Executive Summaries
2020

California Pistachio Research Board
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2020 Manager's Report

Bob Klein

"It certainly was an interesting year" or a similar sentiment is probably the most common comment in every annual report written about 2020. I don't know of anyone who "mourns" the passing of 2020 even as we look at an uncertain 2021.

At the beginning of 2020, the pistachio industry was looking at the 2020 crop as on on-year with a potential yield of over 1.2 billion pounds. As 2020 progressed, there was inadequate chill in many pistachio producing areas and bloom was erratic in these locations. The crop potential was downgraded to about 900-950 million pounds but as the season went on, the crop looked a bit better. When harvest began in late August, initial yields were better than expected and the total California crop reached 1.042 billion pounds. This is the first billion pound crop for California pistachios. However, with 371,000 bearing acres, the per acre yield was only slightly over 2800 pounds which is a far cry from the 3500-3800 pounds per acre we expect in an on-year. A similar situation occurred with the record 2007 crop followed by the crops of 2008 and 2009. The 2010 crop was a record breaker. If a similar event happens in 2021, we could be looking at a 1.5 billion pound crop. If it doesn't happen, we will need to determine why our on-year crops don't meet the "standards" of past on-year crops.

The COVID-19 pandemic and the resulting lockdowns played havoc with our programs. Most universities including UC Davis, UC Riverside, and UC Berkeley as well as all their off-campus locations sharply curtailed their activities as well as faculty and researcher access to laboratories and facilities. Many if not most are still limiting access and personnel. Several projects have consequently been delayed/postponed. There were COVID outbreaks at various food processing/manufacturing facilities, particularly meat and poultry facilities. Fortunately, outbreaks at pistachio plants were limited and none occurred during the harvest period.

Prior to the beginning of the pandemic, the European Union began discussing potential maximum limits on a mycotoxin that has been found in pistachios, Ochratoxin A (OTA). OTA is produced by Aspergillus fungi but not the same species as those that produce aflatoxin. Just at the time we were scheduled to have meetings with the EU regulators, international travel was shut down and our opportunities to engage with the EU were gone. Most of the EU offices are located in Brussels, Belgium and the COVID illness and death rates in Belgium have exceeded those of the US. It has been impossible to meet with the EU regulators, either virtually or in person, and the only information we get has been indirect and second or third hand. The EU has continued to advance their OTA regulations while limiting dissent and the US pistachio industry is facing a potentially severely trade disruptive regulation. We will be conducting a number of OTA projects in the coming year to gain a better understanding of the source of the problem and potential approaches to mitigation.

Twenty years ago, the pistachio rootstock industry was relatively easy to track with only a few producers who were able to tell us at least to which counties and how many rootstock were shipped in any given year. All the rootstock came from seed but when in vitro propagation began and plantlets were shipped to multiple middleman suppliers, we lost the ability to accurately track new plantings. As a result, we had less trust that our statistics were accurately capturing new planting numbers and extending them into the bearing acres. Consequently, the Administrative Committee for Pistachios funded a new acreage survey that has accounted for the new acres as well as the bearing acres. There was a high level of agreement between the new data and our estimates of bearing acres through 2019 but this would not have been the case going forward. The new statistics pages will reflect the significant changes in the estimates of nonbearing acres. The total acreage, bearing and non-bearing, has reached 485,000 acres and will almost certainly exceed 500,000 acres by the end of 2021.

There is a lot of speculation about how the pandemic will change how people work, where they live, etc. but it won't change the basic aspects of farming. There will likely be some changes in the processing plants and how we conduct meetings now that we have been accustomed to meeting online. Regardless, I am looking forward to seeing you all again face-to-face so we can have those conversations that don't happen in virtual meetings.

Best wishes for a productive and prosperous 2021.

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California Pistachio Research Board Event Facilitation

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 shutdowns, in 2020 FNRIC continued and expanded these activities, aiding with planning and promotion of the Pistachio Virtual Short Course, developing promotional materials for the now re-scheduled 2021 ISHS Pistachio and Almond Meeting, assisting with abstract review for the ISHS Meeting, and generally supporting ANR PSU. We are also fielding questions surrounding the re-scheduling of the 2021 ISHS Pistachio and Almond Meeting, particularly from international participants.

The review process for the 2021 CPRB proposals has yet to occur, but we are prepared to once again facilitate this process at the end of December.

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 even in this unusual year we have been able to provide a valuable service to the California Pistachio Research Board and the UC ANR Pistachio Workgroup.

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

At this time, the site is not yet ready for a beta release. We are at the stage of collecting and uploading data for individual workgroup members, upcoming events, ongoing research, historical workgroup information, etc. We anticipate a beta launch for Spring 2021.

Navel Orangeworm Management Survey

Authors: Phoebe Gordon, Orchard Systems Advisor, UCCE, Madera County; **Houston Wilson**, Asst. Coop. Extension Specialist, Department of Entomology, UC Riverside.

Introduction

Navel orangeworm (NOW) (*Amyelois transitella*) is the most significant insect pest of almonds and pistachios and can be a pest of walnuts. This insect can damage nuts directly, and NOW damage has been positively correlated with aflatoxin contamination of nuts. Researchers from the University of California (UC) and United States Department of Agriculture (USDA) have developed multiple tools for managing NOW, however anecdotal reports and observations suggests that adoption of these practices has been unequal. UC's mission is to promote integrated pest management (IPM), thus, understanding industry-wide adoption of the various IPM 'tools' as well as barriers to adoption is of critical importance in order to develop more targeted research, education and/or policy incentives to reduce environmental impacts of pest management associated with tree nut production and decrease NOW damage to nut crops. In this project we sought to verify this anecdotal evidence through a grower survey that was administered at seven UC Cooperative Extension (UCCE) meetings for almond, pistachio and walnut management professionals.

Results and Discussion

The COVID-19 pandemic prevented us from administering the survey at two Southern San Joaquin Valley meetings (pistachio in Madera County, almond in Kern County). Because this survey was intended to understand practices and decision making processes of those directly involved in NOW management, respondents who did not self-identify as PCAs, orchard owners, or ranch managers were excluded, resulting in approximately 480 survey participants. Demographic breakdowns can be found in Table 1, and average responses to monitoring and management questions can be found in Table 2.

Table 1. Asterisks denote "select all that apply" questions. Numbers in parentheses indicate the percent of respondents who selected a response.

Industry role?	**# acres managed**	**Time spent managing NOW?**	**Crops managed***
Orchard Owner (50%)	< 50 (20%)	1 to 5 years (28%)	Almonds (75%)
Ranch Manager (20%)	51 – 500 (33%)	6 to 15 years (32%)	Pistachios (27%)
PCA (30%)	501 – 2000 (18%)	16+ (40%)	Walnuts (61%)
	2000+ (29%)		

Table 2: Responses to monitoring and management questions, averaged across all included respondents.

Response options	Do you evaluate mummy nuts?	Do you use egg traps?	Do you use pheromone traps?	Do you use Peterson traps?
Yes, multiple/yr	47%	N/A	N/A	N/A
Yes, once/yr	27%	57%	64%	35%
Yes, some years	6%	6%	5%	7%
No	20%	37%	31%	58%

Response options	Do you use biofix and degree days?	Do you use mating disruption?	Do you sanitize?	Do you use pesticides?
Yes, once/yr	68%	27%	78%	79%
Yes, some years	5%	11%	13%	12%
No	27%	62%	9%	9%

We used contingency table analyses to examine whether respondents managed or thought about management practices differently based on the demographic categories found in Table 1. In general, a higher proportion of PCAs reported using monitoring and management tools than owners and managers, which is consistent with the main role of a PCA. PCAs also tended to select the same barriers to adopting sanitation, mating disruption, timely harvests, and pesticide use as owners and managers, however they tended to select issues at higher rates than owners and managers. This could be because their responses could be skewed by a single client.

While there were some questions where response was significantly influenced by the number of years of experience managing NOW, this category was not as significant as industry role and the number of managed acres. In general, as years of experience increased, there was a trend for greater adoption of management and monitoring practices.

One important finding in this survey was that adoption of monitoring and management tools generally decreased as the number of managed orchards decreased from 2000+ to <50 (Figures 2a and b show selected results). This could be due to a PCA skew (a large proportion of PCAs also managed more than 2000 acres), however the small survey response size precluded examining interactions between industry role and the number of managed acres. Surprisingly, orchard size had no influence on the use of winter sanitation, although those managing smaller acreages reported fewer issues with orchard access.

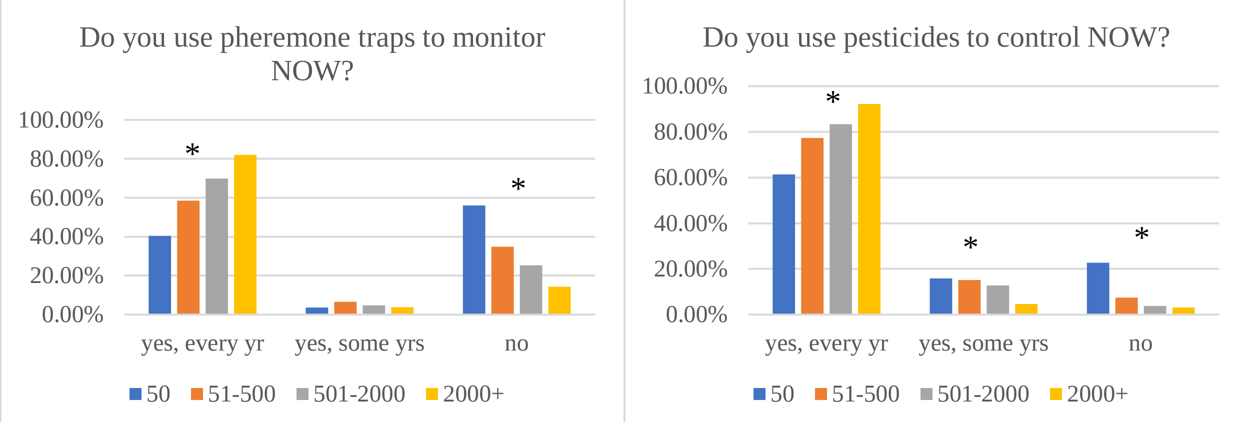

Figure 1a (left) and 1b (right). Responses marked with an asterisk are when contingency table analyses resulted in significant differences between orchard size and response rate.

Conclusion

These preliminary data reveal interesting information about the importance of PCAs as IPM stewards, as well as the need to find ways to make NOW management practices easier to implement for managers of small acreages. It also highlights that while there is room for more traditional research and extension practices on mating disruption, it is clear that the importance of sanitation is understood by the industry, and UC and USDA personnel need to focus on ways to improve orchard access to better facilitate this practice.

However, it is also hard to broadly generalize the results of these data to the wider industry based on the small number of respondents. While there is likely great overlap between the commodity board counts of the number of growers in each industry, it is clear that we reached only a fraction of the industry by only surveying at UC events. More work needs to be done to survey the entire industry, as it may be possible that adoption rates of NOW monitoring and management tools differ across the broader population of industry stakeholders. As such, in Year 2 we are proposing a more wide-ranging survey effort to capture a more complete picture of the tree nut industry.

Raman Spectroscopy to Detect and Measure NOW Pheromones

Authors: **Tiziana Bond**, PhD, Senior Staff Engineer, Center of Micro and Nano Technology, LLNL; **Sarah Sahota,** Junior Staff, LLNL Tulare County**; Houston Wilson,** Asst. Coop. Extension Specialist, Kearney Agricultural Research and Extension Center, Dept. Entomology, UC Riverside **.**

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 be effectively used to reduce crop damage. However, it is not well understood how synthetic pheromones compete with natural pheromones and, in the case of monitoring, how efficaciously the insect follows the diffusing plumes, especially across large blocks and at plot borders. We currently lack the ability to fully understand how these synthetic pheromone compounds diffuse away from their point-source of emission and subsequently how this might affect the efficacy of mating disruption and/or the accuracy of monitoring efforts. In response to these unknowns, Raman spectroscopy (RS) may offer a way to detect synthetic pheromone because of its analytical properties and recent technological advancements. In combination with nanostructured probes, RS has demonstrated the capability to detect volatiles at extremely low concentrations. We proposed to evaluate the use of RS for the detection of synthetic and natural NOW pheromones and, were that to be successful, use this tool in subsequent years to measure pheromone diffusion in orchards.

We have structured the effort to meet two objectives: *Objective 1–Use Raman spectroscopy to Detect Pheromones. Objective 2–Use Raman Spectroscopy to Measure Pheromone Diffusion*.
Our initial efforts have focused on *Objective 1,* on testing the ability of RS to detect emissions from synthetic pheromone lures. We have acquired lures and aerosols, some of which were provided by Dr. Houston Wilson at UC Riverside. At first, we pursued RS calibration of the basic components of the most used lures as it does not exist in the scientific literature, to the best of our knowledge. Secondly, we have performed analysis of how the lures basic component signatures differ from each other, which defines the capability of RS to detect and differentiate one pheromone from another. Our other study has targeted the sensitivity of our systems and we have planned for this year to evaluate the quality of different substrates to register the signal of the various pheromones' sources (i.e. lure vs. aerosol emission). Due to the COVID-19 crisis, the experiments planned for 2020 have suffered a large setback because of mandatory closure of the laboratories from the beginning of March until mid-July, with limited access afterwards due to controlled shifts and use of the lab equipment. Despite all these constraints, we believe we have been able to generate some interesting and promising new data for the basic pheromone chemical components, establishing a solid base for subsequent lure/aerosol studies.

Results

Our system consists of a Raman gun at 785nm with ~30-50mW power shooting into a built-in vial holder where the vials with the chemicals or the lure can be properly set. This configuration offers the best focusing of the laser beam on the samples and thus best Signal-to-Noise Ratio (SNR). We have acquired the basic chemicals that compose a few lures we have identified as used in the fields such as Trece L2L: (Z,Z)--11,1311,13--hexadecadienal hexadecadienal ;(Z,Z)--11,1311,13—hexadecadienolhexadecadienol; (Z,E)Vial #5: (Z,E)--11,1311,13—hexadecadienolhexadecadienol, (3Z,6Z,9Z,12Z,15Z)—tricosatricosapentaenepentaene. We have analyzed the same samples also from other companies as we well (i.e. Alpha Scents). For all of them we have extracted the RS signatures and assigned the peak to different vibration and rotational modes, which is completely new information for the pheromones under study (Figure 1). We have also carried experiments to understand the ability to detect low concentrations. Starting with 100% pure chemicals we have been doing dilution experiment, testing various concentrations down to 0.1%, in ethanol or acetone. The results are summarized below.

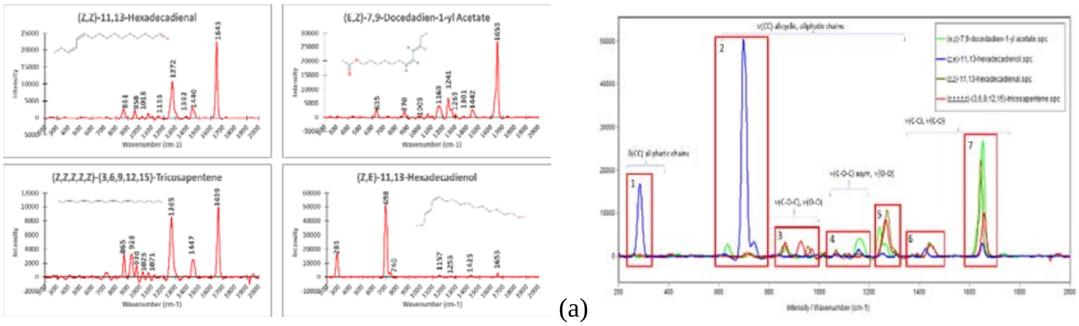

Figure 1. (a) Raman spectra for the four main chemicals showing the peaks that are singularly associated with each chemical thus confirming clear distinguishment among them. (b) Overlay of all spectra with assigned vibrations.

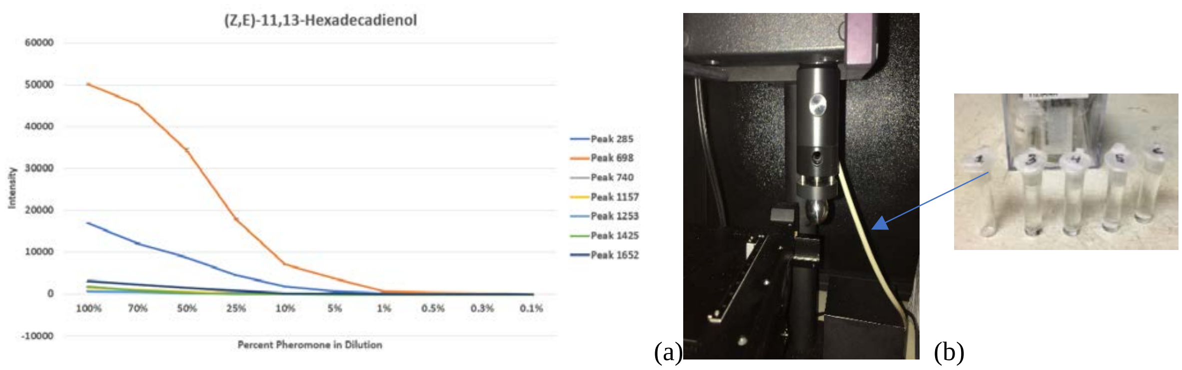

Figure 2. (a) Concentration trend for (Z,E)-11,13-hexadecadienol for several peaks with dilutions in ethanol from 100% to 0.1% (plot of mean with standard deviation over 5 measurements). (b) Picture of the Raman gun holder and of the vials that are positioned inside the holder, for the 4 sample s+ reference empty vials.

Initial testing with lures provided from Dr. Wilson were not conclusive mainly because of initial hardware and software hiccups that required us to modify our system setup, improve operation procedures, and upgrade the software. In the interim, as information is lacking in the literature, we focused on investigating the signatures of the active chemicals of interest and properly assign peaks, as shown. Therefore, we are now set to perform tests with puffers and new lures we are acquiring. Specifically, we are going to use lures from Trece (L2L/L2H) and Suterra (BioLure), and aerosols from Suterra (Puffer NOW ACE). Also, we expect to receive natural pheromones samples from Dr. Wilson. The sources will be arranged in a box containing the Raman gun and the environment will be tested at regular sampling times and at different positions of the lures/puffers to investigate the capability of measuring the diffusion of the emitted pheromones. Initial testing with Surface Enhanced Raman will be carried if time permits and we expect to optimize the limit of low detection.

<u>Conclusion and Practical Applications</u>

This project will develop the use of RS to detect synthetic pheromone compounds emitted by trap lures and mating disruption products, as well as natural pheromone emitted from NOW adults. This detection capability will then be used in a basic experiment (i.e. “orchard in a box”) to quantify pheromone diffusion in a very stable environment. Preliminary data on pheromone detection will be used to attract additional funding from additional agencies (e.g. USDA) to fully mature the technology.

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Use of Pistachio Mummy Volatiles as Lures for Navel Orangeworm

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Introduction

Monitoring is an essential part of integrated management programs for key insect pests such as navel orangeworm (NOW). Currently commercial monitoring tools include pheromone traps attracting males only, ovipositional attractants that trap gravid females or collect eggs, and phenyl propionate (PPO) that captures both sexes in the absence or presence of mating disruption. Challenges include improving the ability to predict damage based on trap counts and developing traps and attractants that are effective regardless of the presence or absence of mating disruption. PPO attracts NOW in either the presence or absence of mating disruption, but has disadvantages including unpleasant odors, necessity of using high concentrations, and captures of large high numbers of non-target insects.

Ongoing studies in our group have sought new attractants to address these challenges. We particularly sought female-specific attractants, since previous studies found that counts of female navel orangeworm (NOW) in traps are the best single predictors of damage. Traps using these baits generally capture few moths compared to pheromone lures or other attractants, so improving attraction to females compared to natural oviposition substrates is also an ongoing goal. A gas chromatography/mass spectrometry (GC/MS) study of pistachio mummies revealed unique volatiles consisting of monoterpenes that had undergone oxidation or microbial transformation, and fatty acid degradation by-products, including saturated and unsaturated aliphatic alcohols, aldehydes, and ketones. Over 120 of these volatiles unique to pistachio mummies were tested for male and female antennal sensitivity using an electroantennogram (EAG) apparatus. In general, female NOW adults had high EAG responses to the degraded monoterpenes and male NOW adults had high EAG responses to the fatty acid degradation by-products. Based on these results, various combinations of unique pistachio mummy volatiles were tested as NOW lures in conventional pistachio and almond orchards throughout the 2019 growing season. These lures were not successful in attracting NOW female adults; however, a 3-component lure increased capture of NOW male adults 30-50% when placed with pheromone lures in conventional pistachio and almond orchards.

In the 2020 growing season our objectives included:

1) Field trials under mating disruption to follow up on the 2019 findings with the three-component blend; and
2) Develop laboratory-based attractiveness assays for adult and larval NOW were developed to improve screening for female-specific attractants for field testing.

Research in 2020 was partially impacted by measures necessitated by the COVID-19 pandemic.

Results and Discussion

A randomized complete block trial compared eight variants of the three-component blend with controls including an empty trap and currently used standards (Table 1). In this trial orchard rows served as replicate blocks, and there was a spacing of 160 feet between traps and 320 between replicate blocks. Capture of a few NOW in the blank trap control and of moderately high numbers in ovibait (Peterson) traps indicated high NOW abundance in these blocks under mating disruption. The only attractants that captured significantly more NOW than the wing trap with no lure was the version of the three-component blend identified as #5, and the Peterson traps. The poor performance in this trial of PPO by itself or with a pheromone lure is inconsistent with previous studies and with a concurrent trial as part of another study.

Table 1. Total navel orangeworm adults per trap (mean ± SE, n = 7) and percent male captured during 9-week trials from March to June 2020 in almond and pistachio orchards under mating disruption.

	Pistachio		**Almond**	
Attractant	**Adults/trap**	**Percent Male**	**Adults/trap**	**Percent Male**
Blank	0.3 ± 0.18	50	0.9 ± 0.55	50
Ovibait	47.7 ± 5.84***	0	38.6 ± 4.74***	0
Phero-Blend1	1.7 ± 0.42	50	1.7 ± 0.42	50
Phero-Blend2	1.7 ± 0.61	75	1.6 ± 0.61	82
Phero-Blend3	0.6 ± 0.30	75	0.3 ± 0.18	100
Phero-Blend4	1.3 ± 0.42	78	1.1 ± 0.46	88
Phero-Blend5	3.4 ± 0.81*	83	2.9 ± 0.80*	85
Phero-Blend6	2.0 ± 0.65	43	1.9 ± 0.67	46
Phero-Blend7	1.6 ± 0.57	64	1.1 ± 0.40	75
Phero-Blend8	1.9 ± 0.46	62	1.1 ± 0.26	75
Pheromone	0.7 ± 0.29	60	0.1 ± 0.14	100
PPO	0.9 ± 0.55	50		
Phero-PPO			0.4 ± 0.30	67

Means in the same column followed by asterisks are significantly different from the mean for the blank control trap (GLMM, negative binomial distribution, Dunnett's post-test). *P < 0.05; ***P < 0.001. All attractants were presented in orange wing traps. "Phero-" indicates that the attractant in question was presented along with a commercial pheromone lure.

This poor performance of PPO is, however, consistent with user complaints. A relatively high freezing point for PPO may a factor in these early-season trials. The capture of only gravid females in the ovibait traps is consistent with previous observations, and the capture of both sexes in the three-component blend is similar to observations from previous studies with PPO and kairomone blends. Dose-response field tests comparing NOW response at different emission might be a helpful follow-up.

Preliminary results were obtained from three different laboratory assays developed to try to improve behavioral screens of female and larval NOW attractants:

1) A Y-tube assay was developed to evaluate adult attraction to individual or combinations of volatiles. The test is run overnight, with adults captures on sticky traps on either arm of the Y-tube as the assay endpoint.
2) A filter paper disk-based two choice test was development to compare attracts for neonate larvae. Preliminary results indicate that neonate larvae are attracted to the same pistachio mummy volatiles that have high EAG responses by adult female NOW.
3) An oviposition assay was compared egg-laying preferences on various dimpled substrates in combination with unique pistachio mummy volatiles with the ultimate objective of improving field assays.

Conclusions

- Field trials indicated that the three-component blend might be a more potent alternative to existing bisexual non-pheromone attractants such as PPO and kairomone blend.
- Laboratory behavioral assays offer promise for improved screening of female-specific attractants prior to field trials.
- Continued development of attractants specific for females and neonates is necessary to make monitoring more effective and improve effectiveness of management tactics for NOW.

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

Authors: **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; **Judith M. Stahl**, Postdoctoral Researcher, Department of Environmental Science, Policy and Management, UC Berkeley.

Introduction

This project investigates the potential of controlling large bug pests in pistachio with annual summer trap crops. The group of large bugs are composed of species 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*). We are revisiting the use of trap crops (cover crops) in pistachio because of the novel middle row irrigation line established at a Nichols Farms organic pistachio block. The middle row irrigation allows for season-long green vegetation (i.e., no dry-down) and this may help hold bug pests in the trap crop or increase the season-long presence of beneficial insects.

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. Different ground covers have differential attraction to plant bugs. Even within one plant species there can be differences due to the plant's phenology with flowering or seeding/fruiting often being the preferred host plant stage. However, information about the attraction to cover crop species of the specific composition of large bugs present in Californian pistachio orchards is currently lacking. In addition to attraction, an important consideration for a using trap crops is the potential for reproduction of the insect pests on the trap crop. This depends on the plant bugs' oviposition preference on the one hand and the suitability of the cover crops as host plants – determined by the nutrient composition – on the other hand.

Working in conjunction with an ongoing, multi-year field study, the primary aims of this laboratory study were to identify cover crop species with the respective phenological stages that attract the majority of the relevant large bug species and determine the possibility of plant bug reproduction on the selected trap crops species and phenological stages.

Results and Discussion

The complex of stink bugs and leaffooted bugs preferred feeding on plants in reproductive stages (flowering and fruiting/seeding) over those in the vegetative stage despite rarely being observed feeding specifically on the reproductive structures during the experiment. They made no distinction between phenological plant stages for first contact. Of the plant species tested so far, the insects favor buckwheat over alfalfa and sunflower, with clover, cowpea, mustard, soybean, sudangrass, vetch and wheat being equally often selected in the cage setup (Figure 1). The tested large bug species, *Chinavia hilaris*, *Chlorochroa uhleri, Euschistus* sp., *Thyanta pallidovirens* and *Leptoglossus zonatus,* did not show differential attraction to the offered cover crop species but sample sizes for combinations of insect species with phenological stages of specific plant species are still being increased to at least twenty, which could slightly alter the results.

Looking at potential oviposition on the cover crops, there were no statistical differences between the feeding selections of male and female insects. Of the more than 350 female insects used in the cage experiment, five *C. uhleri* females oviposited during the experiment and none of them on a plant surface.

Survival from first instar nymphs to the adult stage was not observed for *C. hilaris* or *Euschistus* sp. on either vegetative or reproductive plants: vegetative wheat, mustard, soybean, fruiting cowpea and flowering as well as seeding buckwheat. Not all plant species have been tested yet, but notably even buckwheat, which was the most attractive plant (see above), has so far not supported nymphal development when presented individually.

Collections of overwintering leaffooted bugs have started in November 2020 and will continue over the winter. In early 2021, we will conduct bioassays with collected females to determine their mating status.

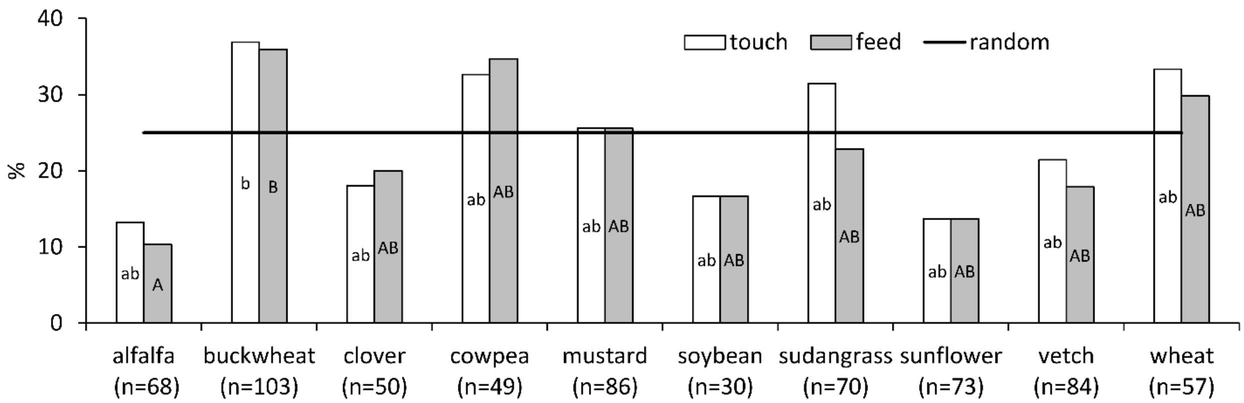

Figure 1: Percentage of presented cover crop species selected by the insects for first contact ("touch") or first feeding ("feed"). Since the insects could choose between four plants, the line at 25% represents the random distribution we would expect without attractive or repellent effects. Responses were compared between plant species within category (first contact lowercase letters and first feeding uppercase letters) with a generalized linear mixed model, bars including the same letters are not significantly different.

Conclusion

Our goal was to screen annual ground covers in a laboratory setting for a future trap cropping program in organic pistachios. This will reduce pesticide application costs and the added environmental costs derived from using broad spectrum insecticides.

Our results confirm large bugs' feeding preference for plants in the reproductive stages, i.e. flowering and fruiting/seeding over the vegetative stage, indicating a functional experimental setup. We saw that of all the tested plant species so far, buckwheat is the most attractive cover crop for the large bug complex. If increasing the sample size does not change that the stink bug and leaffooted bug species largely overlap in their cover crop preferences, it will be easier to select plant species that will attract the whole pest complex.

If the most attractive cover crop is able to sustain large bug nymphal development, targeted pesticide applications of the ground covers would be advised as to not increase pest populations. Our results addressing the suitability of the cover crops for *C. hilaris* and *Euschistus* sp. nymphs indicated no development to the adult stage. This confirms the assumption that these polyphagous pests require, or at least prefer, several host plants for development, which might reduce the need for pesticides further.

We will continue the experiments to further unravel attraction to and suitability of phenological stages of our selected plant species to the individual species that form the large bug complex in California pistachio orchards. This could enable us to select a mixture of cover crop species with different phenologies to sow in the orchard providing a constantly attractive ground cover. If this combination can support nymphal development, targeted insecticide applications on the ground covers would still reduce the treated area and save costs.

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

Authors: **Jocelyn Millar**, Professor, Depts. of Entomology and Chemistry, UC Riverside; **Houston Wilson,** Asst. Cooperative Extension Specialist, Dept. of Entomology, UC Riverside and Kearney Ag. Center; **Kent Daane**, Extension Specialist, Dept. Environmental Science, Policy and Management, UC Berkeley and Kearney Ag. Center.

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. There are typically 2-3 generations. 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 because bug damage may only become apparent after the bugs have moved on, continuous monitoring of bug populations is crucial in timing treatments. Trapping systems based on pheromonal or related attractants for each species would be of great value for monitoring and potentially control purposes.

Based on their behavior and life history, LFB are likely to use at least four types of pheromones:

1. alarm pheromones (and defensive secretions) produced by both sexes, which are not species specific, and so are unlikely to be used in sexual interactions;
2. male LFB produce species-specific secretions from paired, ventral abdominal glands (VAGs) which have an aphrodisiac function, rendering females receptive to mating attempts, but they do not attract adult bugs into traps (Wang and Millar 2000);
3. the rapid buildup of summerform LFB of both sexes in crops suggests that they are using aggregation pheromones. A recent publication has shown attraction of adult bugs to odors of other adults in laboratory bioassays (Franco-Archundia et al. 2018), but the relevant compounds were not identified;
4. non-reproductive winter-form adults form overwintering aggregations in sheltered spots, and it is likely that pheromones mediate the formation and maintenance of these aggregations. These signals are likely to be different from the signals which are used to aggregate bugs into a crop for feeding and mating.

Thus, the goals of this project are two-fold:

1. To develop attractant-based trapping systems for leaf-footed bugs for use in monitoring and possibly control of bugs during the growing season. In parallel, we are optimizing traps and trapping protocols for LFB, and developing new information on summer-form LFB ecology.
2. To develop a better understanding of the biology of leaf-footed bug overwintering, focusing on possible signals mediating the formation and maintenance of overwintering aggregations.

Results and Discussion

During the course of this project, colonies of *L. zonatus* (LZ) have been reared continuously at one or both of UCR and Kearney, to provide insects as a source of chemicals and for use in bioassays. By 2019, we determined that summer-form male LZ produce a blend of 9 compounds, 8 of which had been identified. Our first attempt at identification of the 9th and possibly most important compound failed due to insufficient material, so we collected more crude extracts from ~ 100 cohorts of males for the better part of a year. The 9th compound was purified again in December 2019, and detailed analysis of a series of NMR spectra of the compound finally enabled us to identify it in May 2020. We carried out a quick and dirty synthesis to provide an authentic sample to verify the structure, and are now working on scaling up and optimizing the synthesis to provide multigram quantities for lab and field bioassays in 2021. The remaining 8 compounds have been purchased or synthesized, with the exception of 1 compound, whose synthesis we need to repeat to obtain more material. Thus, we will have the full suite of possible pheromone compounds available for bioassays during 2021. Volatiles from summer-form *L. clypealis* (LC) have also been analyzed and the main components identified, so if we can find populations of LC in 2021, bioassays can be conducted with them as well. In the last couple of years, LC populations have been low and hard to find, for unknown reasons.

In previous years, we had shown that the cuticular hydrocarbon blends of the summer- and winter-forms of LZ and LC vary with the season/physiological state of the bugs. These changes are likely important for the maintenance of overwintering aggregations. The first bioassays testing this hypothesis were not successful, likely due to bioassay chambers being too confining. We were not able to repeat these bioassays as planned because COVID restrictions forced us to destroy the LZ colonies at UCR. We now plan to repeat these bioassays with the large and healthy LZ colonies at the Kearney Ag. Center. In Nov. 2020 colonies of LZ were placed outside to transition them into winter-form, while colonies in the insectary maintain summer-form under long photoperiod and warm temperature conditions. Assays will begin in Dec. 2020 to evaluate mating compatibility of summer- and winter-form males and females. In spring 2021, laboratory olfactometer assays will be used to initially screen attraction of summer-form adult LZ to the new pheromone compounds, followed by field assays over the summer.

Conclusion

We are finally reaching the end game on the search for attractants for summer-form LFB, with all compounds identified, and syntheses of the last compounds in progress, so that lab and field bioassays of the compounds can be carried out in 2021. Along the way, we have identified a suitable trap for LFB (Wilson et al. 2020) for use with a pheromonal or other attractant lure, if the bioassays described above are successful.

Improved control of navel orangeworm: focus on increasing insecticide efficacy using adjuvants

Authors: **Joel P. Siegel**, Research Entomologist, USDA-ARS, Parlier; **Ryan Wylie**, Farm Manager, Agri-World Coop, Madera; **Devin Aviles**, Business Manager, Agri-World Coop, Madera

Introduction

The purpose of this multiyear research project is to improve control of navel orangeworm (NOW) using a combination of increased application efficacy and timing. Any product named is for specific information purposes and does not constitute an endorsement by the USDA. The experiments reported below are part of an ongoing study initiated in 2017 evaluating the ability of organosilicone adjuvants to reduce the amount of water needed for insecticide application by air blast sprayers. For 2020 a sunscreen type adjuvant (Cohere) and two organosilicones were evaluated. The insecticides evaluated included the pyrethroid (Brigade WSB, 1.76 lbs/ac) and diacylhydrazine mixtures (Intrepid Edge at 19 oz/ac, Enkounter at 16 oz/ac). The outcome variable measured is duration of control in the Upper Canopy (14-16 feet) measured using a contact toxicity bioassay. Filter papers were placed in the canopy using hooks hung at approximately 14-16 feet and the filter papers were collected at selected intervals after commercial application by airblast sprayer (Air-O-Fan GB36 with multinozzles) at 100 or at 50 gpa. The filter papers were then placed in petri dishes containing NOW wheat bran diet and challenged by placing 50 eggs laid on paper towel in the center of the filter paper. Newly hatched larvae crawled over the treated surface to reach the diet and mortality was scored 18 days later.

Results and Discussion

Table 1 contrasts the duration of control obtained for Brigade WSB and Enkounter, using a sunscreen adjuvant (Cohere, 12 oz/ac, 100 gpa) assessed over an interval of 28 days. On day 1, treatments were compared at 4 feet (ground) and 14-16 feet (hook), and at 14-16 feet for the remaining days.

Treatment	Mortality	Reduction	Eggs
Control Day 1	44.4%		500
Brigade ground Day 1	77.6%	59.8%	800
Brigade hook Day 1	86.1%	74.9%	800
Enkounter ground Day 1	84.3%	71.7%	800
Enkounter hook Day 1	86.1%	80.9%	800
Control Day 7	51.0%		1,550
Brigade hook Day 7	73.5%	46.0%	1,550
Enkounter hook Day 7	86.8%	73.1%	1,550
Brigade hook Day 14	73.4%	55.7%	1,400
Enkounter hook Day 14	87.8%	78.0%	1,550
Control Day 21	46.4%		500
Brigade hook Day 21	60.2%	25.7%	1,550
Enkounter hook Day 21	92.7%	86.4%	1,550
Brigade hook Day 28	42.8%	NONE	250

Distribution of Enkounter was more uniform than Brigade because there were no differences in mortality between hook and ground. Duration of control lasted 21 days for Enkounter and broke down for Brigade after 14 days, although there still was elevated mortality relative to the control at 21 days. Further studies with other adjuvants combined with the maximum dose of Brigade WSB are needed to determine the length of control for both insecticides.

Table 2 reports the duration of control over 14 days for Intrepid Edge® (19 oz/ac) applied at 50 gpa using two organosilicones, Kinetic and Silwet 719, and Cohere at 100 gpa along with a laboratory control. Silwet 719 was applied at a concentration of 0.1% (12.8 ounces per 100 gallons) Kinetic at 0.12% (16 ounces per 100 gallons), and Cohere at 0.1% (12.8 ounces per 100 gallons).

Treatment	**Mortality**	**Reduction**	**Eggs**
Control Day 4	52.5%		400
Cohere 100 gpa ground Day 4	90.3%	79.6%	350
Kinetic 50 gpa ground Day 4	92.3%	83.7%	400
Silwet 719 50 gpa ground Day 4	94.8%	89.0%	400
Cohere 100 gpa hook Day 4	92.6%	81.9%	500
Kinetic 50 gpa hook Day 4	80.2%	58.3%	500
Silwet 719 50 gpa hook Day 4	94.6%	88.6%	500
Control Day 11	55.4%		500
Cohere 100 gpa hook Day 11	86.2%	74.0%	500
Kinetic 50 gpa hook Day 11	68.8%	43.7%	500
Silwet 719 50 gpa hook Day 11	79.4%	62.8%	500
Control Day 14	55.4%		500
Cohere 100 gpa hook Day 14	86.2%	74.0%	500
Kinetic 50 gpa hook Day 14	69.8%	45.5%	450
Silwet 719 50 gpa hook Day 14	77.8%	59.9%	250

There were some problems with the Kinetic application, indicated by the discrepancy between the mortality for ground and hook. Mortality was satisfactory at 4 feet and fell dramatically at 14-16 feet on Day 4 and did not recover over the two weeks. In contrast the mortality was almost identical for Silwet and Cohere at these two heights on day 4. For all treatments, contact mortality declined over time but the dropoff was the least for Cohere at 100 gpa on day 14. . The treatment with Kinetic at 50 gpa provided the smallest reduction, 45.5%. and the Silwet 719 at 50 gpa was intermediate with a reduction of 59.9% relative to the Control treatment.

Conclusion

These results reveal several of the challenges associated research in this area. There are many different adjuvant rates that can be used as well as insecticides belonging to different families, necessitating multiyear studies. It is quite easy to unfairly praise one adjuvant or criticize another when environmental factors such as wind or spray rig problems may be responsible for the differences noted. Nonetheless, the data in Table 1 clearly demonstrate that the pyrethroid insecticide broke down more quickly than the diacylhydrazine and this is consistent with other studies. However, I have demonstrated better performance for bifenthrin using other adjuvants and this experiment needs to be repeated, especially because the treatment was effective in the field. In contrast, Table 2 clearly indicates that the best performance was associated with 100 gpa despite the use of organosilicone adjuvants.. However, the low rate of 50 gpa was effective for at least 11 days using organosilicone adjuvants according to the label. This needs to be repeated with other organosilicone adjuvants and studies are needed to see if this class of adjuvant can significantly boost duration of control using a higher volume of water.

Improving and verifying quality of mass-reared navel orangeworm for sterile insect technique

Authors: **Houston Wilson,** Asst. Cooperative Extension Specialist, Dept. Entomology, UC Riverside, Kearney Agricultural Research and Extension Center; **Chuck Burks,** Research Entomologist, USDA-ARS, San Joaquin Valley Agricultural Sciences Center.

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 3 years, co-PIs Wilson and Burks have led scientific efforts to evaluate the quality and performance of sterile NOW produced by this Phoenix facility. Previous research by this project determined that male field performance in California is the most limiting factor for achieving the full potential of SIT for NOW. Moreover, handling between collection in the rearing facility is more important factor than the insect strain or irradiation with respect to male performance.

Goals of proposed research in 2020 were to examine cold tolerance of males as it related to operational conditions; and to examine the extent to which release conditions could improve field recovery. Considering COVID restrictions and the discovery of greater recovery in APHIS releases in 2020, the first objective examined field recovery using production aircraft and methods rather than laboratory evaluations of chilling injury.

Results and Discussion

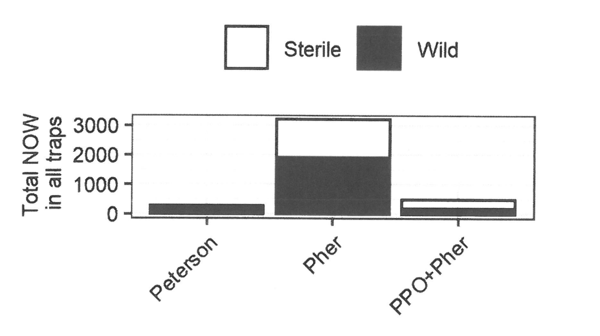

Figure 1. Sterile and wild males captured in an orchard without mating disruption in 64 Peterson traps, 64 pheromone traps, and 49 combination PPO-pheromone traps from August 18-25. Sterile moths captured in the Peterson, pheromone, and PPO+pheromone traps respectively comprised 4%, 40%, and 60% of all adults captured on that date. All sterile moths captured comprised 0.4% of the moths released on August 18.

Objective 1 – Recapture in a non-mating disruption orchard following aerial release

The APHIS/CDFA pilot program noted greater recovery of sterile NOW in 2020 compared to previous years. Between May 5 - Oct. 5 a total of 176 releases of 750,000 mixed-sex NOW were made from a fixed-wing aircraft over 640 acres of pistachios under mating disruption, which resulted in sterile moths forming 10% of the total females captured in a grid of 16 ovibait (Peterson) traps and 17% of the total mixed-sex NOW captured in a grid of 16 traps baited with phenyl propionate (PPO) and pheromone (an attractant combination known to work in mating disruption). This represented a higher number of sterile males recaptured relative to previous years, although it should be noted that as a proportion of total moths released these data still indicate recapture rate of only 0.001%. Regardless, a parallel trial was set up in a 640-acre pistachio orchard with no mating disruption, about 5 miles from the first NOW SIT release site. A 7 × 7 grid of PPO-pheromone traps was superimposed in an 8 × 8 grid of pheromone and Peterson traps spread evenly across the orchard. Releases of 750,000 sterile NOW from the fixed-wing aircraft were conducted every two weeks from Aug. 18 – Oct. 27 and the traps were monitored weekly. Sterile males were captured in the first week after each release (e.g., Figure 1), but not the second week. Generally, these results represent an improvement in performance of mass-reared sterile NOW released from the fixed-wing aircraft. This recovery rate is, however, still low compared to previous studies with NOW prior to this SIT program and the SIT program for codling moth in British Columbia, Canada.

<u>Objective 2 – Evaluate New Transport and Release Mechanisms</u>
Recovery of sterile NOW was compared in 3 small blocks (2 almond, 1 pistachio, 2-5 ac.) and 1 large block (pistachio, 640 ac.) using flight traps baited with pheromone lure (captures males) and ovipositional bait (captures females). Sterile moths were released every 2-weeks between May – Oct. using multiple combinations of transportation and release methods. In the small blocks, a grid of 12 mating tables were placed in the block over the first 3 nights following each sterile moth release. Each mating table contained a virgin female NOW that was replaced daily and examined to determine mating status. If present, male partners were dissected to determine if they were sterile or wild.

Sterile NOW were consistently recovered under field conditions in both flight traps and mating tables. The proportion of moths recaptured from a given release event varied significantly (avg. 2% recapture, range 0.001 - 14%). Variability in recapture rate was influenced by both transportation and release methods. Refinements in these methods increased recapture by 50-150%. Surprisingly the strongest predictor of recapture was background wild NOW population (Fig. 2), indicating that increased activity of wild NOW may somehow lead to increased activity of sterile NOW (or vice versa). This is a unique new dimension to sterile moth performance that will be more closely evaluated in 2021. Here, the total number of sterile moths per release event (avg. 5736 ±168 moths/release) did not influence recovery rate.

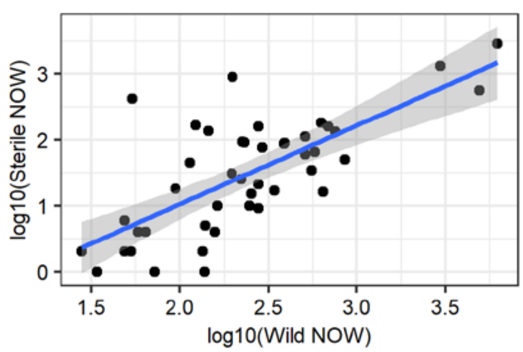

Figure 2. Sterile NOW males captured in pheromone traps in 45 mark-release-recapture assays in small and large plots in 2020; $r^2 = 0.49$. There was a strong association between sterile males recaptured and wild males captured, suggesting that background NOW population and/or environmental factors effecting trap attractancy may play an important role in the recapture rate.

<u>Conclusions</u>

- Field performance of NOW males from the USDA-APHIS mass-rearing facility was improved in 2020 compared to past years, as demonstrated by recapture following releases by program aircraft and experimental ground and drone releases.
- Sterile females attracted wild males in mating tables. For the first time sterile males were also recaptured in mating tables, indicating sterile males/females can locate/attract wild NOW.
- Data from 2020 APHIS releases revealed that recapture plateaus with the number of adults released (consistent with other SIT programs).
- Correlation between sterile and wild NOW recapture suggest that background NOW population and/or environmental conditions may have some influence on recapture rate.
- Increased recapture of sterile NOW following modification of transportation and release methods shows that improvements are possible in these areas.
- Acquisition of an x-ray irradiator shared by the USDA and UC research stations at Parlier will provide higher quality NOW for small-scale experiments for improving monitoring, mating disruption, and SIT.

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

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Introduction

The ability of navel orangeworm (*Amyelois transitella*) (NOW) adults to infest pistachio 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, and 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.

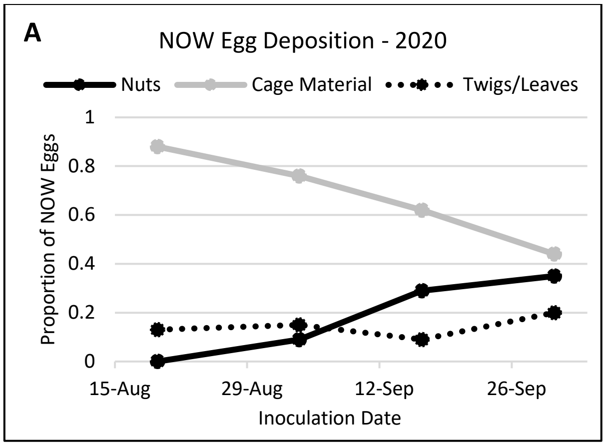
This proposal is about managing hull degradation and shell split itself to reduce crop vulnerability to NOW. While hull degradation and shell split generally take place later in the season as part of the pistachio developmental process, the extent to which this occurs can vary significantly from year-to-year. 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 in a pistachio block at the Kearney Ag. Center (Parlier, CA). Pistachio clusters were caged on May 12 and defoliation treatments applied on June 2. Starting Aug. 19, 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. The first inoculation was on Aug. 19 and additional cages were inoculated every 2 weeks until Sept. 30. Unfortunately, this experiment was terminated early due to mismanagement of the pistachio block, which led to a high incidence of fruit decay.

Logistic regression was used to analyze the proportion of NOW eggs deposited onto pistachio nuts as well as larval infestation. Egg deposition increased over time with each subsequent inoculation (χ^2 = 609.1, P = <0.001, n = 60) and was also influenced by the total number of nuts per cage (χ^2 = 11.0, P = <0.001, n = 60). This indicates that NOW egg deposition increased as the pistachio nuts matured over time (Figure 1A) and in cages with greater quantities of available nuts. Total larval infestation slightly increased over time with each subsequent inoculation (χ^2 = 13.1, P = <0.01, n = 48) (Figure 1B) but was not influenced by the total number of nuts per cage (χ^2 = 0.8, P = 0.39, n = 48).



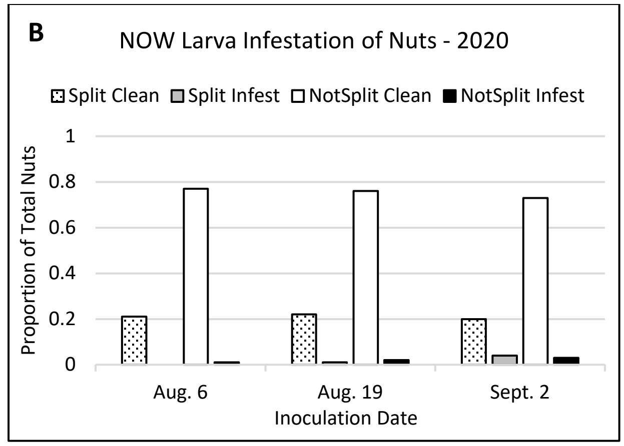


Figure 1. Both egg deposition onto nuts (A) and larvae infestation (B) increased over time.

<u>Objective 2 – Optimize Caging Technique for NOW Egg Deposition Trials</u>

Cage studies using paint strainer bags in 2019 found that this cage material itself was a highly attractive NOW egg deposition substrate, to the extent that it may have skewed data by pulling moths away from the nuts. In 2020 a laboratory assay was designed to evaluate different combinations of cage material and deterrent coatings to identify a more suitable material for constructing cages. Candidate cage materials included paint strainer bags (Supertuff, Trimaco), organza jewelry bags (Hongyitime), and home window screen material (polyester fiber, LazyDog Warehouse Inc.). Each material was evaluated alone and with the addition of a deterrent coating that included fluon (Insect-a-Slip, BioQuip), polyurethane aerosol spray (Varathane) and polyurethan paint (Varathane). Five mated female NOW were placed into a 1-quart glass jar (Ball Co.) which was then covered with one of the twelve candidate materials (5 replicates/treatment, n = 60). Moths were given 48 hours to oviposit onto the material and then all eggs were counted. Logistic regression indicated that the proportion of NOW eggs deposited was influenced by the material/coating ($\chi^2 = 2329.2$, $P < 0.001$, n = 59) (Figure 2). The addition of fluon to any material drastically lowered egg deposition, but the window screen material alone was also very effective. Fluon appears to be a useful deterrent coating, but it is expensive and difficult to work with. As such, window screen material alone was selected for subsequent use in the cage study (see Objective 1).

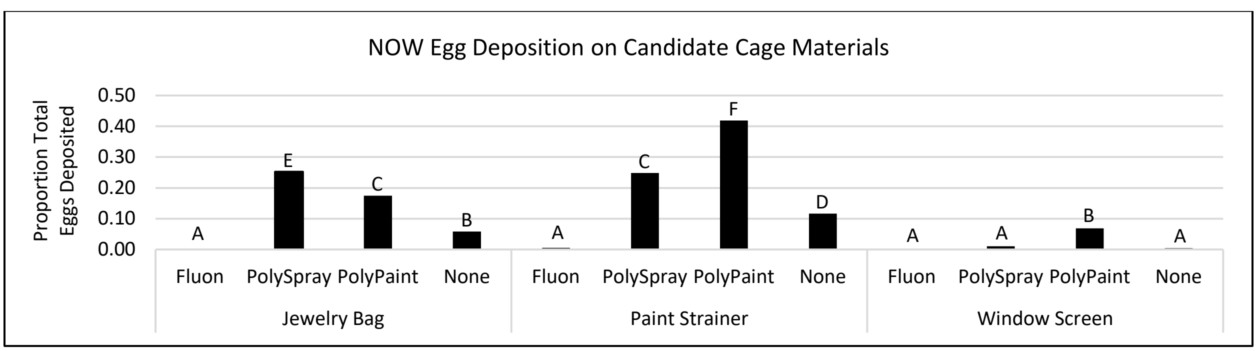


Figure 2. Egg deposition was influenced by the different materials and coatings utilized.

<u>Conclusion</u>

Efforts in 2020 were cut short due to poor management of the pistachio block at the Kearney Ag. Center, which led to significant levels of nut decay and prevented any analysis of treatment effects on NOW egg deposition. That said, efforts to determine a viable cage material were successful (Objective 2, Figure 2) and field trials utilizing these cages demonstrated a clear preference of NOW to deposit eggs onto nuts later in the season (Objective 1, Figure 1A).

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__

Pistachios are occasionally contaminated with mycotoxins, toxic metabolites produced by certain fungi, which constitutes a high economical threat to pistachio industry due to the risk of product being rejected from the market by strict regulations of contamination. The most toxic of the mycotoxins, and highly regulated worldwide, are the aflatoxins produced by fungi in the *Aspergillus* section *Flavi*, which include *Aspergillus flavus* and *A. parasiticus*, the most commonly found in pistachios in California. Even though aflatoxin contamination in pistachio nuts is sparse, aflatoxin contamination is still an issue that needs to be addressed because the high value of pistachio makes any load that is rejected from the market a considerable loss for the grower considering the possibility of product destruction or incurring on extra costs for transportation, re-sorting and additional lab analyses. A successful control of aflatoxin will benefit the growers by avoiding the loss of income with lower risks of crop rejections and the public in general by consuming tree nut products free of aflatoxins. Currently, the only proven method to reduce aflatoxin contamination is the use of *A. flavus* atoxigenic strain technology. The overall goal of aflatoxin management with this technology is to reduce the overall aflatoxin production potential of the population by changing the population structure of the aflatoxin producing fungi *Aspergillus* Section *Flavi* 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, and consequently have aflatoxin contents below the permissible levels in the market. Previous results show that besides the expected increase of the applied atoxigenic biocontrol (AF36) in treated orchards the untreated orchards also had an increase, indicating the capability of the fungus to spread over considerable distances, causing cross effects between treated and untreated orchards. Therefore, the implementation of area-wide long-term aflatoxin control programs might be the best strategy to lower the risks of aflatoxin contamination in almonds and pistachios.

__Results and Discussion__

In an area where tree nuts in risk of aflatoxin contamination (pistachio and almond) are grown together the effect of AF36 applications on area-wide was evaluated. In one part of the area both pistachio and almond were treated, while in another 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 after application and will serve to calculate the percentage of 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. However, data of both the percentage of isolates and the number of propagules belonging to either *Aspergillus flavus* strain L (differential toxin production, including the atoxigenic AF36) or *A. flavus* strain S and *A. parasiticus* (highly toxigenic) in soil samples give a preliminary indication of the effect of the area-wide treatments. Results from the pre-treatment sampling of the 2019 season indicate that the soil from previously treated pistachio orchards have both higher percentage and density of the strain L, and lower percentage and density of the toxigenic strains than the soils from almond orchards that were never treated. Soils from the post-harvest sampling indicate a higher density of both the strain L and the toxigenic strains compared to the pre-treatment sampling. The treated area had higher percentage of the L strain (91%) and lower percentage of the toxigenic strains (9%) in both the almond and the pistachio orchards, compared to the non-treated areas (76%, strains L and 16% toxigenic strains). The samples from pistachio orchards in the treated area had the highest percentage of the strain L (94%) and lowest percentage of the toxigenic strains (6%), while the samples from almond orchards in the not treated area had the lowest percentage of the strain L (69%) and higher percentage of the toxigenic strains (22%). The process of analyzing the isolates obtained from the soil samples, both previous to the treatments and after harvest, to determine the percentage of displacement by AF36 has been delayed due to Covid-19. 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 risks of aflatoxin contamination.

Early applications of atoxigenic biocontrol product might increase the effectiveness of the treatments to reduce aflatoxin contamination. Experiments from this past season 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 the early applications in May was practically non-existent two weeks after application, but good sporulation occurred in applications starting in late June. It is important to consider that this past season was unusually cold and wet in May (minimum temperatures under 10°C and maximum under 20°C) during the time when the early applied product was on the orchard's soil (normal temperatures at this time are 15°C minimum and over 25°C maximum). However, at two weeks after application there was a high percentage of the applied product grains remaining, but if it was still viable and able to sporulate once optimal conditions returned in June was not evaluated. Aflatoxin contamination of pistachio nuts indicate that all the samples from the treated orchards were below the permissible levels of 15 mg/kg, except for a low incidence (0.5%) in samples from orchards treated at the standard time. 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. Population analysis of the post-harvest samples indicate that there were not significantly differences on the density (cfu/gr) of the total population of aflatoxin producing *Aspergillus* and the L strain among the times of application and the not treated control. However, both the density (18.7 cfu/gr) and percentage (8.4%) of toxigenic isolates (*A. flavus* strain S and *A. parasiticus*) were significantly higher in the control, but there were not significantly differences among the times of applications (1.0 to 5.3 cfu/g and 0.8% to 2.8%). Preliminary data of soil samples from both previous to the treatments and after harvest for the density (cfu/g) of the biocontrol AF36 indicate an increase of AF36 in the earlier applications, but not on the late applications after late June. However, Covid-19 has delayed the process of determining the displacement (percentage of the applied AF36) in all replicates of the treatments.

<u>Conclusion</u>

Area-wide programs are intended 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). Establishing founder populations of atoxigenic strains by applying the biocontrol early enough before toxigenic strains are established could increase the likelihood of having a population dominated by atoxigenic strains. Results from both area-wide treatments and timing of application indicate that it might be possible to achieve this goal, but the use of new products with better sporulation under low temperatures (i.e. 60 to 70 ºF) and moisture (i.e. 8 to 12 soil moisture) will certainly help to achieve the goals. However, more research is necessary for conclusive results.

A survey of fungi producing Ochratoxin A in California pistachios and management of contamination

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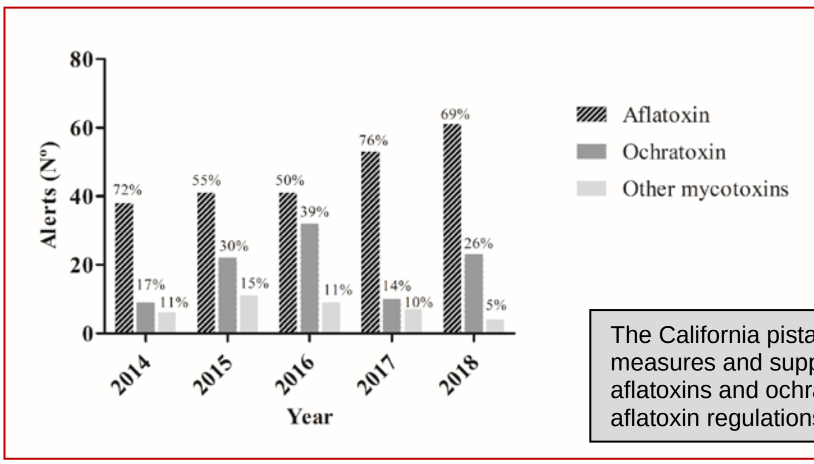
Introduction

Ochratoxin, a naturally occurring mycotoxin, is widespread in certain foods and animal feed. Several fungi in the genera *Aspergillus* and *Penicillium* produce ochratoxin while these fungi are decaying the crops involved. Specifically, of the *Aspergillus*, section *Circumdati* (*A. ochraceus*, *A. melleus*, *A. alliaceus*, and *A. sclerotiorum*) and from section *Nigri*, *A. carbonarius*, are known to have isolates capable of producing ochratoxin A (OTA). From our experience working with *Aspergillus* fungi in fig and nut orchards in California, we frequently find *Aspergillus* in the *Ochraceus* and *Nigri* groups in soil from pistachio and fig orchards and one would expect ochratoxin-producing fungi to occur in pistachio orchards in the San Joaquin Valley. A recent compilation of the Rapid Alters issued by EU in 2014 through 2018 indicates that the aflatoxins is the most frequently encountered mycotoxin (50% to 72% of the Rapid Alerts) followed by ochratoxins (14% to 39% of the Rapid Alerts) (Figure 1). Consequently, the recent notifications by the EU on ochratoxin detection in pistachio loads is problematic and one could expect that soon tolerances for OTA will be set, resulting in rejection of exported pistachios; the EU regulatory limit is 10 ppb (parts per billion) in dried vine fruits. Therefore, developing management measures to reduce OTA contamination in pistachios is urgent and the industry needs to be ready to combat this problem by the time regulations on ochratoxins are established. Figure 1 shows that the incidence of Rapid Alerts for ochratoxins follows those for aflatoxins in magnitude in various crops.



The California pistachio industry has taken extensive measures and supports pre-harvest research to control aflatoxins and ochratoxins to assure compliance with aflatoxin regulations.

Figure 1. Percent Rapid Alerts on aflatoxins, ochratoxins, and other mycotoxins in various crops.

The following objectives were proposed to be accomplished in 2020: a) identify which fungi in pistachio orchards produce ochratoxin A and quantify the levels of the toxin they produce; b) define factors that may influence ochratoxin A content in pistachios; c) determine whether the application of the atoxigenic AF36 Prevail® (registered for use) and Afla-Guard® GR (not registered yet) contributes in displacing ochratoxigenic fungi; and d) investigate additional management approaches to reduce ochratoxin A contamination. In 2020, this project was delayed because our lab assistant who was going to be in charge of this project moved to another job in February 2020 and we were not able (due to COVID-19) to hire another assistant or postdoc on time to complete the various objectives for this project. Therefore, we respectfully request the CPRB that we defer the various activities described in this project to 2021. However, when we analyse the library samples from commercial orchards for aflatoxin contamination, the same samples will be analyzed for ochratoxin contamination and these resutls will be reported to the industry once all these analyses are completed. In addition a competition experiment was performed in the laboratory to determine whether AF36 Prevail® or Afla-Guard® have an effect in reducing ochratoxin contamination when they co-inoculated with an ochratoxin producing *Aspergillus* (OTA 79) and also in the presence or absence of a highly toxigenic *Aspergillus flavus.* All these samples were frozen until analyzed for ochratoxins.

The various treatments include 3 replicated plates of 35 healthy pistachio kernels each inoculated as follows:

a) Ochratoxigenic A*spergillus* species (OTA79) alone.
b) Ochratoxigenic *Aspergillus* (OTA 79) + *A. flavus* AF36 strain (atoxigenic)
c) Ochratoxigenic *Aspergillus* (OTA 79) + *A. flavus* (highly toxigenic)
d) Ochratoxigenic *Aspergillus* (OTA 79) + *A. flavus* (highly toxigenic)+ AF36 (atoxigenic)
e) Ochratoxigenic *Aspergillus* (OTA 79) + Afla-Guard® strain (atoxigenic)
f) Ochratoxigenic *Aspergillus* (OTA 79) + *A. flavus* (highly toxigenic)+ Afla-Guard® (atoxigenic)
g) *A. flavus* AF36 (atoxigenic) alone
h) Afla-Guard® (atoxigenic) alone
i) *Aspergillus flavus* (highly toxigenic) alone
j) Water-inoculated (control)

These inoculated/co-inoculated kernels were incubated at 30 ºC (86 ºF) for a week. All the kernels were then placed in a freezer -16º C (3.2 ºF) until they will be analyzed for aflatoxins and ochratoxins.

Conclusion

Analyses of the library samples (commercial orchards) and samples form the lab inoculation/co-inoculation experiment are in progress and no conclusions can be made at this time.

Evaluation of Salinity, Boron, and Soil Hypoxia on Pistachio Tree Growth

Authors: Phoebe Gordon, University of California Cooperative Extension; **Giulia Marino**, University of California, Davis; **Gary Banuelos**, USDA-ARS; **Louise Ferguson**, University of California, Davis; **Joy Hollingsworth**, University of California Cooperative Extension; **Patrick Brown**, University of California, Davis; **Florent Trouillas**, University of California, Davis; **Bruce Lampinen**, University of California, Davis

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, no published research on pistachio salinity tolerance has examined the oxygen levels of the root zone. 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

Most of the work to date has been preparing the site at the USDA ARS research station in Parlier for the project. The grapevines that formerly occupied the tiles have been removed and the soil tested for nematode populations, which were found to be very low. Because of that, we decided to forgo fumigation. Parts for the irrigation system have been slowly acquired and is being assembled (Figure 1). The UCB-I clonally propagated rootstock pre-budded with Kerman have been ordered from Sierra Gold Nursery and will be delivered in early 2021.

Figure 1: Layout of the trial plot with the irrigation hoses needed for delivering the future salinity treatments. Trees will be planted in every other tile. Photo credit: Baudelio Perez

Future work

Work in 2021 will be to plant the trees in February, install the soil moisture and oxygen sensors, and allow the trees to grow for a year. A small subset of sensors will be installed before tree planting to work on developing a method to try to drop soil oxygen levels before salinity treatments are implemented. Water potential measures, soil moisture, and soil oxygen levels will be collected in 2021 to provide baseline data as well as to ensure all equipment is working properly.

Evaluating new training systems for pistachio

Authors: Bruce Lampinen, UCCE Nut Crops Specialist, UC Davis Dept. of Plant Sciences; **Mae Culumber**, UCCE Nut Crop Advisor for Fresno County, **Elizabeth Fichtner** UCCE Orchard Systems Advisor for Tulare County; **Phoebe Gordon**, UCCE Orchard Systems Advisor for Madera and Merced Counties, **Katherine Jarvis-Shean**, UCCE Orchard Systems Advisor for Yolo Solano Sacramento Counties, **Louise Ferguson**, UCCE Specialist, UC Davis Dept. of Plant Sciences, **Sam Metcalf**, UC Davis, Dept. of. Plant Sciences**, Loreto Contador**, UC Davis, Dept. of Plant Sciences, and **Tran Nguyen**, UC Davis Dept. of Plant Sciences, Daniel Syverson, UCCE Fresno County, Reza Ehsani, UC Merced, and Heraclio Reyes, nurseryman

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 in a 'Golden Hills' block on 'PG1' seedling rootstock. The rootstocks were planted in the fall 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, and 3) an unpruned control. Selected data trees met a minimum height requirement of 50 inches. 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 2020. There were no significant treatment differences in midday stem water potential on any date in any year but the conventionally pruned treatment tended to be the most stressed on most dates. In 2017 through 2019, the conventionally pruned treatment had significantly smaller rootstocks but by 2020, these differences were no longer significant. There were no differences in scion diameter among treatments but the unpruned trees were significantly taller compared to modified central leader or conventionally trained trees. Trees were hand harvested in 2019 with the conventional, modified central leader and unpruned treatments producing 0, 0.5, and 55.3 pounds of good nuts per acre respectively. In 2020, the trees were mechanically harvested and an additional hand harvest was done two weeks later. Cumulative yield for the conventional treatment trees was 535 pounds per acre, for the modified central leader treatment it was 992 pounds per acre and for the unpruned trees 1905 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. Trees tended to run about 2 bars below the fully watered (almond) baseline numbers which was very similar to the range in the Kings County Pruning Trial #1. Once again, the conventionally pruned trees tended to be the most stressed on most dates but the differences were not statistically different. There was a trend for unpruned trees to have the largest scion diameter but the differences were again not significant. The unpruned trees did have significantly larger rootstock diameters compared to the modified central leader tree and both had larger rootstock diameters than the conventionally trained trees. In May of 2018, unpruned, modified central leader, and conventionally trained trees had 46, 20, and 11 shoots opening, respectively. In 2019, there were no significant treatment differences in height, rootstock diameter, or scion diameter. In 2020, unpruned trees were significantly taller than either of the other treatments and scion diameter was significantly smaller for the conventionally trained trees. Trees were hand harvested in 2019 and in 2020 and cumulative yields for conventional, modified central leader and unpruned were 56, 74 and 379 pounds of good nuts per acre 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 raised 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 behaved and these shoots behaved like pruned shoots. 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. By December 2020, rootstock diameter was significantly larger on unpruned and modified central leader treatments compared to the conventional treatment but scion diameter was similar for all treatments. Unpruned treatment trees were significantly taller than conventionally pruned trees. Trees trained to metal stakes were much stronger and were largely strong enough to have stakes removed by the end of the first year. There were no significant differences in rootstock diameter, scion diameter and tree height for the wood versus metal staked trees by Nov. 2020. There was no cropping at this trial in 2019 or in 2020.

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 will be initiated in the winter of 2021. Preliminary irrigation cutoffs were initiated in the fall of 2020.

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 larger rootstock diameters compared to the conventional and modified central leader trees in both Kings County trials as well as in the Yolo County trial. 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 resprouting branches that balance the lean similar to results we have seen in walnut. Yields were significantly higher for the unpruned compared to either of the other treatments at both Kings County sites. Data collection will continue in all three of these trials in 2021.

New CE Specialist Seed Grant

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Introduction

The objective of this seed grant proposal was to partially support my first year of research and extension activity with the aim to: 1) assist Farm Advisors in their trials, identify, with them, important production and environmental issues facing the California pistachio production industry, 2) develop, in an existing trial, preliminary information that can scientifically support future effective research proposals and 3) create a team of campus- and county-based academics to address the identified statewide research and extension needs of this crop

Results and Discussion

Thanks to this seed grant I was able to identify and explore, together with different groups of collaborators, 3 main research priorities of interest to the industry and that fit my personal research focus and expertise.

Research priority 1: cultivar-specific precise management of pistachio orchards

Collaboration: this research priority stem out from the discussion, interaction and collaboration with Craig Kallsen, Phoebe Gordon, Bob Beede Mae Culumber, Elizabeth Fichtner, Patrick Brown, Robert Hutmacher, Louise Ferguson, Douglas Amaral.

Background: the exponentially increasing grower adoption of new cultivars and rootstocks is raising several questions on how to adapt the currently available management strategies, developed for the Kerman cultivar to, for the physiological and horticultural needs of these new genotypes.

Methods: we developed a research proposal to plant a variety trial orchard at the West Side Research Center, that will be replicated randomized sufficiently to investigate management practices on the Kerman, Golden Hills, Lost Hills and Gumdrop cultivars. The proposal will be submitted in the 2021 CPRB funding cycle.

Implication and follow up: the experimental orchard, if funded, will facilitate the new generation of researchers in developing and extending scientific information on pistachio orchard management for the new cultivar and rootstock combinations.

Research priority 2: plant water relation and physiological response of pistachio to salinity

Collaboration: This research priority developed as a result of a collaboration with Gary Banuelos and Phoebe Gordon.

Background: Pistachio growers dealing with salinity have no clear guidelines on how to manage irrigation. The use of an ET-based approach is challenging, due to the difficulty of integrating into a single crop coefficient the multiple and site-specific impacts that soil salinity has on water use, growth and yield. Stem water potential (SWP) measured with the pressure chamber is the gold standard of plant-based water management, but no studies are available on how to use this tool under saline conditions. Preliminary results and interaction with other researchers suggest a lack of sensitivity of SWP to salinity. However, these were mainly observations. Together with project collaborators we agreed to gather preliminary information on how salinity influences pistachio water relations.

Methods: the experiment was performed at the USDA San Joaquin Valley Agricultural Sciences Center, where Gary Banuelos developed a fully randomized experimental design where 4 different saline treatments are applied to young pistachio trees cv Kerman planted on UCB1 and PGI: no salinity (EC 0.3 dS/m), low salinity (EC 4 dS/m+ 6ppm B +0.1ppm Se), medium salinity (EC 8 dS/m+ 6ppm B +0.1ppm Se) and high salinity (EC 12 dS/m+ 6ppm B +0.1ppm Se). We measured SWP every 20 days from June to October. Starting in July we also measured leaf gas exchange, namely photosynthesis and stomatal conductance and collected leaf material to characterize Na accumulation in leaves and leaf osmotic potential.

Results: although results are very preliminary, they seem to support a low sensitivity of SWP to salinity stress. UCB1 had higher photosynthetic rates than PGI. Further analysis of leaf tissues for Na concentration and osmotic potential will be performed, as well as in-depth analyses of other measured parameters.

Implication and follow up: This investigation will be continued within the project of PI Gary Banuelos and Phoebe Gordon, with the objective of increasing our knowledge of pistachio's physiological response to salt stress and test other indicators for precise water management under salinity.

Research priority 3: Physiology and management of pistachio postharvest
Collaboration: This research priority stem out from a collaboration with Maciej Zwieniecki, Paula Guzman Delgado and Emily Santos

Background: postharvest is a critical period for deciduous alternate bearing crop such as pistachio. Trees in this period accumulate resources for next year flowering and leaf out, and experience physiological changes that prepare them for dormancy and winter survival. Hence, determining how to correctly manage trees postharvest is essential for ensuring the next year's production. This requires the development of more accurate information about pistachio postharvest physiology. We conducted a small trial to determine if early leaf senescence, and the associated decay in photosynthetic performance, commonly observed in branches with high crop load is triggered by a nut-driven deficiency of carbohydrate levels in the branch.

Methods: at the beginning of September, in an experimental orchard located in Wolfskill (Winters), pistachio branches were selected based on the level of leaf yellowing/senescence and crop load. Some non-bearing branches with green foliage were then girdled between the 1- and 2-year-old wood. Every 10 days the selected branches were brought to the lab to measure chlorophyll content, green and yellow leaf area and carbohydrate levels above and below the girdling point or the clusters.

Results: the carbohydrate still needs to be analyzed. However, the leaf chlorophyll content promisingly showed that girdling the non-bearing branches behind the current year wood triggered leaf senescence above the girdling point similar to high bearing branches.

Implication and follow up: better management of pistachio in postharvest should consider crop load level and tree carbohydrate status. The research line will be expanded, integrating the work developed in the project with Barbara Blanco, where the impact of branch resources on nut development is studied.

Conclusions
The funding proposal allowed: a) identifying 3 main research lines, b) building 3 teams of collaborators, c) developing promising preliminary data on pistachio physiology and d) writing 2 project proposals. Overall, this will speed up the development of my research program and increase its efficacy and impact for the pistachio industry.

Pistachio Irrigation Monitoring & Training Demonstration

Authors: **Blake Sanden**, UCCE Irrigation Management Farm Advisor (emeritus), Kern**;** **Daniele Zaccaria**, UCCE Agricultural Water Management Specialist, Land-Air-Water Resources, UCD; **Catherine Culumber**, Nut Crop Farm Advisor, UCCE - Fresno County**;** **Louise Ferguson**, UCCE Specialist, Department of Plant Sciences, UC Davis

Introduction

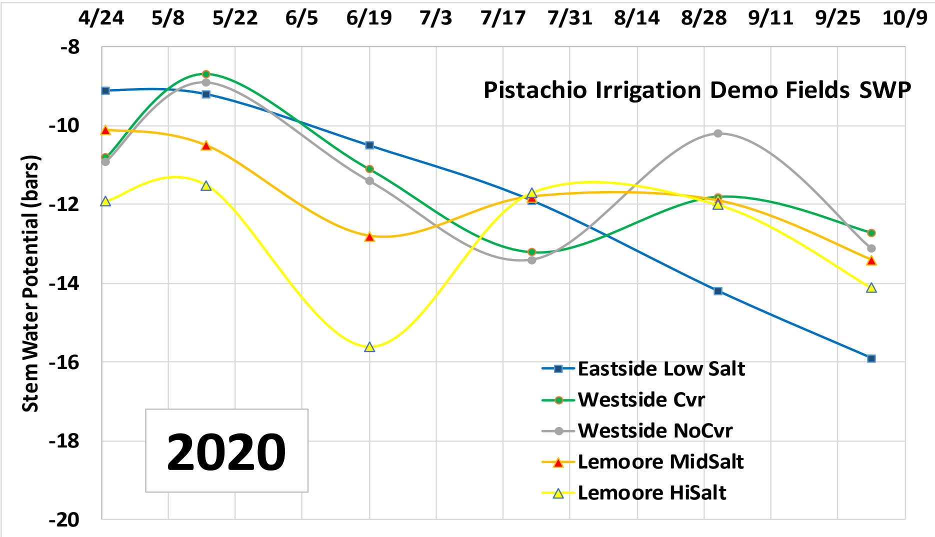
A lot of pistachio acreage is planted to marginal soils due to the high degree of salt tolerance of these trees (Sanden et al., 2004). Production experience farming orchards on these soils and even non-saline soils has shown that real water use for these orchards is often less than that predicted by using the ET crop coefficients (Kc) developed by Dave Goldhamer (1995). On-going research by Daniele Zaccaria sponsored by the Pistachio Research Board (Understanding the impacts of soil-water salinity on water uptake and consumptive use of mature pistachio orchards grown in the San Joaquin Valley with micro-irrigation) and CA DWR from 2016 to present, shows reduced water use by as much as 40% in mature pistachios as salinity increases. So if you combine the impact of salinity/soil structure, protracted juvenility and variability of orchard development (taking 6 to 9 years to reach 50-60% cover), it is absolutely imperative that pistachio growers do real-time soil and tree water status monitoring to achieve optimal irrigation scheduling. Growers are aware of this and eager to find the best options to monitor their orchards to insure optimal growth, yield and efficiency. However, there is a dizzying and expensive array of technology out there that leaves most growers confused. This project is trying to clear the fog and help growers evaluate for themselves what technology would help most for their particular orchard setting. With this goal in mind, we are using state of the art soil and plant monitoring technology in three mature pistachio production fields (Kerman/UCB1) from eastside to westside with increasing salinity to provide real data for irrigation monitoring and training by attempting to achieve the following **objectives:**

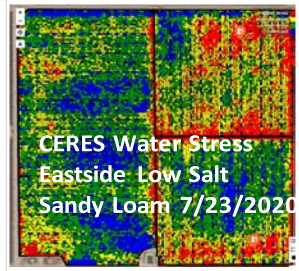
- Comparing automated web-based and field collected data for optimal irrigation scheduling
- Showing the impact of salinity on monitoring and ET
- Provide traditional workshops, field meetings, newsletters and an open-access website for training
- Provide an on-line real-time BLOG for comments/explanation/training

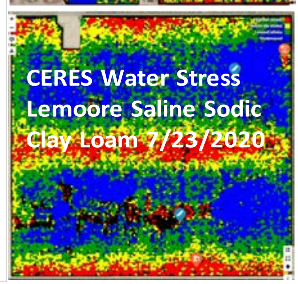
Results and Discussion

This irrigation demo project is loosely connected to the Zaccaria ET project as both use some common field sites. To have the real-time ET data generated from the Zaccaria project is a confirmation of the optimal monitoring and scheduling that comes from this DEMO project. However, each project operates independently. Due to some confusion over this distinction the DEMO project went unfunded until June 2019. This delayed initiating cooperation with the UC Davis Fruit & Nut Research Information Center (UCD-FNRIC) and the establishment of the website until late in the season. This website (https://fruitsandnuts.sf.ucdavis.edu/pistachio-irrigation-training-field-monitoring-demo) has a narrated video and maps explaining the project and field monitoring methodology / equipment. There is summary data mounted to this website and links to the **IRRIGATION BLOG, which** was established 3/25/2020 **(**https://ucanr.edu/blogs/irrigationblog/). There were 6 extensive blogs published roughly once a month comparing the results of field visits (4/25, 5/16, 6/19, 7/23, 8/31, 10/3) using traditional "hand feel" of soil moisture, pressure chamber stem water potential and neutron probe soil moisture to a 9 foot depth to continuous tensiometers readings and web-based measurements as explained below. We had hoped to encourage grower participation and feedback with this BLOG but we received nothing.

Impact of Covid-19: Implementation of isolation/distancing at the UC Davis campus prevented the UCD-FNRIC from being able to recruit a 20% time student assistant for website maintenance of this project. This affected website modification, outreach and exposure. Only in the last month or so does a Google search of "pistachio irrigation demo" lead you to the website. Of course, face-to-face field or classroom meetings were suspended. The only traditional outreach event was a July Pistachio newsletter distributed via the San Joaquin Valley UCCE advisors pistachio grower list explaining the project. **Monitoring equipment:** WaterMark blocks and pressure transducer tensiometer readings at 18, 36 and 48 inch depths readings are recorded hourly by field logger (not on-line). Phytech dendrometers measure tree trunk shrink/swell (real-time web reporting). Pressure bomb stem water potential (SWP) is measured using the wet rag technique. **New site equipment:** Sentek Drill & Drop capacitance probes with Jain Logic reporting and pressure sensors tracking run time (real-time web), CERES imagery (4/25, 5/16, 6/19, 7/23, 8/31, 10/3 was scheduled but smoked out due to fires), additional Phytech dendrometers.







		SATURATION %				EC (dS/m)				Sentek/Jain Logic data	1/1/20 - 10/2/20		Metered	1/1/20 - 10/2/20			Inches/%	[3]Inshell
SITE	SOIL	11/15 2016	2/10 2018	4/25 2019	1/23 2020	11/15 2016	2/10 2018	4/25 2019	1/23 2020	Pressure Sensor Hrs	Calculatd Inches	Soil Mstr Dpltn (in)	Irrigation 2020 (in)	[1]Measrd ET (in)	Avg SWP (bars)	[2]Canopy Cover	Canopy Cover	Yield (lb/ac)
Eastside non-saline	Colpien L	32	37	29	32	2.7	3.8	8.9	6.7	706	29.6 +4.5" flood	0.0	30.9 +4.5" flood	41.7	-11.8	71.8%	0.49	3,632
Westside non-saline	Cerrini SL	--	26	21	24	--	2.8	4.3	1.9	1975	41.5	0.2	43.0	45.7	-11.3	80.8%	0.53	5,344
Westside non-saline	Calflax CL	--	--	New 2019 30	40	--	--	7.7	5.6	1922	40.4	0.0	41.6	48.4	-11.4	76.3%	0.55	5,344
Lemoore semi-saline	Lethent CL	55	60	47	53	7.8	6.7	10.8	6.7	1145	33.1	2.4	33.9	--	-11.8	68.7%	0.49	2,200
Lemoore saline	Lethent SiCL	62	79	54	65	8.2	7.4	11.2	8.3	1068	30.8	0.0	31.9	--	-12.8	34.4%	0.93	1,100

[1]UCCE in-field Eddy-covariance or Surface Renewal energy flux measurement. Lemoore fields not monitored for 2020.

[2]Bruce Lampenin August 2020 mobile undertree survey of % photosynthetically active tree cover.

[3]Block yield or estimated average site total inshell yield (including stained and non-split shells) for 90 trees centered around site instrumention.

Conclusion

There is no substitute for good dirt! Growers do a good job reducing irrigation when saline soils restrict water uptake and increase tree stress, but the result is reduced tree canopy and yield. The best field irrigation monitoring strategy uses technology checking BOTH soil and plant moisture status.

References

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Investigating the effects of winter cover crops on evapotranspiration, water productivity and soil-water functions of mature micro-irrigated pistachio orchards in the water-limited context of the San Joaquin Valley

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Introduction
The use of cover cropping is becoming a common field management practice in several specialty crop production areas of California. However, there is paucity of information on the effects of winter cover cropping on water use efficiency of pistachio orchards, although this information is crucial to inform growers' decisions in water-limited nut production areas. At the same time, cover cropping is among the soil management practices that are being progressively incentivized through climate-smart financial incentives, with the aim to improve soil health and mitigate climate change.

Several studies documented beneficial effects of cover crops on rhizosphere ecology, but there is little information on the effects of winter cover cropping on evapotranspiration and water productivity of nut orchards. In water-limited nut production areas, specific questions are raised by pistachio growers and farm managers concerning the water amounts necessary to establish and maintain cover crops, and if winter cover cropping can lead to water productivity gains.

To fill the existing knowledge gaps, we proposed to establish a field trial, collect datasets, develop, document, and disseminate comparative information on the effects that winter cover cropping and inactive vegetation residues left on the floor of the inter-rows have on evapotranspiration, CO_2 assimilation, water use efficiency, and soil-water functions in commercial, micro-irrigated pistachio orchards grown with winter cover crops versus clean-cultivated orchard floor. The aim of this research project is to develop science-based information to document the resource-efficiency gains that pistachio growers could pursue through the practice of winter cover cropping.

Plan of Activities
The Project team planned to:
1) Establish winter cover crops in sub-blocks of two non-saline pistachio study orchards located in Hanford and Coalinga, and of one saline-sodic orchard in Tulare, and maintain a control clean-cultivated sub-block in each of these orchards;
2) Instrument both the clean-cultivated and cover cropped sub-blocks with ET measurement stations and various other sensors to monitor water use alongside with bio-physical crop and soil parameters during the course of the crop season 2020;

In detail, the Project team planned to split the study orchards in three sub-blocks of adequate fetch area, of which two have two sharply different cover crops (namely the Blando Brome, which has medium-tall vegetation, and the Radix Poa Bulbosa, which has short vegetation), while the other has clean-cultivated soil. In addition, the Project team planned to instrument these three sub-blocks with the same suite of sensors to measure actual evapotranspiration and the amount of radiation reflected and scattered in multiple directions by the cover crop vegetation residues. All this aims to document the differences in water use over the active green vegetation periods (December through April) and in the radiative effects on orchard's ET and the soil-water functions during the period when the vegetation residues cover the floor of the orchard's inter-rows (May through November).

Accomplishments
In February-March 2020, the Project PI and team selected the pistachio study orchards in the areas of Hanford, Coalinga and Tulare, and discussed with grower collaborators the experimental design described

above. Afterwards, at the study orchards in Hanford and Tulare, two of the sub-blocks were seeded with the two different cover crop mixes (Blando Brome and Radix Poa Bulbosa), while the third sub-block had clean-cultivated orchard floor. At the study orchard in Coalinga, the lack of rain and some delay in pruning (i.e., topping and edging of the trees) by the farm management company and field crew did not allow sufficient time to seed the Radix Poa Bulbosa cover crop in one of the sub-blocks, whereas the other sub-block had already the Blando Brome established from late December 2019.

Unfortunately, the spread of Covid-19 in early March 2020 and total lockdown started in mid-March for the entire state of California, which prevented the Project team to proceed with instrumenting the study orchards and all the following research activities.

In July-September 2020, when the University related business allowed, the project team purchased all the necessary field supplies, sensors, mounting structures and miscellaneous parts that are necessary for instrumenting the study orchards to accomplish the project's objectives and conduct the planned set of activities.

Since the release of the lockdown in early June, the project team members have also been able to conduct periodic maintenance of the field instrumentation already installed in the control blocks of the study orchards in order to continue the field data collection on actual evapotranspiration of the clean-cultivated section of the orchards. Finally, the project team also sampled pistachios during the harvest operations in September 2020 to measure nut yield and quality. The nut samples were submitted to the processing plant of the Nichols Farms in Hanford and the results from the quality control analyses are being processed by the project team.

Results

The Project team could only conduct a minimal part of the planned activities due to the spread of Covid-19 pandemic since early March 2020, the subsequent lockdown, which along with UC regulations imposed cancellation of all travels and research activities not deemed essential. As such, in April 2020 the Project PI discussed with the representative of the Pistachio Research Board the need and implications of putting the entire research project on hold until the situation related to Covid-19 pandemic allowed to release the operational restrictions. Following this discussion, the Project PI decided to put the entire set of planned activities on hold, and possibly conduct them during the crop season 2021.

In consideration of the above issues, the Project team could not achieve any of the expected results during the crop season 2020 and will request a one-year no-cost extension to the Pistachio Research Board in order to instrument the study orchards, collect the field datasets, and conduct all the planned activities within the crop season 2021.

Conclusion and Practical Applications

Due to the Covid-19 pandemic, the lockdown decided by the state Governor Newsom and the subsequent restrictive regulations issued by the University of California for all travel and research activities deemed non-essential, the UC Project team only conducted a minimal part of the planned activities, which led to the achievement of none of the expected results during the crop season 2020. In consideration of the above indicated issues, there are also no practical applications of the expected results, as the proposed study could not be conducted within the year 2020. The Project PI will request a one-year no-cost extension to the Pistachio Research Board, which will enable to conduct the study and all planned activities during the course of 2021.

Long-term saline irrigation strategies for pistachios on PG1 rootstock

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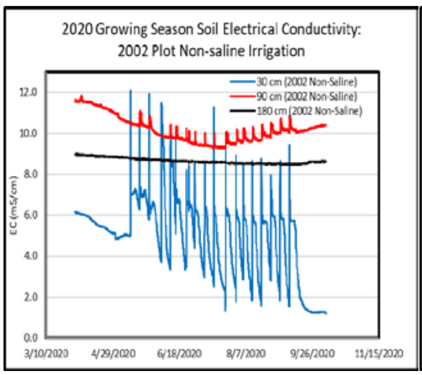
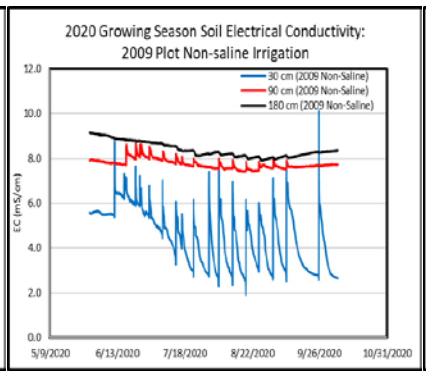
Introduction

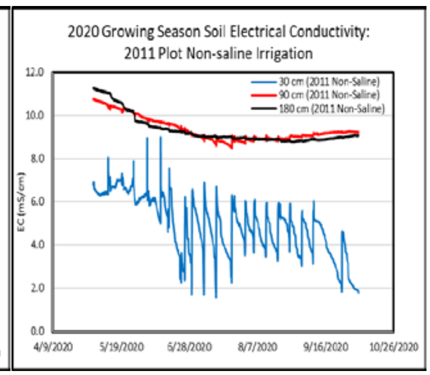
During drought conditions, obtaining the recommended annual 44 inches of irrigation water (Goldhamer and Beede, 2004) has become a major problem for California pistachio growers. Consequently, growers in the westside of Central California are increasingly using poor-quality water groundwater or drainage water with high salinity (Cl and Na), boron (B) and selenium (Se) (Ayars et al. 1993). When good quality waters are not available or are too expensive, growers need to know how other sources of irrigation water, e.g., drainage waters, will affect pistachios' nut yield, quality and tree health, as well short or long term soil quality. Eventually if such waters are used, soil will become saturated with Na, chloride (Cl) and B, and the soil's physical properties will deteriorate (Oster et al., 1999). Once the soil's osmotic threshold has been exceeded, the Na, Cl, and B concentrations can also exponentially accumulate in the tree and negatively affect yields and tree health. All three ions can be readily absorbed by roots, translocated to the scion, concentrate in leaves and fruits, and thereby result in reductions in nut yield and quality (Karima et al., 2009). However, there are differences in rootstock tolerance, with the hybrid UCB1 rootstock demonstrating a better ability to exclude, sequester and recirculate these ions than its more saline sensitive single species parent, PG1. Utilizing lower-quality water on pistachios will, however, likely be a necessity during future drought conditions in Central California. However, this strategy will require obtaining new knowledge about recognizing plant-based stress responses to changes in soil quality with continuous saline irrigation. This information will help both determine the duration and quantity of saline water that can be safely applied, as well as determine how we can effectively reclaim soil from increasing salinity and boron levels (Pistachio Production Manual, 2016). Implementing leaching of salinized root zones with good-quality water can be a management tool for maintaining orchard viability under saline irrigation practices. The availability of inexpensive good quality water for use in leaching can be problematic under drought conditions. Hence, application of water must be applied efficiently in a sparing but effective manner. To more effectively study this strategy, we have built upon the pioneering long-term salinity research by Sanden and Ferguson, and examined the impact of both continued long-term irrigation with saline water and the introduction of non-saline water for soil reclamation. Based upon our last two years of initiating the gentle reclamation of saline soils via micro-jet irrigation with non-saline water, we have observed real-time decreases in soil salinity from 0-90 cm with non-saline irrigation (selected data shown below) with support from the Pistachio Board and CSU Fresno Research Initiative.

Results and Discussion

In 2019-2020, we installed Meter Group Teros soil sensors from 0- to 180-cm depths at 18 different locations in three pistachio blocks of 'Kerman' on PG1 rootstock, irrigated with saline water in Grassland Bypass Authority in Firebaugh CA since 2002, 2009 and 2011, respectively. Our objective was to use these sensors to determine the efficacy of applying non-saline water for reclaiming the saline soils supporting PG1 rootstock pistachios. With our sensors, we obtained real-time data on changes in soil EC and soil moisture at different depths on saline soils receiving saline and non-saline irrigation water, respectively. In addition, we measured biochemical responses in leaves, scions, shoots and in tree core samples collected from trees in selected planting blocks. Most field efforts were not hindered by the invasion of the COVID-19 virus, but mandatory lockdown measures hindered analyses performed in the laboratories at CSU East Bay and Fresno, and USDA-ARS. In the 2020 growing season, analytical results from soils, leaves and tree cores are in progress. Based upon previous analyses, soil salinity and B concentrations have ranged from 8.5 to 11.1 dS/m and 11 to 13 mg B L^{-1} at 0-30, 30-90, and 90-180 cm for 2002 planting, from 7.5 to 8.1 dS/m and 9 to 11 mg B L^{-1} for 2009 planting, and from 6.1 to 7.3 dS/m and 8-10 mg B L^{-1} for 2011 planting. Saline irrigation water averaged 5 dS/m and 6 mg B L^{-1} and non-saline water contained <1 dS/m and <1 mg B L^{-1}. Irrigation water of both saline and non-saline quality occurred respectively within our test sub-blocks established within each planting of 2002, 2009 and 2011, respectively. Our test sub-blocks consisted of 85 trees per each saline and non-saline irrigation treatment (a total of 170 trees), respectively. Water was applied via micro-sprayer, and irrigation scheduling and amounts were determined by JM Lord Consulting. Our soil sensors recorded continuous "real time" data every 15 minutes for changes in soil salinity and soil moisture at different depths. Per data collected from soil sensors, the saline irrigated sub-blocks generally increased in soil EC from 0-90 cm, while soil EC decreased at the 30 and 90 cm depths and had no change at 180 cm with non-saline irrigation (See Figures below on the reclamation effects of non-saline irrigation on soil EC in plantings of 2002, 2009 and 2011). Analyses in progress show that soil EC, B, Cl and Na levels were lower from 0-45 cm with non-saline irrigation compared to saline irrigation. Leaching primarily occurred from 0-45 and thus salt levels and ion concentrations were higher from 45-90 cm. Preliminary tree Na analyses showed highest concentrations in 2002 planting block (mg/kg DM): leaves (5200)>tree core (~1501)> scion (1200)> shoot (1195).







<u>Conclusion:</u>

The results of 2020 effectively demonstrate the use of soil sensors to 'real time' monitor the reclamation of saline soils with the introduction of non-saline water applied via microjet irrigation. These initial observations are defining a beneficial strategy that growers can utilize for gently reclaiming saline soils in pistachio orchards on a PG1 rootstock previously irrigated with poor quality water. Continued support for this reclamation project by Palmer McCoy, the director of Grassland Bypass Authority, provides pistachio growers in Central California's Westside Valley with a practical strategy to sustain pistachio production under saline conditions.

Pistachio Improvement Program

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

Introduction

The goal of this continuing project is to re-establish 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 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

Seedling block establishment. The first set of PIP scion breeding crosses (Table 1) was performed in March 2019, and seeds were harvested in August 2019 and germinated in the greenhouse in January 2020. Leaf tissue was genotyped with a marker linked to the sex-determination locus (Figure 1A). Many of the predicted males from each family were culled. In total, 709 seedlings were genotyped and 460 seedlings were planted, of which 350 were predicted females and 110 were predicted males. Planting density was high, at 4' and 16' within and between rows respectively, or ~700 trees/acre. Forty-six seedlings did not survive and were replaced with clonal *P. vera* individuals from a low-chill Greek source. These clonal *P. veras* will allow us to assess the repeatability of our phenotyping.

Table 1. 2019 PIP crosses planted in Spring 2020.

Family	Female	Male	Females planted	Males tested	Males planted
P19-001	Gumdrop	Zarand	115	110	46
P19-002	Gumdrop	Tejon	96	95	40
P19-003	GoldenHills	Zarand	6	6	2
P19-004	GoldenHills	Tejon	0	0	0
P19-005	GoldenHills	OP	39	28	7
P19-006	WPIE_17_1	Zarand	1	1	1
P19-007	WPIE_17_1	Tejon	5	1	1
P19-008	WPIE_17_1	Randy	5	1	1
P19-009	WPIE_19_7	Tejon1	23	24	6
P19-010	WPIE_19_7	Tejon2	23	24	6
P19-011	Aegina	OP	37	69	0
		Totals:	350	359	110

Establishment and maintenance of germplasm blocks. We continued budding into the new germplasm blocks in Davis and Winters. An additional three rows of seedling UCB-1 rootstocks were added to the Winters site in Fall 2019. These trees have since been budded with diverse material from the USDA collection in summer 2020 with a >90% success rate. The USDA *Pistacia* germplasm collection was hedged for the first time in summer 2020, and this appears to have been successful in rejuvenating the collection to provide better shoot growth for future breeding, propagation, and phenotyping.

Scion breeding crosses. The Famoso site was used in Spring 2020 to perform controlled crosses in a half-diallel design using Golden Hills, Lost Hills, and B15-69 (mother of Gumdrop) as females crossed to six early-flowering males.

Establishing a clonal rootstock trial in the Sacramento Valley. A commercial nursery provided 240 finished trees: 60 Golden Hills on UCB-1 clonal, 60 Golden Hills on Platinum, 60 Lost Hills on UCB-1 clonal, and 60 Lost Hills on Platinum. The 120 Golden Hills trees on clonal rootstocks were planted into a 4 row x 30 tree block in the middle of a Golden Hills orchard on seedling UCB-1 rootstock. The 120 Lost Hills trees on clonal rootstock were similarly planted into a Lost Hills on seedling UCB-1 orchard. Within each orchard, three replicate 20-tree blocks (2 row x 10 tree) of each clonal rootstock were planted. This trialwill enable comparison of all combinations of two scions and three rootstocks at a site easily accessible from UC Davis.

Embryogenic cultures: Attempts were made to generate embryogenic callus from seeds of three different *P. vera* cultivars (Kerman, Aegina, Damghan) at various stages of development using two different media types (auxin- and cytokinin-based). These initial attempts were not successful. The establishment of embryogenic cultures is an important pre-requisite for the development of gene-edited pistachio scion varieties.

Genetics of blanking: P. vera seeds were collected in Fall 2020 for genetic analysis of normal (filled) and blank kernels. The objective is to search for systematic differences in the genetic constitution of male and female gametes that gave rise to blank and filled kernels. We will also test the common assumption that blank kernels represent diploid (heterozygous) embryos rather than unfertilized haploid (homozygous) female gametophytes.

Deployment of markers for salinity tolerance: We developed markers for two salinity tolerance QTL previously discovered in a greenhouse screen of UCB-1 seedlings: psal32-1 in the *P. integerrima* genome, and psal9-2 in the *P. atlantica* genome (Figure 1B-C). Marker results were used to sort UCB-1 seedlings into four groups before planting into a high-chloride site at a commercial nursery (good-good, good-bad, bad-good, bad-bad) depending on whether they carried the good or the bad allele at each QTL.

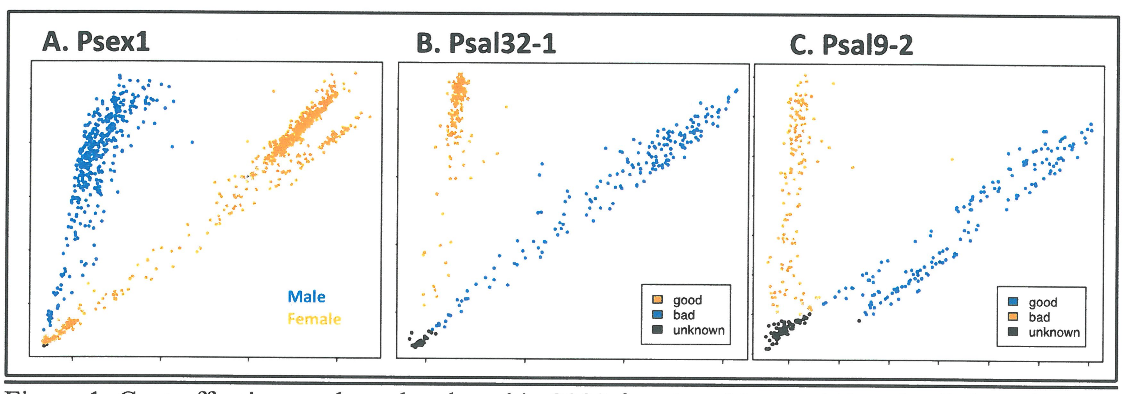


Figure 1. Cost-effective markers developed in 2020 for pistachio sex (A) and salinity tolerance (B-C).

Conclusions

Generating seed from controlled crosses in pistachio is highly efficient, and we now have the ability to cull males using marker data. Critical future considerations for the efficiency of the scion breeding program include the cost of land rental, the maximum planting density at which superior seedlings can still be identified for replicated testing, and perhaps most critically the efficiency and throughput of phenotyping for traits of interest including yield, disease and pest resistance, and nut quality.

Phenotypic and Genomic Diversity of UCB-1 Subclones

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Introduction

Clonal UCB-1 pistachio rootstock has been preferred by growers over seedling rootstocks to produce more uniform, vigorous, and higher yielding orchards. Recently, problems appeared in orchards grafted to clonal UCB-1 rootstock. Stunting, bark overgrowths at the nodes, abnormal growth and cracking at some graft unions became known as Pistachio Bushy Top Syndrome (PBTS). Some researchers have suggested that these symptoms may be caused by *Rhodococcus faciens*, but could also result from bud sporting that occurred in vitro. We previously obtained subclonal shoot culture lines of the original clonal UCB-1 from three California commercial labs where some of the resulting plants reportedly exhibited PBTS symptoms in the field.

Results and Discussion

During the previous three years this project, we acquired and multiplied plant material from three major California rootstock nurseries that were previously selling the UCB-1 rootstock colloquially known as "Duarte Old Clone 1". All this plant material was tested and proved match the genetic profile of Duarte Old Clone 1, suggesting all of our materials are subclones derived from the same original plant. However, some of these subclones are thought to display PBTS symptoms or other "off" phenotypes. Representative samples from each subclone were screened and all tested negative for the presence of *Rhodococcus* isolates. Ten different clonal lines have been multiplied and planted into the field to test if any subclonal lines exhibit PBTS symptoms.

These ten subclones were planted in pairs among 4 different replicated blocks in a randomized complete block design. Also included are seedling UCB-1 from seeds obtained from FPS and germinated in 2020 to match the age and size of the clonal material at planting. With 8 of each of the 10 subclonal lines for a total of 80 clonal trees, and 22 seedling trees, the planting consists of 102 UCB-1 trees. (Fig 1.)

We continue to maintain and propagate trees from subclonal lines such that any trees that fail due to disaster, disease, or otherwise can be replaced. This planting will allow access to all of the trees for measurements to compare and contrast any discernible differences. A further Spring 2021 planting at Wolfskill will add replication to this planting.

Conclusion

Noticeable *in vitro* variations among subclones were reported last year. However, it is critical to remember that *in vitro* traits do not always correlate to field characteristics which necessitated the planting of this study at UC Davis. These trees are a public resource for members of the pistachio community to make their own observations and measure whether differences among subclones can be detected.

Figure 1. Map of Davis planting from October 2020

DAVIS- NORTH

tree	row1	row2	
51	seedling 1	seedling 2	
50	seedling 3	seedling 4	
49	clone 3	clone 3	
48	clone 6	clone 6	
47	clone 8	clone 8	
46	clone 1	clone 1	
45	clone 9	clone 9	**rep4**
44	clone 4	clone 4	
43	clone 10	clone 10	
42	clone 7	clone 7	
41	clone 5	clone 5	
40	clone 2	clone 2	
39	seedling 5	seedling 6	
38	seedling 7	seedling 8	
37	clone 1	clone 1	
36	clone 10	clone 10	
35	clone 3	clone 3	
34	clone 8	clone 8	
33	clone 5	clone 5	**rep3**
32	clone 2	clone 2	
31	clone 7	clone 7	
30	clone 6	clone 6	
29	clone 9	clone 9	
28	clone 4	clone 4	
27	seedling 9	seedling 10	
26	seedling 11	seedling 12	
25	seedling 13	seedling 14	
24	clone 2	clone 2	
23	clone 4	clone 4	
22	clone 6	clone 6	
21	clone 8	clone 8	
20	clone 10	clone 10	
19	clone 3	clone 3	**rep2**
18	clone 1	clone 1	
17	clone 5	clone 5	
16	clone 9	clone 9	
15	clone 7	clone 7	
14	seedling 15	seedling 16	
13	seedling 17	seedling 18	
12	clone 10	clone 10	
11	clone 9	clone 9	
10	clone 8	clone 8	
9	clone 7	clone 7	
8	clone 6	clone 6	
7	clone 5	clone 5	**rep1**
6	clone 4	clone 4	
5	clone 3	clone 3	
4	clone 2	clone 2	
3	clone 1	clone 1	
2	seedling 19	seedling 20	
1	seedling 21	seedling 22	

Integrated Genomic and Conventional Approaches to Pistachio Rootstock Development

Authors: **Pat J. Brown,** Associate Professor, Plant Sciences, UC Davis; **John E. Preece,** Research Leader, USDA, National Clonal Germplasm Repository, Davis; **Malli Aradhya**, Research Geneticist, USDA, National Clonal Germplasm Repository, Davis; **Franklin Lewis**, Assistant Specialist, Plant Sciences, UC Davis; **Florent Trouillas,** Assistant Cooperative Extension Specialist, Plant Pathology, KARE, UC Davis; and **Themis J. Michailides**, Plant Pathologist, Plant Pathology, KARE, UC Davis.

Introduction

The US pistachio industry currently relies on seedlings and clonal selections from only four parents A more diverse array of rootstocks would benefit growers, nurseries, and the pistachio industry as a whole by providing tailored solutions to specific local challenges. The 2020-2021 goals of this project were: 1) to screen seedlings and clones of UCB-1 and novel rootstock breeding populations for resistance to **Phytophthora, Verticillium,** and **salinity**; 2) to generate new seedling diversity and propagate superior clones for further testing; and 3) to discover and deploy markers predictive of superior performance.

Results and Discussion

1A. Phytophthora resistance evaluation. The relative resistance of diverse *Pistacia* clones to Phytophthora crown rot was evaluated. All of these **with the exception of UCB-1 were created** earlier in this project under PI Aradhya. Stems of nine 1-year-old experimental rootstocks (PXK, VA11, VA7, IK 39, VA, VT16, VA15, VA17, and **UCB-1**) were wound inoculated using mycelium plugs of *Phytophthora niederhauserii* isolate KARE465 and 8 plant individuals (8 repetitions) per rootstock type. Plants were inoculated on May 8, 2020 and maintained in a greenhouse at the Kearney Agricultural Research and Extension Center. After 6 months, the length of vascular discoloration produced in the inoculated stems **was** measured and compared among the various **rootstocks** to assess differences in hybrids' susceptibility/resistance to Phytophthora. Results showed significant differences (P<0.0001) in rootstock susceptibility with average lesions length ranging from 12.5 mm (VA-17 and UCB1) to 29.4 mm (PXK) (**Fig 1**). Additional experiments are on-going to evaluate the susceptibility/resistance of UCB1 seedlings to Phytophthora root and crown rot. 200 new UCB-1 seedlings were acquired and are being maintained at KARE following inoculation of roots (using inoculated rice seeds) and stems (using mycelium plugs). In addition, UCB-1 seedling trees from a commercial pistachio orchard suffering severe Phytophthora crown rot were used to phenotype them for resistance and/or tolerance to Phytophthora.

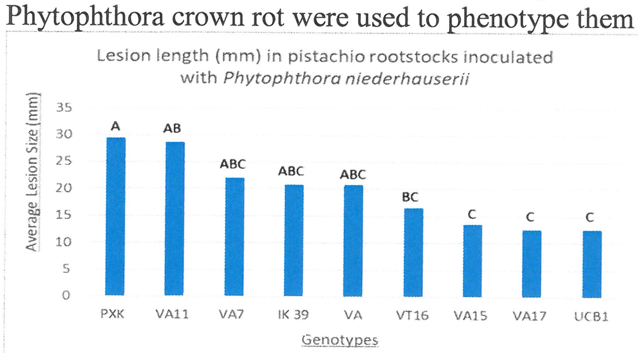

Fig 1. Average lesion length produced in various interspecific hybrid rootstock clones, 6 months after inoculation with *Phytophthora niederhaurserii.*

1B. Verticillium resistance evaluation. The same hybrids as above were also tested for Verticillium susceptibility. Although these plants were older and larger than what is usually used for Verticillium susceptibility studies, they were inoculated with a dense (10^6 conidia /ml) suspension of a virulent *Verticillium dahliae* isolate (11-L83) isolated originally from a diseased pistachio. Each stem was wounded with a scalpel and a drop (50 ul) of the conidia suspension was placed in the wound. None of the plants were killed, indicating that the inoculation of these plants was not successful. Another set hybrids (VA17, IK36, VT16, VA, PxK, VA7, VA11, and VA15) were delivered by Dr. Aradhya on 10 July 2019. Because inoculations in the hot summer are not very successful, we inoculated these plants in the spring of 2020, but so far we have seen no symptoms. In early 2020, we received 20 each UCB1 clonal and Platinum rootstock plants provided by Sierra Gold nursery. These plants were about 2 feet tall. Although, again the age and size of these trees was not suitable for Vert inoculation and infection, we placed these plants in our greenhouse to keep them cool. On 25 June 2020, 15 plants each of UCB1 clonal and Platinum were inoculated with a dense spore suspension as shown above by placing a drop of inoculum on a wound on the stem of each plant. Five non-inoculated control trees were treated similarly using sterile water instead of inoculum. Five each of the rootstocks inoculated with the spore suspension were also inoculated with a very dense microsclerotia suspension (> 10^4 microsclerotia/ml) by pouring 10 ml in each pot after wounding the roots of each plant with a knife (5 cuts per pot). All inoculated and control plants were checked on 13 October 2020. The only plants that showed putative symptoms of Vert infection were 4 out of the 5 Platinum plants inoculated both by the conidial and microsclerotial suspension. It is suggested that in future inoculations with Verticillium, very young plants should be inoculated, as it was originally done to determine the Vert resistance in the UCB1 seedlings.

1C. Salinity resistance evaluation. Two greenhouse salt trials were conducted in summer 2020. First, 12 replicates each of 8 commercial clones (96 total plants) were provided by a commercial nursery and evenly split between salt (200 mM NaCl) and control treatments. The Drakakaki lab performed microscopy on root tips, and the Brown lab is performing sodium and chloride analysis on leaf samples. Second, 288 “UCB-2s” (open-pollinated seedlings from UCB-1 mother trees), consisting of 32 seedlings each from 9 mother trees, were all subjected to salt treatment (200 mM NaCl). Genotype data have been obtained and sodium and chloride analysis of leaf samples is currently in progress.

2A. Generate new seedling diversity. In spring 2020 we germinated seedlings of ~950 UCB-1, ~400 UCB-2, ~200 *P. khinjuk* X *P.integerrima* and ~100 *P. integerrima* X *P. khinjuk*. These last two populations resulted from crosses made earlier in this project under PI Aradhya. Subsets of these individuals were delivered to Kearney for pathology testing and kept at Davis for salinity screening, and tissue from all seedlings has been collected and DNA has been extracted for future genotyping and analysis. In fall 2020, seeds were harvested from new crosses (predominantly *P. integerrima x atlantica* and *P. integerrima x P. vera*) performed in the spring. Maturing seeds from breeding crosses were covered by plastic mesh bags and suffered some minor predation from birds, so we will test lightweight wire mesh bags in Spring 2021. To test whether the removal of the fleshy seed coat helps germination of pistachio species, we compared germination of 100 *P. khinjuk* X *P. integerrima* seed that were cleaned, dried, and stored as outlined by Foundation Plant Services protocols for UCB-1 seeds, versus 100 of these seeds that were stored and dried without the fleshy exterior being removed. The seeds stored without removal of the fleshy seed coat yielded 0% germination under our standard germination protocol.

2B. Propagate superior clones for further testing. Previous salt trials of UCB-1 seedlings yielded 4 high performance clones which are currently being multiplied and rooted. Forty shoots of each clone were provided to a commercial nursery under test agreement for a spring 2021 field trial at a high-chloride site.

3. Discover and deploy markers predictive of superior rootstock performance. DNA was extracted from UCB-1 seedlings in a commercial orchard suffering from severe, but variable, Phytophthora crown rot. Marker-trait analysis will determine whether there is genetic variation in susceptibility.

Conclusions: Certain *P. vera x P. atlantica* (“VA”) clones showed Phytophthora resistance comparable to clonal UCB-1. There is some indication that Platinum may be more susceptible to Verticillium than UCB-1 under some circumstances. Very young plants should be used for future Vert inoculations. Wire mesh bags are necessary to protect maturing Pistacia seeds from bird predation. Removal of the fleshy seed coat improves germination.

Identification of superior UCB-1 rootstocks using DNA markers

Authors: **Richard Michelmore**, Director, The Genome Center, UC Davis; **John Preece**, Research Leader, USDA, National Clonal Germplasm Repository (NCGP), Davis; **Deborah Golino**, Director, Foundation Plant Services (FPS), UC Davis; **Ewelina Jacygrad**, Assistant Project Scientist, The Genome Center, UC Davis.
Collaborators: **Pat J. Brown**, Associate Professor & Nut Crops Breeder, Department of Plant Sciences, UC Davis; **Maher Al Rwahnih**, Lab Director, Foundation Plant Services, UC Davis; **Bob Beede**, UC Farm Advisor, Emeritus.

Introduction

The UCB-1 hybrid is the main pistachio rootstock used in the US and is produced from controlled crosses between specific clones of *Pistacia atlantica* (female) and *P. integerrima* (male). The majority of pistachio rootstocks are currently produced from seed and are genetically variable due to segregation in the gametes of each highly heterozygous parent. This results in differences in morphology among individual rootstocks and in performance in the field. For over 20 years, variation has been observed in orchards planted on seedling UCB-1 rootstock. Reduced vigor and stunting of some trees are of particular concern and stunted tress are often rogued out and replaced. Stunting is a significant economic problem and results in decreased nut yield and/or quality.

In early 2013, 961 UCB-1 seedlings were planted in an experimental orchard at UC Davis. In a collaboration between FPS and NCGP, measurements of phenotypic variability among these F_1 trees have been made annually since January 2014, including measurements of variation in growth, branching, sex, and active growth period. We have also genotyped all of these trees by sequencing. Simultaneously, we have been characterizing the genome of UCB-1 and its parents in order to dissect the causes leading to differences in vigor in commercial orchards that are planted with UCB-1 seedling rootstocks. We are separating out the effects of environment and genetics on vigor and providing molecular markers to allow the early identification of inferior seedlings that need rogueing prior to planting in orchards.

Results and Discussion

Progress has been significantly impacted by Covid-19. The lab was closed mid-March for three months. Analysis of existing datasets continued with lab members working from home but several parts of this project require activities in the lab. Lab activities have gradually ramped up since June but only 50% occupancy is allowed to ensure social distancing. No travel has been allowed for additional sampling.

In 2020, we collected another round of multi-trait phenotyping data in the experimental orchard. These data confirm that tree height and trunk caliper in early years are poor predictors of tree size in later years. Trunk caliper, tree height, canopy volume were highly correlated indicating segregation of a locus for general vigor. We recorded third 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.

Since 2013, we have sequenced and measured rootstock and scion circumferences from 2,009 UCB-1 and 197 PG I trees from ten commercial orchards in the Central Valley. In 2020, we sequenced 368 trees from two more orchards that had been sampled in the Fall of 2019 to increase the age range of orchards studied. The rootstocks in these orchards now range in age from 3 to 18 years, are grafted with either *P. vera* cvs. Kerman or Golden Hills, and are located from Merced to Bakersfield. Rootstock and scion circumference were significantly correlated in all the commercial orchards.

From March 2018, we began 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 2020, we captured multispectral drone imagery for ten of the sampled commercial orchards. We are currently processing this data. The data permitted analysis of canopy volume, canopy perimeter (as an indication of crown irregularity), and tree height. The drone-collected data of tree height and canopy volume were strongly correlated with field measurements. Calculations of tree height and crown diameter was more time efficient than manual collection. These data illustrate the potential benefits of timely and detailed UAV imagery to help agriculture managers estimate commercial yields more efficiently than traditional field measurements.

We have generated chromosome-scale, high quality, genome assemblies for *Pistacia atlantica*, *P. integerrima*, and *P. vera* cv. Siirt (in collaboration with S. Kafkas, Cukurova University, Turkey) as well as generated a draft assembly for *P. vera* cv. Kerman. These genome sequences are finalized and are being deposited in public databases. We also developed high resolution genetic maps for *P. atlantica* and *P. integerrima* using genotyping by sequencing data. These maps confirmed the accuracy of our genome assemblies, as well as refined the location of the genomic regions determining trunk caliper. Data on maturity collected this past year allowed us to identify a chromosome region determining shorter juvenility. These maps also identified a large region of the sex chromosome supporting a ZW-sex determination system. One of the main goals this year was to develop molecular markers for vigor, as reflected by tree size, and shortened juvenility. This has been delayed due to lack of lab access. We are currently testing oligonucleotide primers for PCR-based molecular markers for these traits.

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 our multi-year, multi-trait phenotyping of the experimental orchard in Davis, as well as sequenced four hundred additional trees from young (3 years old) and old (18 years old) commercial orchards. With collaborators, we conducted a second set of multispectral aerial imaging surveys of the sampled orchards. This data will accelerate and enhance our phenotyping efforts. We have finalized chromosome-scale genome assemblies for three *Pistacia* species: *P. atlantica*, *P. integerrima*, and *P. vera* cvs. Kerman and Siirt and have deployed 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. We are using these data to separate out the effects of environment and genetics on stunting, as well as better understand the growth characteristics of UCB-1 and how it relates to canopy volume.

Nurseries commonly rogue as many as 10 to 15% of their UCB-1 seedlings based on early growth parameters, such as tree height and other visual clues. This selection is being made on seedlings that are only a few weeks to months old and before planting in commercial orchards. Our data show that such traits in very young trees are poor predictors of size and vigor in older trees and therefore seedling selection based on phenotypic characteristics is unlikely to be effective. Furthermore, we have observed a bimodal size distribution of trees in all commercial orchards sampled. This reflects a genetic basis for the stunting phenomenon observed by growers. We have identified two chromosomal regions that determine trunk caliper and explain this size distribution in commercial orchards. We are currently testing molecular markers derived from these regions for use as selection tools. 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. It will also allow the selection of vigorous genotypes for clonal propagation.

Evaluation of Pistachio Rootstock-Breeding Selections, 2020-21

Authors: Craig E. Kallsen, UCCE Citrus and Pistachio Farm advisor, Kern County and **Dan E. Parfitt**, Emeritus, Pomologist-AES, University of California, 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. All of these trials are now budded to Kerman, Gumdrop, Golden Hills, Lost Hills or experimental scion selections, the earliest in the fall of 2011, the latest in 2019. The objectives of the rootstock evaluation is 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 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 UCB1 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 distinct, often from crosses with different parents, 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 PVI-2 or PVI-3.

Results and Discussion

The oldest existing rootstock trial consists of Golden Hills grafted to seedling Endeavor-1 and UCB1 seedling rootstocks in the fall of 2011. Unlike the Endeavor-2 rootstocks, the Endeavor-1 rootstocks produce a vigorous full-size tree with the scion and rootstock having similar circumferences. In this trial, planted within a commercial orchard in western Kern County, both the soil and irrigation water are high in boron (up to 5 ppm or more in both cases). Almost from planting, the canopy of Golden Hills on UCB1 rootstock demonstrated large areas of leaf-tissue necrosis with early leaf drop by mid-August, with few or no leaf symptoms on scions grafted to the novel Endeavor-1 rootstocks. Replicated leaf-tissue analyses in late July or August were undertaken in 2017 and yearly thereafter, and demonstrated that leaves of Golden Hills on the UCB1 scion had more than twice the boron concentration of the experimental rootstocks (1767 vs 714 ppm). Over the past four harvests, cumulative edible yields between UCB1 and the experimental rootstocks in this high boron soil have not been significantly different even though the Golden Hills on UCB1 has shown substantial scorching and defoliation every year. Although not cumulatively different, yields on UCB1 rootstocks have consistently tracked below of the Endeavor-2 rootstocks annually, with cumulative yields of 4426 lbs./acre for UCB1 compared to 5459 lbs./acre of edible yield for Endeavor-1 (see Figure 1).

Additional evaluations of Endeavor-1 seedlings and some clones, are being made in replicated trials established using large pots, using both grafted seedling and cloned experimental and commercial rootstocks, to further document differences in boron uptake made in the field trials. Selections have been made from among Endeavor-1 seedlings and provided, under a U.C. Test Agreement, to a commercial nursery so that they may attempt to clone them using tissue culture. If these experimental rootstocks are amenable to cloning, larger numbers of them should become available for testing in large-scale, randomized and replicated trials.

The main objective of the trials with the Endeavor-2 seedling rootstock is to identify individual trees that appear to have the desired characteristics of short stature, moderate vigor, high yield per canopy volume and resistance to root rot. Due to the nature of the genetic crosses, the best of the individual rootstocks from these trials will have to be selected and cloned to produce viable commercial candidates for further testing in scientific trials. The earliest Endeavor-2 rootstock trial to be grafted and to produce yield has been followed the most closely. The scion in this trial in Lost Hills. Yield and nut-quality characteristics of the better performing individual trees in this trial were measured in 2017, 2018 and 2020. In 2019, yield was estimated visually on the tree. These data were compared to UCB1 seedling controls planted within the same trial. Cumulative yield per tree over the past four years has been better for the selected Endeavor-2 rootstocks than for the UCB1 controls, although over the best two years, individual annual yields per tree have been similar. The Endeavor-2 experimental rootstocks, generally, produce a more compact, less vegetatively vigorous tree, with some selections that have produced higher cumulative yields per acre and per tree for its age than UCB1 to 8th leaf. Some selections, although dwarfing, have sufficient new shoot growth to support optimism for its ability to maintain adequate yields in the future. The performance, to date, for some of these experimental rootstocks, suggest that they may be amenable to planting at a much closer tree spacing with reduced future crowding and with an overall cumulative per acre yield greater than a wider space planting on UCB1. These less vigorous rootstocks may have a reduced pruning requirement per pound of nuts produced. Its smaller size may be easier to shake efficiently with less energy. These rootstocks demonstrate little suckering compared to UCB1 seedlings. One of these seedling-selection trials is located near Rosamond, east of the Sierra Mountains, and the rootstocks appear to have good cold tolerance.

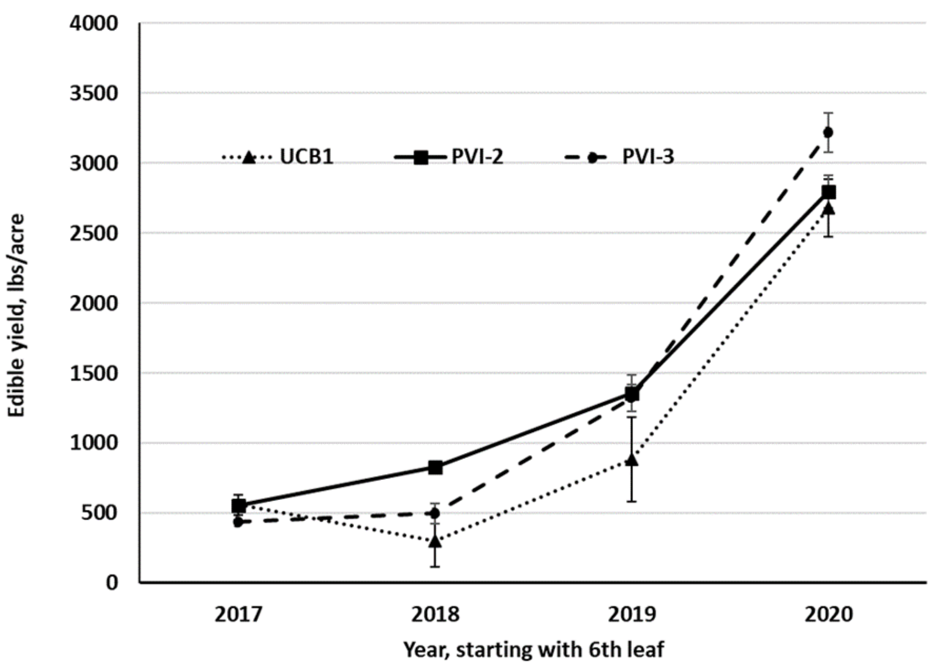

Figure 1. Effect of rootstock on edible yield of Golden Hills pistachio at the Lokern rootstock trial in Kern County from 2017-2020 (error bars represent ±2 SE of the mean)

Evaluation of Pistachio Scion-Breeding Selections, 2020-21

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

The original U.C. breeding program began with original crosses made in 1989 by Dr. Dan Parfitt and Joseph Maranto. Since this time, the program continued with the breeding and evaluation of novel scions, but also, as of 2009, experimental rootstocks (please see separate report for the rootstock summary). With the hiring of a walnut and pistachio breeder, Dr. Patrick Brown, Plant Science Dept. at U.C. Davis, this original program will focus on evaluating the many existing plant materials developed over the years and currently located in scientific trials. New breeding efforts in pistachio will be continued with the work of Dr. Brown. Interested, gracious and generous private cooperating growers have made many of these trials possible with their donations of land, labor, equipment, time and long-term use of land.

In 2020, we continued evaluation of twelve male and/or female advanced scion-selection trials. These trials were planted from 2007 to 2019. Five of these trials have the objective of identifying male advanced selections, several from the precocious seedling trial planted in 2008, which demonstrated precocious, robust flower development and close bloom synchrony with Kerman, Golden Hills or Gumdrop in years with insufficient winter chill/excessive winter heat. Two of these five trials are located near Inyokern, east of the Sierra Mountains in the high desert. Several male selections in some of these trials have demonstrated better bloom synchrony and precocity with Kerman than existing cultivars. Final selection of one or two of these males, as a co-pollinizer with Famoso for Kerman (to completely replace 'Peters') is scheduled to occur after bloom in 2021 after pollen germination tests are completed. The other seven scion selection trials compare novel advanced female scion selections with existing commercial cultivars such as Gumdrop, Golden Hills, Lost Hills or Kerman for yield, nut quality characteristics, bloom and harvest timing. These trials are located in areas with varying amounts of winter chill. In 2020, we continued to evaluate the cultivar Gumdrop in the oldest trial containing this U.C. cultivar and in a demonstration trial within a Lost Hills orchard established in 2014.

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 showed nut characteristics that, based on the past work of other researchers, may make them less susceptible to navel orangeworm damage. The nuts of these individual trees showed these same potentially useful characteristics at harvest in 2019 and 2020. In the summer of 2019, these trees were cloned by grafting buds on Platinum®, UCB1 seedling and/or experimental seedling rootstocks into two separate randomized and replicated experiments. One of these experiments was established within a mature advanced-selection trial that is providing excellent habitat for navel orangeworm and should continue to do so once these experimental selections begin producing nuts.

The evaluation period for the advanced selection 'KB25-78' has been longest at the Jasmine trial. The yield characteristics demonstrated by this selection in this trial have been different from those seen in other selections and cultivars over the years. The Jasmine trial was planted in 2010 and is located in the 'citrus belt' of Kern County where winter chilling can be inadequate. The clean, open, inshell split nut percentage of 'KB25-78' has averaged a modest 70 percent over the past 5 years, similar to that of Kerman. However, despite this relatively low split-nut percentage, cumulative edible yield (also called payable yield) is 3500 lbs. per acre or 23.7% higher than the next highest yielding cultivar Lost Hills (See Figure 1). The lower split inshell percentage of 'KB25-78' has been offset by its high production of harvestable material. A significant proportion of the harvested plant material, is edible yield, and if not open, inshell split nuts, is edible closed shell at 12.8% on average over the past 5 years. 'KB25-78' has two traits that may account for this enhanced yield performance. It blooms early, a week before Golden

Hills, and, usually, harvests later with Kerman, so it takes full advantage of the available growing season. The expansion of the canopy and trunk circumference is faster than other of our existing cultivars. In addition, it produces many branch spurs, especially early in its lifecycle, which provide locations for more flower buds. While these results are interesting, the results pose a couple of questions for this selection. First, would growers and processors be interested in producing pistachios using a tree that has a moderate split nut percentage but a higher potential to produce edible yield? The second questions relates to the observation that this tree grows faster than other existing cultivars. Will the yield advantage continue once full canopy cover is achieved and will the superior spur production continue once these trees are mature? One reason we continue to evaluate ‘KB25-78’ is that split percentages in this trial have been lower than is typical for all of the existing cultivars in this trial. However, results from a trial we have in Arizona, near Bowie, where chilling is excellent, show higher split percentages than at Jasmine. We will continue to evaluate this selection at Jasmin and in Arizona, and begin to collect data from an additional trial just coming into bearing on the valley floor in the southern San Joaquin Valley. ‘KB25-78’ appears to be growing faster at this site than the other selections in this trial, as well.

We continued to evaluate other seedling progeny selections from our breeding program from parents that have displayed greater tolerance to an inadequate winter rest period (i.e. less chilling), have displayed fewer leaf-canopy and flowering symptoms of inadequate chilling themselves, flower very early or have genetics that should reduce their chilling requirement. We identified a number of new candidates in 2019 from our existing progeny selection trials that we grafted into existing rootstocks in a trial first planted in the Coachella Valley of California in 2017. In 2018, we doubled the number of plant entries. Chill at this site is dependably inadequate for current pistachio cultivars. 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 the trees are old enough to bloom.

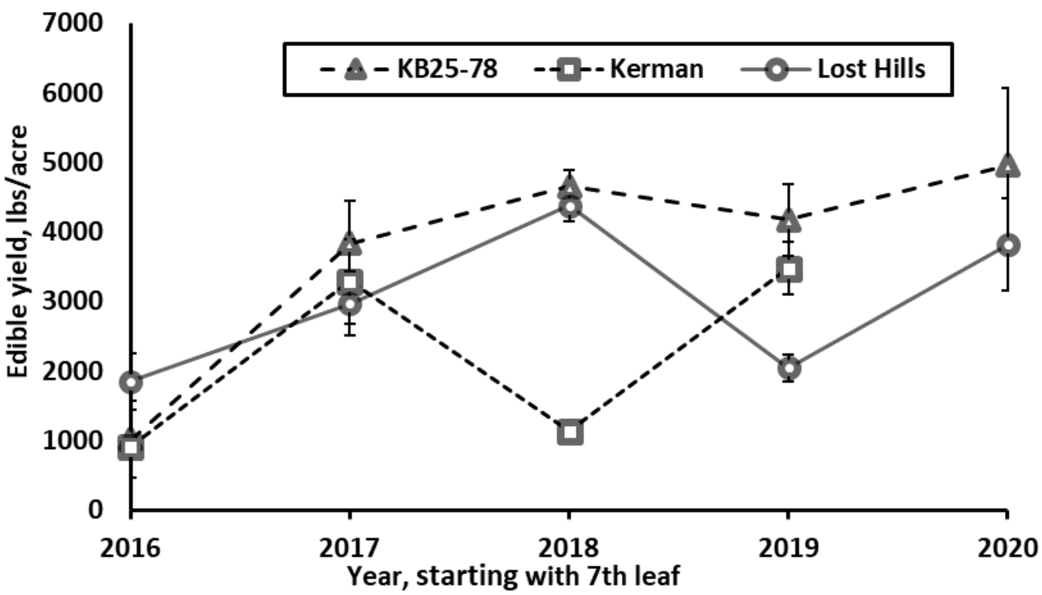

Figure 1. **Differences among the pistachio varieties Kerman, Lost Hills and the selection KB25-78 in annual edible yield in the Jasmine Trial. 2016-2020 (Kerman was not harvested in 2020 due to consistently lower yields than Lost Hills and KB25-78). Error bars represent ± 2 standard errors of the mean.**

Physiological and biochemical factors in pistachio shell split and hull maturity as a function of temperature and tree physiology

Authors: Barbara Blanco-Ulate, Assistant Professor, Plant Sciences, UC Davis; **Giulia Marino**, CE Specialist, Plant Sciences, UC Davis; **Selina Wang**, CE Specialist, Food Science and Technology, UC Davis; **Brian Bailey**, Assistant Professor, Plant Sciences, UC Davis.

Introduction

The impact of temperature and tree physiology on shell split and hull maturity is not well understood in pistachio, even when these developmental processes are directly linked to nut quality. Pistachios that fail to split have a lower value. High percentages of shell split can be achieved when the harvest is delayed until the maximum number of nuts display hull separation (or hull color change). However, if the hull starts deteriorating, there is a higher chance that the nut becomes susceptible to pests. Therefore, extending harvest time needs to be balanced out against the potential risk of insect damage. *Understanding how temperature and source/sink relationships affect shell split is required for better orchard management.* Once the pistachio nut has reached maturity (kernel growth is completed), the hull initiates senescence leading to the deterioration of the tissues and sometimes hull tattering. Senescing hulls are more prompt to fungal decay, favor the entry of insects like the navel orange worm (NOW) to the kernel, and cause shell staining (cosmetic defect). *Knowledge on how the rate of hull ripening and deterioration occurs as a function of changing temperatures and tree physiology is critical to predicting the potential risk of insect infestation late in the season and the best time for harvest.*

Our work addresses how alterations in temperature and branch physiology can differently influence the growth of the whole nut or just the kernel and how they impact hull maturity, ripening, and deterioration. ***The overarching goal of this project is to establish effective orchard management practices for improving shell split and preventing insect damage of pistachio nuts.***

Results and Discussion

The study was conducted weekly, starting at 15 heat units (April 19, 2020) and concluding at 2,577 HU (September 17, 2020), in branches of 'Kerman' trees grafted on UCBI at a commercial orchard in Woodland, California. This period expanded from fruit set to commercial harvest (>70% shell split). Two independent experiments were established: *(1) temperature treatment (**Fig. 1**)* and *(2) a crop load with defoliation treatments (**Fig. 2**)*. In addition, untreated branches (n= 102) were monitored and sampled throughout the season to characterize the normal progression of nut development, compare to the treatments, and validate our phenological data from year 1. For all treatments, we measured photosynthesis of the branches, leaf area, nut number, nut and kernel growth (diameter, biomass), in addition to hull color, texture, and biochemical features (phenolics and volatiles), among others.

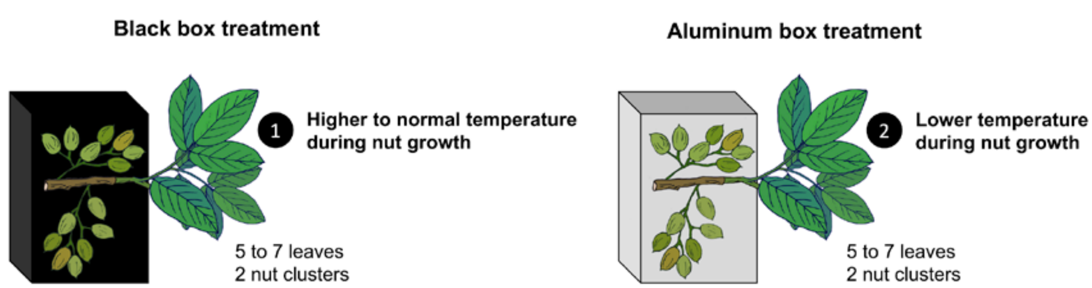

Fig. 1. Temperature treatments established during stage I of nut growth (15 to 520 heat units). Total of 12 branches per treatment. Nuts from black box-treated branches were bigger than those from aluminum box-treated branches, this significant difference was kept throughout the whole season.

At early nut development (stage I), fruit growth rate is driven by temperature and the available carbohydrate resources per nut, which in turn depend on the bearing status of the tree/branch in the previous season and the competition with other sinks. By performing the temperature and crop load treatments at this stage, we ensured to cause a strong effect on nut size and shell growth. Our preliminary data indicate that *higher temperatures (black box treatment) sensed by the branch during stage I significantly increased nut and shell size*. These treatments were performed in a limited number of branches to test the experimental setup, and thus, we did not obtain sufficient data to properly assess shell split or hull maturation. Because our results strongly suggest that the environmental temperature during stage I likely affects nut growth and development, we plan to conduct a follow-up study in year 3 of the project with a larger number of branches in commercial orchards located in two regions of California.

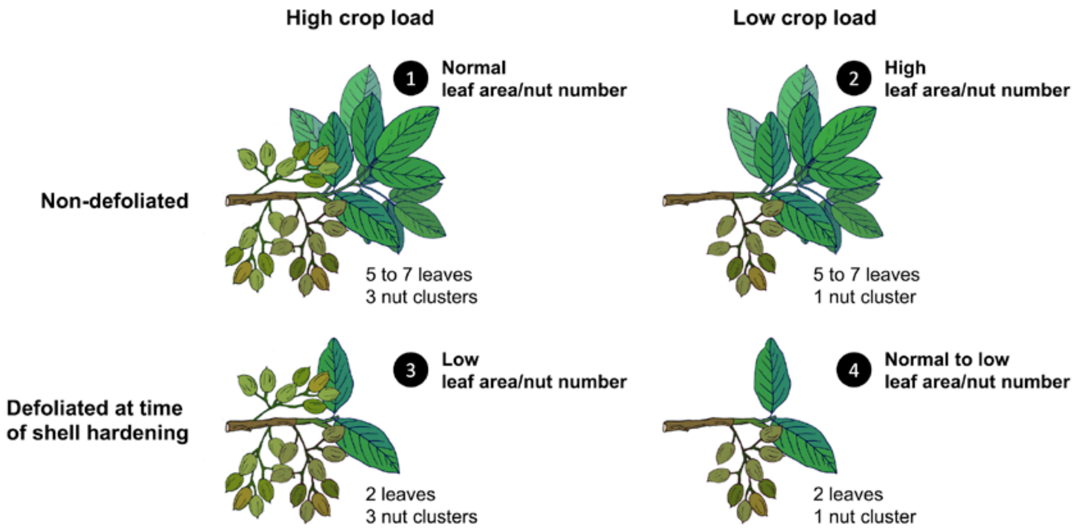

Fig. 2. Crop load treatments established early in stage I of nut growth (15 heat units) and subsequent defoliation in stage II (640 heat units). Total of 40 branches for each of the four treatments. Nuts from low crop load treatments (treatment 2 and 4) were bigger, and the kernels were smaller when the leaves were detached (treatments 3 and 4).

We observed that nuts from branches with high leaf area/nut number had significantly bigger kernels and apparently higher splitting percentage than branches with average to low leaf area/nut number, where the limitation of resources affected embryo growth resulting in smaller kernels and less splitting. We also determined that differences in carbon availability affected hull maturation. For example, the hulls from treatment 2 were softer than the other treatments and had higher levels of volatiles. We plan to validate these results, particularly those of shell split, with a larger sample size during year 3.

Conclusions

We are still analyzing the physiological and biochemical data to develop a mechanistic model of pistachio shell split and hull maturity as a function of temperature and tree physiology. We faced many challenges this year due to COVID-19, including a change of location for the study, reduced personnel, and limited access to lab space. Yet, we still obtained valuable datasets that not only confirm our results from year 1 but also provide new knowledge on how pistachios develop. To date, we have established reliable and novel methods to assess pistachio growth, we have identified biomarkers of nut maturity (hull firmness, nut volatile emissions, kernel color), and we are pinning down when and how specific physiological processes happen during pistachio development. The information generated in this project is groundbreaking and would provide the California pistachio industry with knowledge to develop tailored orchard practices and to ensure high competitiveness worldwide. Moreover, the materials and results from this project support complementary studies on pistachio nut quality and susceptibility to insect pests carried out by Dr. Drakakaki (UC Davis), Dr. Wilson (UC Riverside), and Dr. Burks (USDA).

Cracking the Black-box of dormancy in Pistachios: Tracking biochemical changes in inflorescence buds from dormancy to bud break

Authors: Gurreet Brar, Assistant Professor, Dept. Plant Science, CSU Fresno (PI); **Phoebe Gordon**, Orchard Systems Advisor, Madera & Merced Co., UCCE; **Faranak Hadavi,** Research Scientist, Dept. Plant Science, CSU Fresno; **Louise Ferguson**, CE Pomologist, Dept. Plant Sciences, UC Davis/UCCE (co-PI); **Masood Khezri**, Research Director, MR Research Institute, Fresno

Introduction

Insufficient chill accumulation during the dormant season might disturb some physiological processes and might proceed other processes even though the chilling requirement of the tree has not been fulfilled. However, few studies have focused on the metabolic characterization of buds during the dormancy period, therefore, the physiological and biochemical changes in this period are not well understood. Application of rest breaking agents is widely used to overcome dormancy and advance bud break. The most important dormancy breaking agents are horticultural oil, Dormex, KNO3, gibberellins and cytokinins, in particular the cytokinin analogue thidiazuron (TDZ) and benzyladenine (BA). Winter oil spraying has shown promise as rest breaking agents in different fruit trees and nuts. Preliminary reports have shown that winter oil application on pistachio trees can advance bud breaking when the winter chill accumulation has fallen short. In general, the response to dormancy breaking with horticultural oil is dependent upon timing and application concentration.

In recent years, growers are more interested in winter oil application and trees are usually sprayed in the period of Jan. 15 to Feb. 15. However, there is limited information around the merit of chill based oil spray timing and the physiological changes associated with the buds during dormant and bud swell stages. Therefore, the objective of this study was to determine appropriate oil spray timing in different locations in California on bloom timing, tree yield and yield components. Also, carbohydrate and nutrient changes in the bud and bark of pistachio shoots before and after spray as well as bud swell stage have been investigated.

This study was performed on female and male pistachio trees 'Kerman' and 'Peters' respectively, all on the UCB-1 rootstock. Three locations in California including North (Colusa County), Central (Madera County) and South-Western Fresno County (Cantua Creek) have been chosen and the Oil 440 (6% v/v) was applied during the winter time based on chill portion (CP) accumulation in all locations. 'ON' shoots with ≥ 4 buds and ≥ 3 inch were sampled before and after each spray time and at bud swell stage. Each site was visited regularly to determine the 50% bud swell stage and 80% full bloom stage. Flowering dates (from bud swell to full bloom), tree yield and the yield components (split nut, non-split nuts, blankness, etc.), total non-structural carbohydrates (soluble sugars and starch) and mineral nutrients (macro and micro nutrients) have been analyzed. In this study, the trends of non-structural carbohydrates and mineral nutrients in untreated control trees as well as each oil spraying date were investigated.

Results

Bloom

Results showed that location could significantly affect bloom date and period. In Cantua, all of the oil sprays advanced bud swell on female trees. Oil spray at CP55 and CP60 caused the best male and female overlap and late oil spray at CP60 could enhance bud swell as well as bloom window. Oil spray in Madera sites had little effect on blooming dates and periods.

Yield

In Cantua, oil spray at CP55 and CP60 could significantly increase the yield (60.4 and 50.4 lb/tree respectively) compared to untreated control (13.7 lb/tree). Also blank nuts at CP55 and CP60 were lower compared to untreated trees although that difference was not statistically significant. In Madera, although oil spray at CP55 showed the highest yield (33 lb/tree for CP55 and 20 lb/tree for control), the difference was not statistically significant. There was no difference in blank percentage among treatments. In Colusa, the effect of oil spray on yield was not significant.

Non-structural carbohydrates
The contents of soluble sugars were significantly reduced toward bud swell and the trends of soluble sugars in both bud and bark were almost similar. The content of starch in both bark and bud had a slight increase towards bud swell in untreated control trees however, the difference was not significant. At bud swell stage, the non-structural carbohydrates in the bark and bud of oil sprayed and untreated control trees were compared and it was shown that soluble sugars were reduced toward bud swell in both bud and bark while starch content in bark was increased, However, the starch content of bud was not significantly different among the treatments.

Macro and micro nutrients
Trends of macro and micronutrients in untreated control pistachio shoots in Cantua showed that phosphorus (P), potassium (K), sulfur (S) and zinc (Zn) consistently increased toward bud swell, while calcium (Ca) content was decreased. In the Cantua site, oil sprayed trees at CP55 had significantly lower zinc (Zn) at bud swell compared to untreated control trees. Magnesium (Mn) content increased significantly after the oil spray, however, at bud swell stage Mn was at the same level as before spray. Copper (Cu) for oil spray at CP55 showed a slight decrease in amount but this reduction was significant at bud swell stage. In the Madera site, trends of Nitrogen in untreated control pistachio shoots showed a consistency in N content in CP45, CP50, CP55 and then a significant drop at CP60, that was followed by an increase at bud swell stage. Ca showed a slight decrease in content towards bud swell. Chlorine (Cl) was the highest at bud swell stage. there was no significant difference in other macro and micro nutrients among the CPs. At bud swell stage, K content in untreated control shoots was significantly lower than oil sprayed shoots. In addition, Mg content in CP55 and CP60 was more than other treatments as well as control at bud swell. Oil spray at all the CPs for the most nutrient content was similar to the untreated control. Fe and S contents for oil spray at CP50 and 55 were the most in bud swell, oil spray at CP50 was higher in Mn and Cu content before spray and showed a significant reduction after the spray.

Amino acids
Different amino acids such as alanine (ALA), valine (VAL), leucine (LEU), isoleucine (ILE), threonine (THR), serine (SER), proline (PRO), asparagine (ASN), aspartic acid (ASP), hydroxyproline (HYP), glutamic acid (GLU), phenylalanine (PHE), glutamine (GLN), ornithine (ORN), lysine (LYS), tyrosine (TYR) were detected in pistachio buds. Glycine (GLY), histidine (HIS) and cystine (C-C) have also been seen in some samples in very little amounts. It was shown that buds of oil sprayed trees at Cantua Creek site, had significantly higher amounts of ALA, PHE, GLN and TYR at CP55 compared to untreated Control trees at bud swell stage.

Conclusion
Unlike Madera and Colusa, oil spraying at CP55 and CP60 could significantly boost the yield in Cantua Creek in 2020. It was found that in Cantua Creek site, oil spraying could advance bud break significantly and enhance bloom synchronization between female and male trees. However, little bud break advancement in Madera site led to no significant difference in yield. It seems unlike macro and micro nutrient changes, the conversion of non-structural carbohydrates in bud and bark of pistachio trees in response to oil spraying plays a key role in bud break advancement and improving the yield. Also it seems oil can change the amino acids profile in the bud at bud swell stage. According to the data in 2020, it seems the response of pistachio trees to oil spraying at different CPs needs further investigation.

Winter applied spray amendment impact on winter chill accumulation, flowering, nut development and yield

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Introduction

Increasingly warm winters in the Central Valley in recent years have prompted research to identify methods that mitigate the impacts of decreased chill accumulation. The intent of this project conducted at Fresno State was to measure the effects of two different commercially available sun-reflecting and -refracting amendments on pistachio tree phenological responses and winter chill accumulation. The tested treatments included 25 lb/acre Surround® (kaolin clay), 2 gal or 28 lbs/acre Mask® (calcium carbonate), and untreated control. Each treatment plot had five mature trees. Application rates for the amendment treatments were based on the label recommendation for each product. The products were applied in late December 2018 and 2019, and during January and February in 2019 and 2020.

Results and Discussion

Crop year 2020 was preceded by a low-chill winter (2 to 62 CP, November 1 through February 29 respectively). The month from February 14 to March 14 was slightly warmer than average, with daily highs of 65-82°F, but the month after that was much colder than average, with highs only 55-75°F and even frosty at night. Problems with delayed pistachio leaf-out and scattered bloom were widely reported across California. In this trial, bloom began at the normal time, mid-April, but between 5% and 30% of the shoots on the trees were what we call *laggards*. Laggard shoots showed impaired leaf-out and delayed bloom and lagged for between 1 and 5 weeks. Laggard shoots were more common on female trees than on male trees.

As shown in Figure 1, the test blocks in this trial crossed from a region with poor leaf-out towards the west of the field into a relatively unaffected region in the center of the field. The unaffected region is lower and more clayey than the more affected parts of the field, which are higher, drier, and sandier. Wind breaking and soil water may have influenced yields, but their roles were not specifically explored in this trial design and so remain unknown. In general, interactions between wind and water forcings on trees emerging from dormancy are likely important but are not well understood.

Within partially affected trees, the more exposed and the more apical shoots were more likely to lag. Exposed and apical shoots are the most photosynthetically active parts of the crown, suggesting lagging of these parts likely has outsized effects on yield.

Bloom timing

Both amendment spray treatments delayed 2020 bloom phenology by 1-2 days. This result was unexpected; the previous result was that kaolinite spray had increased chill accumulation, so these sprays were expected to advance bloom. Future winter spray trials should be more carefully controlled for evaporative cooling. Studies of bloom timing & synchrony might also pay more attention to heat accumulation during ecodormancy and early spring as opposed to the chilling requirement.

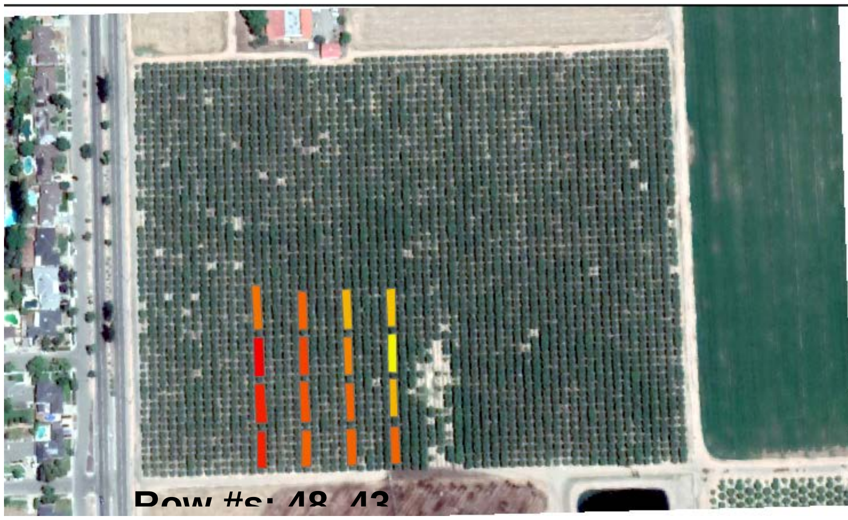

Figure 1. ***Background***: 09 May 2020 aerial photo of test orchard (Google Earth historical imagery). ***Overlay***: this trial's test plots. Filled colors correspond to 2020 green yields ranging from 15 (red) to 120 (yellow) pounds per tree (110 trees/acre).

Nut development

In 2020, slow nut development was observed in many pistachio orchards. In this trial, shell hardening was affected by chill and kernel fill was affected by treatment. The more chill-damaged trees began shell hardening earlier and reached higher maximum hardness. Shells of Mask®-treated pistachio fruit were harder, but embryo weight in Mask®-treated trees did not catch up to Surround®-treated trees by the end of August. Surround®-treated trees also began hull split soonest.

In both years, the nut maturation timing order was not the same as bloom order. Bloom order may be more similar to the previous season's nut development than to the current season's nut development. In a low-chill year, there may be some tradeoff between shell hardness and kernel filling. Due to continuing uncertainty about how pistachio development phenology may mediate or indicate crop failure, obtaining a better understanding of factors that affect the phenology of each developmental event will be important.

Yield

2020 green yields were most strongly affected by position within the orchard. Regions that had leaf-out issues also suffered the greatest yield losses. After plot position was accounted for, no treatment had any operationally or statistically significant effect on either 2020 green yield or 2-year cumulative yield. Considering the low-chill winter, the concurrence of yield loss with poor leaf-out and with exposure, and the disruption to alternate bearing, we attribute this yield loss pattern to low chill.

Conclusions and Practical Implications

Winter sprays of Surround® and Mask® were ineffective in increasing 2-year cumulative yield. Spraying the tested amendments in the winter of 2020 during endodormancy delayed female bloom by 1-2 days and did not protect that year's yield from the warm winter. Instead, this season's observations accentuated how orchard microclimate modulates low-chill yield losses. Wind exposure may aggravate low-chill losses. Future research should investigate how landscape-level topographic and edaphic factors interact with irrigation management to affect orchard susceptibility to warm winters.

Morphological and Cellular Characterization of Pistachio Fruit Development; Shell and Hull Splitting and Kernel Quality Improvement

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Introduction

Shells of Pistachio (*Pistacia vera* L. cv. Kerman) fruits, known as the endocarp, normally undergo dehiscence along the suture line prior to harvest before the degradation of exo-mesocarp (hull). This natural dehiscence makes the kernel of pistachio accessible to consumers and is greatly favored by the industry. Thus, evenly split pistachios with intact hull (preventing pest entry) and fully filled kernel at harvest is a target in the pistachio industry along with the ideal timing of harvest prediction for optimal fruit quality development.

Data of pistachio split ratio and accumulated temperature can be used to predict more accurately the best harvest time. We revisited the relationship between heat and shell split ratio in Woodland this year. We compared data of the year 2020 with that of 2017 and intend to extend the analysis over multiple years in order to model more comprehensively the shell split ratio with the accumulated temperature unit.

In order to understand the basic biology of the pistachio split, anatomical studies are required to monitor pistachio fruit development. Earlier studies were limited by the investigated time period of fruit development, till mid-July, as well as the depth of the structural and polymer analysis of the pistachio endocarp (Polito and Pinney, 1999). We systematically analyzed the anatomically deposition pattern of cell wall polysaccharides during the pistachio fruit development via optical microscopy. Knowledge gained from this study will help to improve harvest timing predictions and provide key references for pistachio fruit development and fruit quality.

Results and Discussion

Comparison between Data of Pistachio Nut Split Rate by Growing Degree Days for Two Years

Growing degree days (GDD) were calculated using a 7°C (45°F) base temperature, as used by Zhang et al. (2017). Twenty or more tip nuts of each branch, with 4-6 branches from each tree being considered, were counted on the sampling day for nut split and blank nut percentage, from the observed initial split to final harvest over a 3-week period. Raw temperature data were retrieved from the Woodland CIMIS station (https://cimis.water.ca.gov/). Overall, the growing season's temperatures of 2017 and 2020 were very similar, with an average April to September temperature of 22.23°C and 22.27°C, respectively. A period of 18 days is necessary for the transition from ~20% to ~70% nut split at harvest for both 2017 and 2020, with a similar gain of ~300°C GDD during these 18 days in both years (**Figure 1** and Figure 1 in Zhang et al. (2017)). After collecting multiple years of data, a correlation analysis of additional possible factors will be incorporated, such as accumulated diurnal temperature difference of the early and late seasons. Recording the nut split ratio from one month before harvest in multiple years will also help in establishing an improved model for an accurate forecast of the harvesting date in the California pistachio production areas.

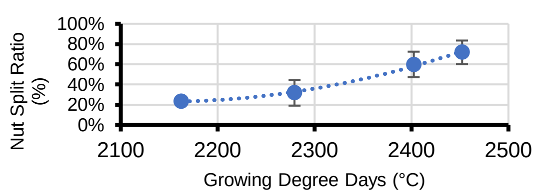

Figure 1. Relationship between nut split ratio and growing degree days of the 2020 season. Data are presented as mean ± SD ($4 \leq n \leq 6$, numbers of branches).

Anatomical Studies of Early and Mid-Season Endocarp and Exo-Mesocarp Development
Branches of pistachio (*Pistacia vera* L. cv. Kerman) samples were collected weekly at a Woodland orchard from April 20th to September 10th 2020, covering the period from the initial fruit setting after bloom to the final harvest day of the orchard. Harvested pistachio fruits were transversely cut into two pieces. Both endocarp and pericarp tissue near and away from the suture line were collected and treated in four combinations of fixatives , dehydrated in ethanol gradient solutions, stained by dyes specific for various biopolymers, and analyzed with bright field and confocal microscopy.

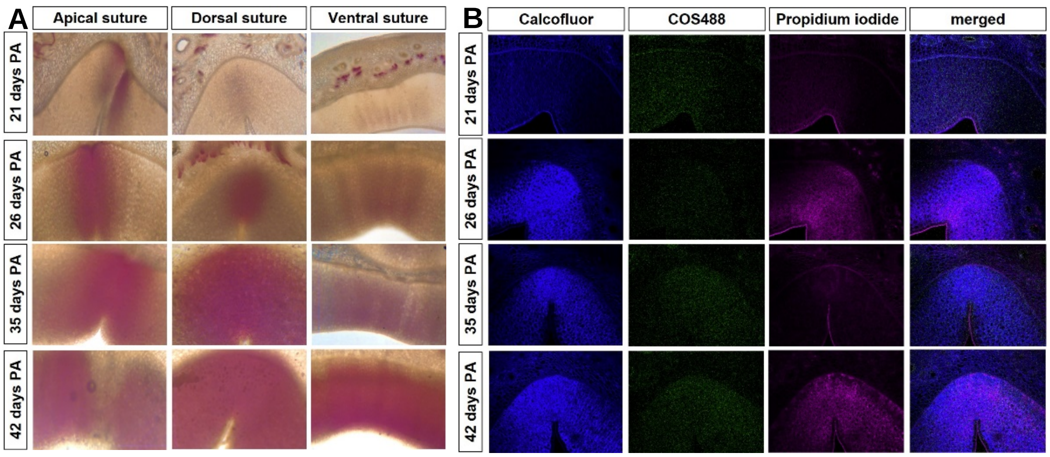

Figure 2. (**A**) Suture endocarp at different stages stained by phloroglucinol. PA, post-anthesis. (**B**) Confocal microscopy of dorsal suture stained by Calcofluor for cellulose and COS488 and propidium iodide for pectin types.

Lignification of cell layers is often concomitant with dehiscence events in plants, like in fruit abscission from the peduncle (Merelo et al., 2017; Parra and Gomez-Jimenez, 2020). Lignification of the pistachio endocarp is initially observed at the apical suture at 3-week post-anthesis (PA) around the transmission track tissue (**Figure 2A**). The dorsal suture and apical suture are sites of the shell split origin (Polito & Pinney 1999), and both locations show a single clear site of lignification initiation, which contrasts the ventral suture. As early as 42-days PA, all regions near the suture line are extensively lignified. Endocarp cell senescence coincides with the site of lignification. Additional cell wall biopolymer depositions were monitored until 42-days PA. Developmental changes in cellulose and pectin patterns were indicated by Calcofluor white, COS488, and propidium iodide and assessed with a fluorescent confocal microscope. Secondary cell wall thickening could be observed at early-season samples of 26-days PA (**Figure 2B**). Detailed attributes of cell wall biopolymer deposition, such as the amount and composition, will provide additional cues on how the dehiscence along the suture line is initiated and regulated in pistachio.

Conclusions

The relationship between pistachio nut split ratio and heat units in 2020 and 2017 shows a high similarity, indicating the importance of multiple year of data for establishing a robust model and its validation Morphological and cellular characterization of pistachio fruit development at early season provides the first developmental timeline reference for the critical cellular events in the endocarp that are related to shell split. Understanding cellular events such as endocarp lignification and cell wall biopolymer deposition can provide useful information toward adjusting timing and the rate of pistachio shell split pre-harvest in future orchard management.

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Trait and marker evaluation for breeding salinity tolerance and climate adaptation in California pistachio rootstocks

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Introduction

High performing pistachio rootstock can increase pistachio nut yield via improved biotic and abiotic stress resistance. Salinity tolerance is of particular importance due to the on-going drought conditions in California, which requires regular irrigation in order to maintain agricultural productivity. However, substantial ground water irrigation brings with it increased soil salinization and minimizes arable land. Pistachio is a nut crop with relatively high salinity tolerance, thus selection of this trait can help maintaining yield. Roots are a first line of defense in salinity tolerance, as they are the organ responsible for absorption of water containing water-soluble nutrients and ions. Various responses contribute to salinity tolerance, including an early osmotic stress phase and a later ion toxicity phase. These mechanisms involve a combination of an increase in osmoprotectants, such as proline, to reduce osmotic stress, increase in apoplastic barrier differentiation, sequestration of toxic ions in the vacuoles and recycling toxic ions out of the shoot via sap.

The increase in deposition of suberin, a waxy, water-impermeable material at the endodermis and exodermis cell layers in the root, has been observed to decrease diffusion in the apoplastic barrier and improve salinity tolerance in multiple species. Sodium vacuolar sequestration in citrus is associated with a higher salinity tolerant phenotype in grafted scions. Our recent work has shown that UCB-1 outperforms *P. integerrima (PG1)* rootstock seedlings, as assessed by leaf senescence and sodium accumulation. Vacuolar sodium sequestration along with increased suberization of cellular barriers contribute to sodium exclusion from the shoot tissue. Interestingly, the differences in sequestration and suberization between the genotypes are at the greatest in the root tips.

However, as a result of genetic heterogeneity, there is considerable variation in salinity tolerance in the commercially available rootstock genotypes, including seedlings of UCB-1 and Platinum (PGII). We hypothesize that there will be differences among the UCB-1 clonal lines in both leaf and root cellular responses. We treated the currently commercially available UCB1 and PGII plants, along with six other UCB-1 clonal lines commercially developed, with salinity stress under greenhouse conditions. We examined the different genotypes based on the leaf phenotypic response and collected root tips across a developmental gradient for microscopy, cell wall polymer deposition and gene expression analysis.

Results and Discussion

Correlation between Salinity Tolerance and Suberin Deposition in *P. integerrima* and UCB-1

We have submitted our manuscript, “Root vacuolar sequestration and suberization are prominent responses of *Pistacia* spp. rootstocks to salinity stress” to the journal *Plant Direct*, summarizing our finding on the association of vacuolar sodium sequestration and root tissue suberization in pistachio rootstock and an provide an atlas of root developmental gradients DOI: 10.22541/au.159225410.03302606). Our study shows a correlation between Na+ vacuolar sequestration and salinity tolerance in the UCB-1 genotype. UCB-1 has higher basal levels of suberization in both the exodermis and endodermis compared to *P. integerrima*. Further, the difference in suberin deposition was accentuated upon salinity stress. Our results indicate that the suberin deposition of UCB-1 contributes to its higher performance under salt stress.

Large-scale Screening of Rootstocks for High Salt-tolerance via Microscopy and Chemical Analysis
We have initiated our salinity stress experiment on a total of eight *Pistacia* spp. rootstock genotypes at 100 mM, increasing to 200 mM over a three-week-long period to reduce osmotic shock. After three weeks of 200 mM treatment, we observed no changes in shoot height growth. However, the current commercially available UCB-1 clonal line, as well as two additional UCB-1 clonal lines, showed a significant decrease in shoot diameter growth. This suggests that under salt treatment, secondary growth is more strongly affected than primary growth (**Figure 1**). There exisits considerable variation between the plants of each clonally propagated line, indicating that there are epigenetic factors at play. We harvested weekly roots during the three-week treatment period and observed, variations in root health and root senescence between the different clonal lines and across time. Our analysis showed, that the current commercial UCB-1 clones and one of the clones being developed are showing the least healthy roots at the end of the treatment. Given that we have observed that secondary growth is affected in the shoots, we reasoned that there is a correlation between the shoot tolerant phenotype and the root phenotype. We have completed sectioning of all 8 clonal lines at three time points, sampling the root tip segment immediately basal to the meristematic region, as this is the area with the greatest difference in vacuolar sequestration and suberin deposition. Quantification of vacuolar sequestration is in progress, and likewise currently shows a change in sequestration ability over time. Preliminary results indicate that, in highly tolerant rootstocks, sodium vacuolar sequestration correlates with root health across time. Suberin analysis is currently in progress, using both chemical and microscopy investigations.

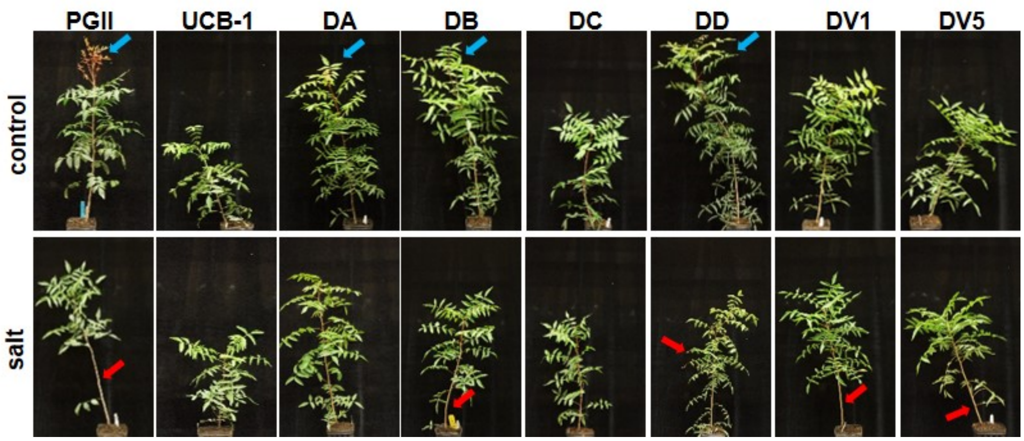

Figure 1. Leaf phenotypes of rootstocks subjected to control and saline irrigation. Blue arrows indicate secondary growth under control condition,and red arrows indicate leaf abscission and senescence induced by salt stress.

Conclusion

Our data show that different UCB-1 lines display significant differences in salinity tolerance, which corroborates with the genetic heterogeneity in seedlings. We identified two clonal lines that show high salinity tolerance and are superior to the current commercial used UCB1 and collected RNA samples, that will assist in the development of molecular markers for salinity tolerance. It is plausible that epigenetic factors contribute to the variability in salinity tolerance in pistachios within clonally propagated lines, though the exact details remain yet unknown. Finally, the temporal response to salinity stress varies between genotypes, with a faster response being beneficial. In summary, our results show that in highly tolerant rootstocks, sodium vacuolar sequestration correlates with root health across time.

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

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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 guiding pistachio tree improvement.

Research efforts described here focus on the development of new approaches to measure trees' physiological status that complements 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 carbohydrate seasonal dynamics specific to pistachios. Our goal is to determine the best carbohydrate management practices in relation to climate, tree age, and geographic distribution. Specifically, we study the pre-senescence accumulation of NSC, dormant carbohydrate 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 data set despite significant delays related to COVID-19
- Tested and parametrize bloom prediction model
- Determined interaction between monthly level of NSC and yield.
- Established know-how to use machine learning for preseason yield potential estimation
- Experimentally tested energy cost (carbon cost) bloom

Results and Discussion

Near real-time results of NSC content 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 fourth full year of data collection.

Thermal conditions during fall/winter/spring are major determinants of pistachio phenology. However, the biological principles governing a plant's internal clock which results in synchronous senescence and bloom remain unknown. Our proposed mechanism detailed in the recent publication (Sperling, et al., 2019) uses spring sugar 'starvation' as a trigger for bloom. Following, analysis of the extensive data set generated by the Carbohydrate Observatory, a mechanism, which is applicable to most nut species, was proposed. The beta version for the project is currently available at http://zlab-chill-heat-model.herokuapp.com/. Using available bloom data from Craigs Kallsen we parametrize the pistachio model and evaluated the role of the fall soluble sugar concentration in twigs impact on bloom time. Interestingly, high levels tend to result in early bloom while lower levels postpone the bloom or predict bloom abandonment altogether (Fig 1). Manuscript (in review) is available upon request

Analysis of over 100 orchards yields and theirs NSC levels in the preceding year show statistically significant positive dependence of yield on high levels of NSC in fall/winter months, no relation May-June and significant negative relation during July-September period. On the average increase of NSC in twigs by 1mg resulted in an increase of the orchard yield by 25 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.

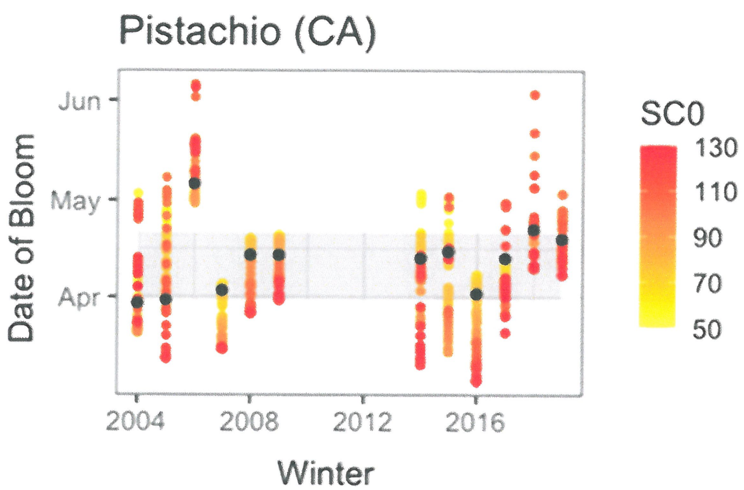

Fig. 1. Impact of soluble sugars concentration (mg/g DW) in the fall (SC0) on bloom date. Real (black dot) bloom date. High fall SC0 (red) tends to force trees to bloom early while lower levels (yellow) result in late bloom.

The presence of correlations between monthly NSC concentration in twigs and yield as well as fact that SC content impacts bloom fecundity and timing provide a promising approach to use winter NSC and their form to improve yield prediction models. Comparative analysis of 20 machine learning algorithms, using 77 orchards for which we had yield data we can predict yield with RMSE at 990 pounds using Extreme Gradient Boosting approach. We expect that including more yield data over a longer time will significantly improve model performance.

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 respiration constitutes only 10% of the cost with major cost (90%) is associated with the structure. Natural senescence and spring thermal conditions promote NSC redistribution across the tree crown and result in synchronous bloom. All conditions that reduce fall NSC content or affect spring redistribution of carbohydrates result in bloom delay and asynchronous bud break that would negatively affect yield potential.

Conclusion

- Developed website provides insight into the current status of NSC in pistachio orchards across California Central Valley and allows growers to compare/evaluate their specific orchards NSC content against similar orchards
- Accumulation-exhaustion pattern of NSC (fall vs summer) can provide mechanistic insight to explain the alternating bearing pattern in pistachio. If proven over the next several years, this insight would offer a path to develop management practices that aim to reduce the exhaustion of NSC (or allow for rapid restoration of reserves) that can reduce an alternate bearing.
- Predictive bloom model suggests that a high level of carbohydrates (soluble sugars) leads to earlier bloom even in warmer winters. This finding suggests a path to reduce the impact of low chill by managing orchard in the fall for high soluble sugar content.
- Cost of bloom exceeds the local NSC reserves. Healthy, synchronous bloom requires fall/spring conditions that stimulate NSC redistribution via phloem/xylem interaction

Two years effect of fungicide programs on Alternaria late blight severity and fruit quality, 2019/2020

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Introduction

In a 31-year-old Kerman commercial orchard located in Tulare County that has historically had conducive conditions for Alternaria late blight (ALB) disease, we tested the effect of two fungicide programs on severity, defoliation and fruit staining. Each program was applied in four blocks of approximately 20 acres each. For two successive years, fungicide programs included three alternating sprays of Quash, Vangard and Inspire Super (program DMI/AP), and Gem, Luna Sensation and Fontelis (program SDHI/QoI). Application dates were on 8 June, 24 June, and 10 July of each year. In total, 40 trees (5 per plot) were flagged and monitored throughout the season. Two weeks after each spray the colony forming unit (CFU) on leaves were counted using the *Over-Night Freezing Technique*. The ALB severity and defoliation were evaluated within one week before commercial harvest when fruit samples were taken from each tree to evaluate staining. ALB 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. Defoliation counted the number of fallen leaves inside a $1m^2$ frame randomly placed at the east and west tree sides. After dehulling, fruits were dried and graded for four staining categories ranging from 0 to 100% fruit stain. Significance levels were determined with the ANOVA test at 5% of probability and means were separated with Fisher's LSD test. The statistical software R-studio (version 1.2.5033) was used to analyze the disease data.

Results and Discussion

The spray program alternating SDHI and QoI resulted in less disease severity, reduced tree defoliation and smaller CFU per leaf for both tested years. The ALB scores revealed significant differences between spray programs in 2019 ($P = 5.8\times10^{-11}$) and 2020 ($P = 1.4\times10^{-8}$) (Table 1). ALB scores were highly consistent among the three graders in both years. Trees treated with DMI/AP spray program had 2-times more defoliation ($P = 5.2\times10^{-8}$) than the DSHI/QoI sprayed trees in 2019, while in 2020 differences were not significant (P=0.31) (Table 2). After collected from the field and tested under conducive lab conditions, leaves treated with DMI/AP fungicides yielded in 1.6- ($P = 1.4\times10^{-5}$) and 1.8-times ($P = 2.2\times10^{-10}$) more *Alternaria alternata* CFU than SDHI/QoI program in 2019 and 2020 respectively (Table 3). Overall, the CFU counts were decreasing from June to July showing that sprays were affecting the disease inoculum. Regarding ALB fruit staining, we observed a prevalence of fruits graded as 0-10% staining in 2019 and a higher distribution of 11% up to 50% in 2020 (Table 4). Some statistical differences were observed in 2019.

Conclusion

Even with widespread presence of SDHI resistant *A. Alternaria* isolates, the SDHI/QoI spray program resulted in higher ALB control than DMI/AP sprays. This result may be explained by different reasons, such as: (i) prevalence of H227Y mutation, which is associated with moderate SDHI resistance levels, (ii) higher intrinsic activity of fluopyram, which is one of the a.i. of Luna Sensation; or (iii) absence of G143A mutation of the *Cyt-b* gene which is associated with QoI resistance. Nevertheless, DMI and AP fungicides are important chemicals that need to be included on the ALB management to avoid the fast SDHI and QoI resistance buildup that may lead to fungicide failure in controlling pistachio diseases. It is suggested in future studies to include the genotyping of *A. alternata* genes associated with QoI and DMI resistance to further explain their impact on fungicide efficacy.

Table 1. Effect of fungicide program on Alternaria late blight severity scores.

Fungicide Program	n[a]	2019			2000		
		Mean[b] (CI 95%)[c]			Mean[b] (CI 95%)[c]		
DMI/AP	20	2.4	(2.3 - 2.5)	a	2.8	(2.6 - 2.9)	a
SDHI/QoI	20	3.1	(2.9 - 3.3)	b	3.5	(3.3 - 3.7)	b

[a] number of trees per treatment
[b] the whole tree score (1-more disease, 5 less disease) were used to determine the ALB severity on individual trees. Means with same letter are not significant different at α=0.05 when compared with Fisher's LSD.
[c] 95% confidence interval of the mean

Table 2. Effect of fungicide program on pistachio tree defoliation.

Fungicide Program	n[a]	2019			2020		
		Mean[b] (CI 95%)[c]			Mean[b] (CI 95%)[c]		
DMI/AP	20	56.53	(48.6 - 64.9)	a	9.65	(8.1 - 11.4)	a
SDHI/QoI	20	26.62	(21.3 - 32.5)	b	8.50	(6.9 - 10.12)	a

[a] number of trees per treatment
[b] Defoliation were square-root transformed prior analysis. Means with same letter are not significant different at α=0.05 when compared with Fisher's LSD.
[c] 95% confidence interval of the mean

Table 3. Effect of fungicide program on Alternaria late blight colony forming unit on leaves.

Fungicide Program	2019				2000			
	n[a]	Mean[b] CFU (CI 95%)[c]			n[a]	Mean[b] CFU (CI 95%)[c]		
DMI/AP	548	6.12	(2.3 - 6.9)	a	900	23.39	(21.5 - 25.3)	a
SDHI/QoI	548	3.93	(1.8 - 4.6)	b	900	12.85	(11.5 - 14.3)	b

[a] number of trees per treatment
[b] CFU were square-root transformed prior analysis. Means with same letter are not significant different at α=0.05 when compared with Fisher's LSD.
[c] 95% confidence interval of the mean

Table 4. Effect of fungicide spray program on fruit stain caused by Alternaria late blight

Year	Program	n[a]	0-10% (CI95%)[b]		11-25% (CI95%)[b]		26-50% (CI95%)[b]		50-100% (CI95%)[b]	
2019	DMI/AP	10,000	84.8 (81.8-86.7)	a	5.5 (4.2-6.9)	a	5.4 (3.7-6.3)	b	4.3 (3.2-5.3)	b
	SDHI/QoI	10,000	89.3 (86.4-91.1)	b	4.9 (3.2-5.7)	a	2.9 (2.0-4.0)	a	2.9 (2.1-3.8)	a
2020	DMI/AP	10,000	47.5 (44.9-49.7)	a	35.1 (33.9-37.3)	a	12.4 (10.6-13.4)	a	4.4 (3.7-5.1)	a
	SDHI/QoI	10,000	46.2 (43.6-48.5)	a	36.6 (34.8-38.3)	a	13.4 (12-14.9)	a	3.8 (3.2-4.5)	a

[a] number of trees per treatment
[b] ALB staining frequency categories. Means with same letter are not significant different at α=0.05 when compared with Fisher's LSD. 95% confidence interval of the mean.

Pistachio Fruit Quality and Stigmatomycosis Survey in Southern SJV

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Introduction

In our first experiment, we aimed to evaluate the frequency of different kernel lesion such as: kernel necrosis caused by hemipteran and *Nematospora coryli*, a yeast transmitted by large hemipterans that causes a decay known as stigmatomycosis (Fig. 1). For that, non-split fruits were randomly taken from four pistachio orchards: Kerman-1 (n=294), Kerman-2 (n=300), Kerman-3 (n=300) and Golden Hill-4 (n=251), located in Ducor, Porterville, Tulare and west Fresno Co., respectively. Sampling occurred during the harvest season of 2017, and fruits were de-hulled and dried before assessment. Descriptive statistic was made.

In a second experiment, we assess different fruit quality parameters harvested from Kerman-3 orchard in 2019 and 2020. Assessments included the weight of 100-fruit, incidence of split fruit, and non-split description for healthy nuts, blanks, nuts with embryo abortion, and presence of stigmatomycosis. Prior to assessment, fruits were de-hulled and dried. As this study was part of a fungicide trial, we maintained the spray main effect as part of the results. Statistical analyses were made with the ANOVA test at 5% of probability and means were separated with Fisher's LSD test. The statistical software R-studio (version 1.2.5033) was used.

Results and Discussion

In 2017, 59% to 89% of the Kerman non-splits yielded healthy nuts, corresponding to 2.8 to 4.2-times the amount healthy fruits in Golden Hills (Fig. 2). Higher kernel necrosis levels were observed in the orchard Kerman-3 with 11%. Stigmatomycosis ranged from 3.7 to 20% in Kerman and accounted for 7% of Golden Hills fruits (Fig. 2). High levels of blanks were observed in Golden Hills, while in Kerman levels were not greater than 15% (Fig. 2).

As expected, there was no main effect by the spray on incidence of splits and weight in 2019. In 2020, a significant spray main effect (P=0.01) on split frequency was observed revealing a positive contribution of SDHI/QoI spray application (Table 1). The weight of 100 fruits was not significantly affected. Among the non-split parameters, no spray main effect was observed in both trial years (Table 2). Among the four non-split parameters, healthy nuts at the Kerman-3 orchard accounted for a maximum frequency of 69.4% (2019) and 49.1% (2020), a slight reduction in comparison with 2017 (Fig. 2). We also observed a progressive increase of stigmatomycosis in infected kernels from 2017 to 2020 for this same Kerman-3 orchard (Fig. 2 for 2017 and Table 2 for 2019 and 2020).

Conclusion

Stigmatomycosis was present in up to 20% of non-split Kerman nuts. However, we observed a variation among the four orchards in 2017. Stigmatomycosis trends on Kerman-3 orchard seems to be increasing as observed in the 2017, 2019 and 2020 data. Kernels were saved to investigate whether aflatoxin and/or ochratoxin mycotoxins occur in these nuts and understand whether hemipteran insects contribute to the dissemination of toxigenic strains of *Aspergillus* spp. in pistachio.

Figure 2. Non-splits showing from left to right, Diffused kernel necrosis, stigmatomycosis, kernel necrosis and healthy nut.

Figure 3. Kernel lesions of pistachio showing from left to right the diffused kernel necrosis (DKN), stigmatomycosis, kernel necrosis (KN), blanks and healthy nut.

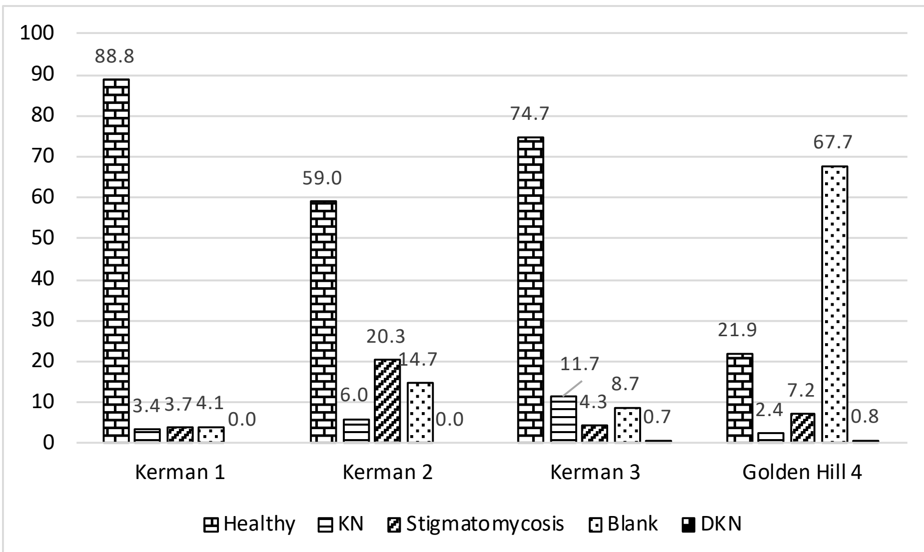

Table 1. Pistachio fruit split frequency and weight

Year	Program[a]	n[b]	Split (%)[c]	CI95%[d]		n[b]	Weight (g)[e]	CI95%[d]	
2019	DMI/AP	10,000	55.3	49.38-60.3	a	100	113.5	111.5-115.4	a
	SDHI/QoI	10,000	60.8	55.8-65.4	a	100	115.1	113.2-116.9	a
2020	DMI/AP	10,000	62.7	59.1-66.3	b	100	153.2	149.7-156.6	a
	SDHI/QoI	10,000	69.5	65.9-73.1	a	100	156.1	252.5-159.4	a

[a]spray program including demethylation inhibitors alternate with anilino-pyrimidines and succinate dehydrogenase inhibitors and quinone outside inhibitor. [b]number of fruits assessed for each treatment. [c]frequency of open (split) nuts. [d]95% confidence interval. [e]weight of 100 nuts, all quality types in 2019 and only splits in 2020.

Table 2. Non-split gradings on kerman pistachio fruit

Year	Program[a]	n[b]	Healty (CI95%)[c]		Blank (CI95%)[c]		Embryo (CI95%)[c]		Stygma. (CI95%)[c]	
2019	DMI/AP	2,000	66.4 (62-70.4)	a	1.6 (0.8-2.4)	a	19.1 (15.9-22.2)	a	12.1(8.6-16.1)	a
	SDHI/QoI	2,000	69.4 (65.3-73.3)	a	1.2 (0.4-2.0)	a	19.2 (16.1-22.3)	a	9.5 (6.4-13.1)	a
2020	DMI/AP	2,000	49.1 (44.3-53.9)	a	1.5 (1.3-1.7)	a	30.8 (25.4-31.5)	a	18.6 (12.9-19.2)	a
	SDHI/QoI	2,000	46.8 (39.1-48.6)	a	1.6 (1.4-1.7)	a	32.9 (27.6-33.7)	a	18.7 (14.3-20.6)	a

[a]spray program including demethylation inhibitors alternate with anilino-pyrimidines and succinate dehydrogenase inhibitors and quinone outside inhibitor. [b]number of fruits assessed for each treatment. [c]frequency of each non-split category and 95% confidence interval.

Managing Anthracnose caused by *Colletotrichum fioriniae* on pistachio, 2020

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Introduction

Five rows of a 32-year-old commercial pistachio orchard intercropping Red Aleppo (RA) and Kerman (KER) cultivars in Glenn County were used to evaluate the efficacy of five commercial fungicides and one grower's spray program. The RA trees were treated with monthly intervals with the following products: Abound (a.i. azoxystrobin), Tebucon (a.i. tebuconazole), Rhyme (a.i. flutriafol – three sprays, see table 1), K-Phite (a.i. Mono- and dipotassium salts of phosphorous acid) and Topsin (a.i. Thiophanate methyl), while treatment used on KER included only Topsin applied at bloom time against Botrytis blossom and shoot blight and Botryosphaeria panicle and shoot blight. All fungicides were applied with a handgun sprayer at maximum label rate and Dyne-Amic was used as surfactant. Trees of both cultivars not sprayed with fungicides served as the untreated control. The trial included 5 replicated trees per treatment. On August 21st, several pistachio clusters were randomly harvested from each replicated tree and brought to the laboratory for efficacy evaluation. All the fruit were removed from the clusters of each replicate tree, mixed well, 100 fruit were randomly selected for each tree (500 total per treatment), and visually inspected for anthracnose lesions and categorized as follows: healthy (no symptoms), spot symptoms, half and full blighted fruit (Figure 1). The frequency of each lesion category was recorded, and statistical analysis was made with ANOVA. Means were separated with Fisher's Least Significance Difference (LSD) at 5% probability. The statistical software R-studio (version 1.2.5033) was used to analyze the disease data.

Results and Discussion

Within the healthy, spotted and full blighted categories, the anthracnose incidence on KER trees differed from the RA results, demonstrating a clear tolerance difference between cultivars (Table 1). No effect on disease incidence was observed on KER trees treated with Topsin in comparison with its untreated trees. Regarding the RA treatments, the only significant difference observed occurred within the healthy category, where K-Phite and Growers program differed from the untreated RA trees. However, these same treatments controlled only 20.3% of the total disease, still not acceptable for the commercial standards. The presence of fruit showing spotted symptoms ranged from 34.3 to 45.9% incidence among RA treatments, and from 6 to 7.9 among KER treatments, revealing no clear spray advantage (Table 1). The frequency analysis of half and full blighted fruit could not reveal a significant difference that justifies the use of any treatment to decrease losses caused by anthracnose. The last two categories when consolidate, add up to near 40% of fruit with severe damage that will drastically affect orchard yield. Because of the prevalence of *C. fioriniae* carrying the G143A mutation at the *Cyt-b* gene, the fungicide Abound obtained the smaller and higher frequency of RA-healthy and RA-full blighted nuts respectively. This mutation was already demonstrated in previous years and may result in reduced control when fungicides formulated with quinone outside inhibitors (FRAC#11) are used.

Conclusion

Treatments made on cv. Red Aleppo or cv. Kerman were not able to inhibit the frequency of symptomatic fruits in relation to their respective untreated control. Differences between cultivars were caused by different plant tolerance for this disease. Even Topsin applied 3 times on RA did not reduce the disease significantly (Table 1). Therefore, presently we do not have any fungicide which is registered to control anthracnose of pistachio in California. Despite the failure of the fungicides to control anthracnose of pistachio in RA trees, this disease seems to be severe only on Red Aleppo and has not spread to commercial Kerman pistachios. Moreover, Kerman, the major pistachio cultivar in California can tolerate high disease pressure and yield more than 80% of healthy untreated fruit even in the presence of this disease.

Table 1. Effect of fungicide sprays to control the pistachio anthracnose caused by *C. fioriniae* in Glenn County.

Treatment[a]	n[b]	Dose	Days after 1st Spray[c]	% Healthy (std)[d]	% Spot (std)[d]	% Half Blighted (std)[b]	% Full Blighted (std)[b]
Topsin-K	500	1.5 lbs/A	0, 23, 58, 86	87.7 (0.33) a	7.9 (0.68) b	1.3 (0.76) c	3.1 (0.59) b
Control-Kerman	500	-	-	82.2 (0.70) a	6.0 (0.54) b	7.3 (0.78) bc	4.5 (1.84) b
K-Phite	500	3 qts	0, 23, 58, 86	20.3 (0.87) b	42.2 (1.01) a	12.3 (1.49) a	25.2 (0.31) a
Growers	500	NA	NA	20.3 (1.73) bc	42.5 (1.31) a	10.4 (0.77) ab	26.8 (0.74) a
Tebucon	500	8 oz/A	0, 23, 58, 86	18.2 (1.27) bcd	40.2 (1.14) a	9.9 (2.23) ab	31.7 (0.63) a
Topsin-RA	500	1.5 lbs/A	0, 23, 58, 86	15.9 (1.82) bcd	45.9 (1.12) a	12.9 (1.38) a	25.3 (0.45) a
Rhyme	500	7 fl oz/A	0, 35, 63	11.9 (1.36) bcd	38.7 (1.30) a	12.1 (1.57) ab	37.3 (0.94) a
Abound	500	12 fl oz/A	0, 23, 58, 86	8.4 (0.87) cd	34.3 (1.67) a	15.9 (2.73) a	41.4 (1.73) a
Control-Red Aleppo	500	-	-	7.2 (0.76) d	41.9 (0.34) a	19.6 (0.42) a	31.3 (0.73) a

[a]Grower's program
[b]Number of fruit evaluated per tree
[c]Spray start date April 21st, excepted for Rhyme, starting in May 14th.
[d]Data were transformed to square roots before analysis. Means were compared using the Fischer's LSD test at 5% probability.

Figure 4. Lesion categories of Anthracnose blight on Red Aleppo pistachios showing full blight (top row), half blight (second row top-down), spots (third row top-down) and healthy nuts (lower row).

Anthracnose of Pistachio in California: Period of Sensitivity and Cultivar Pathogenicity 2020

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Introduction

In this study, we aimed to identify the period when pistachio is most susceptible to anthracnose infection caused by the pathogen *Colletotrichum fioriniae* (isolate 11K11) in Red Aleppo and Kerman cultivars. For that, four successive monthly inoculations, starting in mid-April were made using a suspension of 50,000 spores per ml. For each inoculation event, we used 10 pistachio clusters of each cultivar from three different trees located at the experimental orchards of UC-KARE. Clusters were harvested in Mid-August to evaluate the presence of anthracnose symptoms of each single nut and determine the disease incidence expressed in percentage.

In a second trial, the pathogenicity of *Colletotrichum fioriniae* on four different pistachio cultivars was evaluated by determining the susceptibility differences among Kerman, Red Aleppo, Golden Hills and Lost Hills pistachio cultivars. Inoculations were made in mid-June by using the same methodology previously described for spore concentration, cluster inoculation and lesion evaluation. In both trials, means were compared with ANOVA and separated with Fisher's LSD test at probability of 5%. The statistical software R-studio (version 1.2.5033) was used.

Results and Discussion

No significant infection rate differences were observed among the inoculation months for cv. Red Aleppo ($P = 0.409$) or cv. Kerman ($P = 0.384$). From April to July infections on Red Aleppo and Kerman ranged from 39.8 to 40.8% and 4 to 2.8% respectively (Figure 1). The Red Aleppo results correspond to infection levels obtained in 2017 where similar rates along the trial period ranged from 30-40% (document available online at CPRB website). In 2018 and 2019, the April inoculations on Red Aleppo corresponded to higher infection rates did not observe this year (Lichtemberg et al 2018, 2019 in Pistachio Board Executive Summaries). Instead, in 2020 we observed a high amount of exocarp lesion caused by the citrus flat mite especially on cv. Kerman (Figure 2). Kerman seems to be tolerant to *C. fioriniae* infections confirming data from the last three years. Different from 2017, when the higher infection rate reached 21% in July, the 2020 results did not surpass the 6.5% threshold (Figure 1).

Significant differences ($P = 2.2\times10^{-16}$) for variety pathogenicity were observed in 2020. After inoculations made in June, the cv. Red Aleppo yielded the highest infection rate of 40.8%, corresponding to 6.3-, 7.7- and 19.7-times the incidence found on cvs. Kerman, Golden Hills and Lost Hills, respectively (Table 1). Results corroborate the last three-year results, where cv. Red Aleppo was shown to be the most susceptible cultivar and cv. Kerman, Golden Hills and Lost Hills are mostly tolerant for the anthracnose damage.

Conclusion

In Fresno County, the period of the highest plant susceptibility for cv. Red Aleppo may vary according to the year. In the last four-year trials, May was the month where anthracnose infections resulted is most aggressive lesions, able to collapse an entire cluster. Despite the absence of differences among inoculation period, this cultivar resulted in 40% of disease incidence, and in northern California there were reports of up to 100% of disease in a couple of orchards where Red Aleppo was planted. The disease level in Kerman was very low (<7%) after artificial inoculation, and these results corroborate to our trial results in the commercial pistachio orchard in Glenn Co. where natural infection of untreated plants resulted on 17.7% incidence (See Fungicide Efficacy Study for 2020). All other cultivars tested, Lost Hills and Golden Hills, resulted on low disease incidence, similar to Kerman and smaller than Red Aleppo.

Our results suggest that the pistachio anthracnose is not a threat for California growers growing these tolerant varieties. However, we recommend that growers located at the northern San Joaquin Valley should observe their orchards for lesions caused by the pathogen *Colletotrichum fioriniae*.

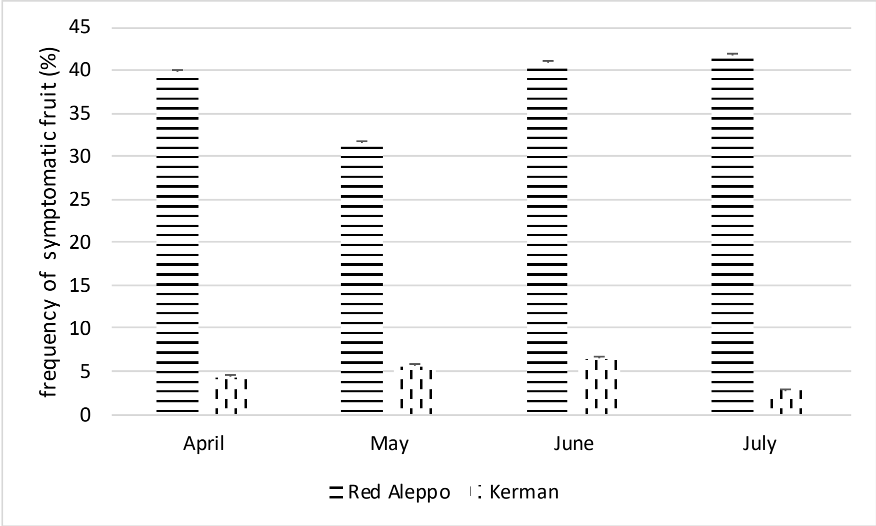

Figure 5. Incidence of anthracnose lesion on artificial inoculations of *C. fioriniae* made on cvs. Red Aleppo and Kerman from April to July 2020.

Figure 6. Lesion on the fruit caused by Citrus flat mite *Brevipalpus lewisi* on Kerman pistachio fruits (not to be confused for anthracnose lesions).

Table 2. Anthracnose pathogenicity on four different pistachio cultivars.

Cultivar	n	% of symptomatic fruit (std)		
Red Aleppo	30	40.89	(0.24)	a
Golden Hills	30	5.27	(0.14)	bc
Kerman	30	6.45	(0.21)	bc
Lost Hills	30	2.07	(0.13)	c

Evaluating pistachio rootstock tolerance to soil-borne diseases

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Introduction

Our laboratory recently identified new soil-borne diseases affecting pistachio trees in California. Phytophthora root and crown rots appeared as emerging new threats to pistachio, particularly in areas where soil conditions or cultural practices promote prolonged soil wetness. The fungal pathogens *Macrophomina phaseolina* and *Fusarium* species also were 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 managed soil-borne diseases of pistachio. Experiments conducted in 2018 and 2019 using mycelial plugs for stem inoculation of the various commercial rootstocks suggested that Platinum is the most tolerant rootstock to crown rot diseases when compared to PGI and clonal UCBI rootstocks. This year, we evaluated the susceptibility/tolerance of clonal UCBI and Platinum rootstocks against Phytophthora, Verticillium and Macrophomina diseases, while emphasizing on soil and root inoculations of the various pathogens. During 2020, several experiments were conducted at the Kearney Agricultural Research and Extension Center on potted trees (1-year-old) in a greenhouse as well as on trees planted in the field (4-year-old). In greenhouse experiments, the soil of potted pistachio rootstocks was inoculated using rice seed inoculum colonized with isolates of *Phytophthora niederhauserii*, *Phytophthora* taxon walnut and/or *Phytophthora cinnamomi* (two experiments). In addition, *Macrophomina* and *Verticillium* inoculations were conducted using microslerotia suspension mixed in the soil of potted clonal UCBI and Platinum rootstocks. Field trials included the inoculation of mycelial plugs of *P. niederhauserii* and *M. phaseolina*, as well as *V. dahliae* in the trunk and branches of UCBI, PGI and Platinum rootstocks, respectively.

Results and Discussion

In 2020, several greenhouse experiments were conducted using clonal UCBI and Platinum rootstocks. PGI trees were only made available to our lab in December 2020 and the comparative susceptibility of PGI to soil borne diseases will be evaluated in 2021 experiments. Following two experiments using soil/root inoculation of *Phytophthora* species with rice seed inoculum, significant differences in susceptibility (as expressed by % plant death) were found between clonal UCBI and Platinum rootstocks. Overall, Platinum was the most tolerant rootstock to Phytophthora root rot as illustrated by the lower plant-death ratio occurring in treated Platinum rootstocks compared to treated UCBI trees, seven weeks after soil inoculation of the various *Phytophthora* species (Fig 1A-C-D). Also, the percent loss of root mass triggered by soil inoculation of *P. niederhauserii* was lower in potted Platinum trees compared to UCB1 trees (Fig 1B). Additional clonal UCBI and Platinum potted plants were root inoculated using microsclerotia of *Macrophomina* and *Verticillium* on May 22, 2020 and August 12, 2020, respectively. Three month following inoculation, 87.5% of Platinum rootstocks expressed wilt symptoms whereas only 12.5 % of UCBI showed symptoms (Fig. 1F). However, none of the inoculated plants were killed in this experiment and all plants had recovered after 2.5 months following the onset of wilt symptoms. Accordingly, more experiments will be needed to be conducted to confirm a higher susceptibly of Platinum to Macrophomina root rot. Plants inoculated with *Verticillium* are still incubating in a greenhouse until the development of symptoms (wilting). In field experiments, 4-year-old UCBI, PGI and Platinum trees were inoculated on June 12, 2020 and August 4, 2020, with isolates of *P. niederhauserii* and *M. phaseolina*, respectively. Mycelial plugs of the various pathogens were inoculated under the wounded bark of trunks just below the soil line, and experiments are still incubating until the expression of visible symptom (crown rot, gumming) in the lower portion of trunks. On June 5, 2020, branches of each of UCBI, PGI and Platinum trees in the field (KARE) were inoculated with mycelial plugs of *V. dahliae*. Plants were sampled 5 months later to assess the length of vascular streaking developing in the inoculated branches. Results indicated that streaking was longer in UCBI plants inoculated with the Verticillium, while lowest in Platinum rootstocks and intermediate in PGI rootstocks (Fig. 1E). Nevertheless, these data are preliminary and similar results will need to be reproduced following soil/root inoculation with the Verticillium pathogen before any conclusion can be drawn.

<u>Conclusion</u>

2020 results indicate differences in the tolerance of clonal UCBI and Platinum (a PGII decent) rootstocks to soil-borne pathogens including *Phytophthora* and *Macrophomina*. Platinum appeared overall as the most tolerant rootstock against Phytophthora root rot. This year data confirmed the superiority of Platinum rootstock and its capacity to better overcome Phytophthora root and crown rot diseases compared to the clonal UCBI rootstock. Hence, Platinum might be a preferred rootstock in areas at risks for Phytophthora diseases. However, uncertainty remains as to the tolerance/resistance of Platinum rootstock to Verticillium as multiple complex factors only may lead to disease expression and several years of experiments following root/soil inoculation with microsclerotia may be required for plants to develop symptoms and to acquire conclusive data. Caution should be taken also before planting Platinum in areas that have been exposed to the Verticillium wilt pathogens. Previous research and recent field observations have indicated the possibility of a higher susceptibility of Platinum rootstock to Verticillium. 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.

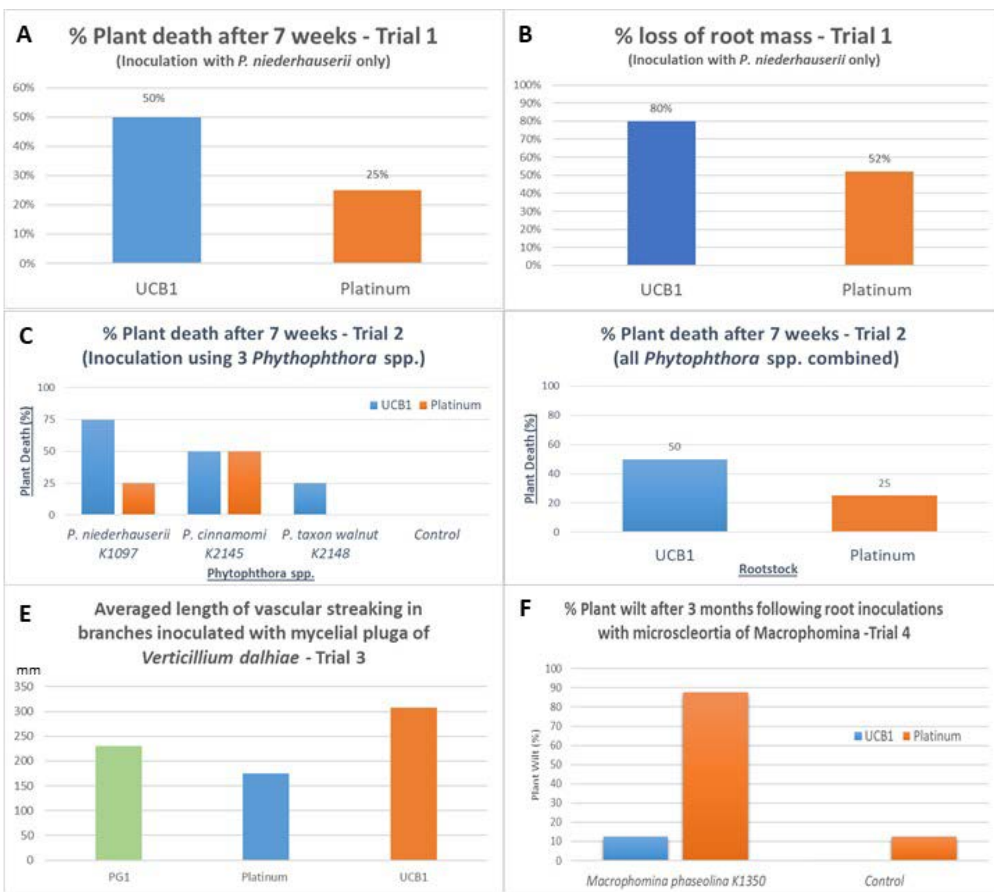

Fig. 1. Summary results of the effect of *Phytophthora*, *Macrophomina* and *Verticillium* inoculations in clonal UCBI and Platinum rootstocks.