce et al. 2021). In California we may have had more problems with *L. zonatus* in the last decade due to increasing populations of the 'widespread' strain. The widespread strain could have a higher fertility (more offspring), or have more generations per year. It will also be important to know if the two strains of leaffooted bug cross attract, and could be attracted to the same pheromone.

The objectives of this proposal include comparing biology (fecundity, longevity, mating behavior) of two strains of the leaffooted bug *L. zonatus*, examining the relative attraction of adult *L. zonatus* to food and pheromone sources, and to relate trap catches of *L. zonatus* to bug damage on pistachios. This is a summary of work from year 1 of a project proposed for two years.

Results and Discussion

The biology was compared between the two strains of *L. zonatus*. First, the number of eggs produced per mating pair was compared between the two strains. We found that the average number of eggs per mating pair in the two strains was not significantly different. The longevity per strain is not complete. To examine mating behavior, we examined cross attraction of the two strains of *Leptoglossus zonatus*, using insects from lab colonies. If there was no preference for male or female mates of either strain, then there would be equal mating between the two strains. Fifty mating pairs were selected from lab colonies, and the mitochondrial DNA COI barcode was sequenced for each male and female pair. We found that there was *some* cross mating between the two strains, in about *one third of the trials*. The rest of the mating pairs were either males and females of strain 1, or males and females of strain 2. This work is still in progress. Colonies of each strain were started from this work. To expedite the identification of the two strains, we identified and tested several DNA probes which can be used with real time qPCR. The probes will save time and help to more quickly identify an insect's strain.

The fertility from mating pairs of the two strains was not found to differ, but there may be preference in attraction between the two strains. We are repeating the above study with fifty additional replicates. This is lab work, and it will be complemented with data obtained from mating pairs collected in the field. We will determine if the two strains cross attract in the field. If the two strains cross attract and mate in the field, this would indicate that a pheromone trap could attract both strains for monitoring this insect.

The second objective was to determine if adult *L. zonatus* have a preference for attractants (odors) associated with food odors (pistachio) compared to pheromones (odors associated with mating pairs). The purpose was to identify which of these may be more productive for finding attractants for this insect. In previous work, we found that pheromones associated with mating pairs were more attractive than odors produced by males alone, or females alone. Following up on our previous finding, and before collecting volatiles from mating pairs, we examined the relative attraction of food odors vs. pheromones, by examining flight of adult leaffooted bugs to odor sources in a wind tunnel. First, we examined attraction of mated and unmated males in the following experiments; 1) mating pairs or a control, 2) pistachios or a control, and 3) pomegranate compared to a control. In each experiment, the male was released and observed, and his first choice was recorded. After these experiments, the two most attractive sources were compared. A similar experiment will be run for mated and unmated adult female *L. zonatus*. We hypothesize that mating pairs may be the strongest attractant. Volatiles are being collected from mating pairs of the two strains. Objective three plans to use the strongest attractant to investigate attraction in the field.

Conclusion

The leaffooted bug species, *L. zonatus*, has become more abundant in the last decade in California. There are two strains of this insect, one known primarily from California, and the second strain which is widespread from California to Brazil. Natural control of the two strains in the field by natural enemies such as parasitoids may vary, this is unknown. The two strains may cross attract, and this could be beneficial if they both are attracted to a similar pheromone blend, which could be used for monitoring. To date, our work found the fertility of mating pairs of the two strains was similar. In addition, the two strains can cross attract in the lab when in close proximity. In will be beneficial to determine if the two strains are cross mating in the field as well. Finally, we compared the relative attraction of adult males to food sources and pheromones. Additional experiments will also examine if there is a female preference to these two types of attractants. It is possible that males may be most attracted to pheromones from mating pairs, while females could be more attracted to other odors/attractants. We will continue this research in year two of the project.

Identification, synthesis, and development of the sex pheromone of the mealybug, *Ferrisia gilli*

Authors: Jocelyn Millar, Professor, Dept. of Entomology, UC Riverside; **Kent Daane**, Dept. of Environmental Science, Policy and Management, UC Berkeley and Kearney Ag. Center.

Introduction

Since the late 1990s, Gill's mealybug *Ferrisia gilli* has caused growing concern in pistachios in California, due to direct damage to nuts and decreased yields, and indirect damage due to growth of sooty mold on the copious honeydew produced by the mealybug. For significant infestations, the mealybug requires careful monitoring so that insecticide applications can be timed for maximum efficacy. Pheromone baited traps could be a useful tool for sensitive and specific monitoring of adult mealybug densities and population dynamics.

Female-produced sex pheromones have been identified from a number of pest mealybug species, and in 2006-7, we demonstrated that *Ferrisia gilli* females do indeed produce a sex pheromone that is highly attractive to males, and that it is likely to be a single component. At the time, we were not able to collect enough of the pheromone to identify, in part because our project was hindered by the necessity of doing all work with the mealybug at UCR in quarantine. Since then, the mealybug has expanded its range in California, and quarantine restrictions have been relaxed so that we can rear the mealybug in secure lab space at UC Riverside, rather than the quarantine facility. In addition, in 2017, a Japanese team identified a pheromone for the congeneric species *Ferrisia virgata* (Tabata and Ichkiki 2017), corroborating that the congeneric *F. gilli* are also highly likely to use sex pheromones, and providing some indication as to the general types of chemical compounds we should be looking for in a pheromone for *F. gilli*. Thus, our project goal is to identify a sex pheromone for *F. gilli*, so that the pheromone can be used to develop simple, sensitive, species-specific, and cost effective methods of monitoring mealybug populations, and for early detection of new infestations as Gill's mealybug infestations continue to expand.

Results and Discussion

The timing of our initiation of this project (spring 2020) was problematic because UC Riverside was locked down from March to mid-June due to covid restrictions. Daane's lab initiated some *F. gilli* colonies in the summer, which were then transferred down to UCR to start fresh colonies and expand the rearing effort. We initially tried rearing the colonies on butternut squash, for several reasons:

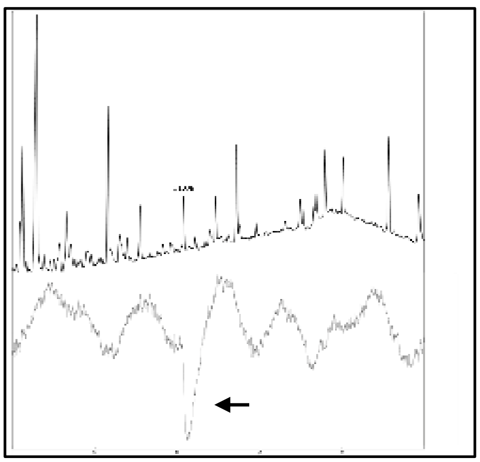
1. We needed a rearing substrate that would last for several weeks to months without rotting, to allow full development of the mealybugs, and having the adults available for periods of several weeks for pheromone collections.
2. Because pheromone collections are made in fully enclosed glass chambers, we needed a rearing host that was small enough to fit in the available chambers.
3. We wanted a host substrate with relatively few cracks and crevices, so that the male pupae could be easily found and removed, thus creating all-female cohorts that would remain unmated and produce pheromone for periods of at least several weeks.

Through the remainder of 2020, the colonies reproduced only slowly on butternut squash, as the insects became adapted to what for them is an unnatural substrate. Colonies had increased in size enough to begin pheromone collections by late 2020, at which point we asked for a no-cost extension to simply roll the 2020 funding into 2021, with no additional request for funding. To date in 2021, we have collected and analyzed 59, 1-week long collections from female mealybugs on butternut squash, continuing collections until the squash rotted or disintegrated.

Because of the slow growth of the mealybugs on butternut squash, we tried two alternative host materials. First, we transferred crawlers to potted, pesticide-free grapevines, donated by Duarte Nurseries, and allowed them to establish. Subsequent transfers to fresh vines were required to rear the mealybugs to the adult stage because infestations rapidly killed the vines. Fortuitously, pupating males moved off the vines and into ridges on the pots to form cocoons, so they could be seen and removed easily, to provide cohorts of mostly virgin females on the vines for pheromone collections. Infested vines were moved into large glass aeration chambers borrowed from Dr. Kerry Mauck, and pheromone collections were continued for one week periods as before. To date, 20, 1-week long pheromone collections have been made from infested grapevines.

The second alternative substrate tried was acorn squash, which were infested with crawlers. After one month, the infested squash were wrapped in tissues to prevent most of the males from migrating off the squash to pupate. Pheromone collections were made from the resulting cohorts of virgin females in analogous fashion to collecting from the infestations on butternut squash, with 5, 1-week long collections made to date.

A total of 57 individual or combined and concentrated extracts have been analyzed by coupled gas chromatography-electroantennogram detection (GC-EAD), followed by coupled gas chromatography-mass spectrometry (GC-MS). As in 2006-7, we have seen one reproducible antennal response in GC-EAD assays, in the same place as the responses obtained in 2006-7. The figure to the left shows a representative GC-EAD trace, with the gas chromatograph trace on top, and the bottom, inverted trace the response from a male antenna to small amounts of the pheromone in the concentrated extract. However, although the active compound is being detected by the insect's antennae, we have not been able to see a corresponding peak in the GC-MS analyses, indicating that the amounts of pheromone being produced are very small. Two possible compounds were identified, but from their chemical structures, both proved to be contaminants. Thus, we are continuing to collect and combine extracts of odors from cultures of virgin female mealybugs on the various substrates, with the goal of obtaining enough pheromone in an extract to at least get a mass spectrum, which would provide a considerable amount of information about its structure.



In additional work in support of this project, we synthesized the pheromone of *F. virgata* and tested it in GC-EAD assays. The *F. virgata* pheromone did not elicit responses from antennae of male *F. gilli*, indicating that the pheromones have markedly different structures. Similarly, on our advice, a colleague in Oregon, where *F. gilli* has invaded, obtained a sample of the *F. virgata* pheromone from Dr. Tabata in Japan, but no *F. gilli* were caught in traps baited with the pheromone, providing further evidence that the pheromones of the two species are different enough that the two species do not cross attract.

<u>Conclusion</u>

The evidence accumulated to date indicates that female *F. gilli* produce very small amounts of pheromone. Thus, our aim for the coming year is to continue to collect and stockpile pheromone extracts, with the goal of obtaining at least a mass spectrum of the active compound, the first major step in the identification of the pheromone.

Tabata, J. and Ichiki, R.T. 2017. (1*S*,3*R*)-*cis*-Chrysanthemyl Tiglate: Sex Pheromone of the Striped Mealybug, *Ferrisia virgata*. *Journal of chemical ecology*, *43*(8), pp.745-752.

Another look at pheromones or related attractants for leaffooted bugs (*Leptoglossus* spp.) infesting California nut crops

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Introduction

Epicarp lesion, nut abortion, and kernel necrosis caused by the feeding of a suite of true bug species is a major source of yield losses in California nut crops. Leaffooted bugs (LFB, *Leptoglossus* spp.) cause some of the worst damage, in part because their mouthparts are robust enough to penetrate maturing endocarp tissues (Daane et al. 2005). In California, LFB overwinter primarily as adults, then move to feeding sites and start to oviposit in spring, and typically complete 2-3 generations per year (Daane et al. 2019). Damage is unpredictable because bugs can rapidly migrate into nut crops from surrounding crops or native vegetation. For the congener *L. australis*, field bioassays suggested that males move into a crop first and begin producing an attractant pheromone which accelerates the aggregation of adults of both sexes (Yasuda and Tsurumachi 1994). Because of these rapid buildups, and since bug damage may only become apparent after the bugs have moved on, continuous monitoring of LFB populations is crucial for timing treatments. Current monitoring relies on beat sampling the tree canopy and/or visual assessment of nuts for damage; both of which are time and labor intensive, and many times fail to detect LFB populations early enough to take action. As such, passive trapping systems based on pheromonal or related attractants for each species would be of great value for monitoring and potentially control purposes.

Several very recent pieces of evidence have strongly supported our working hypothesis that male LFB produce powerful pheromones that attract both sexes. First, Inoue et al. (2019) demonstrated in olfactometer bioassays that adults were attracted to odors released by sexually mature males. Even more important, in 2021, our European collaborators studying the invasive *Leptoglossus occidentalis* trapped >10,000 bugs in trials with synthetic pheromone blends. Thus, our work efforts for the 2021 year have been focused entirely on finishing up the syntheses of possible pheromone components for what is currently the most important species for California (*L. zonatus*), and carrying out laboratory and field bioassays with those components.

Results and Discussion

Our work over the past several years has shown that sexually mature adult male L. zonatus produce ~10 volatile compounds, most of which were previously known chemicals, with the exception of one, dubbed 'leptotriene', which is a compound entirely new to science. Leptotriene is a relatively minor component of the blend, but it elicited the largest responses from antennae of both sexes of LFB in electroantennogram assays. We also found it in volatiles from male *L. clypealis*, probably the second most important species for California (although populations currently appear to be in decline for unknown reasons). Thus, a large part of our effort in the first half of the year was focused on developing a synthesis for this new compound, as well as resynthesizing one of the other components. By early summer, we finally had all the components ready for testing, albeit in very limited quantities.

Olfactometer Assays: Given the small quantities of compounds, we initially tried to evaluate them using a laboratory bioassay with a 2-choice olfactometer. For each assay, an individual virgin LFB adult was placed into the arena and given a choice between an experimental pheromone lure and a no lure control. Males and females were assayed separately and all adults were held for at least 2 weeks prior to testing to ensure sexual maturity. While this initial olfactometer assay indicated consistent attraction of LFB adults to lures that contained the full pheromone blend (Fig. 1), >50% of the assays resulted in no choice at all, which is likely due to unnatural environment of the lab olfactometer arena setup.

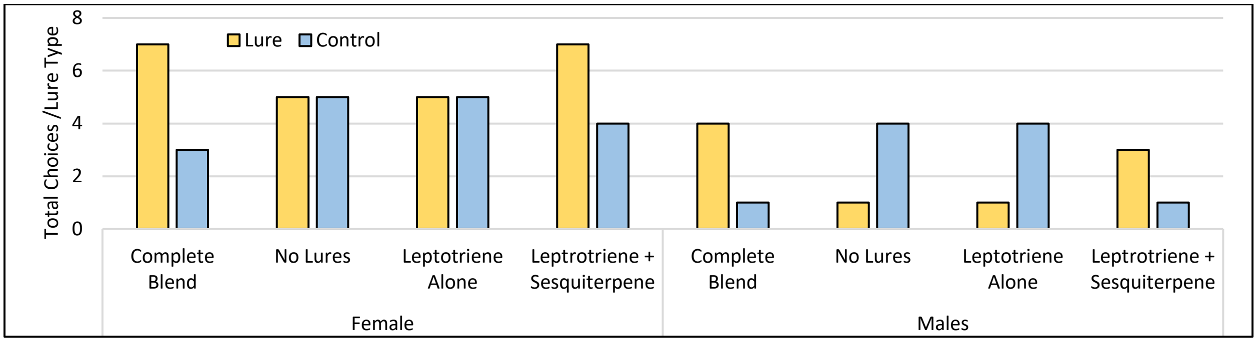

Figure 1. LFB adults preferred lures with the complete blend or just leptotriene + sesquiterpenes.

Field Trials: Given the difficulties with the olfactometer, we also tested the lures in the field using a series of hanging panel traps in an infested pomegranate orchard at the Kearney Ag. Center. A replicated complete block design was used and traps were checked weekly. Each experimental lure was tested for at least two weeks. Findings indicate that various iterations of the LFB pheromone compounds were attractive (Figs. 2-5), especially blends with leptotriene.

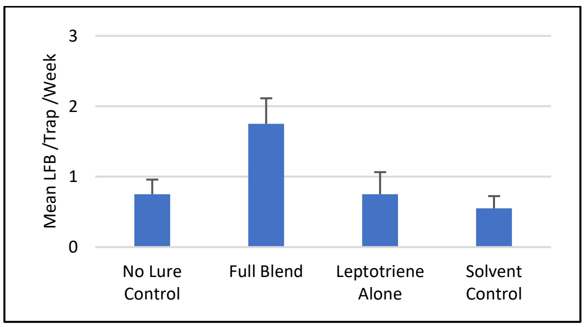

Figure 2. Field trial, Aug. 31 – Sept. 28, females

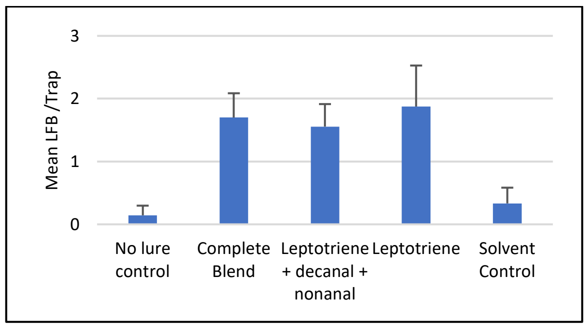

Figure 3. Field trial, Sept. 28 – Oct. 5, females

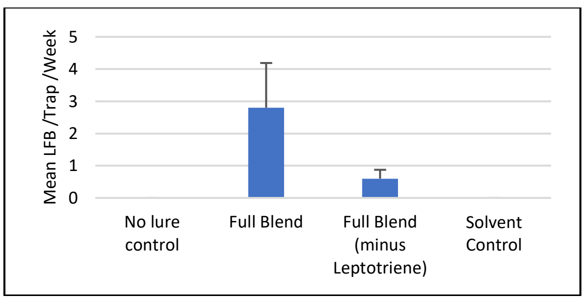

Figure 4. Field trial Oct. 12-26, females

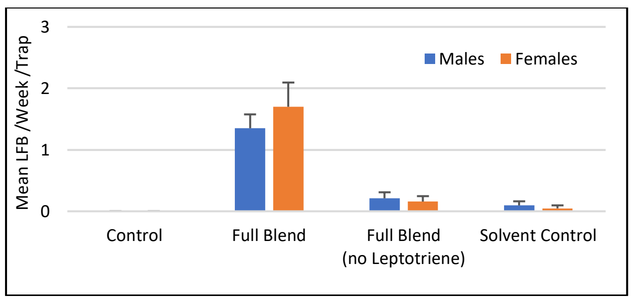

Figure 5. Field trial, Oct. 26 – Nov. 23, both sexes

Conclusion

Our laboratory and field assays to date have shown that the novel compound leptotriene is a key component, and may be the only important component, of the attractant pheromone of *L. zonatus*. Furthermore, placing the lures in a hanging panel trap effectively demonstrated the potential for this new monitoring system in orchards. While additional work is needed to further refine this trap and lure system, findings from 2021 were very promising. In 2022, we plan to continue evaluating new iterations of the lures, as well as conduct field trials in pistachio and almond orchards to (a) relate trap catch to LFB populations in the tree canopy and (b) compare trapping efficacy to traditional beat/visual sampling.

Effective Biological Control of Gill's Mealybug from Drone Releases of Green Lacewing

Authors: **Andreas Neuman,** CEO, UAV-IQ Precision Agriculture, **Robert Starnes**, Director of R&D, UAV-IQ, and **David Haviland**, Entomology and Pest Management Advisor, UC Cooperative Extension, Kern Co.

Introduction

Over the course of the last two years, UAV-IQ Precision Agriculture has developed operational capabilities to fly drones equipped with intelligent release systems over crops of any size and configuration in order to release beneficial insects and mites precisely where they are required. It has generated positive biological control results in multiple crop types using several different species of natural enemies against a variety of crop pests.

The purpose of this project is to determine if drone releases of lacewing larvae can be used as a sustainable practice that can improve integrated pest management programs for Gill's mealybug. This pest is relatively new to the California pistachio industry within the past two decades, but is now well established throughout the industry. Feeding by mealybugs, especially within the nut clusters, can reduce kernel size, decrease the percentage of split in-shell nuts, and cause increases in shell staining and stick-tights. In severe cases, the accumulation of honeydew produced by the mealybugs can cause nuts to remain stuck within harvest trailers when the hopper door is opened at the processer.

Lacewing releases have the potential to assist in biological control programs against the mealybug. There is reason to believe that an inoculative application, timed to deconflict with the application of insecticides, can establish a sufficient population of green lacewings to keep mealybug populations at levels which can be managed with insecticide applications or additional augmentative releases as needed throughout the season. This can improve overall management, but also has the potential to reduce the risks associated with over reliance on pesticides that are becoming increasingly ineffective and heavily regulated.

The primary objective of year one of this project was to evaluate the efficacy of applying lacewing larvae (second stage) by drone during sequential applications during the spring. Theoretically, these inoculative releases would help to establish a population of lacewings early in the season while mealybug populations were low, resulting in reductions in mealybug density and increases in biological control that would last the entire season.

Results and Discussion

During 2021 a trial was conducted within 10 acres of a commercial pistachio orchard in Kern Co, CA. Five random one-acre plots were treated with 5,000 lacewing larvae per acre that were released by drone on 28 April with another 5,000 larvae per acre released approximately two weeks later on 11 May. The other five plots served as a no-release check. Following applications, eleven evaluations were made between 4 May and 30 August to assess the establishment of lacewing larvae within the orchard and potential impacts on mealybug populations. A third application using a rate of 10,000 lacewing larvae per acre was applied on 19 November to evaluate whether an over-winter treatment results in the establishment of a lacewing population as well as a lower emergence of mealybug in the spring. Sampling for that release will begin in spring 2022.

Beat samples failed to recover significant amounts of lacewing larvae on any evaluation date, regardless of whether or not releases were made to the plot. This could be due to poor lacewing establishment, or due to the methodology used and inability to conduct beat samples at locations above reachable locations in the lower tree canopy. Evaluations of the average number of mealybugs per cluster on each evaluation failed to identify any differences between the lacewing release and no-release plots. Likewise, there were no significant differences in the percentage of clusters infested with mealybugs.

We are uncertain about why the lacewing releases did not result in any measurable differences in mealybug density. It is possible that the larvae had poor establishment due to difficulties in finding food while mealybug density was low, difficulties in getting lacewings to land in the tree early in the season while the leaf canopy is sparse, predation of lacewing larvae by predators such as ants, or difficulty finding a trunk to climb for lacewings that landed on the ground. Additionally, it is possible that the density of lacewings released was simply not large enough to make a measurable impact on mealybug density.

The spring applications of lacewing larvae represent one half of this two-year project. The second half of the project is to evaluate post-harvest applications of lacewings for the benefit they might provide the following spring. After harvest lacewings are unlikely to be impacted by insecticide applications, there is plenty of food, the canopy is relatively dense such that it is easier to apply larvae to the tops of the trees by drone, and the weather is conducive to larval survival. At present, the postharvest trials have been established and the lacewing larvae have been released. Evaluations will be conducted during the spring and summer of 2022.

<u>Conclusion</u>

The spring applications of lacewing larvae did not appear to be successful at reducing mealybug densities in pistachios. However, this trial represents only one half of this two-year project. The second half of the project is to evaluate post-harvest applications of lacewings at twice the application rate for the benefits that they might provide the following spring. After harvest lacewings are unlikely to be impacted by insecticide applications, there is plenty of food, the canopy is relatively dense such that it is easier to apply larvae to the tops of the trees by drone, and the weather is conducive to larval survival. At present, a postharvest trial has been established and the lacewing larvae have been released. Evaluations will be conducted during the spring and summer of 2022.

Control of navel orangeworm: focus on increasing insecticide efficacy and reducing application volume using organosilicone adjuvants

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Introduction

The goal is to improve control of navel orangeworm (NOW) by reducing water use. Product names are for specific information purposes and their mention does not constitute an endorsement by the USDA. In two experiments conducted in 2021, we examined the efficacy of an application rate of 80 gallons per acre (80 gal/ac) combined with four organosilicone adjuvants used at their maximum rate, compared to a grower standard of 100 gal/ac with the sunscreen adjuvant Cohere (12.8 oz/100 gal; Helena Chemicals). The first trial in July evaluated the maximum rate of the insecticide Besiege (lambda cyhalothrin + chlorantraniliprole, Syngenta) combined with the Loveland adjuvants Widespread Max (8 oz/100 gal) and Freeway (16 oz/100 gal). The second trial conducted in August evaluated the insecticide combination of Intrepid 2F + Bifenture EC (Corteva, UPL) at their maximum rates combined with the Momentive adjuvants Silwet Eco Spreader and Silwet 636 Super Spreader (0.1%, 12.8 oz/100 gal for both). We measured the duration of control at 14-16 feet using a contact toxicity bioassay. In the first trial filter paper was also placed at 5 feet and collected one day after application to establish maximum percent kill. Filter papers on hooks were hung at 14-16 feet, collected at selected intervals after commercial application by an Air-O-Fan GB36 PTO air blast sprayer (500-gallon tank). The filter papers were placed in petri dishes containing NOW wheat bran diet and challenged by placing 50 eggs in the center of the filter paper. Newly hatched larvae contacted the insecticide when they crawled over the treated surface to reach the diet, and mortality was scored 18 days later.

Results and Discussion

Table 1. Duration of control for Besiege (12.5 oz/ac) applied at 80 gpa with two organosilicone adjuvants, Freeway and Widespread Max, and Cohere applied at 100 gpa, July.

Treatment	Mortality	Reduction	Eggs
Control Day 1	54.7%		300
Cohere 100 gpa **ground** Day 1	83.1%	62.8%	900
Freeway 80 gpa **ground** Day 1	92.5%	72.9%	1,000
Widespread Max 80 gpa **ground** Day 1	94.8%	83.5%	1,000
Cohere 100 gpa **hook** Day 1	81.1%	58.3%	2,000
Freeway 80 gpa **hook** Day 1	81.0%	58.0%	2,000
Widespread Max 80 gpa **hook** Day 1	83.7%	63.9%	2,000
Control Day 7	51.0%		500
Cohere 100 gpa **hook** Day 7	76.9%	54.7%	900
Freeway 80 gpa **hook** Day 7	84.1%	68.9%	900
Widespread Max 80 gpa **hook** Day 7	71.2%	43.6%	900
Control Day 14	54.8%		500
Cohere 100 gpa **hook** Day 14	71.2%	36.3%	900
Freeway 80 gpa **hook** Day 14	81.6%	59.2%	900
Widespread Max 80 gpa **hook** Day 14	72.2%	38.5%	900
Day 21			
Cohere 100 gpa **hook** Day 21	79.0%		2,000
Freeway 80 gpa **hook** Day 21	82.4%		2,000
Widespread Max 80 gpa **hook** Day 21	75.8%		2,000

In terms of overall mortality, the two organosilicone adjuvant treatments were similar averaging 85.3%, while mortality in the Cohere treatment was 81.8%, $P < 0.0001$; the organosilicone adjuvant mortality was significantly higher than for Cohere, $P < 0.0001$; 21,410 eggs total.

Table 2. Duration of control for Intrepid 2F (24 oz/ac) + Bifenture EC (12.5 oz/ac) applied at 80 gpa with two organosilicone adjuvants, Silwet EcoSpreader and Silwet 636, and Cohere applied at 100 gpa, August.

Treatment	Mortality	Reduction	Eggs
Control Day 7	56.2%		450
Cohere 100 gpa **ground** Day 7	97.6%	94.4%	450
Silwet EcoSpreader 80 gpa **ground** Day 7	96.0%	90.9%	600
Silwet 636 80 gpa **ground** Day 7	96.8%	92.8%	600
Cohere 100 gpa **hook** Day 7	98.4%	96.3%	1,100
Silwet EcoSpreader 80 gpa **hook** Day 7	96.3%	91.5%	1,100
Silwet 636 80 gpa **hook** Day 7	93.9%	86.1%	1,100
Control Day 14	57.6%		500
Cohere 100 gpa hook Day 7	94.1%	89.7%	1,100
Silwet EcoSpreader 80 gpa **hook** Day 7	85.4%	74.6%	1,100
Silwet 636 80 gpa **hook** Day 7	77.5%	60.9%	1,100
Control Day 21	57.0%		500
Cohere 100 gpa **hook** Day 21	89.3%	75.1%	1,250
Silwet EcoSpreader 80 gpa **hook** Day 7	86.3%	68.0%	1,200
Silwet 636 80 gpa **hook** Day 7	87.9%	71.9%	1,200

In terms of overall mortality, the two organosilicone adjuvant treatments differed, with the Silwet EcoSpreader treatment having higher mortality (91.1%) than the Silwet 636 treatment (88.0%), $P < 0.0001$, and the Cohere treatment had the highest mortality of all at 94.2%, $P < 0.0001$; 13,740 eggs total.

The grower standard Cohere applied with 100 gpa provided consistent mortality in the contact bioassay for each experiment, although mortality for all treatments was higher in the second experiment. In the first experiment, mortality at 14 feet on day 7 was 81.1% and mortality on day 21 was 79.0%, a decrease of only 2.6%. The organosilicone adjuvants outperformed Cohere and mortality at 14 feet on day 7 was 82.4% and 79.1% on day 21, a decrease of 4.0%. These differences are trivial and performance for all adjuvants was similar three weeks after application. In the second experiment, mortality in the canopy for the Cohere treatment was almost absolute at 98.4% on day 7 and dropped down to 89.3% on day 21 a decrease of 9.2%, but this mortality was still 10% higher than the first experiment. Although the overall mortality for the two Silwet adjuvants was significantly different, the separation between the two occurred on day 14 (85.4% vs 77.5%) and there was no meaningful separation on days 7 and 21. In this experiment Cohere at 100 gpa always did a bit better than the Silwet adjuvants at 80 gpa for each collection date, but in practical terms this difference was minimal and there is a clear advantage to 80 gpa.

Conclusion

Some variability within and between experiments is expected, but overall, there was no loss in contact toxicity when 80 gpa was used in both trials. Reducing water volume enabled each tank load to treat an additional acre before refilling (100 gpa treats 5 acres, 80 gpa treats 6 acres), an increase in efficiency of 20%. This is an important saving in time. I have been testing adjuvants for over 5 years and this is the first time that I have been able to demonstrate no loss in control using a water volume below 100 gpa. Several promising candidates were identified and organosilicone adjuvants produced by other manufacturers should be equally effective. Future efforts will focus on determining if the water volume can be further reduced to 60-65 gpa (7-8 acres per tank load) without sacrificing duration of control.

Producing Sterile Navel Orangeworm on Demand for Improvement of Pest Management

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Introduction

Recently, the pistachio industry made a significant investment in the development of sterile insect technique (SIT) for navel orangeworm (NOW) that leverages the availability of a preexisting mass-rearing and irradiation facility operated by USDA-APHIS in Phoenix, AZ and developed for a sterile pink bollworm program. The program goal is to develop SIT as a complementary strategy to augment existing IPM tools for NOW. Over the past four years, co-PIs Wilson and Burks have led scientific efforts to evaluate the quality and performance of sterile NOW produced by this Phoenix facility. Initial research efforts documented very poor performance of the sterile NOW generated by this facility, and subsequent efforts have focused on identifying key bottlenecks in the production, transportation and release process. Goals of the research in 2021 were to continue evaluating field performance of sterile NOW generated by the Phoenix mass-rearing facility, as well as begin to explore the use of x-ray sterilization as a potential alternative route to low-cost production of sterile moths.

Results and Discussion

Objective 1 – Verify and Optimize X-ray Sterilization of NOW

With support from the CPRB, co-PIs Wilson and Burks have been able to acquire an x-ray irradiator unit (RS-1800Q, RadSource Inc.) for use at the Kearney Agricultural Research and Extension Center. Unfortunately, the postdoctoral scholar (Culbert) hired for this work sustained an injury in July 2021 that has precluded her from conducting research with this x-ray unit. The work will resume in January 2022.

Objective 2 – Effects of Environmental Variables (Cold, Motion) on NOW Field Performance

This element was re-directed to examine rearing regimes with the objectives of (i) permitting collection of unmated NOW adults from cultures of mixed-sex moths in the first day after eclosion, (ii) maintaining the cultures in an unmated condition, and (iii) prolonging adult longevity and vigor. An environmental regime was developed with a 24-hour thermoperiod of (9:13 hr) (25°C:13°C) nested in a (14:10 hr) (L:D) photoperiod. First-night mating was approximately 10% and holding moths at 13°C slowed but did not stop mating. Adults were then held at 10°C, and preliminary evidence indicates that adult longevity and vigor is maintained longer at this temperature than at 4°C or 25°C. Moths reared in this manner were tested June – Aug. using mark-release-recapture experiments. In the first two trials only males were released, but subsequent trials used mixed-sex release of fertile NOW. Data from remain to be analyzed, but recovery of 5-10% was obtained.

Objective 3 – Determine Sources of Variability in Field Mark-Release-Recapture Experiments

(Experiment 3A) At the Kearney Ag. Center, small pistachio (2 acre) and almond (5 acre) orchards were used to evaluate the influence of different combinations of rearing (mass-rear vs. lab), release (paper bags vs. drone) and irradiation (sterile vs. non-sterile) on performance of NOW adults. Moths were released weekly (May 4 - Sept. 14), and recapture monitored with wing-traps (10 traps/block) baited with pheromone lure (attracts males) or an oviposition bait (attracts mated females). Mating success was measured with mating tables, which exposed sentinel virgin female moths for 1-3 nights.

Recapture of females was too low to merit analysis, so here we focus on sterile males only. Recovery of sterile males was highest when locally reared, non-irradiated moths were released using the paper bag or drone system, whereas recovery was lowest when releasing mass-reared moths regardless of irradiation or release method. At the same time, equivalent recovery of locally reared moths using the paper bag and drone release systems, indicating that drones may be a viable option for a future SIT program. Mating table data indicate that (a) males arrive at the mating tables most frequently in the first 1-2 nights after release, whereas (b) mass-reared females tended to have more mating success on nights 2-3 of exposure. The declining mating frequency of released males may have to do with their dispersal outward from the block, whereas the delayed mating success of females may be due to photoperiod adjustment in the field.

<u>(Experiment 3B)</u> Three pairs of 160-acre almond blocks (no mating disruption) were used to evaluate the influence of APHIS sterile NOW releases on NOW populations, egg deposition and crop damage. Sterile moths from the Phoenix facility were released weekly using the modified APHIS airplane June 16 - Oct. 15. Adult NOW populations and egg deposition were monitored weekly, and crop infestation documented at harvest. Each block contained 5 paired wing-traps (10 traps total), one with a pheromone lure and the other with ovipositional bait. Ten egg traps were also placed into each block and replaced weekly. Data indicate that activity of both wild females and wild males tended to be lower in the blocks that received the SIT treatment, although recovery of sterile moths was highly variable. Egg deposition followed a similar trend in some but not all weeks. Crop damage data is still being processed.

<u>(Experiment 3C)</u> This experiment evaluated dispersal of APHIS sterile NOW across three large (640-acre) pistachio blocks (no mating disruption). Each week June 16 – Oct. 1, aerial release using the modified APHIS airplane took place over the same 160-acre section in the northeast corner of each block. The total amount of moths released each week varied between a low, medium and high abundance release to measure the influence of total sterile moth population on dispersal. A grid of paired wing-traps (1 trap per 10 acres), one with pheromone and the other with ovipositional bait, measured NOW across the entire 640-acre block following the weekly release event. Data from this experiment are still being processed.

<u>Objective 4 – Use Sterile NOW to Compare Recapture in Mating Disruption (MD) and Non-MD Fields</u>
Traps baited with phenyl propionate (PPO) can capture NOW in either the presence or absence of mating disruption, but have disadvantages due to capture of large numbers on non-target insects. Preliminary data indicated that a high-rate pheromone dispenser (designed for mating disruption rather than monitoring) also captures NOW regardless of mating disruption. A field experiment in 2021 found dose-dependent reduction of NOW capture in traps baited with pheromone monitoring lures in plots treated with 0.11, 0.33, 1, or 2× rates of mating disruption. In contrast, both PPO and high-rate pheromone captured the same number of NOW in all intensities of mating disruption. The rate of non-target capture using PPO was approximately 50%, whereas non-target capture was <5% for both pheromone lures. These findings suggest that a high-rate pheromone lure could provide consistent and robust detection of NOW regardless of mating disruption, and do so with fewer disadvantages than PPO. This experiment was with a wild NOW population rather than recaptures of mass-reared sterile moths. However, it demonstrates a bioassay that provides useful comparison of the effect of mating disruption intensity on the response of NOW. In addition to providing new data on attractants, it demonstrates a practical method for future experiments examining the effect of mating disruption intensity on sterile vs. wild males following a sterile release.

<u>Conclusion</u>

Data from 2021 demonstrate the negative influence of mass-rearing on quality and performance of NOW. That said, release of sterile moths into almond orchards was associated with reductions in wild NOW activity, although more analysis of the data are needed to understand the specific mechanisms. Mating table data from small-plot experiments indicate that possibly due to necessary adjustments to the local photoperiod, sterile male and female NOW may be able to coexist by occupying staggered temporal niches, which could reduce the likelihood of sterile moths mating with each other. Data from the x-ray experiments and some large-block trials were unfortunately limited due to a series of logistical constraints.

Spatiotemporal Models to Evaluate the Potential Value of Sterile Insect Technique for Control of Navel Orangeworm

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Introduction

Recently, the pistachio industry made a substantial investment in the development of sterile insect technique (SIT) for NOW as an additional control strategy. With SIT, large numbers of the target pest are mass-reared and sterilized (typically with gamma irradiation) and then released into crop fields or orchards. Pest population control occurs when sterile pests mate with wild pests, which negates the ability of the wild pests to reproduce. SIT was first implemented in the United States in the 1950s, with great success, and has since been used around the world to control a wide range of agricultural pests that includes flies (Diptera), beetles (Coleoptera) and moths (Lepidoptera) in both annual and perennial cropping systems. This recent investment in SIT takes advantage of a pre-existing USDA mass-rearing and irradiation facility in Phoenix, AZ that was originally designed for a pink bollworm (*Pectinophora gossypiella*) SIT program. The goal now is to see if this facility can be retrofit to produce large numbers of sterile NOW, and then determine the best ways to use these sterile moths in California tree nut orchards. Since the inception of this NOW SIT project, co-PIs Wilson and Burks have been leading the scientific efforts in California to evaluate and improve quality and field performance of sterile NOW coming from this USDA facility.

Development of SIT for any species x crop combination presents a unique set of challenges, from the radiation biology to the mass-rearing, transportation and release process. A key concept in SIT is the 'overflooding ratio', which refers to the ratio of sterile: wild pests per acre necessary to achieve acceptable control levels. Although the concept is straightforward, determining the appropriate timing and frequency to release sterile organisms is a function of multiple interacting factors, such as background wild pest population, crop phenology, mating behavior, and pest dispersal/diffusion rates. As such, the overflooding ratio is likely not a static number, but rather a ratio that changes over the course of the season. Once the necessary overflooding ratio is understood, a mass-rearing process/facility must then be capable of producing (and delivering) an adequate number of sterile organisms to achieve this overflooding ratio over the total acreage desired – and to do so in a way that is economically feasible.

Development of SIT for NOW in California faces many unique challenges. Tree nut acreage is extensive (>1.5 million acres) and spread throughout the entirety of the Central Valley. Furthermore, key management strategies like mating disruption and insecticide sprays may be incompatible with SIT. For instance, SIT works when sterile NOW locate and mate with wild NOW, but in a mating disruption environment this process may be hindered. While some SIT projects focus on eradication of invasive insects, use of SIT for many agricultural pests, including NOW, should be seen as a complimentary tool within the IPM toolkit. In this way, SIT may actually be complimentary with NOW management strategies. For example, mating disruption works best when implemented over large contiguous acreage (i.e., square blocks >100 acres) whereas SIT works best when used in small, isolated areas (i.e. blocks that are like islands). The former is to reduce colonization by mated females from outside of the mating disruption area, while the latter is to reduce dispersal/diffusion of sterile organisms away from the target release site. Similarly, certain orchards may have more severe restrictions on pesticide use due to their proximity to schools or residential areas, or because they are certified organic. In these situations, depending on block size, SIT may provide an alternate strategy.

Here we propose to develop spatiotemporal agent-based models to explore multiple scenarios for the use of SIT in tree nuts as a means of understanding the full potential of SIT for NOW. Models will estimate SIT program requirements, costs and potential impacts under various scenarios that incorporate a range of key variables, such as orchard size, overflooding ratios, dispersal rates, area requirements, mass-rearing production levels, and costs of sterile moth production, transportation and release. This is not an exhaustive list. Each modeling scenario will assume different values for key variables, and in this way identify the most important program features that influence costs and efficacy (e.g. production vs. transportation costs), highlight areas where more research is needed (e.g. dispersal rate, overflooding ratio), and most importantly estimate the total viable acreage for SIT and total number of moths needed to be successful. Findings from this effort are intended to generate a roadmap forward for the most logical and cost-effective development of SIT for NOW in California pistachios and almonds. Similar work has been conducted successfully with other sterile Lepidoptera programs, including painted apple moth in New Zealand (Wee et al. 2006) and sugarcane borer in South Africa (Potgieter et al. 2013, 2016).

Results and Discussion

Objective 1 – Use scenario modeling and cost analysis to evaluate the potential for SIT to improve control of NOW in almonds (Wei/Goodrich)

Co-PIs Wilson and Burks initially defined a series of key environmental, biological and production variables, as well as interactions between them, that would potentially drive regional NOW population development. Those variables are now being incorporated by co-PIs Wei and Zhang into a spatio-temporal agent-based model. Initial runs of the model are now being used to refine the definition of key variables and their interactions, with a lot of attention currently on estimation of key tree phenological events such as bloom, nut maturation, degradation of hull integrity and harvest. The prototype model is focused on an area of 7.8 x 9.8 km in west Fresno County was selected as the current test site (Figure 1). Each patch/pixel in the map equals 1 hectare. The NOW degree-day model was successfully integrated into the agent-based model to track insect phenology, and initial simulations used the daily max/min temperatures at the Five Points CIMIS weather station from 2020-03-15 to 2020-07-30.

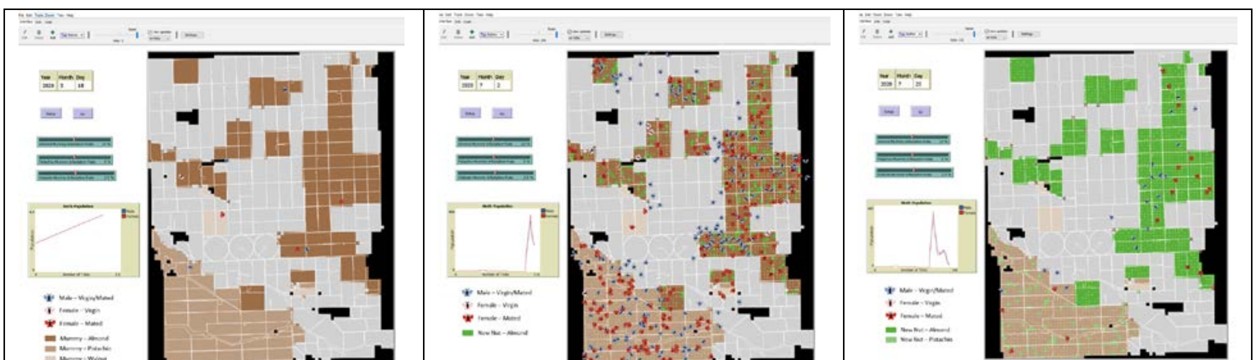

Figure 1. Images from the prototype model demonstrating population changes over time.

Conclusion

This project requested a no-cost extension due to delays hiring a postdoctoral scholar to execute the spatial modeling effort. As of December 2021, the postdoc (Yujia Zhang) has been hired and commenced work with the project PIs to develop and refine the model. While this project was catalyzed by the need to define priority areas for deployment of SIT, the global NOW population model that is required to answer this question actually provides a platform for a series of other useful projects, such as pest forecasting and estimating priority areas for other technologies such as mating disruption.

Influence of Pistachio Hull Degradation and Shell Split on NOW Egg Deposition and Infest

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Introduction

Infestation of pistachios by navel orangeworm (*Amyelois transitella*) (NOW) is contingent on the ability of this insect to gain access to the kernel, which is protected by both the hull and shell of the nut. Over the course of the season, degradation of the hull and/or shell split provides access to NOW larvae. It is thought that NOW adult females can detect when such changes in the nut begin to take place, which subsequently triggers them to increase egg deposition on degraded nuts.

This project is about managing hull degradation and shell split itself to reduce crop vulnerability to NOW. While hull degradation and shell split generally take place later in the season as part of the pistachio developmental process, the extent to which this occurs can vary significantly from year-to-year. Unfortunately, growers currently have no way to predict the extent to which this is going to occur each year, much less manage it. Hull degradation and shell split are likely driven by interactions between tree physiology and environmental conditions, and better understanding of these interactions could allow for the development of management strategies to specifically influence these processes. In this way, it might be possible for growers to better predict and even manage hull integrity and shell split. A CPRB-funded project in 2019 (Blanco-Ulate, Wang, Ferguson, Wilson) evaluated the relationship between the accumulation of heat units, pistachio nut physiology, and NOW egg deposition. Here, our goal is to experimentally manipulate trees in different ways to see if we can alter the timing and extent of hull degradation and shell split, and subsequently NOW egg deposition and infest of kernels.

Results and Discussion

Objective 1 – Evaluate NOW Egg Deposition and Infest Under Different Crop Management Regimes

In 2020, experimental treatments to adjust the crop load and crop:foliage ratio were applied to replicate trees (var. Kerman) in a pistachio block at the Kearney Ag. Center (Parlier, CA). Unfortunately, this experiment was terminated early due to mismanagement of the pistachio block, which led to a high incidence of fruit decay. In 2021, similar experimental treatments were applied to replicate trees (var. Golden Hills) in a commercial pistachio block in west Fresno County (near Cantua Creek). Treatments included a low and high crop:foliage ratio, with low crop load cages consisting of 11.0 ±1.9 nuts/cluster and high crop load with 70.1 ±5.2 nuts/cluster. Low and high crop load pistachio clusters were identified on April 28 and then caged on May 12. Starting Aug. 6, subsets of cages were inoculated with 5 mated (gravid) NOW females and allowed to oviposit for 1 week, after which 50% of the inoculated cages were removed and inspected for NOW egg deposition. The remaining cages were allowed to stay in the field for 3 additional weeks to give NOW larvae a chance to infest nuts, at which point the remaining 50% of cages were removed and all nuts evaluated for NOW infestation. Inoculations took place on Aug. 6, Sept. 3 and Oct. 1. At the time of inoculation, a subset of pistachios were sampled to record data on key chemical and textural properties to characterize changes that may be occurring in hull composition.

Overall total egg deposition varied between the low (6.7 ±2.0 eggs/cage) and high (22.1 ±5.4 eggs/cage) crop load treatments. Within a given cage, the total proportion of NOW eggs deposited onto the pistachio nuts tended to be greater in the high crop load treatment (Figure 1A), but this did not translate to any differences in NOW infestation between the two treatments (Figure 1B). It is surprising to see such dramatic differences in overall egg deposition between the high and low crop load treatments, which may indicate some sort of behavioral effect related to the total availability of pistachio nuts in the cage. Additionally, overall egg deposition onto pistachio nuts was much lower overall in 2021 than it was in 2020 (Figure 2), which could be the result of moving the project to a new orchard site and/or differences in environmental conditions between crop years. While samples for pistachio nut textural and chemical properties are still being processed, these data may reveal important differences between the pistachio nuts under these two treatments, and could explain observed differences in NOW egg deposition on nuts.

<u>Objective 2 – Optimize Caging Technique for NOW Egg Deposition Trials</u>
This objective was completed in Year 1 of the study. Window screen material was identified as the best cage material since it was the least preferred egg deposition substrate for NOW. For more details please see the Wilson/Burks 2020 Executive Summary for this project.

Conclusion

In 2020, egg deposition onto pistachio nuts increased over time, likely due to changes in hull integrity, but a similar trend was not observed in 2021. Instead, egg deposition tended to generally be higher in the ‘high crop load’ treatment, and egg deposition onto pistachio nuts in particular. Analysis of pistachio nut textural and chemical data may explain the observed differences between high and low crop load.

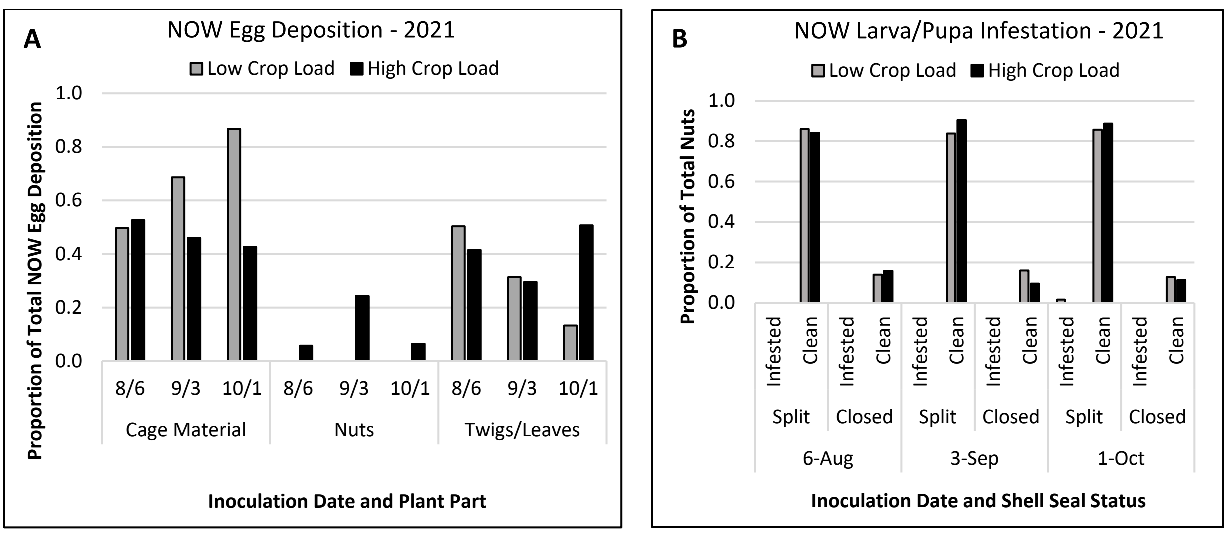

Figure 1. Total egg deposition (1A) onto the nuts was higher in the ‘high crop load’ treatment, but there were no differences in total infestation (1B) between the two treatments.

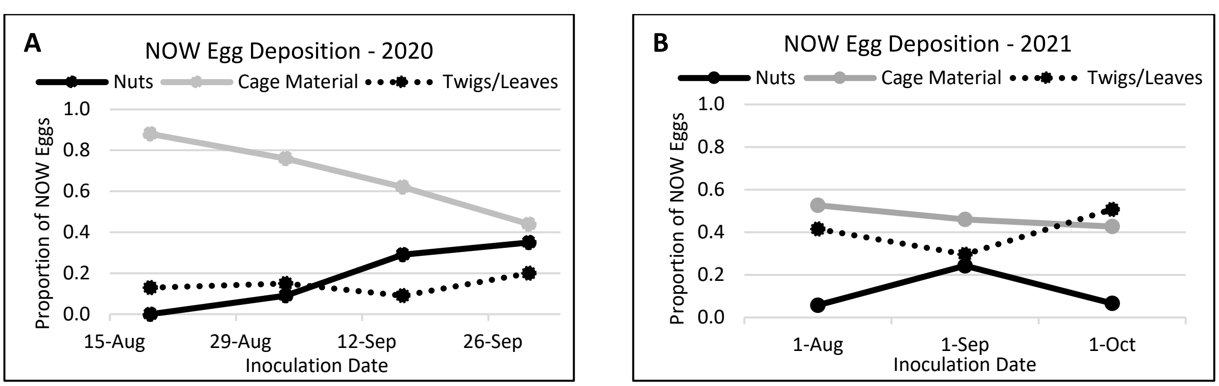

Figure 2. Egg deposition onto nuts over time increased in 2020 (1A) but not in 2021 (2B).

Factors Affecting the Efficacy of AF36, Improvement of the Biocontrol Agent, and Establishing an Area-wide Long-term Mycotoxin Management Program

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Introduction

Mycotoxins are toxic metabolites produced by certain fungi and constitute a high economical threat to pistachio industry due to the risk of product being rejected from the market by strict regulations of contamination. Aflatoxins are the most toxic mycotoxins, and they are highly regulated worldwide. They are produced by fungi in the *Aspergillus* section *Flavi*, which include *Aspergillus flavus* and *A. parasiticus*, which are the most commonly found in pistachios in California. Aflatoxin contamination, even though is sparse in pistachio nuts, still it is an issue that needs to be addressed because of its high toxicity and the high value of pistachio makes any rejected load from the market a considerable loss for the grower, since it will imply the possibility of incurring on extra costs for transportation, re-sorting and additional lab analyses or even product destruction. Pistachio nuts free of aflatoxin contamination due to successful control of aflatoxin will benefit both the consumers and the growers. Consumers will have a lower risk of being affected by aflatoxins and the growers will have a lower risk of income loss by avoiding crop rejections. Aflatoxins control in crops are difficult and unpredictable. Management of the nut crops to avoid damage by insects, including Navel Orange Worm (NOW), help to reduce the risk of aflatoxin, but it does not completely avoid contamination. The use of *Aspergillus flavus* atoxigenic strain technology is currently the only proven method to reduce aflatoxin contamination. The overall goal of aflatoxin management with this technology is to reduce the overall aflatoxin production potential of the aflatoxin producing fungi *Aspergillus* Section *Flavi* population in a crop system. This can be achieved by changing the population structure of the fungi in the soil of all crops susceptible to aflatoxin contamination (including pistachio and almond) from a population dominated by toxin producers to a population dominated by atoxigenic fungi. Previous results show that besides the expected increase of the applied atoxigenic biocontrol (AF36) in treated orchards the untreated neighboring orchards also had an increase, indicating the capability of the fungus to spread over considerable distances, causing cross effects between treated and untreated orchards. However, toxigenic isolates from untreated areas will also move to treated areas reducing the effectiveness of the treatments. Therefore, the best strategy to lower the risks of aflatoxin contamination in almonds and pistachios, which usually are grown together, is implementing area-wide long-term aflatoxin control programs.

Results and Discussion

Since the registration of the biocontrol *A. flavus* AF36 for applications in California pistachios in 2012 most pistachio orchards have been treated with the biocontrol. However, past research indicates that the applications have not been completely successful. The success of the biocontrol is measured by the percentage of displacement of toxigenic isolates by the applied atoxigenic isolate and it is considered that a displacement of over 80% will successfully reduce aflatoxin contamination. In general, the average displacement in treated pistachios in California has historically been around 70%. We hypothesize that the causes for the biocontrol not reaching its full potential are that some orchards are applied late when some nuts have already been infected and that there are toxigenic isolates from untreated areas causing infections in treated areas. Early applications of atoxigenic biocontrol product might increase the effectiveness of the treatments to reduce aflatoxin contamination. Experiments from past seasons indicate that the biocontrol *A. flavus* AF36 Prevail® might not satisfactorily sporulate at the earlier applications intended to establish a founder population of atoxigenic strains. Results from last season indicate that sporulation of AF36 Prevail® in early May applications was slightly better than past seasons (no sporulation), with a 25% of grain starting to sporulate two weeks after application. Sporulation improved in later applications reaching up to 70% in applications made in late June. Aflatoxin contamination of pistachio nuts from 2020 indicate that all the samples from the treated orchards were below the permissible levels of 15 mg/kg, except for a low incidence (3.0%) in samples from orchards treated at the July application. Population data from soil samples taken before application indicate that the population structure on all the orchards under the study was similar, with no significant differences on the percentages of the different *Aspergillus* strains. Post-harvest samples results indicate that there were no significant differences on the density (cfu/gr) of the total population of aflatoxin producing *Aspergillus* ranging from 154 cfu/g in the early May application to 284 cfu/g in the application made in July. Results from 2019 of the percentage of displacement given by the percentage of AF36 and toxigenic isolates indicate significant differences in displacement between the nontreated control (25% AF36 and 61% toxigenic strains) and the treated orchards with an average of 65% AF36 and 22% of toxigenic isolates. There were no significant differences among the times of applications for both the percentage of AF36 and the toxigenic isolates. Population data from 2020 indicate no significant differences among the treatments, including the control, on both the percentage of *A. flavus* strain L, and the toxigenic *A. flavus* strain S and *A. parasiticus*. However, the density of the population was higher in the late application with 360 cfu/g compared to the control and the early application with 57 and 59 cfu/g, respectively.

The effect of area-wide applications of the biocontrol *A. flavus* AF36 was evaluated in an area where tree nuts in risk of aflatoxin contamination (pistachio and almond) are grown together. The area was divided in two parts with one where both pistachio and almond were treated, and other where only pistachios were treated. Soil samples from the orchards under this study were taken both before application and after harvest. Samples taken before application served as a base line of the population of the aflatoxin-producing *Aspergillus* fungi in both areas. Samples after harvest indicate the change of the population structure during the season as the displacement of toxigenic isolates by the applied biocontrol AF36. Comparing the population structure of *A. flavus* between the almond treated and not treated areas after harvest will indicate the influence of the treatments in an area-wide basis. Results from 2019 postharvest soil samples indicate a higher percentage of AF36 isolates (94%) and lower percentage of toxigenic isolates (6%) in pistachios from the treated area, while the not-treated almond had the lowest percentage of AF36 (69%) and higher percentage of toxigenic isolates (22%). The pistachio in the nontreated area had similar percentage of AF36 (87%), but lower percentage of toxigenic isolates (8%) than the almond from the treated area (12%). Data from 2020 postharvest soil samples indicate a higher percentage, but not significant, of the *A. flavus* strain L in the treated area (96%) compared to the nontreated area (90%), and a significantly lower percentage of the toxigenic strains (*A. flavus* strain S and *A. parasiticus*) in the treated area (0.5%) compared to the nontreated area (5.7%).

Analysis of the AF36 and toxigenic isolates in the *A. flavus* strain L to determine the percentage of displacement in the 2020 data and population of the fungi in the 2021 data are underway. This analysis will give a better indication of the effectiveness of area-wide applications in establishing a population dominated by atoxigenic strains and consequently reduce the risk of aflatoxin contamination.

Conclusion

Displacement data, as the percentage of both the applied atoxigenic AF36 and the toxigenic isolates, from the 2019 season indicate no significant differences among the times of application, even though the product in the earlier applications had an extremely poor sporulation. Improving sporulation in the earlier applications might help to establish a founder population of atoxigenic strains before toxigenic strains start infecting nuts and could increase the likelihood of having a population dominated by atoxigenic strains. The use of new products with better sporulation under low temperatures and soil moistures will certainly help to achieve the goals. Results from 2019 indicate that area-wide programs might help to increase the efficacy of the atoxigenic strain technology to modify the population structure of the aflatoxin-producing fungi from a population dominated by toxin producing strains to a population dominated by strains that do not produce toxin (atoxigenic).

Characterization of fungi contaminating Pistachios with Ochratoxin A in commercial orchards of California

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Introduction

Ochratoxin A (OTA) is a naturally occurring mycotoxin that contaminates a wide variety of foods and beverage. Because of health risks associated with consumption of foods contaminated by OTA, the European Union (EU) has adopted stringent regulations for OTA in foods intended for human consumption and has proposed a maximum level of 5 µg/kg (ppb) OTA in pistachios. Mycotoxin analysis of 209 pistachio library samples in 2018 reported 26% positive for OTA. While 8% of these samples were contaminated with > 15 µg/kg OTA, the highest level of OTA was recorded in one sample with 357.9 µg/kg. This can be a huge threat to the export of pistachio and the pistachio industry in the United States. There is an urgent need for management of OTA in pistachios so that the industry can meet the EU standards and circumvent economic losses due to possible rejections.

Fungi in the genera *Aspergillus* and *Penicillium* can infect a wide range of crops and produce OTA while decaying them. Within the genus *Aspergillus*, section *Nigri* and section *Circumdati* contain species known to produce high levels of OTA in liquid lab culture and dried vine fruits. While these fungi were frequently detected in soils of pistachio orchards in California, an in-depth characterization of fungi responsible for OTA contamination of pistachio in California orchards was not done. The current report summarizes isolation and characterization of OTA producing fungi from canopy, soils and pistachio nuts collected from 14 orchards across California. Overall occurrence of *Aspergillus* section *Flavi* was also quantified. Levels of OTA detected in commercial pistachio samples from California in 2021 and library samples in 2020 are reported. Fungal incidence and characterization, along with their OTA producing ability on pistachio nuts will help in elucidation of etiologic agents, which is the first step towards OTA mitigation in pistachios.

Results

Twenty-five percent of commercial pistachio samples contained detectable levels of OTA and 10% exceeded 10 µg/kg OTA. Of the 200 library samples evaluated, 61% were positive for OTA, whereas 41% and 32% were contaminated with more than 5 and 10 µg/kg OTA, respectively. Six samples contained more than 100 µg/kg OTA with the highest being 1170 µg/kg in one sample. These samples fail to meet the maximum permissible levels for these mycotoxins in the EU. OTA levels did not differ between orchards treated with or without aflatoxin biocontrol AF36 Prevail ($p = 0.4$).

Orchards in the Chico (n = 5), Fresno/Madera (n =3) and Bakersfield (Wonderful orchards; n = 6) areas were sampled for soil, leaf and pistachio nut samples in 2021. Composite samples of soil (n = 3), leaves (n = 1), early split and normal nuts (150 each) were collected from each orchard. While isolation of potential OTA producers from soil and leaf samples is complete, fungal isolations from early split and normal nuts is in progress. Irrespective of sampling location, of the potential OTA producers, only *Aspergillus* section *nigri* were recovered from leaf samples. We quantified sections *Nigri* and *Flavi* to assess for correlation between the occurrence of these fungi. Overall quantities (colony forming units/ gram) of section *Nigri* were higher than section *Flavi* in all leaf samples. Section *Nigri* was present in significantly higher quantities than section *Flavi* in samples from the north and south but similar levels were detected in samples from central CA. While the greatest quantities of section *Flavi* were detected in samples from central CA, followed by southern and northern regions, quantities of section *Nigri* did not differ among regions. Additionally, a significant positive correlation was detected between the occurrence of sections *Nigri* and *Flavi* in central CA; however, correlations in the northern and southern regions were not significant. All soil samples (n = 42) contained section *Nigri* fungi while section *Circumdati* was recovered from six samples originating from central and southern CA. Soil samples showed similar trends as leaf samples and were dominated by section *Nigri* with greatest CFUs detected in the south, followed by central and northern regions. Similar results were observed for section *Flavi* in terms of region wise occurrence. While a significant negative correlation was detected between occurrence of sections *Nigri* and *Flavi* in soils from the north, non-significant correlations were detected in the remaining regions.

A total of 300 section *Nigri* fungi from leaves and 511 from soil were isolated and saved. Within section *Nigri*, isolates of *A. niger* rarely produce OTA and they do so at low levels. However, *A. carbonarius*, which is morphologically similar to *A. niger*, produces higher levels of OTA and has been implicated as the causal agent of OTA contamination of grapes and raisins. We utilized the resistance of *A. carbonarius* to the fungicide boscalid to differentiate it from *A. niger* and discovered that 16 and 14 percent of section *Nigri* isolates from leaves and soils, respectively, were *A. carbonarius*. The incidence of *A. carbonarius* was the highest in leaf samples and statistically similar to the incidence of *A. niger* in northern CA. All isolates identified as *A. carbonarius* and representative isolates of *A. niger* are being subjected to OTA analysis on sterilized pistachios. Thirty-one isolates of fungi belonging to section *Circumdati* have been recovered from soil samples and are being evaluated for OTA production. The beta-tubulin and calmodulin genes of representative isolates of *A. carbonarius, A. niger* and the yellow *aspergilli* will be sequenced for molecular identification.

Conclusion and Practical Applications

The results of the current study, along with analysis of pistachio library samples show that pistachios can be frequently contaminated with >5 µg/kg of OTA. Considering EU regulations, OTA management in pistachios is imperative. Fungal characterization for identification of potential OTA producers indicates that *A. carbonarius* may be an important causal agent of OTA contamination of pistachios. The potential of registered biopesticides for aflatoxin management in pistachio orchards will be evaluated against ochratoxigenic *A. carbonarius* and *A. ochraceus*. We will further evaluate fungicides registered for use in pistachio orchards for efficacy against OTA producers and ochratoxin management.

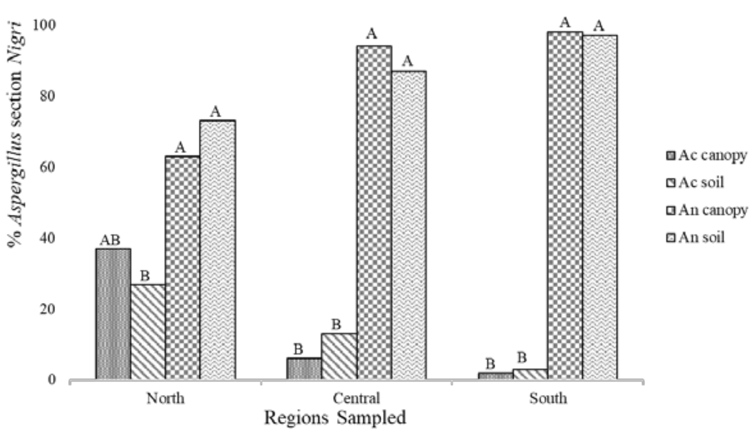

Figure 1. Incidence of *Aspergillus carbonarius* (Ac) and *A. niger* (An) in California pistachio orchards Different upper-case letters indicate significant differences ($p > 0.05$) in incidences of Ac and An among canopy and soils within different regions in CA as per Tukey's HSD.

Assessing Nitrogen Uptake to Develop Best Management Practices and Early Leaf Sampling Protocols for Pistachio Cultivars 'Lost Hills' and 'Golden Hills'

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Introduction

California pistachio growers have traditionally grown only one female cultivar 'Kerman' until the release of the first pistachio cultivars from the UC breeding program in 2005. Although 'Kerman' remains an important commercial cultivar, it is not a perfect selection for all conditions. According to a recent report, the new cultivars, such as 'Golden Hills' and 'Lost Hills', demonstrated a range of earlier bloom and harvest dates than 'Kerman' and some improved nut quality characteristics, such as a higher percentage of split, in-shell nuts and fewer closed shell and blank nuts. Due to these characteristics and to alleviate the worst of the peak harvest demand problem, newer cultivars 'Golden Hills' and 'Lost Hills' are now widely planted; in 2015 85% of new plantings were 'Golden Hills' and about 86,000 acres of 'Golden Hills' and 10,000 acres of 'Lost Hills' were in the ground as of 2018. To help growers achieve the goal of efficient and profitable nitrogen application, a new method of tissue testing and yield-driven fertilization has been developed for 'Kerman'. However, this approach has not been validated for other pistachio cultivars in California. Furthermore, due to relatively high chilling requirements of pistachio cultivars, dormancy-altering treatments in fall and spring often are applied that further impact nutrient (particularly N) storage in, and demand by, tissues and organs. Therefore, growers may not have the most reliable fertilizer management decision tools to apply the right rate of fertilizer at right time to optimize productivity and avoid environmental losses if the methodology has not been validated for modern pistachio cultivars. The project will address these knowledge gaps and will be relating all N budget and removal data to actual individual tree yield and will also be monitoring proportional yield to vegetative growth.

This project aims to develop demand curves for nitrogen and other nutrients which will guide the quantity and time of fertilizer application allowing growers to match fertilizer supply with crop demand. It also aims to provide a sound and practical 'early-warning' and monitoring tool for 'Golden Hills' and 'Lost Hills' growers to optimize N management by developing an early leaf N prediction model. This tool will improve plant tissue sampling protocols to diagnose excessive, sufficient, and deficient nitrogen levels early in the season. Efficient and responsive fertilizer strategies are essential if we are to protect the Californian environment from non-point fertilizer pollution and are an economic imperative as consumers increasingly demand sustainable production techniques.

Results and Discussion

The study is being conducted in two high yielding commercial pistachio cultivars "Golden Hills" and "Lost Hills" orchards in the California San Joaquin Valley. Both varieties were grafted on UCB1 rootstock. We have been monitoring two replicated blocks of trees (3 trees per block, totaling 9 trees per orchard) for each cultivar for changes in nutrient concentrations in annual (leaves and fruits) and perennial organs (roots, trunk, scaffold, canopy branches and small branches) six times during the season at different phenological stages. Samples collected are being processed for analysis.

Figure 1. Highly productive groves of pistachio were selected in Madera and Kings Counties for sampling. Photo: Joy Hollingsworth.

Additional 15 orchards of each cultivar are being monitored for leaf nutrient content. Leaf samples were collected during spring and summer and are being processed for analyses. At the end of second season, an early leaf N prediction model will be developed and validated.

In addition, to determine tree biomass at the beginning and end of season, trees that represent optimum leaf N concentrations and not showing any deficiency of other nutrients will be excavated in February 2022.

Samples of water and soil from all locations are being collected at every 6-month periods. The samples are being analyzed for Organic Matter (OM), soluble and mineralizable N pools and other critical parameters.

Conclusion

The first year of this project was assigned to establish the experiment and to collect samples, thus no data have been analyzed. Preliminary results (N demand curve) will be shared when the first full season is completed by May 2022. Project success will be evaluated by the development of demand curves for ‘Golden Hills’ and ‘Lost Hills’ cultivars over two growing seasons.

Improving Productivity of Pistachio Harvesting Machines Using Automation and Artificial Intelligence

Authors: Reza Ehsani, Professor, Mechanical Engineering Department, UC Merced; **Arash Toudeshki**, Research Specialist, Mechanical Engineering Department, UC Merced; **Louise Ferguson**, Professor, Department of Plant Sciences, University of California, Davis

Introduction

Trunk shakers are the most widely used mechanical harvesting machines for California pistachios. Currently, the field manager or machine operator sets the shaking frequency, pattern, and duration for trunk shakers. It is possible to mathematically determine for each individual tree unique optimum shaking frequency for maximum fruit removal and minimal damage. However, the governing mathematical equations require some very tree-specific parameters such as tree's mass and damping frequency which are a function of other factors such as leaf density and branch configuration. Therefore, determining those parameters for every tree is not practical. Recent advancements in machine learning have enabled us to develop a data-driven model to predict each tree's correct optimal shaking parameters. To develop this model, sensors that can record the tree's responses to the shaking energy need to be developed. Also, another sensor is needed to monitor the fruit removal rate to determine shaking duration and avoid tree damage from excessive shaking. This sensor can determine when shaking should be stopped. We have developed a wireless sensor system to collect trees' canopy vibration data during harvesting. The vibration distributed from the trunk to the tree's primary, secondary and tertiary scaffolds was measured in 2018-2019. In 2020, we developed a mathematical model based on a limited number of data we collected to show the optimum shaking frequency for different sizes of trees. More data is needed to build a more general model.

In the 2021 research project, we developed and tested a faster, more rugged simpler sensor system that can be mounted on the shaking head (Fig. 1). Data were collected from 102 pistachio trees with trunk diameters from 9 and 17 ½ inches. The duration and shaking pattern were the same for all trees and set by the field manager. In addition to manually measuring trunk size, we scanned the canopy from both sides with a 3D lidar scanner and camera. This data could better characterize each tree canopy and better estimate optimal shaking patterns in the future.

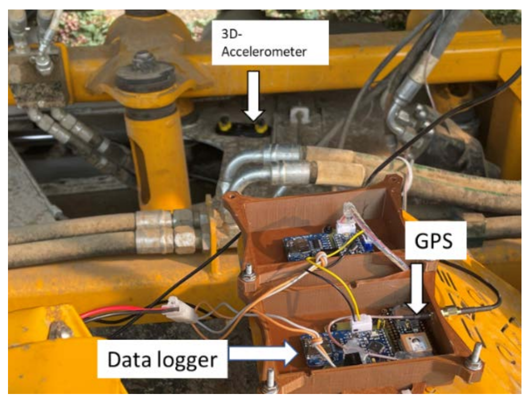

Figure 1- Sensor installed on pistachio harvester

We have also developed a new prototype sensor and associated data logger to monitor the rate of fruit removal. This system includes a sonic sensor, a signal conditioning system, a counter algorithm, and a data storage module. This system can count the number of harvested nuts and measure the nut removal rate.

Results and Discussion

Collected data were analyzed using a python code developed in our lab. For each tree, we calculated the shake duration from the sensor recorded data. This analysis demonstrated the shaking duration was the same for all the trees; 3 ± 0.1 seconds throughout the trial. We were also able to record the location coordinates of the machine for each harvested tree. Occasionally, the tree canopy blocked the sky and we lost the GPS coordinates. However, using the coordinates of known trees, we could successfully extract the exact location of every tree harvested. Since the shaking duration and pattern were constant, we examined the shake acceleration applied to trees and the number of effective shakes for each tree. We observed the average acceleration generated by the machine was different for each tree. We mapped the acceleration data with the tree trunk size and found that the larger trees damped the trunk applied vibration. However, small trees damp very little the trunk applied vibration and had a higher shaking frequency. More importantly, the number of effective shakes, which is defined by the number of shaking frequency above a threshold, was more in trees with smaller trunk sizes than trees with large ones (Fig 2). This variability could cause variability in fruit removal and tree damage. These results suggest that trunk harvesters should be able to change their shaking frequency for different trunk sizes.

For measuring the optimum duration of shake for harvesting, we studied different techniques for measuring fruit removal rate and decided to develop a prototype of an acoustic-based fruit removal rate sensor. This sensor consists of a microphone and a specially designed analog circuit that is tuned to be only sensitive to the sound of pistachio fruit drop on a plate. For evaluation, the prototype fruit removal rate sensor was temporarily installed near the conveyor belt of the catch frame machine without a shaker head. It was able to record the approximate number of harvested nuts as a function of time on the surface area of the sensor. We were able to extract the fruit drop rate as a function of time with this newly developed sensor. A high removal rate indicates that a large number of fruits are being harvested. When the majority of fruit are harvested, the fruit removal rate reduces significantly, indicating the tree shake should be stopped.

The 3D lidar scanner was able to scan tree canopy factors such as branch size and distribution with good detail. However, the field of view and resolution of the camera in this sensor system was not suitable for large pistachio trees. The collected data from 3D lidar scanner can be used in the data-driven model to select the optimum shaking frequency.

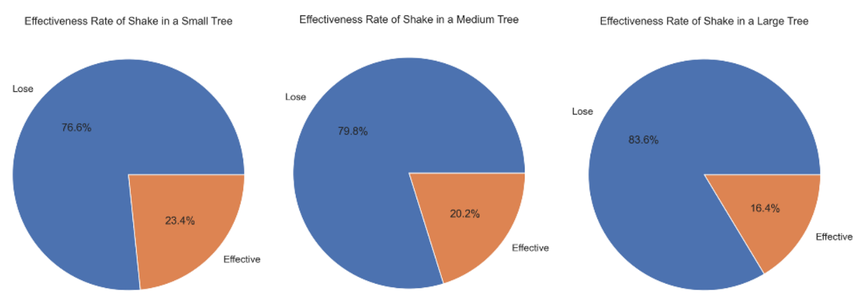

Figure 2 Effectiveness of shaking for different size of trees when shaken with the same shaking frequancy

Conclusion

- A new prototype sensor system with GPS was developed and its performance was tested under field conditions. The sensor was rugged and able to collect data without interrupting or slowing the harvesting operation.
- A new acoustic-based nut removal rate monitoring sensor system was developed and its performance was tested under field conditions. This sensor can be improved and be mounted on a harvester to collect data continuously during the next harvesting operation.
- The trunk diameters of all shaken trees were measured and the relationship between the of shake frequency applied by the machine on each tree and its trunk dimension was studied
- GIS map for shaken trees for better visualizing the data were generated. The data clearly shows how trees with different trunk sizes responded differently to similar input shaking energy.
- Both sides of shaken trees were scanned using a new moving multi-level LIDAR to record the tree morphological data for future experiments.

Evaluation of salinity, boron, and soil hypoxia on pistachio tree growth, year 2

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Introduction

Soil salinity is known to depress pistachio growth, however recent research suggests that the traditional method for measuring salinity, soil electroconductivity, does not correlate well with in-field growth. While high sodium destroys soil structure and can result in saturated conditions, it is not fully understood how this affects pistachio growth. Salinity is also linked with high boron, particularly on the west side of the San Joaquin Valley, however there has been no published interaction between salinity and boron for pistachio. This trial seeks to examine the interaction of soil salinity with boron, as well as attempt to separate the effect of periodic, short-term low oxygen conditions from salinity and examine tree responses to both.

Results and Discussion

Plot establishment:

The pistachio trees were planted in April 2021 (Figure 1). An early, too-heavy fertilization resulted in mild foliar burn and a cessation of growth, however after leaching and a more conservative fertilization approach, the trees recovered and nearly all were tall enough to head, which will occur at the end of December, 2021. By the end of the growing season, the trees gained an average of 1 cm in trunk diameter.

Figure 1: Golden Hills on UCBI clonal, April 1 at planting (left) and October 7, 2021 (right)

Sensors and soil oxygen:

Work has not progressed as quickly as hoped in this area. While we purchased a small quantity of sensors in early 2021 to assess their accuracy and potential use for the trial, the number is too small to do a replicated study to examine whether it is feasible to drop oxygen levels in soils. In laboratory tests, the sensors are accurate and responsive to changes in oxygen concentration, though there does seem to be a slight depression in output at low temperatures (Fig. 2). One issue that has come up in testing is that the sensors are easily clogged (Fig. 3). Initially we created chambers out of PVC piping with mesh covering the opening, however field testing showed this does not work (Fig. 3). Protecting the opening with a hydrophobic membrane seems to be more effective (Fig. 3), however we need more replicated tests. We were able to test a few sensors in situ with the totally impermeable film (TIF) installed on the soil surface to try to artificially drop soil oxygen levels (Figure 3), however due to clogging issues and low replications, it is unknown if the data reflects sensor errors or a successful trial. In late 2021, we were finally able to complete a purchase for all the needed sensors and will do more field testing this winter.

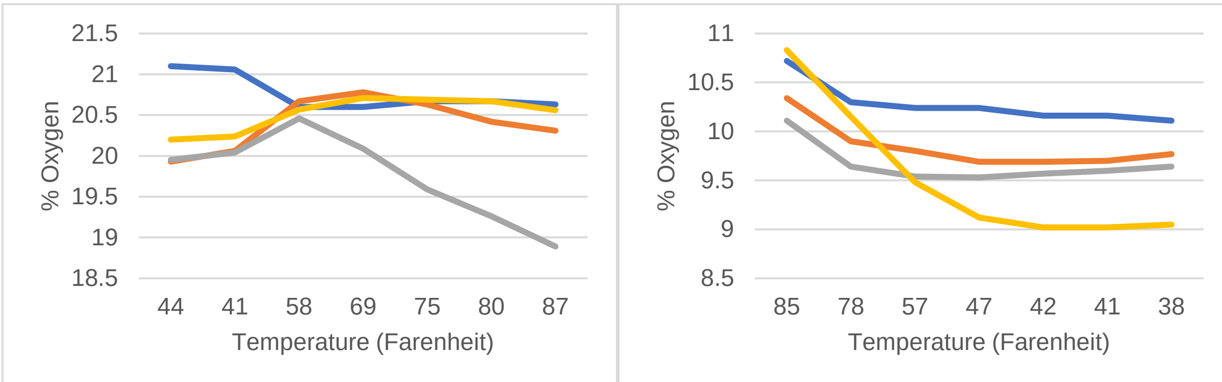

Figure 2: Calibration graph of the Figaro KE-50 sensors at 20% oxygen (left) and 10% (right), featuring the check (blue line) and three different sensors, including one with a hydrophobic membrane sealed to the base to try to prevent clogging (gray line). Data is recorded in linear order as temperatures were decreased, then increased (at 20% oxygen), or started at a high temperature, then dropped (10% oxygen).

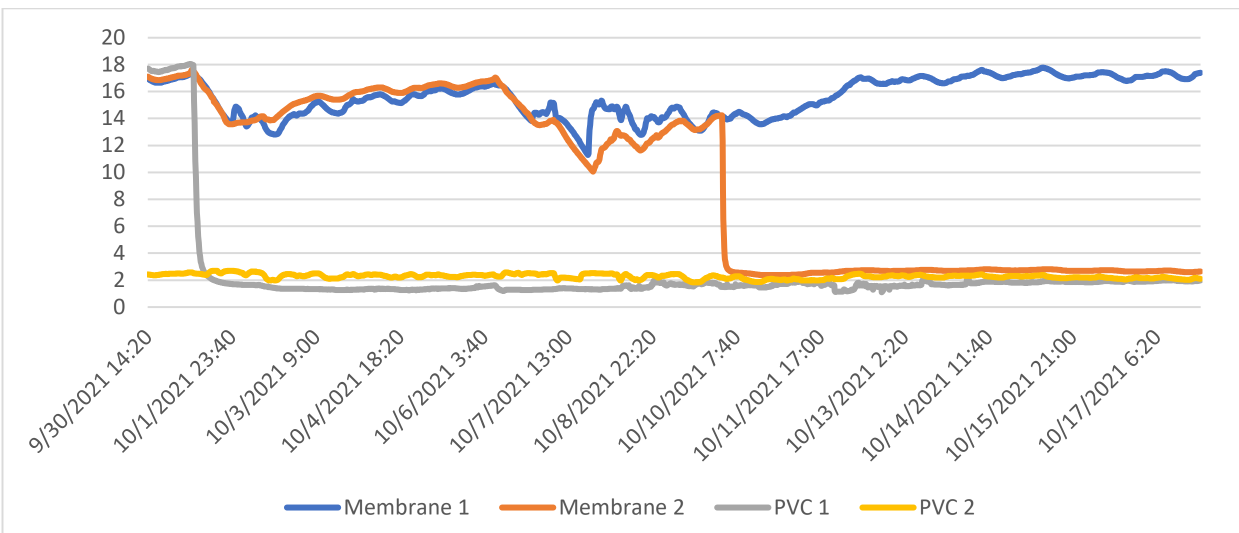

Figure 3: Output of Figaro KE-50 sensors buried 12" into the soil with TIF over the soil surface. Sensor names reflect how we tried to prevent clogging. Water was applied to the soil for five hour increments on October 1, 6, and 7. There is a decrease in sensor output following these readings, followed by a recovery, however we would like to do more testing to ensure these are not erroneous readings . PVC 1 and 2 likely had soil clogging the emitters. Membrane 2 was found to be damaged when it was recovered from the soil; it is unknown when this damage occurred and if this resulted in the low oxygen results.

Conclusion

At this point in time the experiment has not been formally initiated, however we are excited to finally initiate data collection next year.

Acknowledgements: Thanks to Carlos Crisosto for his help in testing the accuracy of the KE-50 sensors.

Understanding the biology and improving the management of alkaliweed in pistachio

Authors: **Brad Hanson,** Cooperative Extension Specialist, UC Davis Department of Plant Sciences; **Andres Contreras,** graduate student researcher, UC Davis Graduate Group in Hort and Agronomy; **Phoebe Gordon**, UCCE Farm Advisor, Madera and Merced Counties; **Elizabeth Fichtner**, UCCE Farm Advisor, Kings and Tulare Counties; **Douglas Amaral**, UCCE Farm Advisor, Kings and Tulare Counties

Introduction

Pistachio growers in several parts of the San Joaquin Valley have been reporting the presence of a rapidly spreading invasive weed species, alkaliweed (*Cressa truxillensis*) in and around pistachio orchards. In some cases, alkaliweed has become the dominant weed within only a few years after being introduced. Alkaliweed is a very difficult weed to control because (1) little is known about its biological and ecological characteristics; (2) it is a perennial plant with reduced sensitivity to pre-emergence herbicides; (3) it is not very sensitive to glyphosate, the most commonly used translocated post-emergence herbicide in orchards; and (4) it is very tolerant to saline soils which can let it outcompete many other weed species in orchards.

The rapid spread and limited control of this weed species are of great concern to growers because of interference with field operations, cost of repeated control efforts, and unknown effects on pistachio productivity. As a rule, weedy plants that escape control measures often have the potential to reach reproductive maturity and set seed, which could replenish or enhance the seedbank. As a perennial weed, alkaliweed may also re-grow from belowground stems.

This research was initiated in 2021 as a first-step for this team to conduct replicated field observations and evaluating herbicide treatment performance on alkaliweed in commercial pistachio orchards. The proposal aimed to (1) understand emergence pattern and phenology of alkaliweed in orchards; (2) field screening of registered herbicides and potential products for control of alkaliweed in pistachio during fall versus spring, (3) conduct greenhouse and laboratory study to understand the role of surfactants and herbicide mixtures for control of alkaliweed.

Results and Discussion

A series of field experiments were established in commercial pistachio orchards near Alpaugh, Lemoore, and Five Points, CA in summer 2021. Treatments in these studies focused on herbicides with potential to translocate or move within the plant under the assumption that contact herbicides are not likely to provide adequate control of this perennial species. In addition to the registered active ingredients (glyphosate, glufosinate, halosulfuron, flazasulfuron, rimsulfuron, and 2,4-D) two unregistered herbicides under consideration for future registration in orchard crops were included (florpyrauxifen and quinclorac). Plots were treated June 10, 2021 and evaluated at monthly intervals during the remainder of the season. The same plots were retreated on November 5, 2021, to evaluate the effects in fall, which is often an effective time to treat herbaceous perennial plants that go dormant in winter.

By the end of summer 2021, none of the treatments provided commercially-acceptable levels of control as a single-shot program. Given grower and PCA experience with this weed in recent years, that is not unexpected. Also as expected, glyphosate (with various surfactant or tankmix combination) did not provide adequate control. Although they can move within the plant and are often used as POST herbicides in other cropping systems, the ALS-inhibiting herbicides halosulfuron, flazasulfuron, and rimsulfuron provided only moderate levels of control in this study. The best-performing treatments on established alkaliweed populations were the 2,4-D treatments (2,4-D amine as Orchard Master or 2,4-D choline as Embed Extra); although regrowth occurred, alkaliweed was suppressed to a greater degree than with the other treatments. The spring 2022 evaluations will provide much greater insights into the success of these herbicide programs.

The original laboratory objective was intended to evaluate the mechanism of tolerance to glyphosate that is apparent based on grower reports and on our initial results. One research question to be addressed was whether this tolerance is due to an inherent biochemical tolerance or simply due to insufficient uptake related to the plant's extremely hairy leaf surfaces. We proposed collecting plant material from the field and growing it in the greenhouse at UC Davis for systematic evaluation of herbicides and herbicide-surfactant combination. We also proposed an initial study to evaluate uptake (or lack thereof) of glyphosate using radiolabeled herbicide. Progress on both of these objectives has been delayed due to the unexpectedly difficult task of propagating and cultivating alkaliweed in non-field conditions. If sufficient plant material can be generated, we will conduct the smaller herbicide uptake study in spring and summer 2022.

Conclusion

This research is ongoing. The bulk of the observations on emergence patterns and phenology will occur during 2022 due to a late start in 2021 caused by a staffing change with the UC Davis team. The greenhouse and laboratory work will continue into 2022, assuming we will be able to successfully grow the plants outside of the orchard setting. The herbicide efficacy trials are anticipated to continue for several years with the collaboration of the local UCCE Advisors and cooperating pistachio growers to develop a robust data set.

Evaluating new training systems for pistachio

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Introduction

This study was initiated to investigate alternative training systems for pistachio. The current trials are designed to compare the conventional practices (as outlined in the Pistachio Production Manual) with two other tree-training strategies, a modified central leader and an unheaded/unpruned treatment. The conventional training method involves heading the trees at approximately 43 inches and then doing in-season tipping and dormant heading cuts to generate the desired tree structure. Some California growers have been using a modified central leader training system and the results of these orchards look promising with good tree structure and the first commercial harvest being moved up by 1 to 1.5 years. The unpruned treatment was untouched except for removing any branches that were too low or in the way of tractor traffic.

Three pruning trials were initiated as part of this project in 2017 and 2018. The first was initiated in a ‘Lost Hills’ on ‘PG1’ seedling rootstock orchard on double line drip irrigation in Kings County. The rootstocks were planted in early winter of 2016 and budded in July of that year. Treatments were imposed in the spring of 2017. The second trial, also in Kings County, and is also in a ‘Lost Hills’ block on ’PG1’ seedling rootstock. The rootstocks were planted in the summer of 2016 and budded in the summer of 2017. Treatments were imposed in the spring of 2018. In 2018, a third site was established in an orchard in Yolo County near Woodland. The orchard used nursery budded ‘Golden Hills’ on seedling ‘UCB1’ rootstock and was planted in mid-February 2018. Irrigation was supplied with double line drip with in-line emitters. Dataloggers with Watermark and temperature sensors were installed in one replication of each treatment at all three pruning sites. Pruning treatments were: 1) The industry standard for training young orchards, as described in the Pistachio Production Manual, including in–season tipping (Beede and Ferguson, 2016), 2) a modified central leader training system (developed by grower Jeb Headrick and consultant Brian Kempf), and 3) an unpruned control. Selected data trees met a minimum height requirement of 62 inches at the time treatments were imposed. The conventional trees in the two Kings County trials had metal stakes rather than the traditional wooden stakes while the Yolo County trial had metal stakes for the unpruned and modified central leader treatments but traditional wood stakes for the conventional training treatment.

Results and Discussion

Trial #1 Kings County- Midday stem water potential was measured approximately every two weeks to one month in 2017 to 2021. There were no significant treatment differences in midday stem water potential on any date in any year. By 2021, the unpruned treatment trees had significantly higher light interception compared to either the modified central leader or conventionally pruned treatments. Trees were mechanically shaken onto tarps in 2021 with the conventional, modified central leader and unpruned treatments producing 1761, 2168 and 2589 pounds of good nuts per acre respectively. Nut removal was similar among treatments. The second shake was missed due to the trees being accidentally harvested by the grower. Cumulative yield for the conventional treatment trees was 1982 pounds per acre, for the modified central leader treatment it was 2559 pounds per acre and for the unpruned trees 3592 pounds per acre.

Trial #2 Kings County- This orchard was only flood irrigated on 3 dates in 2018, and on two dates in both 2019 and in 2020 yet trees were generally not stressed. All treatments had significant levels of stress during 2021 but the unpruned trees tended to have less stress with significant differences on several dates in mid-summer. Similar to Kings County site #1, in 2021, the unpruned treatment trees had significantly higher canopy light interception compared to either modified central leader or conventionally pruned trees by 2021. Trees were hand harvested in 2019 and in 2020 and cumulative yields for conventional, modified central leader and unpruned were 55, 72 and 317 pounds of good nuts per acre respectively. In 2021, trees were mechanically harvested for the initial harvest with a secondhand harvest and total yields were 1398, 1672 and 1961 pounds per acre of good nuts for conventional, modified central leader, and unpruned treatments respectively. This resulted in cumulative yields of 1435, 1737, and 2295 pounds of good nuts per acre for conventional, modified central leader, and unpruned trees respectively.

Trial #3 Yolo County- This trial utilized nursery grafted trees. There were more problems with leaning trees than at either of the other trials described above. This has been previously observed by others and likely these trees are more flexible due to having been grown in crowded conditions in the nursery. There was extensive cold damage from the nursery in these trees at planting. This did not impact the conventional or modified central leader trees since the damaged tips were pruned off during the dormant season. However, approximately 50% of the shoots on the unpruned trees were damaged and these shoots behaved like pruned shoots with the central leader often being lost. Approximately 10 conventionally trained trees broke loose of ties to the wooden stakes on extreme north wind days and bent over towards the ground as if they were made of rubber. Similar to both Kings County sites, canopy light interception was significantly higher in the unpruned treatment compared to either the modified central leader or conventionally pruned treatments by 2021. There was no cropping at this trial in 2019 or in 2020. In 2021, trees were hand harvested since the grower did not harvest the rest of the conventionally trained orchard due to lack of crop. Yields were 7, 16 and 71 pounds per acre of good nuts for the conventional, modified central leader, and unpruned trees respectively.

Westside Field Station Trials- Additional pruning trials (Golden Hills on UCB1 seedling and Plantinum rootstock) and a fall irrigation cutoff trial were planted in the spring of 2019 and trees were budded in early September 2019. Dataloggers for monitoring soil moisture and time lapse cameras were installed in 2020 and pruning treatments were initiated in the winter of 2021. In-season tipping to establish secondary and tertiary branches was imposed on conventional trees before mid-July. Preliminary irrigation cutoffs were initiated in the fall of 2020 and in 2021 and tissues were sampled for carbohydrate analysis in fall and spring just prior to bud break.

Preliminary Conclusions

Although these trials are in their infancy, the results to date look encouraging. Trees in all treatments grew well with the unpruned treatment tending to have higher canopy light interception at both Kings County as well as the Yolo County pruning trials. Conventionally pruned trees tended to be more stressed on most dates. Although some unpruned trees had tops that were bending over (since they were often taller than the stakes), they appear to be straightening themselves out by re-sprouting branches that balance the lean similar to results we have seen in walnut. Annual and cumulative yields were significantly higher for the unpruned compared to either of the other treatments at all three trial sites in Kings and Yolo counties. Nut removal from mechanical shaking in 2021 was similar among treatments at both Kings County sites. Percentage of good splits was similar among all treatments at all sites in all years. Data collection will continue in all three of the original trials as well as the new trials at Westside Field Station in 2022.

Upscaling new findings on Pistachio water use (ET and Kc) to enhance irrigation scheduling and demand estimation in the water-limited context of the San Joaquin Valley.

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Introduction

According to a recent survey (CDFA, 2018), about 96% of the California pistachios are produced in the counties of Madera, Fresno, Kings and Kern, where the pistachio acreage has rapidly expanded on land affected by salinity and sodicity. In the water-limited context of the San Joaquin Valley, improving the accuracy of irrigation demand estimation is of critical relevance to pursue climate-adaptive water resource planning, secure reliable water supply for pistachio production, and manage on-farm irrigation efficiently.

Although in the last decades significant scientific and technical advancements have made accurate ETo data timely and publicly available throughout California, resource-efficient irrigation management for pistachio requires accurate Kc values by growth stages for the current cultivars, farming and nutrient management practices, and for high-frequency micro-irrigation methods used by most pistachio growers. However, there is a paucity of information on how salinity and sodicity affect the actual evapotranspiration, nut yield, and water productivity of micro-irrigated pistachios, which is crucial to inform resource-efficient irrigation scheduling and management practices.

In the period between 2016-2020, scientists from UC Cooperative Extension conducted applied research to fill the knowledge gaps on actual water use of pistachios grown on non-saline and increasingly salt-affected soils. The present project aims to upscale the updated ET and Kc information for pistachio and enhance the capability for modeling/forecasting pistachio irrigation demand and devising irrigation scheduling based on research findings from the UC studies of 2016-2020, which are crucial for maintaining profitable pistachio production in the valley.

Results

Since the start of this project, the research team worked on two main specific tasks: Task 1) analysis and interpretation of datasets on actual evapotranspiration (ETa) and crop coefficient (Kc) values determined from micro-meteorological measurements collected from the various pistachio study orchards during 2016-2020; and Task 2) calculation of the evapotranspiration of applied water (ET_{AW}) of micro-irrigated pistachio for the purpose of mapping the irrigation water demand in the pilot area of Kings County.

The information on consumptive water use for pistachio obtained from the Task 1 are being used to calculate ET_{AW} and the spatially-distributed irrigation water demand in Task 2. The Task 2 also included the analysis of yearly cumulative rainfall data to identify a wet, average, and dry water years and to determine ET_{AW} for these various water supply conditions through the use of the Cal-SIMETAW model, which was developed jointly by scientists from UC Davis and the California Department of Water Resources.

Prior to performing the model simulations, the research team intersected spatially-distributed information on soil texture, pistachio acreage, and atmospheric water demand (ETo) to generate simulation units that represent various unique combinations among these parameters. Afterwards, the Cal-SIMETAW model was run with Kc information obtained in Task 1 in order to determine the necessary irrigation water to apply for meeting ET_{AW} for the farmland planted to pistachio throughout the pilot area of Kings County.

Table 1 reports results from the data analysis of Task 1, i.e., bi-weekly Kc values from the for the different study sites calculated as Kc = ETa/ETo. The Kc values of the different study sites were averaged over the bi-annual cycles of field data collection. The table also reports the Kc values from the UC ANR Pistachio Production Manual of 2005, which were determined from a study conducted by UC researchers in a sprinkler-irrigated pistachio orchard located in the south-western side of the San Joaquin Valley during the late '90s.

Table 1 – Bi-weekly Kc values for the non-saline (S0 – Nichols; S0 - Gebhardt) and the increasingly salt-affected pistachio study sites (S1, S2, S3 – Flores) for the relative periods of field data collection.

	Averaged Crop Coefficient (Kc) values					
Bi-weekly period	S0 - Nichols (2016-2019)	S0 - Gebhardt (2018-2019)	S1 - Flores (2016-2019)	S2 - Flores (2016-2019)	S3 - Flores (2016-2019)	UC ANR (2005)
Apr 1-15	0.37	0.52	0.44	0.26	0.44	0.07
Apr 16-30	0.59	0.86	0.65	0.52	0.52	0.43
May 1-15	0.79	0.94	0.86	0.71	0.66	0.68
May 16-31	0.82	0.91	0.89	0.66	0.70	0.93
Jun 1-15	0.89	0.94	0.80	0.67	0.58	1.09
Jun 16-30	0.89	1.05	0.86	0.72	0.60	1.17
Jul 1-15	0.90	1.04	0.83	0.70	0.60	1.19
Jul 16-31	0.84	1.03	0.81	0.70	0.60	1.19
Aug 1-15	0.86	0.97	0.77	0.69	0.58	1.19
Aug 16-31	0.86	0.96	0.73	0.64	0.55	1.12
Sept 1-15	0.82	0.92	0.59	0.52	0.45	0.99
Sept 16-30	0.78	0.81	0.50	0.41	0.38	0.87
Oct 1-15	0.62	0.78	0.35	0.27	0.30	0.67
Oct 16-31	0.59	0.58	0.32	0.33	0.39	0.50
Nov 1-15	0.41	0.41	0.23	0.28	0.35	0.35

Figure 1 shows the cumulative annual precipitation (mm) for average (2013), dry (2015) and wet (2011) years, whereas Figure 2 illustrates the cumulative values of ET_{AW} (mm) for these years, determined only for non-saline pistachio orchards.

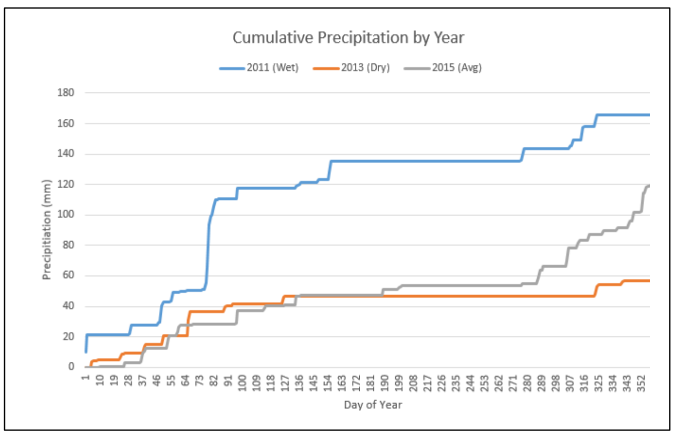


Figure 1: Cumulative precipitation (mm) over the calendar year for dry (2013), average (2015), and wet (2011) years.

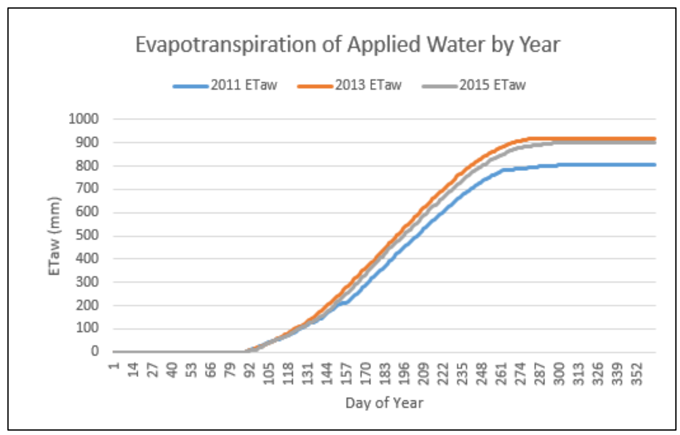


Figure 2: Evapotranspiration of applied water (mm) for non-saline pistachios on dry (2013), average (2015) and wet (2011) years.

Conclusion and Practical Applications

The results obtained from the activities conducted in Tasks 1 and 2 are preliminary information that must be complemented with values of irrigation water demand for pistachio orchards grown in salt-affected soils. The research team is currently analyzing additional field datasets collected during the growing seasons 2020 and 2021 in non-saline pistachio study orchards, which will allow obtaining ETa and Kc information averaged over multiple bi-annual growing cycles to smooth out the water-related effects of alternate bearing behavior. The final information, methods and tools that will be developed from this project are of practical use by pistachio growers and orchard managers to improve irrigation-scheduling decisions, but will also enable water resource managers to pursue more accurate water resources planning, allocation, and delivery in the water-limited context of the San Joaquin Valley. The broad adoption of updated ET and Kc information will contribute to addressing water supply shortfalls and uncertainties imposed by increasingly stringent environmental regulations, recurring droughts, and climate variability.

Collaborative Pistachio Rootstock Breeding

Authors: **Pat J. Brown,** Assoc. Professor, and **Franklin Lewis**, Asst. Specialist, Plant Sciences, UC Davis; **Themis J. Michailides**, Plant Pathologist, Plant Pathology, **Alejandro Hernandez**, Graduate Student, and **Florent Trouillas,** Asst. Cooperative Ext. Specialist, Plant Pathology, KARE, UC Davis.

Introduction

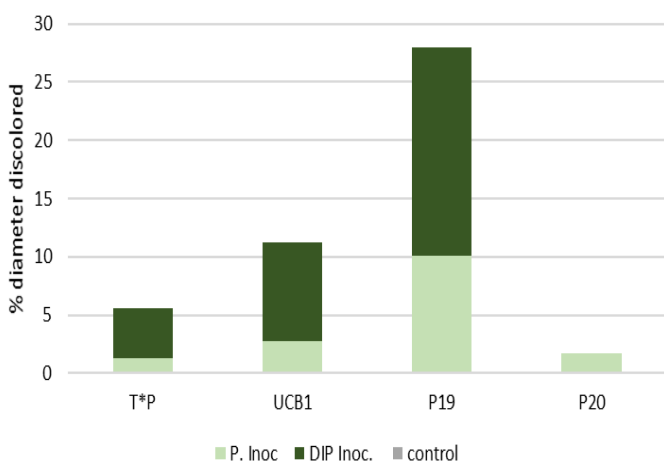
The California pistachio industry currently relies on rootstocks from a single seedling population and clonal selections from only four parents. These established rootstocks perform very well in most situations, but emerging soil-borne pathogens, variation in quantity and quality of available water, and climate change all create uncertainty about future production. Creating more diverse pistachio rootstock options is an efficient insurance policy that benefits growers, nurseries, and the entire pistachio industry. Understanding the genetic control of Verticillium tolerance/ resistance in existing rootstocks is a pre-requisite for confident adoption of new rootstocks to address challenges such as Phytophthora, salinity, and reduced winter chill. Our major goals for 2021 were: **A)** to generate and genotype new diverse seedling populations; and **B)** to establish seedling assays for resistance/tolerance to Verticillium, Phytophthora, and salinity.

Results and Discussion

A.1 Generation of new diversity: 20 controlled crosses were performed; predominantly using a P. integerrima female with pollen from P. atlantica, P. terebinthus, or P. khinjuk. Open pollinated seed was also collected from over 20 females representing the species diversity in the USDA repository. Altogether several kg of seed have been cleaned and readied for planting in 2022.

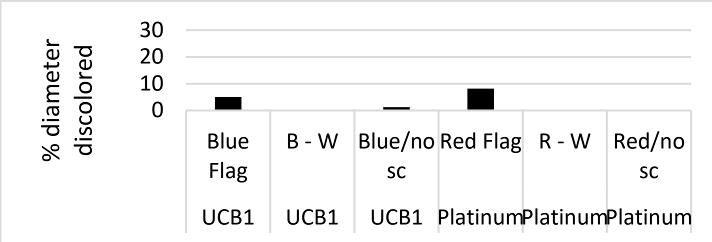
A.2 Genotyping & genomic work: Leaf tissue was collected for genotyping from 23 experimental rootstocks (complex hybrids of P. vera and P. integerrima) at Westside created by Craig Kallsen and inoculated with Phytopthora by Flo Trouillas. Rootstock cambium tissue was collected from ~360 mature trees at KARE ("Project 812") and is currently being genotyped.

B.1. Verticillium. Several experiments were performed, with inoculation of small seedlings (Figure 1) yielding higher rates of infection than inoculation of larger seedlings (Figure 2).



P.inoc = pipette inoculated, with a drop placed in a 3 mm wound made with a knife. **DIP inoculated** = dipped for ~30 minutes in inoculum. Inoculum for both treatments was a suspension of 1x 10^7 conidia per milliliter. Y axis represents % discoloration of the stem cross-section.
T*P is a terebinthus X palestina cross created by Malli Aradya; P19 represents an integerrima x atlantica cross; P20 is open pollinated integerrima seed; all are seedling populations.

Figure 1. Verticillium inoculation of small seedlings using pipette & dip methods



UCB1 and Platinum rootstocks were inoculated with both conidia and microsclerotia (Blue/Red Flag) or conidia only ("no sc") or neither (B-W and R-W). Platinum inoculated with microsclerotia and conidia developed some dead shoots.

Figure 2. Verticillium inoculation of large seedlings using conidia and microsclerotia

B.2. Phytophthora Resistance Evaluation. The relative susceptibility of diverse UCB-1 seedlings to Phytophthora root and crown rot was evaluated using mycelium stem inoculations of 79 two-year old potted UCB-1 seedlings. Sixty-nine random seedlings were wound inoculated using mycelial plugs (6 mm) of *Phytophthora niederhauserii* isolate KARE446. An additional ten seedlings were inoculated with clean APDA plugs (6 mm) to serve as controls. Plants were inoculated on March 9th, 2021 and maintained in a lath house at the Kearney Agricultural Research and Extension Center (KARE). Lesion size after 7 months varied from 7 to 83 mm among UCB-1 seedlings. Seedling lesion lengths were grouped into four categories (Figure 3): highly susceptible (green, 3%) > 83 mm > susceptible (yellow, 17%) > 16 mm > tolerant (blue, 55%) > 7mm ≥ resistant (orange, 25%).

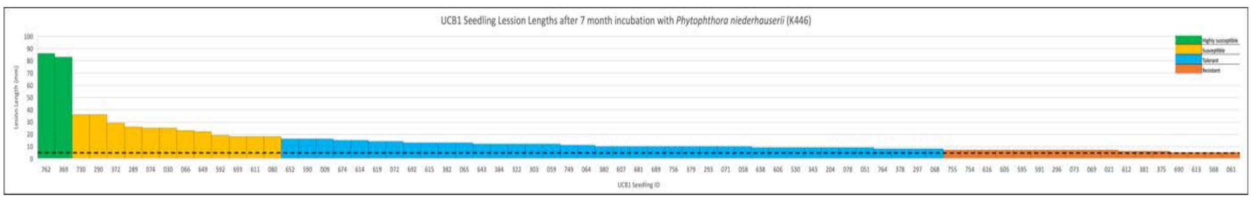


Fig 3. Lesion Lengths of Inoculated UCB-1 Seedlings with *P. niederhauserii* after 7 months.

Twenty-four complex hybrids of *P. vera* and *P. integerrima* created by Craig Kallsen and 3 seedling UCB-1 were subjected to stem inoculation with Phytophthora mycelia in 2020 and 2021. Individual branches were inoculated with *P. niederhauserii* plugs and clean APDA control plugs (8 mm each) on October 30th, 2020 and on June 9th, 2021 at Westside. Lesion length after 5 months varied from 11-193 mm and 25-142 mm in 2020 and 2021 (Figure 4). Rootstocks with lesions consistently lower or equal to UCB-1 included Exp-10 R1 and Exp-10 R2.

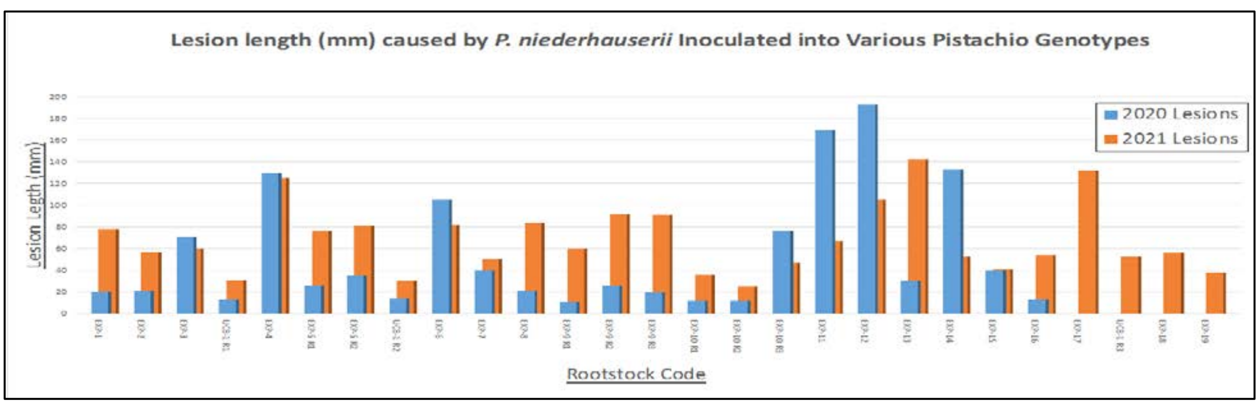


Figure 4. Lesion Lengths of *P. niederhauserii*-inoculated Kallsen/UCB-1 rootstocks after 5 months.

Conclusion

UCB-1 seedlings show variation in Phytophthora resistance. Open pollinated *P. integerrima* seedlings showed less discoloration from *Verticillium* than UCB-1 or other populations tested. Salinity results will be presented in our forthcoming proposal.

Pistachio Improvement Program

Authors: Pat J. Brown, Associate Professor, **Chuck Leslie**, Specialist, and **Franklin Lewis,** Assistant Specialist, Dept. of Plant Sciences, UC Davis.

Introduction

The goal of this continuing project is to advance public pistachio breeding and related activities at UC Davis. This project is anticipated to serve important functions including the release of new varieties; establishment of germplasm blocks and *in vitro* germplasm resources to facilitate research by physiologists, pathologists, entomologists, and farm advisors; and trait discovery to address biotic and abiotic challenges and to access new growing environments and markets. The long-term vision for this project is a genomics-assisted breeding program in which low-cost genotyping is used to reduce field costs by culling inferior individuals and skewing the sex ratio in favor of females.

Results and Discussion

Germplasm blocks in Davis and Wolfskill: Flowering and seed production were observed for the first time in 2021 in these blocks. The only entries to produce significant quantities of seed were (from highest production to lowest): S-51, Gumdrop, and C2-35. These trees are currently being phenotyped for phenology and chill requirement. We continued to bud material from the USDA repository C block --in which own-rooted *P. vera* trees are dying rapidly, apparently due to soil-borne pathogen—onto UCB-1 rootstocks. Hedging or pruning of some very old, overgrown trees is needed to stimulate budwood production and complete the establishment of our germplasm blocks. We were unable to perform hedging last winter due to a purchasing issue that has now been resolved, and we hope to finish this task in 2022.

Seedling blocks at Wolfskill: The 2020 seedling block (n=437) consists of crosses of Zarand, Tejon, and Randy pollen onto Golden Hills, Gumdrop, and several unnamed females at Wolfskill. Most crosses were performed at the Buttonwillow site with the cooperation of Craig Kallsen. A sex marker was used to cull males before planting and to plant the block in a repeating pattern of three females followed by one male in each row (3:1 ratio). These trees still show no signs of producing floral buds. Sticks from this block will be used for a chill requirement assay this winter. The 2021 seedling block (n=630) consists of crosses of Famoso, Randy, B15-43, B15-58, and B22-20 pollen onto Golden Hills, Lost Hills, and B15-69 (mother of Gumdrop). Crosses were performed at the Famoso site with the cooperation of Craig Kallsen. This site contains a great diversity of males, and some open-pollinated seed from each of the four females was also harvested and planted. Similarly to the 2020 seedling block, a sex marker was used to cull males and plant in a 4:1 predicted female:male ratio. Seed for 2022 planting consists of diverse crosses between young trees in our germplasm blocks and mature trees at Wolfskill.

UCB-1 subclone experiment: In vitro microshoots of ten UCB-1 subclones were obtained from three major nurseries. Subclones were verified to be genetically identical (or nearly so; identical across ~50K GBS markers) and verified to be free of *Rhodococcus* bacteria. Subclones were multiplied, rooted, and planted into a field experiment on UC Davis campus in fall 2020. The experiment contains four 2-tree reps of each subclone, interspersed with seedling UCB-1 for comparison. The clones are randomized and anonymized so that the nursery origin of each clone cannot be deduced. Several independent sets of observers agreed the three of the ten clones show abnormal phenotypes: two are “cracky”, and one is “bushy”. This experiment is open to visitation and/or sampling from interested researchers.

In vitro germplasm maintenance and propagation: We continue to maintain microshoot cultures of UCB-1 (multiple subclones of a commercial clone), Platinum, several UCB-1 selections identified in a salt screen, and additional diverse material from crosses performed by Malli Aradhya.

Development of embryogenic cultures: Immature kernels of *P. integerrima*, *P. vera*, and *P. atlantica* were collected throughout the season (n~150 total), extracted in sterile conditions and placed on embryogenesis media in darkness. Two *P. vera* kernels generated embryogenic tissue from kernels collected June 18th, and we have started germinating one of these. Other experiments are underway with various concentrations of cytokinins and auxins to test if adventitious shoot regeneration protocols can be established for UCB-1, or if embryogenic callus can be derived from tissues other than immature kernels.

Development of a chill requirement assay: Budsticks were collected from 119 mature trees at Wolfskill on four collection dates: December 14th, January 1st, January 22nd, and February 12th. On each collection date, 3 sticks were cut from each tree and brought back to the laboratory, where a fresh cut was made immediately before placing each stick upright in a plastic tub containing a solution of ~75ppm (1g/13L) NADCC to prevent microbial growth. Tubs were placed in a 25C growth chamber with a 16/8 hour light/dark cycle, and sticks were monitored at weekly intervals for 3 weeks for signs of terminal bud break. If sticks collected on a given date eventually break bud in the growth chamber, then we infer that their endodormancy requirement has been met. The speed with which sticks break bud in the growth chamber provides information on their ecodormancy requirement, or how many heat units are needed to break dormancy once the endodormancy requirement has been met. The proportions of terminal buds broken on each collection date were used to calculate an "area under the chill progress curve" similar to the AUDPC measurement for plant disease progression. Five pairs of genetically identical trees in different blocks (Aegina, Red Aleppo, Trabonella, Damghan, and Kaz95-16-02) were used to estimate the repeatability of various alternative measurements (considering budbreak on lateral buds; calculation of AUCPC using data from 2, 3, or 4 collection dates). Repeatability was highest using terminal bud data for all collection dates (AUCPC3). Correlations with population structure (4 subpopulations inferred from faststructure on GBS genotype data) were also highest for AUCPC3, with the Mediterranean subpopulation showing the lowest average chill requirement, the Central Asian subpopulation showing the highest, and the Iranian and USA/mixed groups showing intermediate values. We expect that the repeatability in younger, more even-aged trees in a single block will be much higher, and we plan to test this in 2022. Kerman shows an intermediate, but lower than average, chill requirement in our growth chamber assay. We did not test Golden Hills, Lost Hills, or Gumdrop in 2021, but will do so in 2022. While much germplasm has a lower chill requirement than Kerman, we anticipate that most genetic diversity in pistachio will be found in high-chill Central Asian varieties from the center of origin, suggesting that development of a marker to predict chill requirement in breeding crosses will be invaluable. Bark and wood for carbohydrate analysis were also sampled from a subset of 20 trees on 4 collection dates. These samples are currently dried and in storage awaiting analysis by the Zwieniecki lab.

Genetics of blanking and IKD: In a small pilot study, 95 filled nuts and 95 blanks were collected from a Golden Hills orchard in Woodland, CA in the first week of September. Incidence of internal kernel discoloration (IKD) in the filled nuts was 28% (27/95). Our two-fold goal is to see whether blanking and/or IKD are associated with the zygotic genotype of individual nuts.

Cold-tolerance screen: 234 two-month-old *P. vera* seedlings were moved to a 4C cooler with a long night/short day cycle for 3 weeks, then 196 seedlings were transferred to a -2.5C cold room for 96 hours, and 38 control plants were left at 4C. All trees were then moved back to a lath-house for a month before being rated as healthy, damaged, or dead. This cold treatment was not severe enough to significantly damage a higher percentage of plants than the control. Future cold screens should impose more severe cold and/or should do so with less prior acclimation.

Conclusion

We developed a chill requirement assay that is simple, inexpensive, scalable, and repeatable. The Mediterranean and Central Asian subpopulations of *P. vera* have the lowest and highest average chill requirements, respectively.

Dissection of Pistachio Fruit Development Towards Optimal Hull Split

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Introduction

The pistachio "nut" is the fruit of *Pistacia vera*, a tree that is native to Iran. Its relatively high tolerance for drought and salinity makes it an increasingly more popular crop in areas of USA where drought is a prominent threat. The pistachio fruit is botanically classified as a fleshy drupe, characterized by the presence of fleshy exocarp and mesocarp tissue layers. Together, these two tissue layers correspond to tissues commonly known as the "skin" and "hull" of the pistachio fruit (**Fig. 1A**). These two tissue layers surround a woody, lignified endocarp tissue layer that is commonly known as the pistachio "shell".

The development and maturation of the hull and shell are of significant interest to pistachio growers. High shell split rate is a trait that is preferred by the consumers. However, in contrast to the preferred split shell, an intact, unsplit hull is desirable since it is associated with decreased pest and pathogen infestation of the fruit. Earlier studies by Pinney & Polito (1999) hypothesized that the kernel (embryo) expansion, generates some of the force necessary to induce shell split. Since the shell is immediately interior to the hull, a larger kernel size may also be associated with higher hull split rate, and thus increased rate of pest / pathogen infestation at maturity. However, no experimental evidence exists, at the structural level, which compares cultivars with different shell split rate to test if there is a significant association of kernel size, shell and hull split rate and pest / pathogen infestation frequency. Additional traits that may affect shell and hull split rate, such as shell lignification and programmed cell death in the hull, also remain largely unstudied.

Results and Discussion

We examined, for the first time, the anatomical differences of shell, hull, and skin tissues between the relatively high shell-split rate cultivar Golden Hills (GH) and a lower shell-split rate cultivar Kerman.

Shell split analysis Our data indicate that: 1) shells lignify approximately at the same time in both cultivars, despite of the shorter growing season of GH. 2) Kerman have thicker shell, but there is no difference in the morphology of the shell sclerenchyma cells compared to GH. This suggests shell thickness rather than differences in cell shape properties contributes to the mechanical strength difference and shell split rate. 3) In addition, we noticed that kernels of GH may undergo a rapid thickening near the end of stage III of fruit development, resulting in a kernel that is rounder in cross section at the same degree days as Kerman. We are investigating whether the kernel diameter expansion (the "girth"), rather than the length, is associated with pistachio shell split.

Hull breakdown analysis We further characterized the anatomical changes of hull breakdown during the stage III of fruit development. Our data showed that: 1) at 133 days post anthesis (DPA), which is the time of GH harvest, GH fruits have a higher rate of skin split. However, when we compared the final harvest dates for these cultivars, 133 DPA for GH and 154 DPA for Kerman, Kerman showed a higher rate of skin split. 2) Hull-split was rarely observed in either cultivar at their final date of harvest. 3) In addition, we analyzed a third cultivar, Lost Hills, which showed a different pattern of hull maturation with minimal skin split but with high rate of hull split (**Fig. 1**).

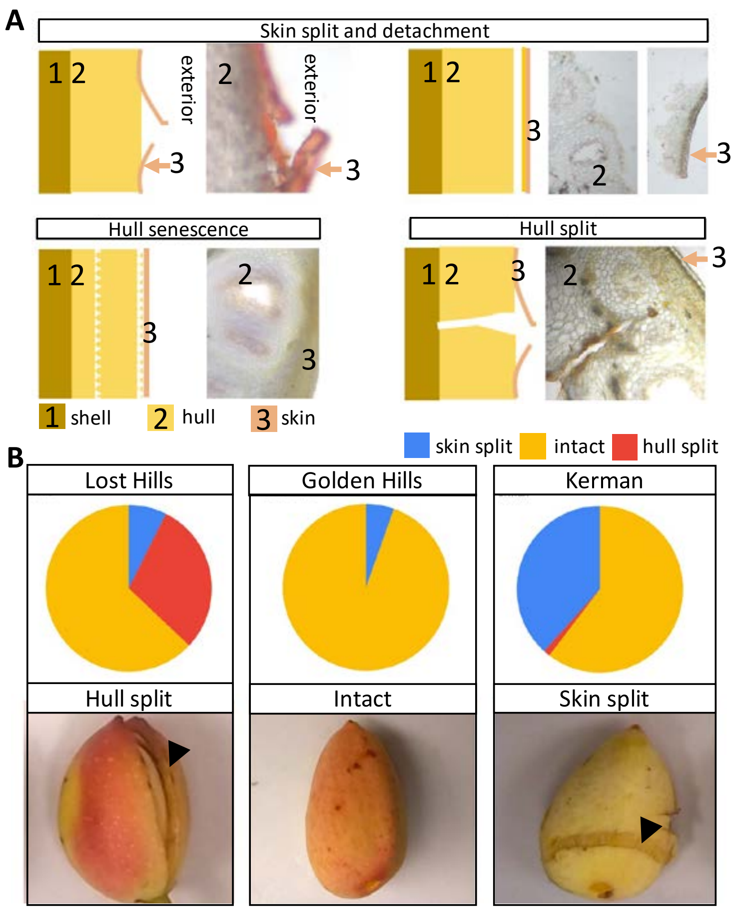

Figure 3. Events of pistachio hull breakdown as observed at final date of harvest in Lost Hills, Golden Hills, and Kerman. **A.** Hull tissue sections of the 3 common type of breakdown events. Hull senescence is indicated using Evan's blue stain (blue color indicates senescing tissue). **B.** The hull phenotypes of 3 cultivars at final date of harvest show traits characteristic to each cultivar. Site of split is indicated by black arrowhead.

Cell viability To further understand the process of hull breakdown, we assayed the viability of the hull tissues in GH and Kerman fruits. Increased hull senescence was observed in GH compared to Kerman. Interestingly, unlike observations in soft fruits such tomatoes, there appears to be no obvious change in cuticle thickness and integrity during the last stage of pistachio fruit ripening regardless of skin split. We are currently quantifying anatomical and cellular changes of the hull, with an emphasis on the difference of intact and split hulls during the final harvest days.

Due to the relatively low rate of Navel Orange Worm (NOW) infection rate this year, we were able to obtain limited samples of NOW-infected fruits. NOW infestation only occurred in fruits that remained on the tree long after the date of commercial harvest, or post stage III of fruit development. Interestingly, all the NOW infection occurred in fruits with intact skin but withered, or dissected hull tissue ("mummies"). Work is currently in progress to assay whether the collapse of hypodermal parenchyma cells and the enlargement of filler parenchyma cells in the hull tissue, which is observed to some degree in stage III hull senescence, is associated with NOW infestation. Additional work using fruit samples collected from the USDA pistachio germplasm is being performed to assess whether the traits for hull senescence is also associated with increased overall insect and pathogen infestation in different genotypes.

<u>Conclusion</u>

Shell split and hull breakdown has previously been implicated with the susceptibility of pistachio fruit to pest and pathogens. We have identified the cell types involved in each process and the stage of fruit development where key changes in cellular features occur. We compared the anatomical differences in shell hull and skin between different commercial cultivars and their association with hull and shell split. Finally, we have begun to dissect the anatomical traits that may be associated with insect and pathogen infestation.

Development of molecular markers and biotechnological approaches for pistachio to improve agricultural traits

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Collaborators: **Pat J. Brown**, Associate Professor & Nut Crops Breeder, Department of Plant Sciences, UC Davis; **John Preece**, Research Leader, USDA, National Clonal Germplasm Repository (NCGP); **Bob Beede**, UC Farm Advisor, Emeritus, Kings County.

Introduction

There is significant variation in tree size, which determines productivity, in commercial orchards planted with UCB-1 seedling rootstocks. It has been unclear to extent to which this is due to genetic differences or environmental variation. Nurseries have tried to tackle this problem by rogueing young seedlings before they are planted in orchards; however, our data has demonstrated that performance in the first year is a poor predictor of later tree size. Over the last six years, we have developed a comprehensive collection of genomic resources for *Pistacia* spp. We have sequenced and assembled the genomes of the three key *Pistacia* species: *P. atlantica*, *P. integerrima*, and *P. vera*, cvs. Kerman and Siirt (the last in collaboration with Salih Kafkas, Cukurova University, Turkey). In addition, we have collected a large amount of phenotypic and genetic data from both experimental and commercial orchards and now have DNA sequence-based marker data derived mainly from bark cambium for more than 3,000 trees. We developed a novel high-throughput, low-cost, genotyping and trait association approach that allows us to handle data from thousands of trees and dozens of traits simultaneously. Trunk caliper, tree height, and canopy volume were highly correlated indicating segregation of loci for general vigor. These data allowed us to identify two major loci that control vigor of UCB-1 rootstocks in commercial orchards and hence determine tree size. Therefore, the variation in tree size has a large genetic component. This project aims to provide molecular assays for these loci that will allow nurseries to select UCB-1 seedling rootstocks that will grow into more uniform, vigorous adult trees and productive orchards.

Pistachio has a juvenility period of at least five years and orchards take at least seven years to mature and become commercially productive. The long juvenility period plus the years necessary to evaluate yield phenotypes makes conventional pistachio breeding a lengthy process and progress in genetic improvement has been slow. Consequently, the genetic potential for improving pistachio production in the Western US is largely unexplored and untapped. We aim to develop and deploy biotechnological technologies to shorten the maturity time of pistachio to just a few years. Combined with marker assisted breeding, rapid-cycling genotypes would have a major impact on the speed of pistachio improvement.

Results and Discussion

Genotyping by sequencing data from experimental and commercial orchards and genome wide association studies (GWAS), combined with chromosome-scale, high quality, genome assemblies for the parental *Pistacia atlantica* and *P. integerrima* trees have provided two highly informative molecular markers for vigor. In 2021, based on the genomic sequence information, we developed an inexpensive, quick, and easy qPCR protocol for single nucleotide polymorphism (SNP) marker analysis. This TaqMan assay involves two fluorescent probes to detect specific genotypes. The strong predictive value of these assays was validated with previously collected samples from the experimental and commercial orchards as well as with samples provided by Duarte and Sierra Gold nurseries. We were also able to calculate the improved size distribution that extant orchards would have had if these markers had been used to rogue seedlings. Therefore, these molecular markers can be used by nurseries to rogue out trees with low vigor.

In 2021, 93% of trees flowered and we recorded fourth round of the sex, flowering time, and seed development for the trees that had reached sexual maturity. Early blooming was correlated with larger trees, regardless of sex. Data on maturity collected past years allowed us to identify a chromosome region determining shorter juvenility in UCB-1. It is unknown whether this locus affects juvenility in *P. vera* or whether segregation of this locus in rootstocks influences juvenility of the scion. We were able to design markers for this locus and we would like to analyze rootstocks in orchards that are just starting to flower.

Since March 2018, we have made drone flights over the experimental and commercial orchards to capture multispectral imagery in collaboration with Sean Hogan (UCANR), Robert Johnson (UCANR), and Alireza Pourreza (UC Davis). In 2021, we captured another round of multispectral drone imagery for nine of the sampled commercial orchards. We were able to process all the data and we are currently compiling it into one large GIS project. A segmentation model was built to delineate the crowns of each of the pistachio orchard trees and following this segmentation, zonal statistics for each of the orchard trees were combined with structural field measurements via pre-assigned unique IDs for each tree within the study sites. The drone derived structural metrics include measurements of height, crown area, and perimeter length of each tree crown. Additionally, min, mean, median and maximum NDVI values were calculated from the multispectral datasets for each tree crown. These data illustrate the potential benefits of timely and detailed UAV imagery to help agriculture managers estimate commercial yields more efficiently than conventional field measurements.

In a collaboration with Pat Brown and Grey Monroe, we initiated experiments aimed at transformation and ultimately genome editing of pistachio. Pat Brown's lab has established tissue culture of pistachio that we are going to use for transformation with *Agrobacterium*. We are currently testing multiple *Agrobacterium* strains for their ability to infect pistachio.

Conclusion

This project is providing the foundational resources needed for next-generation rootstock development. Together with collaborators, we have developed genetic and phenotypic tools to enable next-generation pistachio genetics. These tools and resources are being made available to the wider pistachio research community in order to accelerate the deployment of superior rootstocks.

Over the years, we have collected a large amount of phenotypic and genetic data from both experimental and ten commercial orchards and have now data for more than 3,000 trees. This year we continued to record sex, flowering time and seed development data in the experimental orchard in Davis. With collaborators, we also conducted a third year of multispectral aerial imaging surveys of the sampled orchards. These data will accelerate and enhance our phenotyping efforts. We used genotyping by sequencing data from experimental and commercial orchards and GWAS, combined with chromosome-scale, high quality, genome assemblies for the parental *P. atlantica* and *P. integerrima* trees to identify two highly informative molecular markers for vigor. We developed and validated the predictive value of Taqman assays using samples from the experimental and commercial orchards as well as samples provided by two nurseries. Our previous data show that seedling selection based one early growth parameters such as tree height is a poor predictor of tree size and vigor in older trees and therefore unlikely to be effective. These markers will enable the selection of young UCB-1 seedlings that will result in rootstocks with predictable sizes and the culling of inferior genotypes prior to planting in orchards. They also allow the selection of vigorous genotypes for clonal propagation.

We are transitioning the project to develop transformation and editing technologies to generate rapid-cycling pistachio in collaboration with Pat Brown and Grey Monroe.

Pistachio Pan-Genome for Accelerated Breeding

Authors: **J. Grey Monroe**, Assistant Professor, Department of Plant Sciences, UC Davis

Introduction

Crop losses due to stresses from emerging pests, new diseases, and warmer winter temperatures are reminders of the need to accelerate the development of pistachio varieties adapted for the needs of California growers. However, breeding with traditional methods in long-lived, long-generation species such as pistachio could be too slow in the face of emerging environmental stresses confronting California.

Fortunately, next generation breeding approaches (e.g. genomic selection), are now possible with the advent of genome sequencing and pan-genome analyses in crops. For example, pan-genomes (whole genome sequence data for several varieties) now exist for numerous crops such as maize, tomato, rice, soybean, wheat, canola, pepper, sunflower, and walnut - transforming breeding efforts in these species. Genome-enabled breeding approaches in pistachio will be made possible with the creation of the first pistachio reference genome and pan-genome. Genome resources in pistachio would provide broadly useful tools such as marker assisted selection, genomic prediction, and gene discovery for the California pistachio research and breeding communities. New breeding approaches will accelerate efforts to improve pistachio varieties adapted for California's unique challenges, with the aim of speeding up the arrival of varieties with long-term value to California's pistachio growers, ensuring continued and enhanced productivity.

The pistachio genome also contains all the genetic information influencing pistachio biology, agronomic traits, interactions with pests, and susceptibility to environmental stresses. Unlocking the genetic code of pistachio by sequencing their genomes will enable discoveries useful the large network of California pistachio researchers working to understand the biology responsible for productive, valuable, and resilient pistachio crops.

This project aims to create a pistachio reference genome and pan-genome, critical resources to aid genome-enabled breeding and current and future pistachio research. This work is supported by investment from the California Pistachio Research Board.

Results and Discussion

We used a new genome sequencing method, HiFi sequencing from Pacific Biosciences, to generate pistachio genomes. We created platinum quality genome sequence data for a reference genome in pistachio based on the widely grown Kerman genotype. DNA from Kerman leaves was extracted and sequence using HiFi method, yielding 1.5 million unique sequenced DNA molecules for a total of over 25 billion DNA base pairs of data. We then assembled these DNA molecules into "contiguous sequences" (contigs) – the building blocks of a reference genome useful for breeding and genomic discoveries. Our assembled reveal the Kerman genome to be 603 million base pairs in length. The assembled genome was also fully "phased" meaning heterozygosity can be accurately studied.

When we set out to create the Kerman reference genome, we defined a number of quantitative metrics to measure the success of our data generation approaches. Specifically, we aimed to generate a reference genome with a contig N50 size (common metric of sequence assembly quality) of 1 million DNA base pairs (Mb) in length. The reference genome that we created resulted in a contig N50 size of 30 million DNA base pairs in length, meaning our result was 30 times higher quality than our original goals.

In addition to sequencing the Kerman genome we also wanted to identify places in the genome that code for genes important for pistachio biology and traits. To do this we measured genes that were expressed by sequencing RNA (IsoSeq) in the buds, leaves, flowers, and fruits of Kerman growing in the field. These data yielded over 4 million RNA molecules, reflecting actively expressed genes in the Kerman genome for a total of over 9 billion base pairs of data used to discover more than 20,000 genes. With the addition of sequence-based machine learning gene prediction (*ab initio*) we identified a total gene count of approximately 30,000 genes in the Kerman genome. To ensure the quality of our gene discovery approach, we set out to achieve at least a 95% BUSCO value, a commonly used measure of the genome completeness. Our Kerman genome has a greater than 98% BUSCO value, confirming that the gene discovery pipeline was successful.

A major aim of our project is to facilitate the improvement of pistachio varieties that are adapted for challenges facing California growers, such as the threat of warming winters that threaten production in high-chill varieties. We therefore sequenced 5 additional genotypes of pistachio that originate from diverse climates including warmer climates where winters experience fewer chilling days. Low-chill genotypes adapted to these environments contain genes that allow them to thrive despite warming winters. By sequencing their genomes, we aim to discover genes that are useful for accelerated breeding for winter warming temperature adaptation. We repeated the above DNA and RNA work for these 5 additional genotypes, generating over 1 trillion DNA and RNA base pair data points. This data set underlies the first pan-genome compiled in pistachio. These genomes were also of exceptional quality – all had a contig N50 score greater than 25 million base pairs and a BUSCO score greater than 95%.

Result highlights:

- **Created the first Pistachio reference genome for Kerman.**
    - Genome quality: HiFi sequencing. Contig N50 > 30Mb; BUSCO score > 98%, Fully phased genomes (heterozygous genomic regions assembled independently).
    - Tissue specific (buds, leaves, flowers, fruits) IsoSeq expression complete + *ab initio* gene prediction identified ~30,000 genes.
- **Generated complete Pistachio pan-genome data.**
    - Created reference-quality genomes for 5 additional genotypes.
    - Genome qualities: HiFi sequencing. Contig N50 for all are > 25 Mb; BUSCO Scores for are > 95%. Fully phased genome assemblies.
    - Tissue-specific (buds, leaves, flowers, fruits) Iso-Seq expression profiling complete + *ab initio* gene prediction identified ~30,000 genes in each genome.

Conclusion

In summary, we created the first reference genome pistachio, from the Kerman variety. We also sequenced 5 additional genomes in pistachio, providing the foundation for the first pan-genome. This work will aid in transformative genomics breeding and discoveries for improved and accelerated solutions to challenges confronting California's pistachio research growers. To put this in perspective, the first human genome to be sequenced in 1999 required 15 years in cost $1 billion. This forever changed our understanding of human biology. With the use of new genome sequencing methods, we were successful in generating multiple genomes in pistachio, which will change our understanding of pistachio biology and aid in community efforts by breeders and researchers to improve pistachio in California.

These data are available for the pistachio research community to freely use. Future efforts will continue to extend their value, by discovering genes responsible for performance and resilience traits. We are also working with breeders to launch pistachiome.org which will be the home for pistachio genomic resources and tools and data for free use by the larger pistachio community.

Evaluation of Pistachio Rootstock-Breeding Selections, 2021-22

Authors: **Craig E. Kallsen**, Pistachio Farm advisor, UCCE Kern County and **Dan E. Parfitt**, Emeritus, Pomologist-AES, University of California, Davis. **Giulia Marino**, Orchard Specialist, UC Davis and **Florent Trouillas,** Plant Pathology Specialist, U.C. Davis.

Introduction

The U.C. scion-cultivar breeding program began with the original crosses made in 1989 by Dr. Dan Parfitt and U.C. Farm Advisor Joseph Maranto. Since 2009, the breeding program has included development of experimental rootstocks (please see a separate report on our scion breeding program).

As part of this breeding program, potential seedling rootstocks originating from breeding crosses made in 2009, 2011 and later, have either been planted in rootstock-selection trials (four of these) or in randomized and replicated evaluation trials in comparison with UCB1 seedling and other rootstocks (five of these). These nine trials are located in Kern County (one east of the Sierras) or at the Westside Research and Extension Center in Fresno County. Many of these trials are in orchards with high sodium, chloride and boron salts. These trials were budded to Kerman, Gumdrop, Golden Hills, Lost Hills or experimental scion selections beginning in the fall of 2011 to the summer of 2019. The objectives of these trials were the identification of breeding lines or individual rootstocks that produce higher early yields, have a reduced pruning requirement with a closer tree spacing, may confer greater cold and salt tolerance, a smoother graft-union and comparable Verticillium wilt and Phytophthora root and crown rot resistance to that possessed by existing commercial rootstocks. The first nut harvests of any trials occurred in two of these trials in 2017.

The rootstocks from the U.C. breeding program are novel in that the parentage is different from UCB-1 or pure *P. integerrima* rootstocks. Based on the parentage, the rootstocks are identified as either being Endeavor-1 or Endeavor-2 class rootstocks. Each of the experimental rootstocks in this trial originated from a seed and, generally, each was grafted to a commercial cultivar. While each experimental rootstock is genetically unique, they are somewhat similar based on the interspecific crosses in their parentage within each of the two classes of Endeavor-1 and Endeavor-2. Endeavor-1 rootstocks, again based on parentage, are further identified as being either PVI2 or PVI3.

Results and Discussion

Evaluation of the original seedling Endeavor-1 and 2 rootstock trials are largely complete. Since individual Endeavor-1 and Endeavor-2 seedling rootstocks demonstrate considerable variability in appearance and performance within each class, the next objective has been to produce clonal lines of selected individual seedling rootstocks. The selections have been based on the performance of individual rootstocks in our existing trials. These trials have been described in Full Reports to the California Pistachio Research Board in previous years. Clonally produced rootstocks will allow the project to begin large-scale test trials to evaluate in more detail the possible strengths and weaknesses of these novel rootstocks compared to existing commercial rootstocks.

Endeavor 1 rootstocks: Three Endeavor-1 rootstocks were selected for cloning, largely based on their apparent vigor, resistance to Verticillium wilt disease and cold tolerance. The cloning is being conducted by a commercial nursery under a U.C. Test Agreement. Sufficient clones for two large trials should be available for planting into trials in early 2023. In general, the following characteristics of Endeavor-1 rootstocks have been observed in existing trials compared to UCB-1 seedlings.

1. They demonstrate reduced concentrations of boron in the scion including the leaves, shoots, and nuts by about 50% compared to existing rootstocks. An article comparing boron uptake between

Endeavor-1 and Endeavor-2 rootstocks with UCB-1 seedling and Platinum rootstocks was accepted recently for publication in the Journal of HortScience entitled, "Leaflet boron concentration reduced with hybrid *Pistacia vera* rootstocks".

2. These rootstocks showed very little rootstock suckering, which would greatly reduce the tree-training requirement and sucker passes that need to be made by tree-training crews. They readily take a graft.
3. The growth in circumference of the rootstock was more similar to that of the scion than existing rootstocks.
4. Rootstock leaves appear to enter fall senescence and push in the spring more similarly to those of the grafted scions indicating more similar dormancy periods. This character may reduce the problem with Winter Juvenile Tree Dieback.
5. At the same location for similar aged trees, the Endeavor-1 rootstocks were ready for harvest a few days before cultivars on UCB-1 seedling rootstock.
6. The parentage of these rootstocks is different from current commercial rootstocks, which increases the diversity present in the pistachio industry's rootstocks.

Endeavor-2 rootstocks: Some selections of seedling Endeavor-2 rootstocks were made in 2021 based on their performance in seedling selection and advanced selection trials this past season and as described in previous Full Reports to the California Pistachio Research Board. Selection criteria included yield, dwarfing-attributes, vigor and health. UC Plant Pathologist Florent Trouillas has assisted the project by testing possible selections for relative degree of *Phytophthora* susceptibility. Endeavor-2 rootstocks are designed to be planted as high-tree density orchards to potentially increase early yields and reduce pruning, pest management and harvesting costs. Attempts to clone the selected seedlings will begin in 2022. In general, the following characteristics of seedling Endeavor-2 rootstocks have been observed in existing trials compared to UCB-1 seedlings.

1. These rootstocks have demonstrated high early nut yield (precociousness), short stature, moderate vigor for reduced pruning requirement and high early nut yield per unit of leaf-canopy volume making them suitable candidates for testing as dwarfing rootstocks for high-density plantings.
2. These rootstocks demonstrated reduced concentrations of boron in the scion leaves compared to existing rootstocks.
3. These rootstocks showed very little rootstock suckering and grafted well.
4. Rootstock leaves appeared to enter fall senescence and push in the spring more similarly to those of the grafted scions indicating more similar dormancy periods.
5. At the same location for similar aged trees, these rootstocks are ready for harvest a few days before cultivars on UCB-1 seedling rootstock.
6. The parentage of these rootstocks is different from current commercial rootstocks, which increases the diversity present in the pistachio industry's rootstocks.

Some of the yield and dwarfing characteristics of these rootstocks were presented this summer at the 2021 International Society for Horticulture Meeting sponsored by Washington State University and a manuscript submitted for possible publication in Acta Horticulturea. The title of the manuscript is "Conceptions of a Novel Crop Ideotype for Pistachio Production in the San Joaquin Valley (SJV) of California".

This project is long term. UC Orchard Specialist Giulia Marino has agreed to be part of this rootstock project to provide the necessary continuity of research that will be necessary as original researchers retire.

Evaluation of Pistachio Scion-Breeding Selections, 2021-22

Authors: **Craig E. Kallsen**, UCCE Pistachio Farm advisor, Kern County and **Dan E. Parfitt**, Emeritus, Pomologist-AES, University of California, Davis.

Introduction

The original U.C. breeding program began with parent crosses made in 1989 by Dr. Dan Parfitt and Farm Advisor Joseph Maranto. Since this time, the program continued with the breeding and evaluation of novel scions, and as of 2009, experimental rootstocks (please see separate report for the rootstock summary). Private cooperating growers who donated long-term use of land, labor, equipment and time made much of this research possible. The current focus of this program is evaluating the existing plant materials developed over the years located in diverse locations. New breeding and research is continuing with the hiring of a walnut and pistachio breeder, Dr. Patrick Brown, Plant Science Dept. at U.C. Davis.

Results and Discussion

In 2021, we continued evaluation of 12 male and female advanced scion-selection trials. These trials were planted from 2007 to 2019, although some of these trials have had, and will continue to have, novel plant material added to them from existing seedling selection trials in 2022. The Jasmine trial is our longest currently monitored female advanced selection trial. This trial was planted in 2010 in the "citrus belt" of Kern County where winter chilling if often borderline or inadequate. This trial has been noteworthy due to the performance of the advanced selection 'KB25-78'. Over the past six years, the clean, inshell split nut percentage of 'KB25-78' averaged a modest 72.4%, similar to that of Kerman at 68.1%. However, despite this relatively low split-nut percentage, cumulative marketable yield (also called payable or edible yield) was 5804 lbs. per acre or 32% higher than the next highest yielding cultivar Lost Hills (see Figure 1).

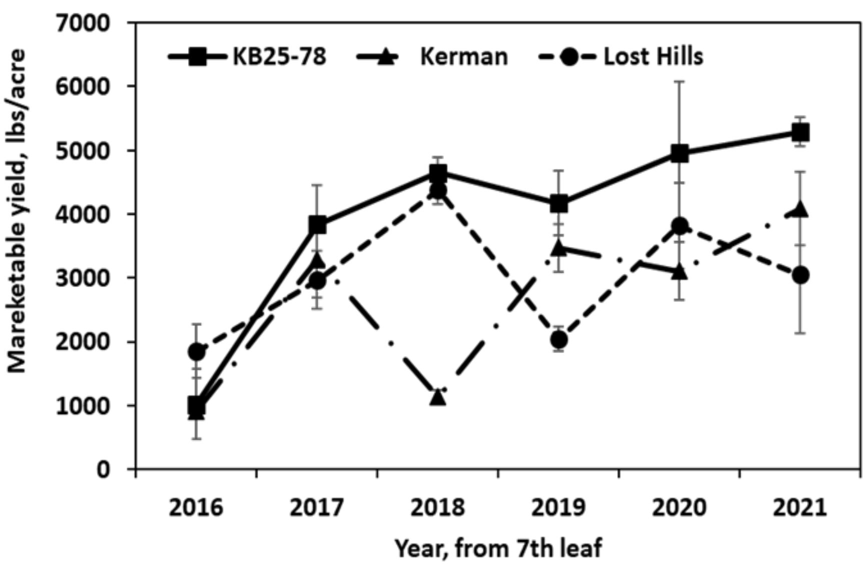

Figure 4. Differences among the pistachio varieties Kerman, Lost Hills and the selection KB25-78 in annual marketable yield in the Jasmine Trial from 2016-2020. Kerman was not harvested within the trial in 2020. However, yield data was obtained from the same aged Kerman trees in which this trial is located. Error bars represent ± 2 standard errors of the mean.

The lower split inshell percentage of 'KB25-78' compared to Lost Hills (84.1%) has been offset by its high production of harvestable material. A significant proportion of the harvested plant material is marketable yield, and if not open, inshell split nuts, is edible closed shell kernels at 11.1% . 'KB25-78' has two traits that may account for this enhanced yield performance. It takes full advantage of the available growing season by blooming a week before Golden Hills or Lost Hills, and, has harvested only four days earlier, compared to Lost Hill's ten days, than Kerman. The expansion of the canopy and trunk circumference was faster in this trial than the existing cultivars. In addition, it produces many branch spurs, especially early in its lifecycle, which provide locations for more flower buds. However, questions remain as to whether the apparent yield advantage will continue once full canopy cover is achieved and if the superior spur production will continue once these trees are fully mature.

In 2020, we began collecting harvest data from the Westside REC advanced selection trial, now at seventh leaf, which includes a range of early and late maturing advanced female selections and existing cultivars such as Golden Hills, Kerman, and Gumdrop. The advanced selections include ‘KB25-78’ and another selection with good yield and nut characteristics that in 2021 was ready for harvest in between Golden Hills and Gumdrop. The very precocious Gumdrop was the only trial entry to produce enough yield to harvest in 2020 (847 lbs. /acre), but ‘KB25-78’ performed well in 2021, and its comparatively large tree size bodes well for good production in 2022. A desirable characteristic of both Gumdrop and ‘KB25-78’ was that kernel discoloration was not found in random observations of nuts from either of these selections at our trials, but could be found in Lost Hills and Golden Hills. In general, nut quality has been higher in Gumdrop in 2020 and 2021 than it has been in other trials in the past and the trees appear to be performing well on Platinum rootstock in the West-of-Wasco Trial planted in 2014.

Three of our pollenizer advanced-selection trials were initiated in 2014 and 2015 in response to the poor response of Kerman and Peters to the very low winter chill in these years. Many of the experimental male selections in these trials originated in the seedling breeding trial planted at the Westside Research and Extension Center in 2008. The objective of these randomized and replicated advance-selection trials was to find a male pollinizer replacement for Peters, the historical pollenizer for Kerman. Peters is not precocious, with few flowers in 4- and 5 year-old trees, and tends to bloom much later than Kerman in years with low winter chilling. In the spring of 2021we completed our evaluation of the experimental male pollenizers, including pollen germination, in these trials. As a result, we have made a male selection that we plan to put forward to the U.C. Davis Cultivar Release Committee as a possible new male cultivar for Kerman. This selection, in combination with the previously released Famoso cultivar, will cover the bloom period of Kerman in both low chill and high chill years. Plant breeding is a slow process, especially with pistachio, and when these trials began, we had not foreseen the degree with which new plantings of Golden Hills and Lost Hills would replace Kerman. However, Kerman is still being planted, and if this pollenizer were released to the industry, we would recommend that it and Famoso be grafted into new and existing Kerman plantings in the San Joaquin Valley.

In 2018, we identified several selections from the seedling breeding trial, planted in 2012, that not only displayed harvest maturity equal to or in advance of Golden Hills, but also demonstrated a nut hull that appeared to be less susceptible to early tatter. Based on the past and current work of other researchers, a hull less prone to tatter may make them less susceptible to navel orangeworm damage. These selections were grafted into commercial and experimental rootstocks in replicated trials with Kerman and Golden Hills in 2019.

We continued to evaluate other seedling progeny selections from our breeding program that have displayed greater tolerance to an inadequate winter rest period by demonstrating fewer leaf-canopy and flowering symptoms of inadequate chilling or that flower very early in the spring or have very novel parental genetics that could reduce their chilling requirement. We continue to introduce selections into our very low-chill trial in the Coachella Valley of California established in 2017. The seedling trees on their own roots, and grafted trees, both experimental and existing scion and rootstock cultivars, are demonstrating a diversity of vegetative growth under low-chill conditions. This trial will become more interesting once bloom begins.

Pistachio hull breakdown and its relation to insect damage (Year 3)

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Introduction

Navel orange worm (NOW) causes significant crop losses in California pistachios. NOW infestation is mainly influenced by insect density, environmental conditions, and host susceptibility. The physiological and biochemical processes of pistachios that result in hull breakdown and higher susceptibility to insect damage have been understudied. Further, little is known about how environmental differences in growing conditions can affect pistachio ripening and NOW susceptibility. Hull ripening occurs once the pistachio nut has reached full size and maturity (kernel growth is completed). The hull ripening process includes tissue softening and changes in color from green-yellow to yellow-pink. Following ripening, senescence occurs, leading to hull breakdown and sometimes hull tattering. Deteriorating hulls are more susceptible to fungal decay, favoring the entry of insects like the NOW to the kernel. The primary goal of this project was to assess the timing of hull ripening and deterioration in different varieties in response to chill and heat accumulation. Using this data, we can identify biomarkers for harvest time and help anticipate hull deterioration and its coordination with insect flight patterns.

Results and Discussion

Nut sampling began July 22, starting with kernel growth, and ended Sep. 22, after hull ripening or breakdown occurred. We selected two commercial orchards in Fresno County managed by Maricopa Orchards LLC with similar geographical locations and management practices: one Golden Hills cultivar, and one Kerman cultivar, both grafted on UCBI rootstock. The Kerman orchard terrain features an elevation gradient that produces a significant difference in winter chill accumulation and therefore bloom date. In this orchard, we chose two locations differing in bloom date. Thus, we established two experimental comparisons of hull ripening: 1) Golden Hills vs. Kerman and 2) normal bloom vs. late bloom in Kerman. For each sampling, eight branches with three or more clusters collected from each of the comparisons. NOW insect inoculations were done on Aug. 6, Sep. 3, and Oct. 1 by our collaborators Drs. Wilson and Burks in the same Golden Hills orchard. Egg oviposition was evaluated one week after inoculation, while larva infestation was assessed two weeks after inoculation.

We first compared nut development of Golden Hills and Kerman. Consistent with previous reports, the Golden Hills orchard bloomed one week earlier and was harvested three weeks before Kerman. We quantified hull color using the L*a*b* color scale and found that Golden Hills nuts became redder earlier in the season and at a quicker rate than the Kerman nuts (**Fig. 1A, C**). Hull texture was measured with a Texture Analyzer and a perforating probe. Both varieties began to soften starting Aug. 6, but Golden Hills softened faster than Kerman (**Fig. 1B)**. Golden Hills kernels also were consistently significantly larger than Kerman and grew faster, reaching their full size three weeks before Kerman (**Fig. 1D**). Similarly, the shell split occurred sooner and at a faster rate in Golden Hills than in normally blooming Kerman (**Fig. 1E**). Our previous research demonstrated that Kerman nuts produced a peak in volatile compounds at the initiation of hull ripening. We are currently testing if this peak of volatiles is also present in Golden Hills. We continued assessing Golden Hills nuts for hull degradation and observed continuous changes in color and texture after harvest (**Fig 1A, B, C**). We are also measuring the volatiles and phenolics at these sampling dates for Golden Hills to determine if any later peaks correspond with NOW insect flights.

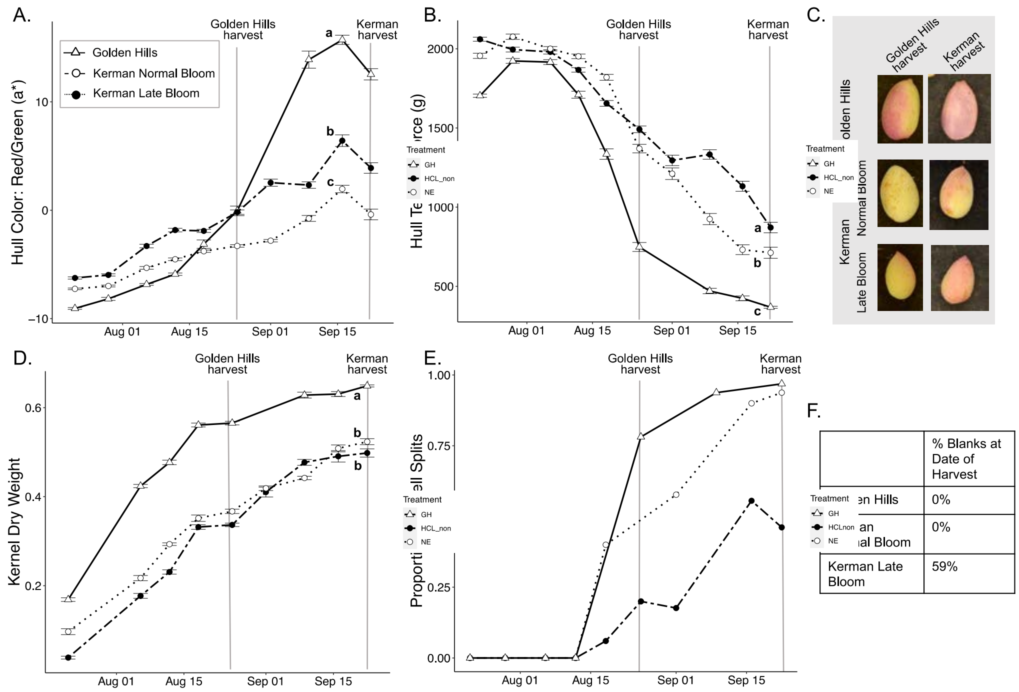

Fig 1. *Comparison of pistachio hull quality and other harvest indices between Golden Hills, normal bloom Kerman, and late bloom Kerman*. Average and standard error of color (**A.**), texture (**B.**) and kernel dry weight (**D.**). Letters represent significant difference by ANOVA and Tukey test. Harvest dates of each cultivar are indicated by dashed lines. **C.** Pistachios at harvest for each variety and bloom time. **E.** Proportion of nuts with shell splits through time, **F.** Percentage of blanks sampled at the date of harvest.

To assess how bloom date can affect hull ripening and breakdown, we compared two Kerman locations within the same orchard that displayed significantly different bloom times. We confirmed that the Kerman trees that bloomed late (about one week later) were exposed to more heat year-round, leading to lower chilling hours and higher spring and summer temperatures. The normal-blooming Kerman trees experienced average temperatures and bloomed in coordination with other Kerman orchards in the area. The late-blooming Kerman trees produced nuts with significant changes in hull ripening and harvest indices. Although the hull color followed a very similar pattern for both locations, the nuts from late-blooming trees were consistently and significantly redder (**Fig. 1A, 1C**). In addition to this color change, the hull texture of these nuts was significantly firmer, indicating the ripening process was altered and could lead to poor nut release during harvest. There was a high proportion of blank nuts from late-blooming trees (Fig. 1F). There was no significant difference in kernel size between filled nuts and the normal- or late-blooming trees. However, the filled nuts from late-blooming trees split later and in lower proportions than those from normal-blooming trees. We are also measuring volatiles in these samples and expect that the late-blooming nuts also have delays and/or decreases in the volatile peak that coincides with hull ripening.

<u>Conclusion</u>

We are still analyzing the physiological and biochemical data and finding correlations with insect flight patterns. So far, we have found that because Golden Hills hulls ripen earlier and breakdown quickly compared to Kerman, Golden Hills should be harvested without delay to avoid further damage from coinciding insect flights. We also gained better information on how warmer winters can negatively impact pistachio nut development. Trees produced many blank nuts and had delayed hull ripening and shell split. The information generated in this project will provide the California pistachio industry with the knowledge to develop tailored orchard practices based on cultivars used and in response to changes in bloom time and cultivation temperatures.

Metabolomics analysis of Pistachio bud and bark samples during the dormant period under oil and Dormex spray

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Introduction

The purpose of the investigation is to utilize a nuclear magnetic resonance (NMR) spectroscopy metabolomics approach to investigate metabolic changes that occur in the bud and bark tissue on pistachio rootstock treated with rest-breaking agents (RBAs). Most pistachio cultivars require low-temperature chilling requirements during winter months to efficiently break dormancy when temperatures rise in spring. When crops do not receive adequate chilling portions, bud development is erratic, and fruit yield is reduced. In the past several decades, California's winter months have been warmer, leading to inefficient bud breaking inducing pistachio yield reduction. RBAs break bud dormancy in crops that do not receive adequate chilling potions during the winter months. Growers utilize RBAs to induce bud breaking, but the physiological mechanism of action and optimal timing of application is unknown. Metabolomics identifies and quantifies all metabolites present in biological tissues or fluids. This allows researchers to identify metabolites that are prominent or lacking in treated tissues compared to a control; thus, altered metabolic pathways can be identified. This study aims to identify the physiological changes induced by RBAs utilizing metabolomics and identify the optimal treatment timing.

Bud and bark samples from Cantua and Madera sites with bud swell treatments and the respective controls collected in 2019 were assessed. Each consisted of three intervals of IAP440 oil treatment, with the Cantua site having one extra Dormex treatment. Bud and bark samples were stored at -80 °C until analysis. Samples were ground finely using liquid nitrogen with a mortar and pestle. 250 mg of ground tissue was transferred into 5 mL Eppendorf tubes. 3 mL of methanol was aliquoted into each tube, and samples were sonicated for 15 minutes. Sonicated samples were centrifuged at 18 G for 30 minutes. The supernatant was transferred into clean 5 ml Eppendorf tubes with two 1 mm holes drilled on the cap for a means of evaporation during lyophilization. The methanol was removed via evaporation, and any remaining solvent was removed via lyophilization. 650 uL of D_2O stock solution with 0.2mM imidazole, .05 mM sodium trimethylsilyl [2,2,3,3-d4] propionate (TSP), and 90 mM KH_2PO_4 with the pH adjusted to 6.8 was added to each tube, and samples were vortexed for 20 seconds. Samples were centrifuged at 18 G for 30 minutes, and the supernatant was transferred into clean, pre-labeled 5 mm NMR tubes.

Samples were assessed with a 600 MHz JEOL NMR Spectrometer at 25 °C at 512 scans each. The data of each was processed using MestReNova utilizing baseline correction, phase correction, and normalization with regard to the TSP peak. Data were further processed using ChenoMX NMR Suite 8.4, which included calibrating the pH of each sample utilizing the imidazole peaks and calibration of the TSP peak with regard to the targeted TSP concentration. Metabolites were identified and quantified by referring to metabolites identified in the literature and the ChenoMX and Human Metabolome Database spectral libraries available in the program. Partial Least Squares-Discriminant Analysis (PLS-DA) was performed on the metabolite data generated by ChenoMX utilizing MetaboAnalyst 5.0, and data were normalized via log transformation and Pareto scaling.

Results and discussion

28 metabolites were identified from each tissue at both sites consisting of 4 sugars, 11 amino acids, six organic acids, and seven other metabolites. **Figure**, **1a and 1b depict PLS-DA plots comparing Cantua and Madera sites and each site's bark and bud controls**, respectively.

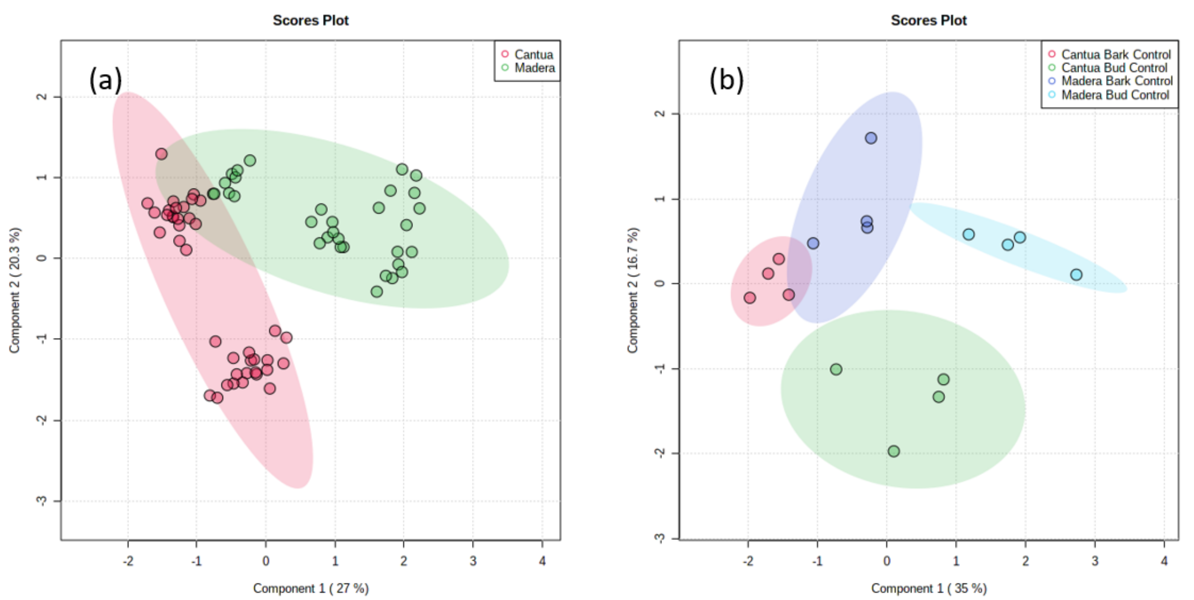

Figure 1: Classification based on NMR metabolite data: PLS-DA plot comparing (a) Cantua and Medera Sites, (b) Bark and bud control treatments for each site

Based on the data collected, NMR spectroscopy can be utilized as a tool for discriminating between common pistachio cultivars at different geographic locations and tissues within the plant. Some notable metabolites that have distinct compositions between the two geographic sites include alanine, arginine, fumarate, tyrosine, and uridine. In addition, several metabolites were identified that could be used to differentiate bud and bark tissue, including alanine, asparagine, aspartate, creatine, formate, fructose, fumarate, leucine, malate, myo-inositol, shikimic acid, sucrose, threonine, trans-4-hydroxy-proline, tyrosine, uridine, and valine. There was a substantial overlap of clustering when assessing the PLS-DA plots of the different spray treatments (data not shown), suggesting that the samples that were assessed for all treatments have similar metabolite compositions. Considering that the samples assessed have all broke dormancy, they are all in a similar physiological state, possibly explaining the similar metabolic profiles throughout.

Conclusion

The samples from the 2019 season and the samples from the 2020 and 2021 seasons will be assessed in 2022. Metabolite differences between the calendar years within sites can be determined with the data collected. Further, assessing the temporal samples will determine if the spray treatments induce a change in metabolites.

Evaluation of pistachio rootstocks for high root carbon storage, water use efficiency and salinity tolerance

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Collaborators: Patrick J Brown[1], Judy Jernstedt[1], Louise Ferguson[1], Thomas Wilkop[2]
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Introduction

Pistachio is a woody perennial nut crop known for its relatively high salt and drought tolerance. It is a genus with considerable genetic heterogeneity, and there are considerable interspecific and intraspecific variations in its abiotic stress tolerance in salinity and drought. Of the two abiotic stresses that it is tolerant of, salt tolerance is a complex trait which includes the response of many tissues to the stress caused by salt ion toxicity and osmotic stress, in which salt accumulation lowers the soil water potential. Since drought tolerance is primarily a response to osmotic stress, there may be an overlap between the traits that are associated with salt and drought tolerance. In addition, drought tolerance is associated with multiple traits at both the physiological and the anatomical level, such as changes in deposition of the apoplastic barrier or the diameter of the vessel elements in different species. The relative importance of these various traits in salt and drought tolerance in pistachio is unknown.

Pistachio is grown as a grafted crop. Since plant roots are the main organ responsible for water absorption and the organ is directly in contact with salinized soil, the root plays a significant role in both drought and salt tolerance. The mechanism of salinity and drought tolerance in pistachio rootstock is therefore of considerable interest to pistachio breeders. We previously showed that increased suberization at the two apoplastic barriers, the endodermis and the exodermis, is associated with the high salt-tolerance rootstock, UCB1 (Zhang et al., 2021). Increase in suberin deposition has been associated with drought response in other species, and it remains to be explored whether a similar response occurs in pistachio rootstock and how it contributes to drought tolerance.

Results and Discussion

Our recent studies showed that the vacuolar sodium sequestration and suberin deposition in the root is a prominent response of UCB1 in salinity stress (Zhang et al., 2021). Following these studies, we investigated salinity and drought tolerance in several commercially available clonally propagated rootstocks. Our study included the commercial rootstocks UCB1 and Platinum (PGII) along with several other clonally propagated *Pistacia atlantica x Pistacia integerrima* lines available in nurseries in an extensive phenotypic analysis.

Salinity stress response We used a correlation matrix to calculate the significance of each phenotypic trait in contributing to improved salinity tolerance, such as shoot trunk diameter and leaf loss under salinity treatment. Our data identified selected rootstocks with desirable QTL alleles that tend to have more live root cortical cells after salt treatment and maintain thicker trunk. In addition, we used both biochemical and microscopy-based approaches and found that suberin deposition in roots is increased after salinity stress in these commercially available genotypes. Work is currently in progress to assess the sodium content in relation with the other quantitative and qualitative physiological values.

Drought stress. We subjected the genotypes that we previously identified in our salinity experiment as genotypes of interest to a water-withholding, short-term drought treatment in a greenhouse setting. We examined the drought stressed plants for differences in water potential, leaf wilting, root cell health, and overall plant size. Our phenotypic analyses showed that wilting more commonly observed in the lines abbreviated as D110 and V215, while specific genotypes such as V211 maintained a robust root growth under drought (**Figure 1**).

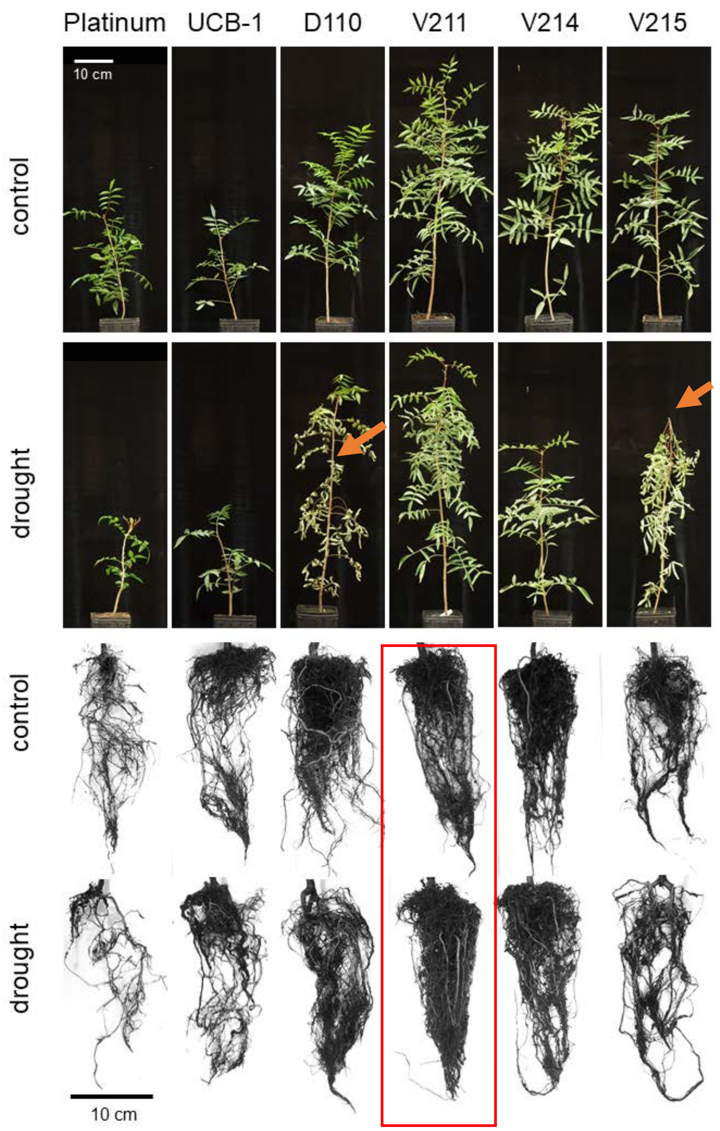

Figure 5. Pistachio rootstock clonal lines after short term drought treatment. Clonal lines show difference tolerance to drought stress with line v211 shows the highest total water content after treatment. Quantification is in progress to assess the difference in root mass and root system architecture. Wilting phenotype is indicated with arrows. Phenotype with robust root growth after drought is indicated with red box.

In addition, we harvested root tips and are currently analyzing the samples for suberin and lignin deposition in apoplastic barriers using our previously established microscopy-based approach. These results will be supplemented with biochemical analysis of suberin that is currently in progress. Preliminary microscopy based analysis, showed that drought stress leads to an increase in suberin deposition in endodermis and exodermis and that drought may induce a stronger response in exodermis.

Conclusion

Soil salinization and drought often go hand-in-hand under field conditions, and both types of stress are known to cause a complex series of responses in both the root and shoot. Although osmotic stress is a key feature to both salt and drought stress, our data suggests that there may be a difference under drought and salinity in the cellular signals that control suberin deposition in root apoplastic barriers. In order to account for the complexity of the traits and the different responses in roots and shoots, we started to dissect the association between the traits using correlation and hierarchical analyses that allow us to better perform QTL identification. We are currently quantifying the changes in the different traits of drought response.

Together, our study can form a framework for analysis of complex traits in salinity and drought tolerance and assist breeding efforts on rootstock selection.

Exploratory research for a better understanding of the biochemical and physiological responses of Pistachio buds to winter chill

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Introduction

California is the largest producer of pistachios in the U.S., providing over 98% of the nation's total production (https://quickstats.nass.usda.gov/). Buds of pistachios enter into dormancy/rest in the Fall and are released from their dormancy state ("bud break") in the Spring, following exposure to a certain number of low-temperature hours ("winter chill"). However, the ongoing changes in weather patterns have led to fluctuations in winter temperatures in recent years. Insufficient winter chill causes poor or non-uniform bud break, which in turn results in reduced pistachio yields (note that yields can also be affected by additional environmental factors). To mitigate the delay in bud break, rest-breaking enhancing chemicals, such as horticultural oil, have been applied to pistachios to overcome bud endodormancy/rest, though with varying degrees of success thus far (sometimes ineffective or counterproductive if applied at the wrong time). Much remains to be learned about the physiological and biochemical responses of endodormant pistachio buds to winter chill, and the mechanistic basis of bud break induction by rest-breaking enhancing chemicals when winter chill is inadequate. Such knowledge is critical for developing molecular and metabolite markers to facilitate successful selection and application of rest-breaking enhancing chemicals.

The overall goal of our research is to develop simple molecular and/or metabolite diagnostic assays that can be used to facilitate the selection and application of rest-breaking enhancing chemicals in pistachios. For this one-year exploratory project, we determined the transcriptomic (global gene expression) and metabolic responses of pistachio buds experienced various periods of winter chill to investigate the processes surrounding bud endodormancy release, a critical phase of bud development that impacts downstream flowering and fruit production.

Results and Discussion

Identification of genes with expression levels strongly correlated with winter chill portions and bud endodormancy break

Buds of the high-yielding commercial pistachio cultivar "Kerman" were collected in the 2018-2019 growing season at 45 Chill Portion (CP), 50 CP, 55 CP, 60 CP, 65 CP, and 70 CP from triplicated blocks of five trees for a total of 18 plots. The triplicated blocks were located at three orchards: Couture, Rose, and Scroggs. We extracted total RNA from these bud samples and conducted transcriptome analysis using the Illumina Next Generation Sequencing platform. High quality transcriptome data were obtained (error rate was less than 0.03% and Q20 was 97% or higher) and mapped to the pistachio genome (total mapping rate was 80% or higher). Representative results of comparative gene expression analysis for buds collected at the same CP at different orchard locations (A) or buds collected at different CPs at the same orchard location (B) are shown in Figure 1.

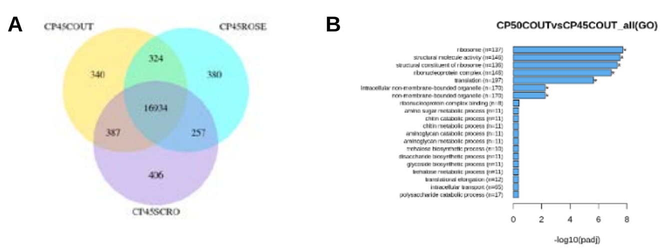

Figure 1. Representative results from the transcriptome analysis. **A.** Coexpression Venn Diagram of genes expressed at CP45 at the three orchard locations. **B.** Gene ontology enrichment diagram of genes expressed at CP50 relative to CP45 for buds collected at the orchard Couture.

We then evaluated gene expression changes in pistachio buds subjected to various periods of winter chill to identify processes that are potentially involved in bud endodormancy break. We were excited to identify genes that showed significantly changed transcript levels during bud endodormancy break and were correlated with winter chill portions. The transcriptome results were not only validated by real-time quantitative PCR analysis (Figure 2), but also supported by analysis of metabolites both upstream and downstream of the biochemical pathway.

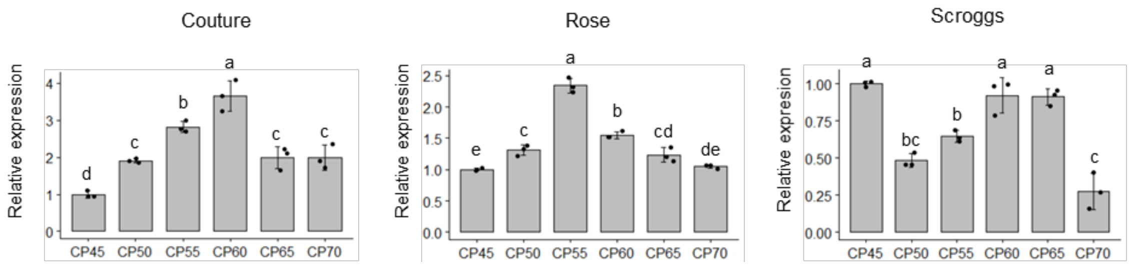

Figure 2. Representative results of a gene that showed significantly changed expression in pistachio buds experienced different chill portions as determined by real-time quantitative PCR analysis.

<u>Characterization of phenolic metabolite profiles of endodormant buds experienced various chill portions</u>
Of the secondary metabolites produced in woody perennials, phenylpropanoids (aka. phenolics) have been associated with bud dormancy in several plant species, such as black currant and apple. A recent study in apricot showed that concentrations of phenolic metabolites in buds correlated closely with bud dormancy progression and transition from endodormancy to ecodormancy. To determine the association of phenolic metabolites in winter chill and bud endodormancy release, we extracted soluble phenolics from an aliquot of the bud samples (the other aliquot was subjected to transcriptomic analysis) and separated them on a reverse-phase HPLC. The representative results are shown in Figure 3. Variations in phenolic accumulation were detected at certain CPs for buds collected at each orchard location. However, unlike the gene expression analysis and its associated metabolite analysis results, there was not a clear trend of phenolic metabolite changes associated with CP variations and bud endodormancy break.

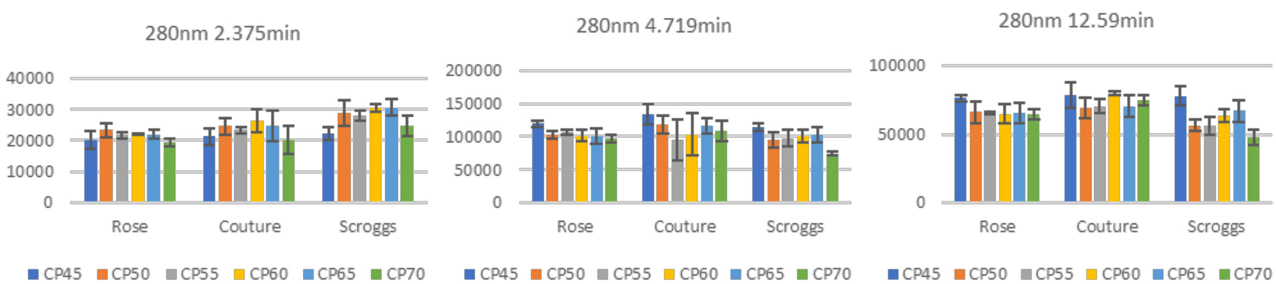

Figure 3. Representative HPLC analysis results showing quantification of phenolic metabolite peaks eluted at different retention time.

<u>Conclusion</u>

We have completed all the tasks planned for this year. Our transcriptome analysis established a baseline understanding of the physiological and biochemical responses of pistachio buds to winter chill. More importantly, we identified genes showing transcript changes that are strongly correlated with winter chill portions and bud endodormancy break, which suggests their role in bud dormancy development in pistachios. These genes will be used to develop gene expression markers for selection and application of rest-breaking enhancing chemicals next year.

Development of Physiology Based Methods for Sustainable Management of Pistachios under Changing Central Valley Climatic Conditions

Authors: **Maciej Zwieniecki (Professor) and Paula Guzman Delgado (Postdoc)**, Department of Plant Sciences, UC Davis

Introduction

The overarching goal of this research is to characterize the physiological responses of pistachio trees to abiotic stresses with the aim of using this knowledge to improve production and guide pistachio tree improvement.

The research efforts described here focus on the development of new approaches to measure trees' physiological status that complement current methods (analysis of water and nutrient status of trees). Specifically, we aim to understand tree non-structural carbohydrate (NSC – sugars and starch) management in the context of dormancy, chilling requirements, and yield performance. We have taken a state-wide approach to rapidly and thoroughly study NSC seasonal dynamics specific to pistachios. Our goal is to determine the best carbohydrate management practices in relation to climate, tree age and rootstock/scion combination, and geographic distribution. Specifically, we study the pre-senescence accumulation of NSC, dormant NSC dynamics, and the role of NSC in orchards to determine practices that promote orchard performance. Our large-scale observations are supplemented by physiological experiments that aim at resolving specific questions arising from the Carbohydrate Observatory data. This year we have:

- Expanded the data set despite significant delays related to COVID-19.
- Published bloom prediction model for pistachio: Sperling. O., Zwieniecki, M.A. 2021.
- Determined interactions between monthly level of NSC and yield and determined that December content of NSC in twigs has the highest relative importance in affecting yield.
- Experimentally tested the energy cost (carbon cost) bloom
- Developed collaboration with growers to provide knowledge on NSC content in individual experiments (test of reflective chemicals on NSC contents in twigs)

Results and Discussion

Near real-time results of NSC contents are presented via the Carbohydrate Observatory data website: http://zlab-carb-observatory.herokuapp.com/. Our website is freely accessible and allows participating growers to review and compare NSC content in their orchards with the overall values observed throughout the entire Central Valley. Carbohydrate content in the form of soluble sugars and starch can be analyzed across varying aspects, ranging from large spatial dimensions encompassing geographical parameters (county) down to individual tree components (rootstock, scion, age). We are currently completing the fifth full year of data collection. Patterns and dynamics of the NSC content were published in: Davidson, A.M., Le, S.T., Cooper, K.B, Lange, L., **Zwieniecki, M.A.** 2021.

Thermal conditions during the fall/winter/spring are major determinants of pistachio phenology. Our proposed mechanism (spring sugar starvation) detailed in a recent publication (Sperling et.al. 2019) was tested and parametrized for pistachio (Sperling. O., **Zwieniecki, M.A.** 2021). The beta version of the model is currently available at http://zlab-chill-heat-model.herokuapp.com/, the model is presented with comparative analysis of classical approaches (chill hours, chill portion and dynamic model). Interestingly, high levels of sugar content in the fall tend to result in early bloom while lower levels postpone bloom or predict bloom abandonment altogether.

Analysis of over 100 orchard yields and their NSC levels in the preceding year show a statistically significant positive dependence of yield on high levels of NSC in the fall/winter months, no relation in May-June and a significant negative relation during the July-September period. On average, an increase of 1 mg in total NSC content in twigs (i.e. considering sugars and starch in both wood and bark) resulted in an increase of orchard yield by 30 pounds per acre, but if the content was predominantly in the form of soluble sugars in wood this resulted in an increase of 75 pounds per acre per mg of sugar. A negative correlation between NSC and yield in late summer suggests the potential for tree NSC exhaustion and explain alternative bearing. December content of NSC was determined to be the most important predictor of orchard yield potential.

The positive impact of fall NSC concentration on yield and the role of fall soluble sugar concentration on bloom time underlines the need to better understand the energy cost of bloom. Our findings determined that the spring amount of NSC in twigs is inadequate to sustain the initial phase of bud growth, especially in male trees, and bloom requires the transport of NSC from limbs and trunk. We further determined that winter and spring respiration constitutes only 10% of the cost while the majority of the cost (90%) is associated with the structure. The observed discrepancy suggests that NSC redistribution and supply to growing buds is required for a synchronous bloom (Amico Roxas, A., Orozco, J., Guzmán-Delgado, P., **Zwieniecki, M.A.** 2021)

<u>Conclusion</u>

- There is a positive strong correlation between yield and twigs' NSC content in the winter (Dec, Jan, Feb). For each increase of 1 mg of total NSC in twigs there is a 30 pound increase in yield per acre. Orchards with 200 mg of NSC in December vs. 100 mg will produce ~900 pounds more. This, together with the negative correlation between NSC and yield in late summer, constitutes a strong incentive to develop techniques that allow trees to accumulate high levels of NSC prior to entering dormancy.
- The analysis of only December NSC might be sufficient to evaluate the orchards' crop potential, allowing for use of NSC analysis to be an efficient part of the management 'toolbox' in assisting growers.
- Effect of winter chill and spring heat on bloom timing and synchrony is impacted by the level of soluble sugars in the fall. Knowledge of both chill accumulation and initial levels of sugar might guide the decision making in respect to the application of dormancy-breaking treatments.
- Grower, CE, FA performing small- or large-scale experiments, or trying to compare different fields etc. are encouraged to include NSC analysis in their enquiries. If the project scope is relatively small such analysis is free, but results will be shared within pistachio community as it is being paid by the pistachio research board.
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Published works with support from Pistachio Research Board in 2021 (available online or upon request from Zwieniecki):

1. Amico Roxas, A., Orozco, J., Guzmán-Delgado, P., **Zwieniecki, M.A.** 2021. Spring phenology is affected by fall non-structural carbohydrates concentration and winter sugar redistribution in three Mediterranean nut tree species. *Tree Physiology* 41(8):1425-1438 DOI: 10.1093/treephys/tpab014
2. Sperling. O., **Zwieniecki, M.A.** 2021. Winding up the bloom clock—do sugar levels at senescence determine how trees respond to winter temperature? Tree Physiology https://doi.org/10.1093/treephys/tpab051
3. Davidson, A.M., Le, S.T., Cooper, K.B, Lange, L., **Zwieniecki, M.A.** 2021. No time to rest – seasonal dynamics of non-structural carbohydrates in twigs of three Mediterranean tree species suggest year-round activity. Scientific Reports **11**, 5181 https://doi.org/10.1038/s41598-021-83935-1

Efficacy of fungicides against Alternaria late blight, 2021

Authors: **Boris X. Camiletti**, Postdoctoral Scholar, Department of Plant Pathology, UC Davis and UC Kearney Agricultural Research and Extension Center (KARE); **Juan A. Paredes,** Graduate Student, UC Davis, and KARE; **Victor M. Gabri,** Staff Research Associate, UC Davis and KARE; **Themis J. Michailides**, Principal Investigator, Department of Plant Pathology, UC Davis & KARE.

Introduction

The principal measure to reduce Alternaria late blight (ALB) of pistachio is the application of multiple sprays per season. Thus, field trials were performed to evaluate the efficacy of several commercial fungicide products and the best timing for sprays. The fungicide screening was performed in a 10-year-old Kerman experimental plot located at Agricultural Research and Extension Center, in Parlier, CA. Treatments consisted of three applications approximately four weeks apart (3 Jun, 2 Jul – a critical time for spray – and 5 Aug). All fungicides were applied at the maximum rate as indicated on the label. Each treatment consisted of five-single trees replications. Sprays were applied with a handgun sprayer at 400 gallons per acre, except for Rhyme which was applied through the drip. Disease symptoms develop only very late in the season and for this reason evaluation was performed on 15 Oct. The severity was evaluated using the whole tree evaluation method or efficacy score, where 1 = the least control, 5 = the best control, and 2, 3, and 4, are the intermediate levels of increasing disease control. A separate field trial was also performed to determine if the Disease Severity Model can be used to time fungicide applications and, subsequently, improve the performance of the current calendar-based fungicide program. The effect of an early spray was also explored. The field trial was conducted in a 31-year-old Kerman commercial orchard located in Tulare County that has historically had conducive conditions for ALB. The selected treatments were the current calendar-based program, early spray + current calendar-based program, and early spray + DSV. The calendar-based program consisted of Merivon, Switch, and Luna Experience sprayed on 8 Jun, 29 June, and 10 July, respectively. The early spray consisted of Flint Extra sprayed on 14 May. For the DSV model, fungicides were also sprayed when 7-day DSV exceeded 10 units (Table 1). The DSV model determined 4 sprays: Merivon on 8 June, Switch on 1 July, Luna Experience on 14 July and Switch again on 8 August. Immediately before harvest (3 Sep), defoliation was recorded as the number of fallen leaves inside a $1m^2$ frame randomly placed at the east and west sides of the trees. The difference vegetation index (DVI) was monitored using Google engine at the end of the season as a quantitative indicator of disease control. Higher DVI values indicate higher biomass and, subsequently, higher disease control. The Kruskal-Wallis one-way nonparametric test was performed on these data, and means were compared at P =0.05. The statistical software R studio was used to analyze the data.

Results and Discussion

The fungicide treatments using Mibelya (mefentrifuconazole-FRAC#3 + fluxapyroxad-FRAC#7) and Luna Experience (tebuconazole-FRAC#3 + fluopyram-FRAC#7) in combination with Serenade Opti (microbial-FRAC#BM02) showed the best efficacy scores (Fig. 1). Seven fungicides products showed a similar performance to the above-mentioned treatments according to the statistical analysis. The remaining fungicides had a mean score below 3.5 and were the least effective in controlling the disease. It should be noted that the drip application of Rhyme showed no efficacy against ALB, as observed in previous years. An early spray seems not to improve the efficacy of the calendar-based program, based on the results obtained during the first year of experiments (Fig. 2). Conversely, sprays based on the DSV model resulted in a significant reduction in defoliation with respect to the calendar programs. Although differences were not statistically significant, the DVI showed higher values for the program involving the DSV model.

Conclusion

Fungicide products formulated with active ingredients that belong to FRAC groups #3 and #7 resulted in higher control of ALB. The use of the DSV model to time sprays showed potential to improve the efficacy of the current fungicide programs. The DVI index seems to be correlated with defoliation and could be used as an indicator of disease control.

Table 1*. Disease severity values (DSV) as a function of leaf wetness period and average ambient air temperature during the wetness period.

	Leaf-wetting time (hours) required to produce daily disease severity values ** of:				
Mean air temp ºC	0	1	2	3	4
13-17	0-6	7-15	16-20	21+	
18-20	0-3	4-8	9-15	16-22	23+
21-25	0-2	3-5	6-12	13-20	21+
26-29	0-3	4-8	9-15	16-22	23+

* Source: Madden, L., Pennypacker, S. P., and McNab, A. A. 1978. FAST, a forecast system for *Alternaria solani* on tomato. Phytopathology 68:1354-1358.

**The scale of S-values ranges from 0 (environmental conditions unfavorable for *Alternaria solani* spore formation) to 4 (highly favorable conditions).

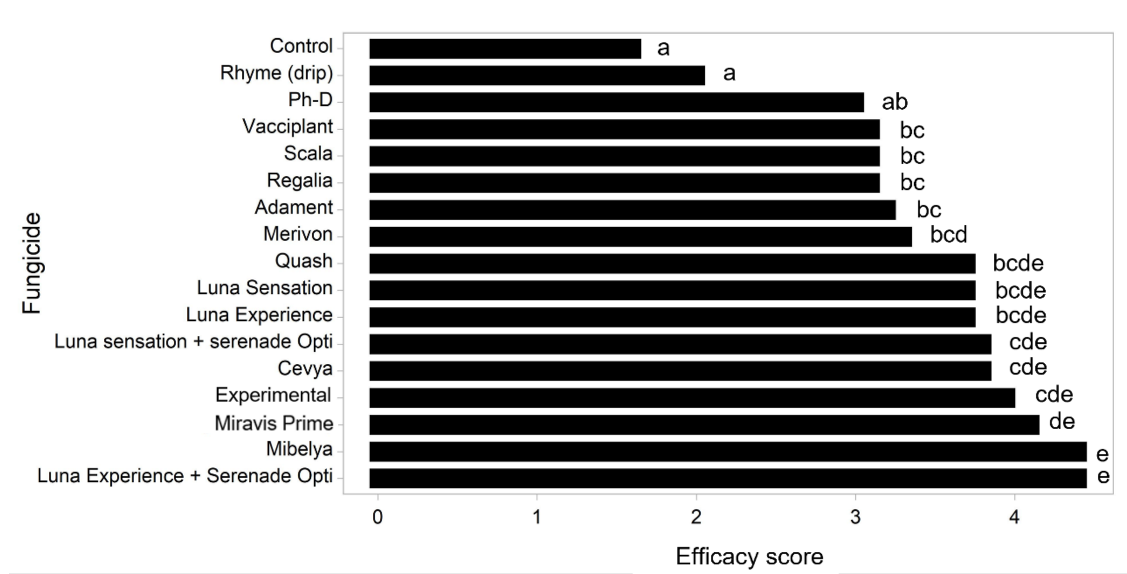

Fig. 1. Efficacy of fungicides against Alternaria late blight in an experimental pistachio orchard in Parlier, CA. (The higher the efficacy score the better control.)

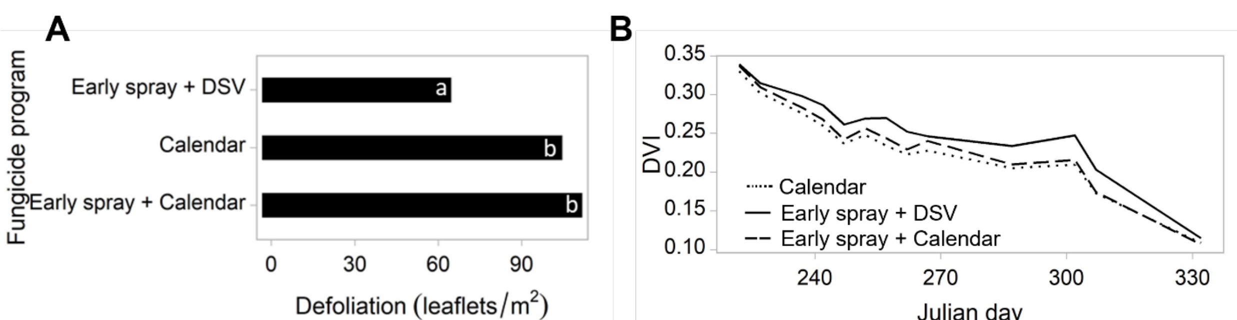

Fig. 2. (A) Effect of fungicide programs on defoliation caused by Alternaria late blight. Different letters indicate significant differences. (B) Effect of fungicide programs on the difference vegetation index (DVI). Higher DVI values indicate higher biomass and, subsequently, higher disease control.

Evaluating Pistachio Rootstock Tolerance to Soil-Borne Diseases

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Introduction

Our laboratory recently identified Phytophthora root and crown rot as an emerging new threat to pistachio in California, particularly in areas where soil conditions or cultural practices promote prolonged soil wetness. *Macrophomina phaseolina* also was found occasionally in association with crown rots in UCBI rootstocks. In the last three years, we have conducted experiments to determine the relative tolerance of UCBI, PGI and Platinum commercial rootstocks to these new soil-borne pathogens. This work aims to identify tolerant/resistant rootstocks that can be used to sustainably manage soil-borne diseases of pistachio. Experiments conducted in 2018, 2019, and 2020 using mycelium stem inoculations and soil/root inoculations show Platinum as the most tolerant rootstock towards Phytophthora crown rot. This year, we evaluated the susceptibility/tolerance of UCBI seedlings and Platinum rootstocks against Phytophthora, Verticillium and Macrophomina diseases, while emphasizing on soil and root inoculations of the various pathogens. In 2021, several experiments were conducted at the Kearney Agricultural Research and Extension Center (KARE) on 5-month-old to 1-year-old potted trees in a greenhouse, and 2-year-old potted trees on a gravel bed. **Greenhouse Experiments**. For *Phytophthora* inoculations, the soil of potted pistachio rootstocks was inoculated using either colonized rice seed inoculum or zoospores of isolates of *Phytophthora niederhauserii*, *Phytophthora* taxon walnut, and/or *Phytophthora cinnamomi*. For *Macrophomina* inoculations, a microsclerotia suspension at a concentration of 10 MS/g of soil was used. For *Verticillium* inoculations, a conidia suspension ($1x10^7$) was used for root dip inoculations. **Gravel Bed Experiments**. For *Phytophthora* inoculations, soil of potted pistachio rootstocks was inoculated using rice seed inoculum colonized by all three *Phytophthora* spp. For both *Macrophomina* and *Verticillium* inoculations, a soil microsclerotia suspension at a concentration of 10 MS/g of soil was used. Mycelium plug inoculations were conducted on Platinum, and UCBI rootstocks, before acquiring PG1 rootstocks.

Results and Discussion

Phytophthora Inoculations. Following three experiments using soil/root inoculation of *Phytophthora* species, differences in susceptibility (as expressed by % loss of root mass and % crown rot incidence) were found among UCBI, PGI, and Platinum rootstocks. Overall, Platinum was the most tolerant rootstock toward phytophthora root rot as shown by the lower averaged loss of root mass in all three Phytophthora trials (Fig 1A-B-C). Platinum was also the only rootstock to not develop crown rot lesions in zoospore inoculations after 6-month incubation (Fig 1D). PGI rootstock had shown a greater loss of root mass compared to UCBI rootstocks (Fig 1C). However, fewer PGI seedlings developed crown rot lesions (67%), compared to 100% of the UCBI seedlings developing crown rot lesions (Fig 1D). Phytophthora inoculations of the three potted rootstocks on the gravel bed did not develop any symptoms after 8 months. These rootstocks were replanted and inoculated in the field at KARE in October 2021, and will be re-inoculated with *Phytophthora* spp., in the spring of 2022 and plants will be evaluate monthly for symptom development. **Macrophomina Inoculations**. Mycelium plug inoculations on clonal Platinum and UCBI potted trees after 10 months resulted in UCBI rootstocks developing longer lesions (45.42 mm) compared to Platinum inoculated plants (30.92 mm). Microsclerotia inoculations of the three rootstocks under gravel bed and greenhouse conditions were inoculated March 4th, 2021 and June 24th, 2021, respectively. They are still incubating until the development of symptoms (wilting). **Verticillium Inoculations**. Root dip inoculations for 30 minutes of 5-month-old seedlings in a verticillium conidia suspension ($1x10^7$) resulted in disease development in seedlings after 3 weeks (Fig 2). Disease severity was measured on a scale from 0 to 4. Zero was a healthy plant, 1-mild wilt, leaf reddening, 2-moderate wilt, leaf necrosis, or defoliation, 3- severe wilt, leaf necrosis, or defoliation, and 4-complete wilt, death, or defoliation. After 6 to 7 weeks, we saw the peak of disease severity in all seedlings (Fig 2). Overall, Platinum showed relative disease severity similar to that of verticillium resistant PGI rootstocks and lower disease severity than UCBI and the verticillium susceptible seedlings of *P. terebinthus x P. atlantica* (Fig 2).

Conclusion

2021 results indicate differences in the tolerance of UCBI, Platinum, and PGI rootstocks toward soil-borne pathogens: Phytophthora, Macrophomina, and Verticillium. **Phytophthora**. Overall, Platinum appeared as the most tolerant rootstock against Phytophthora root and crown rot in all the greenhouse soil/root inoculations. This work supports Platinum's superiority and recommend planting in areas at high risk for Phytophthora disease. **Macrophomina**. Mycelium plug inoculations resulted in lower lesions in Platinum when compared to clonal UCBI rootstocks, indicating Platinum may have a greater tolerance. Soil/root inoculations of potted plants in greenhouses and on gravel beds with Macrophomina have yet to develop symptoms on either rootstock after 6 to 9 months post inoculations. Overall, Macrophomina infection in pistachios seems more of a secondary pathogen to a primary stress and does not pose as big a problem to pistachios as *Verticillium* or *Phytophthora* infections. **Verticillium**. Platinum appears to be tolerant toward verticillium infection based on conidia root dip experiments. Our results, showed its tolerance to be similar to PG1 resistant rootstocks and lower than the, *P. terebinthus x P. atlantica,* susceptible rootstock. However repeated experiments of root/soil inoculations will need to be conducted to confirm and attain conclusive data. Research continues in our laboratory to provide the California pistachio industry with knowledge of the tolerance of commercial rootstocks to soil-borne pathogens and to identify most tolerant rootstocks as a sustainable management strategy against soil-borne diseases.

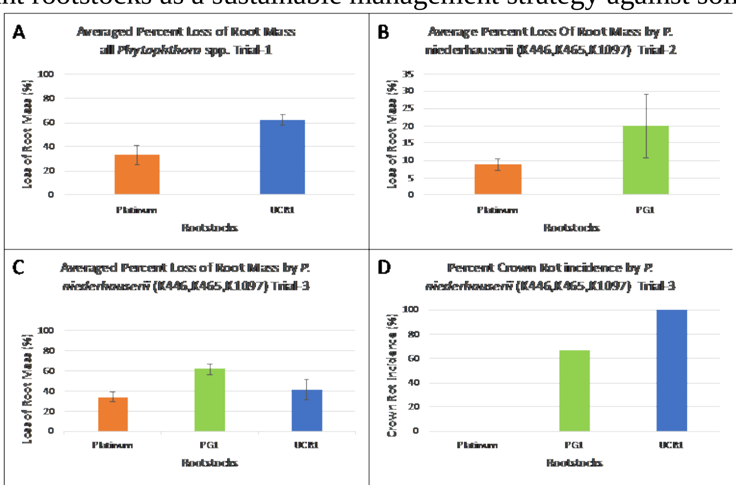

Fig. 1. Summary results of the effect of Phytophthora inoculations after 6 months in a greenhouse. (A.) Zoospore inoculations with all *Phytophthora* spp. (B.) Rice grain inoculations with *P. niederhauserii* (K446, K465, K1097). (C,D.) Zoospore inoculations with *P. niederhauserii* (K446, K465, K1097).

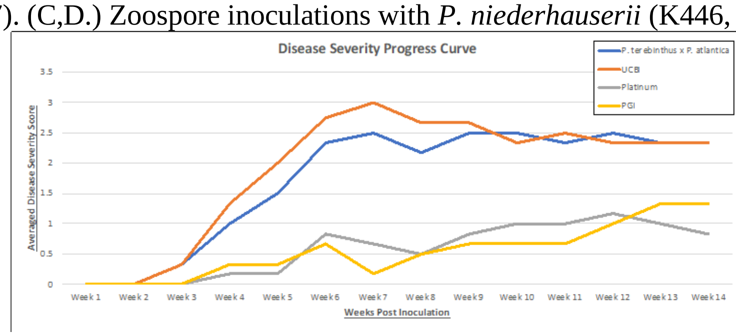

Fig. 2. Verticillium inoculations of small seedlings using root dip method in conidia suspension (1x10^{7}) after 14 weeks.

Is there a risk of plant-parasitic nematodes in pistachio on current and future rootstocks?

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Introduction

Pistachio is a high value nut crop of expanding acreage in California. Traditionally the female cultivar 'Kerman' and the pollinating male 'Peters' were grafted onto a common rootstock. New female cultivars become available, e.g., 'Golden Hills' but the genetic width of rootstocks is somewhat limited. At the beginning of the California pistachio industry, scions were grafted onto rootstocks of *Pistacia atlantica* and *P. terebinthus.* While apparently resistant to *Meloidogyne* spp. and *Pratylenchus vulnus* these rootstocks were highly susceptible to Verticillum wilt that occurred widely in California (Michailides and Teviotdale, 2014; Crane and Maranto 1988; McKenry and Kretsch, 1984). Different genotypes of a controlled cross of *P. atlantica* x *P. integerrima* all called 'UCB1' clonal rootstock are used to mitigate increasing challenges with Verticillium.

Nematode problems in pistachio are considered minimal perhaps because in a California survey, only low population densities of plant-parasitic nematodes were found (McKenry and Kretsch, 1984). Susceptibility to *Meloidogyne* spp. (root-knot nematode, RKN) is generally reported as low (Westerdahl, 2015). *Xiphinema index* was found to infect *Pistacia vera* and *P. mutica* (Weiner and Raski, 1966), and recently was found associated with weak pistachio trees, compared to more vigorous trees (McKenry, unpublished). The susceptibility to dagger nematodes needs clarification.

In preliminary screens of UCB1 clones, large differences between defined clones of this cross were identified (McKenry, unpublished). In recent work, interaction of *Pratylenchus vulnus* with *Mesocriconema xenoplax* (ring nematode) on pistachio illustrated the susceptible host status of one clone of UCB1 under greenhouse conditions (Westphal et al., 2016). With the expansion of pistachio, these orchards frequently follow a crop of cotton, grapes or another nut crop, all crops that likely leave behind populations of plant-parasitic nematodes.

In this project the relative host suitability to *Pratylenchus vulnus*, *Meloidogyne incognita,* and *Mesocriconema xenoplax* of currently available pistachio rootstocks, including multiple clones of UCB1 is determined. In addition to the UCB1 genotypes, there were *P. atlantica*, Pioneer Gold I, and genotypes provided by Dr. Mallikarjuna Kuma Aradhya (USDA-ARS Davis) are being tested along with *Prunus* or *Juglans* rootstocks with known susceptibility. Newly developed rootstock candidates from the program of P.J. Brown are also included in the field testing.

Results and Discussion

<u>The field screen for susceptibility to RLN and RKN planted in 2017</u> included nine clonal UCB1, one seed derived UCB1, two *Pistacia atlantica*, one *P. terebinthus*, one *P. integerrima* and one hybrid of *P. atlantica* and *P. integerrima* (15 genotypes total). Started in 2017, these plants are annually examined for susceptibility for RLN and RKN by collected root samples for nematode extraction. Every dormant season, trees are pruned back to optimize space of the experimental planting. Root population densities of root lesion nematode were overall low in the roots of UCB1 trees. There were non-significant trends, and numerically higher population densities were observed for multiple years in particular lines of UCB1.. Two of the three genotypes with *P. atlantica* in the parentage had similarly low numbers as Krymsk 1. In 2021, experimental rootstocks (>200) were planted and inoculated. After one growing season, plant heights varied from 9 to 88 inches under nematode infestation illustrating vigor differences.

Experiments for determining the susceptibility to *Mesocriconema xenoplax* were planted in single-plant plots in tanks with sandy soil in 2017 and 2018 and were repeatedly examined. Low infection rates were observed in soils in these plots. In the final evaluation in 2021 of the 2017 planting, pistachio rootstocks had grown differently vigorous. Among the different pistachio genotypes, there were trunk diameter differences of the medians of three-fold, top weight median differences of ten-fold, and root weight median differences of almost 19-fold in these sandy soils with ring nematode infestations. Ring nematode population densities appeared to decline from year to year for undetermined reasons, and nematode numbers were low overall.

Microplot experiments for damage potential of RLN on pistachio rootstocks. In one trial planted in 2018, the most susceptible, and the most resistant UCB1 genotypes were exposed to increasing infestation levels of RLN either in sand or sandy loam. Plant growth responses to nematode numbers were variable. In both soils, the pre-classified “resistant” cultivar grew more vigorously. When the same plant lines were cultivated in greenhouse pots, increasing infestation levels with RLN on the resistant cultivar reproduced relative to the infestation levels whereas all infestation levels resulted in similar final population densities on the susceptible rootstock. The entire trial is currently being repeated in a new series of microplots with different RLN infestation levels and the same pistachio rootstocks, which will be budded to ‘Kerman’ after one year of growth. This experiment will be continued during the following years. In this trial in sandy loam soil, the susceptible rootstock genotype trended with reduced growth with increasing levels of nematode population densities. Planned infestation levels are reflected in a gradient towards higher population densities in the pre-planned plots. It will be possible to draw inferences from these replicated trials, on what population density of the *Pratylenchus vulnus* can be tolerated at planting with limited risk for plant damage

Microplot experiments with dagger nematodes. In 2021, one pistachio orchard was identified where *Xiphinema index* was associated with weakly growing pistachio trees compared to other trees in the same orchard. Soil from the root zones of these weak trees contained *X. index.* Such soil was collected and transported to KARE. Soils were either left untreated or autoclaved before they were added to the microplot soil. Half of each soil category was treated with an experimental nematicide, the other one left untreated. One series of plots was left non-amended. All plots were planted to pistachio ‘Golden Hills’ grafted on UCB1 was cultivated for one growing season. None of the trends were significant, but trees grown in plots amended with autoclaved soil grew thicker during the first growing season.

Conclusion

So far, the role of plant-parasitic nematodes in pistachio rootstocks overall seems minor. Differences in response to RLN based on greenhouse and field experimentation were detected between the different UCB1 genotypes. It is not clear if this translates into plant damage in commercial orchards. Threshold level microplot experiments are set to further illustrate the differences among UCB1 clones. The examination of novel rootstock genotypes has just started. New experimental genotypes illustrated large differences in growth under nematode-infested conditions, and nematode evaluations will follow. It appears crucial to safeguard the development of novel rootstock genetics so the currently favorable nematode status of pistachio in not encumbered.