
# Executive Summaries 2022

California Pistachio Research Board
4938 East Yale Avenue, Suite 102
Fresno, CA 93727

# 2022 Manager's Report

Bob Klein

The research direction of the California Pistachio Research Program is influenced strongly by past and current cropping problems. Consequently, the California Pistachio Research Board (CPRB) meets after the crop is in to discuss what problems were observed, how it might have affected the total crop, what went right, and what production research might be able to address. The 2022 crop year did not disappoint with the number of problems that developed.

While December 2021 was very wet and we entered the 2022 growing season with about 200% of average snowpack, the spring months were dry, dry, dry. We knew water would be in short supply even with a wet year and growers had to struggle with water supplies for all of 2022. The dry spring was also relatively warm, and some areas struggled with inadequate chill. After the warm winter, some areas also had a late spring freeze that caught a lot of orchards in late bloom or just after bloom. The freeze was hard enough to reduce crop loads significantly and severely in the affected areas. Temperatures during the growing season tended to be high – the number of days that exceeded 100F wasn't necessarily unusual but there seemed to be more days with temperatures above 105-110F. Insect and disease problems were not out of the ordinary with the exception of mealy bug problems due to a limited number of efficacious insecticides. The crop looked like an early harvest was in the works and there was talk of harvest beginning as early as August 10, but the nuts would not come off the trees when shaken. This problem continued throughout the harvest season. Growers routinely reported crop sizes 25-30% lower than expected. Pre-season crop estimates of over one billion pounds did not come to pass and the 2022 crop totaled 884 million pounds. Nut size was much smaller than the previous year, closed shell percentages were up by about 10% over the level seen in 2021. Stain was higher as was insect damage due to navel orangeworm (NOW).

There are two emerging issues that will need to be addressed as the industry moves forward. The first is the mycotoxin, ochratoxin A (OTA). The OTA causal fungi are closely related to the fungi that produce aflatoxin. OTA and aflatoxin "control and mitigation" look a lot alike: control NOW and sort for adhering hull, insect damage, and dark stain. We do not yet know if the aflatoxin biocontrol agents Prevail AF36 and Afla-guard also serve to control OTA. OTA has become important because the European Union has established a maximum level of 5ppb, compared to 10ppb for total aflatoxin. This is likely to double the risk of rejection when tested at the EU port of entry. Consequently, to maintain an acceptable rejection rate and to keep processing costs stable, the acceptable level of NOW damage will be reduced.

The second issue is internal kernel discoloration (IKD). This has been observed in Golden and Lost Hills kernels and is characterized by brown semi-circular concentric arcs on the internal cotyledon surface of the kernels. The arcs may extend the length of the kernel but usually do not affect more than about 25% of the cotyledon surface and the discoloration is very shallow, not penetrating more than 2-3 cell layers into cotyledon surface. There are no shell or kernel surface irregularities associated with IKD and IKD can only be observed by splitting the kernel. There are no taste effects nor are there any effects on kernel nutritional composition. IKD does not appear to be a result of insect feeding nor of a pathogen but is most likely caused by as yet undetermined environmental conditions affecting a particular genotype of pistachios. Nevertheless, the USDA believes that IKD should be assessed and scored as a kernel defect. Scoring IKD would increase both incoming and outgoing inspection costs and is problematic because there is no sortable characteristic. Investigations are underway to determine the cause of IKD and develop mitigation strategies.

Prospects for the 2023 crop look good. After an off-year, bud counts are high, indicating the potential for large and likely record crop. December has been wet (as was the previous December) but we will likely still be water short. Chill has been good with more hours below 45F to date than any of the last 10 years and particularly good conditions with clouds, fog, and rain instead of sunshine. My tune may change in another month or two but 2023 currently looks very promising.

Wishing you all a gloomy, wet, and chilly January- March and a moderate 35 days over 100F for the 2023 growing season.

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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

Beginning in 2019, FNRIC has provided support for upcoming Pistachio events, worked with ANR PSU to develop a social media presence for Pistachio Day and facilitated the review process for the CPRB proposals.

In 2022, FNRIC was primarily responsible for coordinating abstract submission and agenda development for the VIII International Symposium on Almond and Pistachio.

## Conclusion

Overall feedback has suggested that our support has proven especially valuable for the proposal review process and the promotion of virtual events. We believe that we have continued to provide a valuable service to the California Pistachio Research Board and the UC ANR Pistachio Workgroup. With the VIII International Symposium on Almond and Pistachio coming up in 2023, we are prepared to continue providing these services.

# Raman Spectroscopy to detect and measure NOW pheromones

**Authors:** **T. Bond**, PhD, Senior Staff Engineer, **S. Sahota**, junior staff, and **A. Chang**, PhD, staff, Lawrence Livermore National Laboratory**; H. Wilson,** PhD, Asst. Coop. Extension Specialist, Dept. Entomology, UC Riverside; **C. Burks**, PhD, Research Entomologist, USDA Ag research Center.

## Introduction

We have shown significant progress in evaluating enhanced Raman spectroscopy for the detection of synthetic and natural NOW pheromones and its potential use to measure pheromone diffusion in orchards. 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 become an essential component of monitoring and/or managing some key pests of agricultural crops, including navel orangeworm (*Amyelois transitella*) (NOW) in California tree nuts1-3. It is not well understood how synthetic pheromones compete with natural pheromones and, in the case of monitoring, how effectively the insect follows the diffusing plumes and how those evolve from emission points, especially across large blocks and at plot borders. We proposed to continue our evaluation of the use of enhanced Raman spectroscopy for the detection of synthetic and natural NOW pheromones. In Year 1 we were able to generate the Raman signatures for the main pheromone components in synthetic pheromones, and in Year 2 we have been able to provide a clear distinction of such signatures in mixed samples via Principal Component Analysis (PCA).

## Results and Discussion

The following components of pheromones were used: (Z,Z)-11,13-Hexadecadienal, (E,Z)-7,9-Docedadien-1-yl Acetate, and (Z,Z,Z,Z,Z)-(3,6,9,12,15)-Tricosapentene. we are able to show that we can detect Raman signatures down to 0.1% concentration of pheromone diluted using 100% acetone. Raman spectra of each dilution shows that the system is able to detect signatures of the significant pheromone peaks at even the lowest concentrations. As the pheromone concentration decreases, the signature peaks of the pheromone are also decreasing and increasing as the amount of acetone used to dilute the pheromone increases.

The complex signatures of the pheromones were analyzed with Principal Component Analysis (PCA) in order to help isolate the response of each concentration and differentiating the Raman signatures. Using principal component analysis trends are visible of significant peaks that are present in each individual pheromone component. PCA was conducted first on each concentration made of individual pheromone component, then all concentrations of the three pheromone components were plotted against each other. We were able to easily discriminate the various diluted concentrations, validating the power of the PCA versus simple Raman peak analysis. Each diluted sample falls closer to one or another depending on the pheromone concentration levels. As more mixtures are made, identify the component will be easier, due to the visible trendlines of the existing compiled PCA data. As the pheromone component concentration lowers, the percent of acetone increases, so in the PCA plots, the dilutions with lower concentrations of each pheromone component are found to be plotted closer to the acetone point. As the pheromone component increases in concentration, the further away it is seen on the plot from acetone. This compiled data can be used for trending and calibration in determining unknown concentrations in the fields. A database of these dilutions and mixtures will continue to be built for reference.

Due to the low concentrations of pheromones released by moths in the field, we pursued boosting the detection capacity by implementing two approaches: preconcentrate with Solid Phase Micro Extraction (SPME) fibers following GC-MS approaches applied to Raman. SPME fibers appropriate for the molecular weights of semiochemicals (50/30um DVB/CAR/PDMS, Stableflex 24Ga by Sigma-Aldrich) were first tested with diluted toluene uptake. With additional tests we concluded that we measured a

compounded signal with the SPME components instead of just the absorbed chemical of interest. It proved difficult to differentiate the SPME and toluene components. GC/MS was then performed on the fiber. Principal and parent ions of 91 and 92, which are the most abundant and highest mass ions from the fragmentation of the toluene, respectively, were signatures that were observed to isolate toluene signatures from other ions. Although toluene is absorbed by the SPME fiber, it is difficult to identify which Raman peaks coincide with the toluene versus the components that make the SPME fiber. There is great potential to measure headspace using SPME fibers but, further testing and deconvolution would be required to use this method of measuring the headspace of toluene or the components of pheromones.

We continued exploring surface enhanced Raman spectroscopy, SERS for increasing the sensitivity. We have pursued two methods of experimentation: comparing SERS in film (liquid) and headspace (gas). Individual pheromone components were made using acetone. For the SERS method using liquid, Raman measurements were taken by first evaporating a drop of the pheromone dilution onto the silver SERS substrate creating a thin film. For the SERS method measuring the headspace, a SERS substrate was attached to the cap of a vial containing the pheromone component, the pheromone was heated to 200°C, then the SERS substrate was removed and measured for Raman signatures. PCA conducted on the SERS substrate samples of pheromone dilutions show groupings as well as a trend of the higher concentrations found towards the top of the plot and lower concentrations towards the bottom. If a mixture was made with each pheromone, we would be able to determine, based on the PCA of the measurements - where the points are found on the plot, the concentration as well as the pheromone. PCA might be different from the bulk since analytes in Raman signatures change when they are interfaced with the substrates. The vibrational modes of the molecules are affected by the affinity of the chemical to the substrate.

We are working towards a small-scale pheromone diffusion experiment. This set-up was created at an even smaller scale, with a vial containing (E,Z)-7,9-Docedadien-1-yl Acetate and a SERS substrate placed into the cap in an attempt to measure any diffused pheromone in the headspace that can be captured by the SERS substrate. After confirming that 100% concentration of (E,Z)-7,9-Docedadien-1-yl Acetate was detected on the SERS substrate during heating, we further reduced the concentration, to determine the lowest concentration that can be observed. Individual SERS substrates were placed into the cap of each pheromone dilution. After 1.5 hours of heating, all samples Raman spectra were measured and plotted against each other. Each blank SERS substrate was found to be very consistent with each other. Peaks found at 700cm-1 to 1000cm-1 and at 1350cm-1 appeared after the heating procedure. It's evident that each substrate was detecting the pheromone in the headspace. However, after remaining idle for a time duration after the heating procedure, the peaks decreased or disappeared altogether, as expected.

## Conclusion

By successfully completing the next set of planned experiments, we can validate the enhanced Raman approach as a tool for pheromone emission profiles measurements. These are all critical initial steps to overcome in order to develop the "orchard in a box" experiment in which we will incorporate diffusion models. Moving forward the best method of detecting diffusion is apparently using SERS substrates in measuring headspace. We are able to generate a database calibration of semiochemical mixtures and high/low concentration pheromones via principal component analysis which will help deconvolve unknown mixtures and concentrations. We intend to replicate these studies using live moths and to measure pheromone emission in real-time using our Raman system. Acquiring a better confocal Raman system will allow more accurate and precise measurements of the pheromone signatures as we move into the next phase of this research.

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# Cover crops and trap crops to support biological control of large bugs

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

## Introduction

'Large bugs' can be a damaging group of insects in California pistachio production. They 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*). Large bugs can cause the same damage as their smaller relatives during the first half of the season. However, during the latter half of the season (from shell-hardening until harvest) they can continue to puncture the shell, causing kernel necrosis (damage to the nut meat) and stigmatomycosis (a mold that infests the nut).

In this project, we are revisiting the use of groundcovers to support large bug control in pistachios. Groundcovers might serve as trap crops that attract and retain large bug populations to reduce pest occurrence in the tree canopy. Previous work funded by the CPRB showed that sown and irrigated groundcover strips can successfully decrease large bug populations in the canopy, although the reduction was slight (Stahl et al. 2021 internet link: 10.1007/s11829-021-09869-7). They also led to increased numbers of natural enemies in the trees. Biological controls of large bugs consist of generalist predators and more specialized parasitoid wasps attacking large bug eggs. While naturally occurring predation and parasitism alone cannot keep up with stink bug population surges that result in pistachio nut damage, delayed buildup of parasitoid populations can lead to a 50% reduction of nymph hatch in the late season in organic pistachio production (Stahl et al. unpublished data). If this natural parasitoid population were to be supported by timed mass releases and sustained with groundcovers providing alternative food through nectar and pollen, large bug control might be improved significantly. In both conventional and organic production, releases of parasitoids would need to be coordinated with chemical control. Here we tested the use of groundcovers to impact stink bug densities, as well as the augmentation of egg parasitoids.

## Results and Discussion

Overall, eggs of four pest species of stink bugs (*Chinavia hilaris*, *Thyanta pallidovirens*, *Euschistus* sp., *Chlorochroa* sp., as well as the beneficial predaceous stink bug *Brochymena* sp.) and most likely only one species of leaffooted bug (*Leptoglossus zonatus*) were found using visual surveys of seventeen tree canopies in each of the six sites. All species were recovered from sites with groundcovers, while in sites with bare soil only *T. pallidovirens* and *Brochymena* sp. were found. In addition to increased diversity of species, there were also overall more eggs per tree (Poisson GLM, df = 1, 55, $\chi^2 = 1150$, $p < 0.001$) in the groundcover treatment (2.61 ± 0.87, mean ± SE) than in the bare soil treatment (0.23 ± 0.15). However, while no parasitoids were recovered from the bare soil orchards, more than 60% or the eggs in the groundcover plots were successfully parasitized (see Fig. 1). While those differences may be due to the (present or absent) groundcover, other management factors might also have played a role. The majority of the parasitoids found to attack stink bug eggs were *Trissolcus* sp., and those attacking the few recovered leaffooted bugs were *Gryon* sp. (recently renamed *Hadronotus*). The parasitoid samples have been sent to a taxonomist to be identified to species level.

To test augmentative release, 100 *Ooencyrtus* sp. adults per site were released on flagged trees. Freeze-killed *L. zonatus* egg masses from our colony (=sentinel egg masses) were glued onto branches on seventeen trees radiating out from the release tree for five days until recovery into the laboratory to

measure the impact of the release. There was no evidence of the egg parasitoid *Ooencyrtus* sp. presence inside the six sites before its release. No *Ooencyrtus* sp. offspring emerged from those egg masses, and there was no indication that *Ooencyrtus* sp. established in the orchards since none of the naturally laid egg masses recovered (described above) yielded *Ooencyrtus* sp. *Leptoglossus zonatus* egg masses were only chosen because the stink bugs in colony were not producing enough egg masses. Since *Ooencyrtus* sp. seem to prefer stink bug egg masses over *L. zonatus* in the laboratory, we could be underestimating the effect of the releases. Furthermore, the release timing was based on stink bug egg laying data from other sites, and seems to have been too early for the sites in this study: during the *Ooencyrtus* sp. release, no naturally laid stink bug egg masses were yet present, and they were not abundant until more than a month after the release (see Fig. 1). With their short lifespan, by this time, the released parasitoids would already have died. This lack of hosts would hinder their establishment.

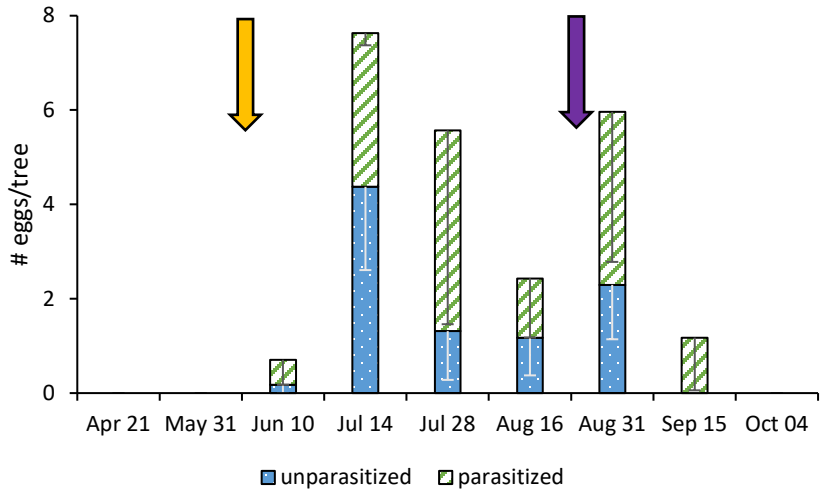


**Fig. 1** Stink bug (most data) and leaffooted bug (only represented as a small fraction on Sep 15) eggs per tree over the season in three pistachio orchards with groundcover [mean ± standard error (SE) between the three sites]. Arrows indicate *Ooencyrtus* sp. release against stink bugs (yellow) and *H. pennsilvanicus* release against leaffooted bugs (purple).

In contrast, there were already *Hadronotus* sp. present in the orchard before our release, which makes measuring the impact of our releases difficult. With the *H. pennsilvanicus* releases, two of the sites for each treatment (groundcover or bare soil) received 100, and one of the sites received 300 parasitoids. The only site with recovery of parasitoids was the groundcover site with the higher release value, but parasitism (=number of successfully parasitized eggs as a proportion of all recovered sentinel eggs after the release) was less than 2%. Parasitism was also recorded almost a month after release on naturally laid *L. zonatus* eggs, and the taxonomist might be able to answer if that was a result of our release.

In a laboratory study evaluating the effect of organic insecticides on the survival of adults of the leaffooted bug egg parasitoid *H. pennsilvanicus*, most products were not found to have a negative effect on this potential biological control agent. However, if *H. pennsilvanicus* adults came into contact with 1h old Pyganic residues, 100% of those beneficials died within the day. If the Pyganic residues were 24h old, no negative impacts on survival were reported.

## Conclusion

Groundcovers can be used to impact stink bug densities and increase natural enemies, although the cost effectiveness suggest the addition of ground covers must also include management benefits beyond pest control. An initial test of egg parasitoid releases suggests release timing needs to be optimized for every year and site to ascertain the presence of target pests/hosts so as to not waste beneficials and create the opportunity for establishment, and with that longer term impact on pest populations. *Hadronotus pennsilvanicus*, and possibly other egg parasitoids, can be negatively impacted by insecticides, including organic materials such as the pyrethrin Pyganic. This is a future area of study to increase levels of natural enemies in pistachio orchards.

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

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

## Introduction

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

The increasing problems over the last few years, as well as 2019-2020 insecticide studies showing poorer performance (D. R. Haviland, presentations in November 2020), suggest that this program is losing efficacy. Two possible explanations are insecticide resistance and asynchronous population development. Insecticide resistance is unlikely as both the IGRs and the neonicotinoids have seen a reduction in their performance, and it would be surprising for resistance to develop concurrently to materials with different modes of action. The more likely culprit is asynchronous population development, such that there is a prolonged period of crawler emergence, which thereby reduces the performance of IGRs and neonicotinoids, both of which tend to better kill the smaller mealybug stages. In 2021 we found that the first generation was largely synchronous, however, the peak crawler emergence was in early- to mid- May instead of late May with later generations becoming asynchronous. We repeated the study in 2022 to confirm those results.

We also note that natural enemies, especially parasitoids, have been important in Gill's mealybug control in other crops. We continued to assess natural enemy populations in pistachio as well as surveyed for Gill's mealybug parasitoids in vineyards.

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

## Results and Discussion

The seasonal phenology of Gill's mealybug was evaluated every two to four weeks in six sites throughout the San Joaquin Valley from April to November 2022. Branches with visible infestation were cut around fifteen inches from the tip and mealybugs and natural enemies were counted in the laboratory. Three distinct crawler peaks could be identified, with larger nymphs and adults present throughout the season (Fig. 1). Crawler generations were largely synchronous among sites, although not every site had a noticeable first-generation crawler peak. Adults and nymphs were at low numbers during the first crawler peak and increased greatly toward the second half of the season. This persistent population of nymphs and adults likely reduces the effectiveness of any insecticide applied targeting the crawlers. This agrees with the previous year's findings and further supports asynchronous populations as a likely cause for the lack of insecticide effectiveness. The initial crawler peak was again earlier in May than reported previously, possibly representing a change in life history of the mealybug (Haviland et al. 2012). Growers who are following the recommendations to scout in late May would likely miss the first generation of crawlers. At

the end of the season, large populations of nymphs could be found on the clusters that remained in the trees after harvest.

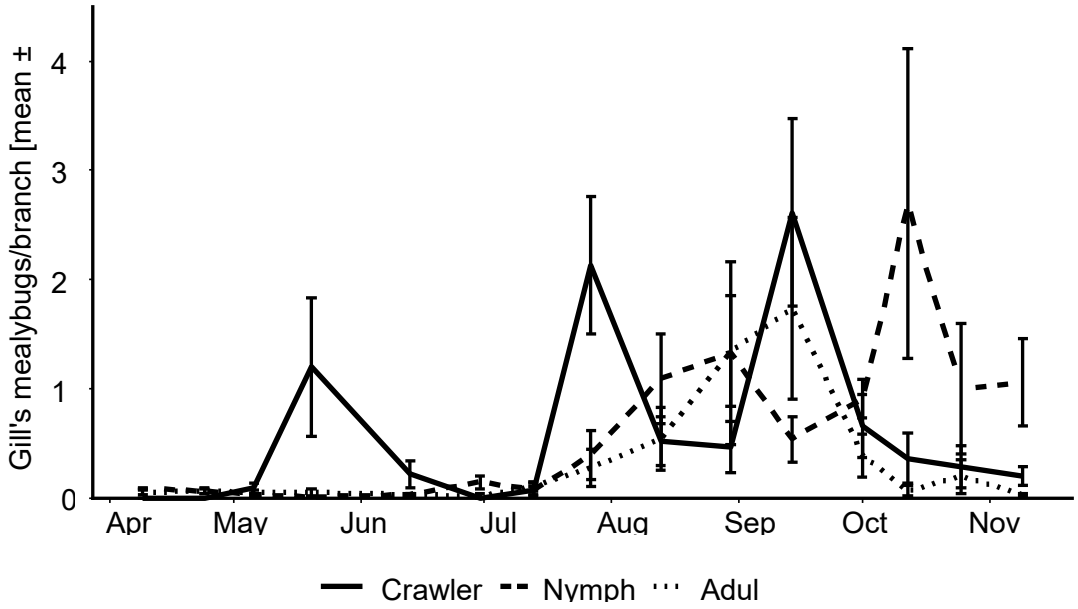


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**Fig. 1** Gill's mealybug phenology in five sites in the SJV. Crawlers were counted up to 100 per branch.

The most common natural enemy was the green lacewing, 3,386 were found on sampled branches. No parasitoids were recovered from pistachio orchards. Surveys of vineyards in the Sierra Foothills for parasitoids recovered over 100 Gill's mealybug, only two were parasitized both were *Acerophagus* sp., which is the parasitoid species of interest that had been found controlling Gill's mealybug in almonds but rarely ever found in pistachios. Attempts to establish a parasitoid colony from field collections were unsuccessful. We had proposed to test Gill's mealybug resistance to the commonly used insecticides. However, trials are likely to be economically unfeasible. Potted pistachio plants proved difficult to maintain with Gill's mealybug infestations. Larger trials on established orchards with infestations would be too costly for the needed quantities of insecticide and sampling needed. Simpler laboratory-based trials for Gill's mealybug resistance would be too unrealistic because most insecticides used are systemics and topical application would not yield realistic results.

## Conclusion

All life stages of Gill's mealybug were present from April to November. Three distinctive peaks of crawlers occurred, similar to last year, and the original Gill's mealybug studies. However, larger nymphs and adults were found during those peaks and insecticides targeted at crawlers would be ineffective on these older life stages. Our findings agree with our hypothesis of asynchronous generations of Gill's mealybug in pistachios and could be leading to the reduction in insecticide control. Like in 2021, green lacewings were the predominate natural enemy. Parasitoids remained absent from pistachio orchards and at very low levels in Sierra Foothill vineyards. In the coming year, the population dynamics will be written up for publication. Based on the large numbers of green lacewings found later in the season, we will propose to shift our focus to augmentative releases of green lacewings early in the season to help manage Gill's mealybug.

# Optimizing chemical control programs for Gill's mealybug, *Ferrisia gilli*, in pistachio

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

## Introduction

Gill's mealybug is a significant pest of pistachios throughout California that is associated with reductions in crop yield and quality. Growers have traditionally achieved control through a single insecticide application in late May to early June. However, increasing pest pressure now sometimes requires multiple applications of insecticides at a time that insecticide options have become increasingly limited due to highly restrictive tolerance levels for residues on crops sent to certain export markets.

The goals of our project were to 1) to evaluate new insecticides for Gill's mealybug, 2) evaluate new timings for insecticide applications that could alleviate issues with residues, and 3) evaluate single versus two-spray programs for mealybug control.

## Results and Discussion

A large trial was established following harvest in 2021 that contained a total of 30 different treatments, treatment timings and combinations of treatments that were each replicated 8 times using randomized single-tree plots. Plots that received post-harvest treatments were sprayed on 21 October 2021 while plots receiving early spring applications were sprayed on 1 April 2022. Evaluations through mid-April showed that post-harvest applications of Movento, Assail, Sequoia, and Centaur did not provide any benefit towards mealybug control. This was primarily because overwintering mortality is naturally so high that the effects of an insecticide spray after harvest were undetectable in the spring. The efficacy of early spring insecticide applications could not be determined due to the impacts of an insecticide application that was accidentally sprayed over the top of our entire trial in late March. We did not become aware of this incident until late May such that we had to disregard all data collected following the spring treatment.

Once we were informed that our trial had accidentally been oversprayed, we scrambled to find a new research site where insecticide applications in May could be evaluated. A site near Buttonwillow, Kern Co. was quickly identified, and a trial was set up to evaluate 13 different insecticide treatments when applied on 2 June 2022 using six replications of single-tree plots with insecticides applied at a water volume of 200 GPA. Evaluations every 2 to 3 weeks through harvest showed that Movento, Senstar, Centaur, Assail and Sequoia were effective at controlling Gill's mealybug (Fig. 1). Other products that caused moderate reductions in mealybug density included Sivanto, Fujimite, PQZ (pyrifluquinazone), Aza-Direct, MBI-306, and Sefina.

## Conclusion

The most effective timing for Gill's mealybug control continues to be late May through the first few days of June. Post-harvest treatments are not effective, and we were unable to determine the effectiveness of applications during the early spring. Pistachio growers should continue to work closely with their processors regarding the disposition of their crop to determine limitations on maximum residue limits (MRLS) while cross-referencing our data on efficacy when making decisions on which products to use and when to apply them.

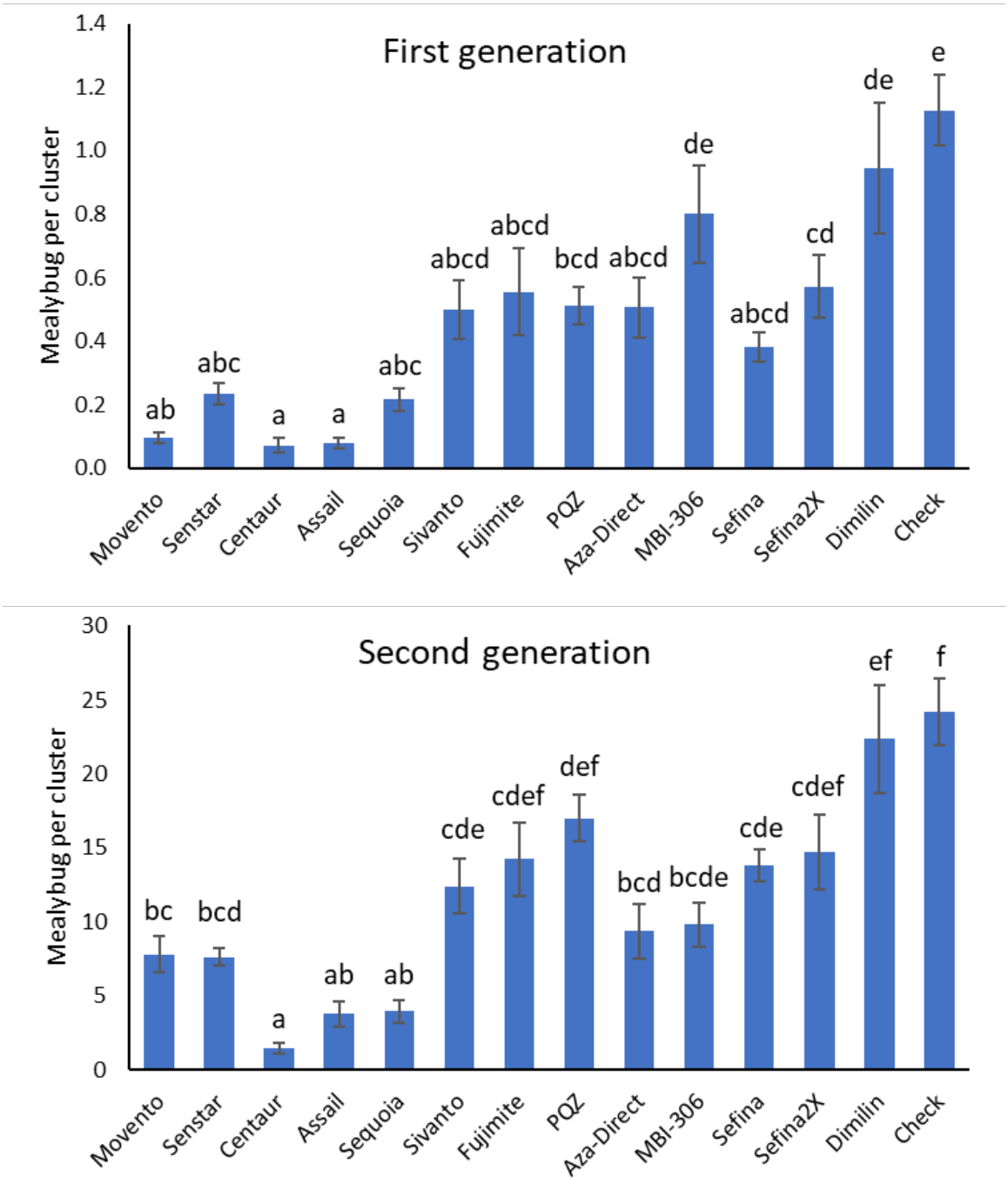


Figure 1. Mean ± SE Gill's mealybugs per cluster during the first (June-July) and second (August) in-season mealybug generations.

# Attractants for leaffooted bugs in pistachios

**Authors:** **Andrea L Joyce** Associate Professor, University of California Merced, **Kylie McMillan**, Staff Research Associate, University of California Merced.

## Introduction

Leaffooted bugs can be a pest in pistachio and bug feeding can result in crop damage (Daane et al. 2008). There is a need to develop traps or monitoring devices for early detection. In addition, few insecticides control these large bugs in conventional or organic orchards. The overall goal of this proposed work is to further our understanding of potential attractants for leaffooted bugs in order to aid in monitoring of this pest. Previous studies of potential attractants for *L. zonatus* include investigating host plant associated products such as pistachio, almond meal, and pomegranate volatiles (Beck et al. 2018), while others have investigated insect-produced odors such as pheromones (Wang and Millar 2000, Joyce et al. 2017a). We previously investigated adult *L. zonatus* flight behavior to attractants in a wind tunnel and found that mating pairs of *L. zonatus* were attractive to both *L. zonatus* males and females (Joyce et al. 2017a). Panel traps were investigated by Wilson et al. (2020) who found they could be used for monitoring leaffooted bugs. Others are working on male produced attractants for *L. zonatus* (Millar et al. 2018). A commercial attractant is not yet available for leaffooted bugs. We are continuing our research to examine the potential for an attractant produced my mating pairs of *L. zonatus.*

Another factor which could influence monitoring or control of *L. zonatus* is that there are two genetically distinct strains of the leaffooted bug *L. zonatus* in California (Joyce et al. 2017b). One strain is primarily found in California, while the second strain is found from California all the way to Brazil; this second strain will be called the 'widespread' strain (Joyce et al. 2021). It will also be important to know if the two strains of leaffooted bug cross attract and could be attracted to the same pheromone. In addition, it will be interesting to determine if the mating pairs of the two strains produce the same pheromone. Objectives of this two-year project include comparing the biology (fecundity, longevity, mating behavior) of two strains of the leaffooted bug *L. zonatus*, examining the relative attraction of adult *L. zonatus* to food and pheromone sources, and relating trap catches of *L. zonatus* to bug damage on pistachios. Previous results were reported last year and will be briefly summarized here as well.

## Results and Discussion

*Fertility and Cross Mating of Strains*

Colonies of *L. zonatus* are maintained in the lab which are a mix of the two strains. From those colonies, separate colonies were produced for each of the two strains of *L. zonatus* which are found in the Central Valley, the 'California' strain and the 'Widespread' strain. This year we determined the strain type for an additional 170 mating pairs using two qPCR probes to rapidly identify adults. We have colonies of the two strains of *L. zonatus* and we continue to set up mating pairs and then screen their offspring to determine whether they are the California or widespread strain type.

*Relative attraction of adult L. zonatus to food or pheromone sources*

Food sources and pheromone odors are both potential attractants for *L. zonatus.* We suspect that pheromones may attract adult leaffooted bugs more than food sources. However, this may depend on the time of year and mating status of the insects. We are also interested in whether mating pairs of each strain are equally attractive to adult *L. zonatus* of both strains, or whether each strain is preferentially attracted in nature to its own strain. Last year we began work in the wind tunnel to examine relative attractive of food odors compared with those produced by mating pairs. This year the goal was to determine whether both strains of *L. zonatus* cross attracted each other from a distance.

*Cross Attraction Between L. zonatus Strains*

Cross attraction between the two strains of *L. zonatus* (California strain and Widespread strain) could suggest that a pheromone from one strain would attract insects from the second strain. If so, this would be beneficial from a pest management perspective. Research on this goal had delays this year and we have extended the research with a no-cost extension from year two into year three. The goal remains to determine if both strain of *L. zonatus* cross attract. We will examine if males of both strains are attracted to mating pairs of the widespread strain. At this time, the widespread strain is known to have the more widespread distribution. To complement the experimental portion of this question, we are collecting volatiles from mating pairs of the Widespread strain and comparing them to volatiles from mating pairs of the California strain. We began by using 10 mating pairs of *L. zonatus* and holding them in a 1L glass flask overnight. The next day, volatiles were pushed out of the flask into a volatile collection trap. Samples were eluted with hexane and then run on a GC-MS machine. This work will continue into next year.

## Conclusion and Practical Applications

We have found that the two strains of *L. zonatus* cross mate and produce offspring under lab conditions; the two strains may cross attract in the field. In addition, trials in the wind tunnel have shown that mating pairs of *L. zonatus* are attractive to adult male and female *L. zonatus*. Mating pairs were more attractive to adult *L. zonatus* than pistachio odors. This work continues to examine whether there is cross attraction at a distance between the two strains. This research complements other research being conducted on *L. zonatus* pheromones. There are two *L. zonatus* strains in nature, and we will contribute to understanding whether a pheromone produced for one strain of *L. zonatus* will also attract the second strain.

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

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

## Introduction

Gill's mealybug *Ferrisia gilli* is of increasing concern in pistachios in California, due to direct feeding damage to nuts and decreased yields, and indirect damage due to growth of sooty mold on the copious honeydew produced by the mealybug. For significant infestations, the mealybug requires careful monitoring so that insecticide applications can be timed for maximum efficacy. As with many other agricultural pests, pheromone baited traps could be a useful tool for sensitive and specific monitoring of adult mealybug densities and population dynamics.

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

## Results and Discussion

We began this project just as the Covid pandemic started, which delayed the start by most of a year. Thus, 2021 was our first full year of work on the project. During 2021, we initially tried rearing the mealybugs on butternut squash, for several reasons:

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

However, the colonies only reproduced slowly on butternut squash, as the insects became adapted to what for them is an unnatural substrate. Nevertheless, we persisted, and collected and analyzed 59, 1-week long collections from female mealybugs on butternut squash.

Because of the slow growth of the mealybugs on butternut squash, we tried two alternative host materials, grapevines, and acorn squash. Thus, we carried out 20, 1-week long pheromone collections on infested grapevines, and 5, 1-week long collections on acorn squash. All extracts were analyzed by gas

chromatography coupled with electroantennogram detection (GC-EAD), in which the antennae of a live male mealybug are used as a living pheromone detector. GC-EAD is usually more sensitive than the GC detector. From all these extracts, we obtained a few antennal responses at the same spot, but the amounts of the active compound were too low to be seen clearly by gas chromatography-mass spectrometry. In short, the data showed that there is a pheromone, but under our rearing conditions, it was produced in amounts too small to work with. Thus, our goal for the 2022 year was to try every possible means of increasing the pheromone production by females, to produce enough to analyze.

Previously we had reared the mealybugs on butternut or acorn squash for roughly a month in complete darkness, as is commonly done with other mealybug species. Male cocoons were removed before the adult males emerged, to prevent females mating. In 2022 we began rearing the mealybugs on squash in a summer equivalent 14:10 light:dark cycle, and after about 3 weeks females were manually moved from these rearing squash onto new uninfested host substrates that were kept separate from the rearing squash. This was done to reduce the risk of a few unnoticed males mating with many females on the host substrate, as well as providing fresh host substrate for the females being used for pheromone collections. The hosts used were again butternut and acorn squash, but we also tested sprouting potatoes, another substrate that has often been used for lab rearing of mealybugs and scales.

A total of 17 pheromone collections have been carried out so far using these substrates. Ten were on butternut squash, 3 on acorn squash, and 4 on sprouting potatoes. We also collected for longer periods of time, 10-14 days for each aeration, to try and increase the amount of pheromone in the extracts. These aerations went from December 1, 2021-May 2, 2022. Extracts from these aerations were first analyzed by coupled gas chromatography-mass spectrometry as before, both in unconcentrated and concentrated form. To try and further increase detectability, samples from the same host substrate were combined, concentrated down again, and reanalyzed to increase the chances of seeing a pheromone. In total, 24 extracts or combined, concentrated extracts (13 butternut, 3 acorn, 8 potato) were analyzed by GC-MS and GC-electroantennogram detection.

Because the mealybugs did very well on grapevines as a host plant in 2021, we also retested grapevines as a host substrate for pheromone collections. 75 Zinfandel grapevines donated by Duarte Nursery were infested, starting in September. The first set of pheromone collections of infested grapevines started in October and are ongoing. Initially aerations were carried out with a single infested vine, collected from for 72 hours, but we have now started aerating the infested vines for a week at a time. So far, 6 aerations have been completed and 14 samples (due to concentrating and combining) have been analyzed by GC-MS and GC-EAD. However, from all of these analyses carried out in 2022, we have not seen any antennal responses indicative of pheromone presence, nor have we seen any peaks in the relevant part of the chromatograms that might possibly be pheromone components. We intend to continue to collect and analyze extracts from infested grapevines until the end of the grant period in February 2023, in the hopes of finally detecting a pheromone peak.

**Conclusions**

At this point, it seems unlikely that we will be able to find and obtain enough of the pheromone to have a chance at identifying it. Thus, unless things turn around quickly, we intend to close this project down at the end of February. We are extremely reluctant to do this given the funding and effort that have gone into the project, but we can see no way forward at this time.

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

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

## Introduction

Epicarp lesion, nut abortion, and kernel necrosis caused by the feeding of a suite of true bug species are 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). 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 that accelerates the aggregation of adults of both sexes (Yasuda and Tsurumachi 1994). Because of these rapid buildups, and since bug damage may only become apparent after the bugs have moved on, continuous monitoring of LFB populations is crucial for timing treatments. Current monitoring relies on beat sampling and/or visual assessment of nuts for damage, both of which are time and labor intensive, and many times fail to detect LFB populations early enough to take action. As such, passive trapping systems based on pheromonal, or related attractants would be of great value for monitoring and potentially control purposes.

Several recent pieces of evidence strongly support our working hypothesis that male LFB produce powerful pheromones that attract both sexes. First, Inoue et al. (2019) demonstrated in olfactometer bioassays that adults were attracted to odors released by sexually mature males. Even more important, in 2021, our European collaborators studying the invasive *Leptoglossus occidentalis* trapped >10,000 bugs in trials with synthetic pheromone blends. In 2021, we successfully synthesized all components of the LFB pheromone and were able to demonstrate its ability to attract LFB adults in the field. Now, in 2022, we continued efforts to (1) improve the efficiency of the synthesis process and (2) compare different blends and ratios of key pheromone compounds – all aimed towards development of an effective pheromone lure for LFB.

## Results and Discussion

[Improved Synthesis of Leptotriene] In past years, we have shown that sexually mature adult male *L. zonatus* produce ~10 volatile compounds, including "leptotriene", a compound entirely new to science. Leptotriene is a relatively minor component of the blend, but it elicited the largest responses from antennae of both sexes of LFB in electroantennogram assays. We also found it in volatiles from male *L. clypealis*, probably the second most important species for California (although populations currently appear to be in decline for unknown reasons). Thus, a large part of our effort in 2021 focused on developing a first synthesis for this new compound. By early summer 2021, we had all the components ready for testing, albeit in limited quantities, and the initial lab and field trials produced promising results (see Executive Summary for Millar et al. 2021). In 2022, careful optimization of the synthesis conditions resulted in a ~five-fold increase in the yield, providing more material for bioassays. Our initial route (in 2021) to the key component leptotriene started with a cheap starting material, beta-caryophyllene, which has most of the structural elements of leptotriene, and the correct stereochemistry. Leptotriene can be made in four steps from this starting compound (a short synthesis minimizes labor costs). However, the overall yield was only 1%, primarily because the first and last steps had yields of only 11% and 24% respectively. In 2022, we carefully optimized these two steps, improving the yields to 30% and 40% respectively, thus increasing the overall yield 4.6 times. Further savings in labor and materials costs may

be possible by using less pure, "technical grade" pheromone, because purification to high and possibly unnecessary levels of purity is time- and materials-intensive.

[Field Trials with Candidate Lures] With the new pheromone compounds in hand, we conducted a series of field trials in 2022 to evaluate leptotriene, both alone and in combination with other possible components. Pheromone lures were formulated at UC Riverside and shipped to the UC Kearney Ag. Center for testing. In all orchard trials, lures were placed into black hanging-panel traps coated with fluon. The first trial measured LFB response to the compounds in three almond orchards during the typical spring colonization period (4/5/22 – 5/10/22), with lures replaced every 2 weeks. Treatments included leptotriene, leptotriene + aldehydes, leptotriene + bergamotene, leptotriene + aldehydes + bergamotene, and a solvent only (hexane) control. While all of the candidate pheromones attracted female LFB adults (Figure 1), the most attractive lure contained the more complete pheromone blend (leptotriene + aldehydes + bergamotene). The second trial evaluated attractancy of leptotriene alone over the course of the entire year in three different orchard types (almond, pistachio, pomegranate) at the UC Kearney Ag. Center, with lures replaced every 4 weeks over the sample period 4/5/22 – 11/1/22. Leptotriene was consistently more attractive to adult female LFB relative to the hexane control lure (data not shown). While populations of LFB adults varied over the course of the season in each orchard, this generally reflected their phenology in each of these three crop types. The third field experiment evaluated different pheromone blends in a pomegranate block at the UC Kearney Ag. Center from 8/2/22 – 10/25/22 when populations of LFB were especially high. Treatments included leptotriene, leptotriene + aldehydes, leptotriene + bergamotene, leptotriene + aldehydes + bergamotene, and a solvent only (hexane) control. A set of higher-dose lures also were evaluated in the last week 10/18/22 – 10/25/22. Relative to the control lures, LFB adults appeared to respond most frequently to "leptotriene + bergamotene" and "leptotriene + aldehydes + bergamotene" (Figure 2).

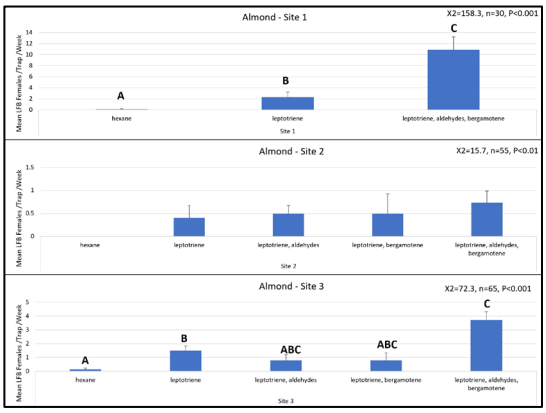



**Figure 1.** In almond orchards, the full blend of pheromone compounds was the most attractive to LFB adults.

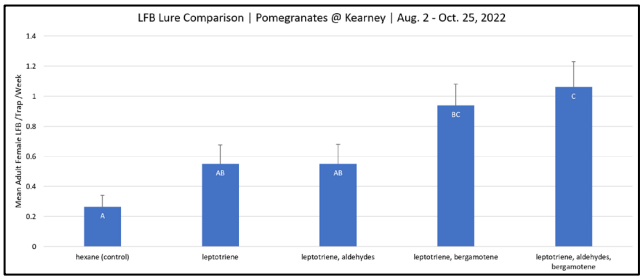



**Figure 2.** Adult LFB were attracted to leptotriene, especially when blended with other possible pheromone components.

## Conclusions

We have now identified and synthesized all possible components of the *L. zonatus* pheromone. Confirming what we saw towards the end of 2021, our field assays in 2022 have shown that the novel compound leptotriene is a key component of the attractant pheromone of *L. zonatus* and is likely synergized by one or more additional components. Furthermore, placing the lures in a hanging panel trap effectively demonstrated the potential for this new monitoring system in orchards. While additional work is still needed to further refine this trap and lure system, findings from 2021 and 2022 were very promising. In 2023, we plan to continue evaluating new iterations of the lures, as well as a further improved trap for use with these lures.

# Effective biological control of Gill's mealybug from drone releases of green lacewing

**Authors:** **Andreas Neuman**, CEO, UAV-IQ; **David Haviland**, Farm Advisor, University of California Cooperative Extension Kern County; **Thomas Grandperrin**, Project Manager, UAV-IQ.

## Introduction

Biological control has historically been lacking as a viable control method for Gill's mealybug. This has been true within production systems that are both conventional and organic. Lacewings are the most common predators seen feeding on Gill's mealybug, however, populations are generally low and not sufficient to provide the levels of control needed. Likewise, predatory coccinellid beetles are rare and usually only found in the fall. Additionally, parasitoids that readily attack Gill's mealybug when it infests grapes and almonds have had a negligible presence within pistachio orchards.

This project attempted to address this problem by doing inundative releases of lacewing larvae to augment the natural population already present in a pistachio orchard. During the first year of the two-year project, applications of lacewings were made by drone at a rate of 5,000 per acre on 28 April and 11 May. Beat samples and cluster evaluations conducted on ten evaluation dates following releases were unable to document clear recovery and establishment of the lacewing larvae and there were no significant differences in mealybug densities on any evaluation date through harvest ($P > 0.32$).

During the second year of the project, we made an attempt at doing inundative releases after harvest. This timing was chosen because ample food would be available so lacewing larvae could readily feed, grow, and overwinter, such that they would be available the following spring to feed on the very small percentage of mealybug crawlers that survive the winter.

## Results and Discussion

Beat samples failed to document any establishment and recovery of the lacewing larvae. Only one larva was found across all plots on 27 Jan, and it was in a control plot. No lacewing larvae were found in beat samples collected on 23 Feb or 16 Mar. Mealybug evaluations showed that there were no differences in pest density in release plots compared to the control, nor in the percentage of infested clusters.

During both years of the study, we were unable to document any significant impacts of lacewing releases by drone in pistachios. This included spring-time releases of split applications of 5,000 per acre, as well as a single fall application of 10,000 per acre. In both cases we failed to document that the larvae became established in the orchard and found no effects on mealybug density.

We do not know why the releases were not successful. Following the first year of the study we hypothesized that released larvae could have starved to death before finding food due to the relatively low mealybug density that was present in the spring. We debunked that idea with our fall applications where mealybug density was extremely high, as is common after harvest, such that the availability of food would not have been a limiting factor. Additionally, at this time of year many mealybugs move from clusters to the more permanent woody parts of the tree where lacewing larvae that landed on the ground after releases would have easier access to prey.

Another possibility is that the tree structure is not highly conducive to lacewing releases by air. Releasing lacewing larvae over a pistachio tree is much different compared to making releases over a short-statured crop such as strawberries where drone releases of larvae have been successful. Within the pistachio system, most of the larvae falling from the drone were likely to either fall through the tree canopy, either by missing the canopy altogether, or by landing on a leaf and sliding off to the ground, especially due to

air currents caused by prop wash from the drone overhead. As a result, larvae would have landed on the ground where they needed to find a tree trunk to climb in order to find food. It is possible that the resources involved in this process could have been exhaustive to small larvae, or that they could have succumbed to predation by ants foraging on the orchard floor.

Another idea is that the lacewing larvae did not survive the release process, but that was also debunked. During the fall applications we put out large blue tarps under areas where releases occurred, and we were able to find live larvae that made it to the ground safely.

It is also possible that the release rates were insufficient to have an impact, although we lack data to prove it. The release rate of 10,000 per acre corresponds to approximately 65 lacewing larvae per tree - assuming they all remain in the tree after release. We don't know what percentage of the released larvae fell to the ground instead of the canopy. At this rate we believed that we should, at minimum, have been able to recover larvae during the weeks immediately following releases. But we've seen that beat samples tend to recover low sample numbers, so our results do not conclusively support a finding that lacewing larvae were not present. Even if the sampling methods had proven to be effective to collect lacewings, the time interval between the fall release and sampling was longer than the time required for lacewing larvae to become adults. Low sample counts could have been the result of the larvae maturing to adulthood and then flying out of the control fields before laying eggs for the second generation.

Over the years we have made numerous observations of pistachio orchards where there has been a significant variation in the number of lacewing larvae established naturally. We have suggested that this variation was likely influenced by pesticides that were used to control small bugs (lygus, phytocoris, calocoris, etc.), large bugs (stink bugs, leaffooted bugs) and navel orangeworm. However, our experimental orchard was not sprayed for small or large bugs during the length of our trial, and navel orangeworm sprays were limited to products containing methoxyfenozide and chlorantraniliprole that would have negligible impacts on lacewings. As a result, we have ruled out impacts of insecticides as a contributing factor for the lack of establishment of lacewing larvae.

## Conclusion

In summary, at the conclusion of our study, we have failed to document any impact of releasing small lacewing larvae by drone. If future efforts are made towards inundative releases of lacewings in the pistachio system, we suggest that research focus on alternate release methods or methods using different stages of lacewings, such as through cards with lacewing eggs placed near clusters with known infestations. If those are successful, it would be worthwhile exploring whether methods such as wetting the leaves and using different substrates when releasing from drones to increase the percentage of product remaining in each tree can achieve positive results with the additional benefit of reducing labor requirements.

# Control of navel orangeworm: focus on increasing insecticide efficacy and reducing application volume using organosilicone 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 goal is to improve control of navel orangeworm (NOW) by reducing water use. Product names are for specific information purposes and their mention does not constitute an endorsement by the USDA. In an experiment conducted in 2022 at Agri-World Coop, we examined the efficacy of an application rate of 70 gallons per acre (70 gal/ac) combined with Altacor eVo (2.25 oz/ac; FMC), an organosilicone adjuvant used at a rate of 12 oz per 100 gal (Kinetic; Helena Agri-Enterprises), compared to a grower standard application rate of 100 gal/ac with a combination of Intrepid (24 oz/ac, Corteva Agriscience) +Bifenthrin 2EC (12.8 oz/ac, Helena Agri-Enterprises) as the insecticide application and the sunscreen adjuvant Cohere (12.8 oz/100 gal; Helena Agri-Enterprises) as adjuvant. The trial was conducted in August in Madera County. We measured the duration of control at a height of 14-16 feet using a filter paper contact toxicity bioassay. Filter paper was also placed at 5 feet and collected 5 days after application to establish maximum potential percent kill in case of loss as the insecticide rose in the canopy. All filter papers were collected on days 5, 12 and 19 after commercial ground application using an Air-O-Fan GB36 PTO air blast sprayer (500-gallon tank). The filter papers were then placed in petri dishes containing NOW wheat bran diet and challenged by placing 50 eggs in the center of the filter paper. Newly hatched larvae contacted the insecticide when they crawled over the treated surface to reach the diet, and mortality was scored 18 days later.

## Results and Discussion

Table 1. Year 2022 duration of control for Altacor eVo (2.25 oz/ac) applied at 70 gpa with the organosilicone adjuvant Kinetic compared to a mixture of Intrepid + Bifenthrin and the adjuvant Cohere applied at 100 gpa, August.

| Treatment | Mortality | Reduction | Eggs |
|---|---|---|---|
| ***Control Day 5*** | 52.40% | | 450 |
| Cohere 100 gpa **ground** Day 5 | 99.50% | 98.95% | 600 |
| Kinetic 70 gpa **ground** Day 5 | 97.33% | 94.39% | 600 |
| Cohere 100 gpa **hook** Day 5 | 99.00% | 97.90% | 2,000 |
| Kinetic 70 gpa **ground** Day 5 | 97.80% | 95.37% | 2,000 |
| ***Control Day 12*** | 59.80% | | 500 |
| Cohere 100 gpa **hook** Day 12 | 97.65% | 94.15% | 2,000 |
| Kinetic 70 gpa **ground** Day 12 | 95.05% | 87.69% | 2,000 |
| ***Control Day 19*** | 56.22% | | 450 |
| Cohere 100 gpa **hook** Day 19 | 99.35% | 98.52% | 2,000 |
| Kinetic 70 gpa **ground** Day 19 | 97.80% | 94.97% | 2,000 |

In practical terms there was no difference in mortality between 70 and 100 gpa, and the insecticides used for both adjuvants were stable for 19 days. Our data for 70 gpa were identical to the data reported last year for 80 gpa, demonstrating that the combination of organosilicone adjuvants and Air-o-Fan spray rigs equipped with multi nozzles could successfully treat pistachios at water volumes of 70-80 gpa. Other organosilicone adjuvants should be equally successful under these conditions.

It is worth reviewing our data from our initial study in 2020, using a water volume of 50 gpa and organosilicone adjuvant rates of 16 oz/100 gal for Kinetic, 12.8 oz/100 gal for Silwet 719, and 12.8

oz/100 gal for Cohere. Intrepid Edge (19 oz/ac) was used as the insecticide. Note that at 4 days after application there were no differences among treatments in the canopy, but by day 11 the applications at 50 gpa began to break down compared to 100 gpa, although the application using Silwet 719 had higher mortality than the application using Kinetic. It is dangerous to put too much weight on any single trial because Kinetic was quite effective in 2022; the best way to evaluate the efficacy of organosilicone adjuvants at rates below 100 gpa is to pool the information from multiple trials. At 50 gpa, organosilicone applications were not as stable as they were at 70-80 gpa, while at 100 gpa (Grower Standard) and the adjuvant Cohere, the applications were stable regardless of insecticide used.

Table 2. Year 2020 duration of control for Intrepid 2F (24 oz/ac) + Bifenture EC (12.5 oz/ac) applied at 50 gpa with Silwet 719 compared to Cohere applied at 100 gpa, August.

| Treatment | Mortality | Reduction | Eggs |
|---|---|---|---|
| *Control Day 4* | 52.50% | | 400 |
| Cohere 100 gpa hook | 92.60% | 84.00% | 500 |
| Kinetic **50 gpa** hook | 80.20% | 58.00% | 500 |
| Silwet 719 **50 gpa** hook | 94.60% | 89.00% | 500 |
| *Control Day 11* | 55.40% | | 500 |
| Cohere 100 gpa hook | 86.20% | 69.00% | 500 |
| Kinetic **50 gpa** hook | 68.80% | 30.00% | 500 |
| Silwet 719 **50 gpa** hook | 79.40% | 49.00% | 500 |
| *Control Day 14* | 55.40% | | 500 |
| Cohere 100 gpa hook | 86.20% | 69.00% | 500 |
| Kinetic **50 gpa** hook | 69.80% | 32.00% | 450 |
| Silwet 719 **50 gpa** hook | 77.80% | 50.00% | 500 |

In terms of overall mortality, the two organosilicone adjuvant treatments differed, with the Silwet 719 consistently producing higher mortality that was stable for 14 days. However, the 100 gpa treatment with Cohere was equally stable and produced even greater mortality, making it the superior treatment.

## Conclusion

Some variability within and between experiments is expected, and organosilicone adjuvants from three different manufacturers produced satisfactory results when paired with Air-o-Fan multinozzles in 2021 and 2022. These results should extend to organosilicone adjuvants produced by other manufacturers. Reducing water volume to 70-80 gpa enabled each tank load to treat an additional acre before refilling (100 gpa treats 5 acres, 70-80 gpa treats 6 acres), an increase in efficiency of 20%. This is an important saving in time and in turn, money. Future efforts will focus on determining if similar results can be achieved using spray rigs from other manufacturers, as well as determining if there is an optimal organosilicone adjuvant concentration for air application.

# Producing sterile navel orangeworm on demand for improvement of pest management

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

## 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. The program goal is to develop SIT as a complementary strategy to augment existing IPM tools for NOW. Over the past five years, co-PIs Wilson and Burks have led scientific efforts to evaluate the quality and performance of sterile NOW produced by this Phoenix facility. Initial studies documented poor performance of the sterile NOW, and subsequent efforts focused on identifying key bottlenecks in the production, transportation and release process. Goals of the research in 2022 were to continue evaluating field performance of sterile NOW from the Phoenix mass-rearing facility, as well as explore the use of x-ray sterilization as a potential alternative route to lower-cost production of sterile moths.

## Results and Discussion

### Objective 1 – Verify and optimize x-ray sterilization of NOW (Wilson)

Initially, this objective was delayed in 2021 due to an injury to the postdoctoral scholar assigned to work on the x-ray project. In 2022, that individual subsequently left the program prematurely, and so work on this objective has been further delayed. Over the summer we hired a new postdoctoral scholar to work on x-rays and as of November 2022 the work has resumed, but it is too early to report any results at this time.

### Objective 2 – Effects of environmental variables (cold, motion) on NOW field performance (Burks)

(2A) Pheromone Dispensers for Prevention of Mating the First Night after Adult Emergence:
A postdoctoral scholar was hired to work on this objective in fall 2022. Currently preliminary experiments with Y-tube and pheromone exposure chambers are underway.

(2B) Fluctuating Thermoperiods to Extend Adult Longevity and Vigor
This second subobjective was postponed pending additional progress on subobjective 2A.

### Objective 3 – Determine sources of variability in field mark-release-recapture experiments (Wilson/Bansal)

(3A) Sterile NOW Field Dispersal and Release Rate (Wilson)
*[Experiment 1 - Strain Comparison]* Our previous studies demonstrated that the procedures utilized by the Phoenix Facility have significant negative impacts on NOW performance. Recently, a new strain of NOW (the 'MCS' strain) was developed by the Phoenix Facility to better tolerate rearing and handling conditions. As such, a series of lab and field assays were carried out at the UC Kearney Agricultural Research and Extension Center (Parlier, CA) to compare the performance of the new 'MCS' strain to the existing 'Phoenix' strain. Data from flight cylinder assays and field recapture studies in two small orchards (pistachio and almond) found that the 'MCS' strain consistently outperformed the 'Phoenix' strain.

*[Experiment 2 - SIT Impacts in Paired Almond Blocks]* For the second year in a row, three pairs of 160-acre almond blocks (6 blocks total, no mating disruption) were used to evaluate the influence of APHIS

sterile NOW releases on wild NOW populations and crop damage. Activity of both wild females and wild males was unchanged in blocks that received sterile NOW, and recovery of sterile NOW was highly variable. For the second year in a row, levels of NOW infestation were equivalent between the blocks with and without sterile NOW releases.

*[Experiment 3 - Sterile Moth Dispersal in Pistachios]* This experiment is also in its second year and was designed to evaluate dispersal of APHIS sterile NOW across three large (640-acre) pistachio blocks (no mating disruption). Data on the recovery of sterile NOW demonstrate that while sterile males and females can disperse throughout the entirety of the block, approximately 50% of them remain in the release area.

(3B) Recapture of X-ray Sterilized NOW (Wilson)
As mentioned in Objective 1, the postdoctoral scholar working on the x-ray component of this research program left prematurely, and so this subobjective was unable to be completed. We have hired a new postdoctoral scholar and as of November 2022 has resumed this x-ray sterilization work.

(3C) Develop a Marker for NOW Spermatophores (Bansal)
The goal of this experiment was to determine NOW's molecular response to ionizing radiation, which could be used to identify sterile male spermatophores in mated wild females. Male adults were irradiated at a dose of 300 Gy and compared to a non-irradiated control. Ten adults from each group were collected at 6, 12, 24, 48, 72, 96 h after the irradiation. Currently, these insects are being processed for RNA extraction which will be followed up by RNA-sequencing. As mentioned above, this study was delayed and started only after X-ray standardization work by new postdoctoral scholar.

**Objective 4 – Use sterile NOW to compare recapture in mating disruption and non-mating disruption fields (Burks)**
(4A) Sterile NOW Female Response to Ovipositional Baits
Laboratory two-choice experiments were conducted to compare locally reared non-irradiated females (Mendota strain, 55 tests) to a mass-reared strain (Phoenix) with (52 tests) or without (49 tests) irradiation. Under the test conditions, about 146 eggs per test were laid by mated locally reared females, compared to about 10 per test laid by mass-reared mated females with and without irradiation. In all cases about 70% of the eggs were laid on an egg trap (more favorable to development) and 25% on an egg trap. These results indicate that irradiation does not impact ovipositional choice.

(4B) Recapture of Sterile NOW Under Mating Disruption
A series of 7 field releases were conducted in the summer of 2022, each consisting of 10,000 to 20,000 moths released at the center of adjacent 160-acre blocks heavily impacted by NOW mating disruption that was being used in an adjacent orchard section. Females with internal dye, indicating mass-released sterile females, were consistently recaptured in each release event and comprised approximately 10% of the NOW females in the 80 oviposition bait traps in the two 160-acre plots. Taken with the laboratory study above, these results indicate that irradiated mass-released females are consistently recaptured with ovipositional baits in the presence of mating disruption.

**Conclusion**
The new 'MCS' strain of NOW looks promising and could help improve the field performance of sterile NOW released in larger blocks. This is important within the context our two-year study with paired almond blocks, in which weekly releases of the 'Phoenix' strain of sterile NOW did not lead to any change in NOW populations or crop damage. It may be that use of the 'MCS' strain could generate different outcomes. Similarly, it would be of interest to evaluate dispersal of this new strain in large blocks akin to the two-year study that was just completed with the 'Phoenix' strain. Data from the x-ray experiments and some large-block trials were unfortunately limited due to a series of logistical and personnel constraints.

# Influence of pistachio hull degradation and shell split on NOW egg deposition and infest

**Authors:** **Houston Wilson**, Asst. CE Specialist, Dept. Entomology, UC Riverside; **Chuck Burks**, Research Entomologist, USDA-ARS, San Joaquin Valley Ag. Sci. Center, Parlier, CA; **Selina Wang**, CE Specialist, Dept. Food Sci. and Tech., UC Davis. **Collaborators:** **Barbara Blanco-Ulate**, Asst. Prof., Dept. Plant Sciences, UC Davis; **Giulia Marino**, Asst. CE Specialist, Dept. Plant Sciences, UC Davis; **Georgia Drakakaki**, Dept. Plant Sciences, UC Davis; **Louise Ferguson**, Dept. Plant Sciences, UC Davis; **Joseph Coelho**, Director of Agronomy, Maricopa Orchards LLC.

## Introduction

Infestation of pistachios by navel orangeworm (*Amyelois transitella*) (NOW) is contingent on the ability of this insect to gain access to the kernel, which is protected by both the hull and shell of the nut. Over the course of the season, degradation of the hull and/or shell split provides access to NOW larvae. It is thought that NOW adult females can detect when such changes in the nut begin to take place, which subsequently triggers them to increase egg deposition on degraded nuts. This project is about both characterizing and 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 timing and extent of these events can vary significantly from year-to-year. Unfortunately, growers currently have no way to predict this, 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, the goal is to experimentally manipulate trees in different ways to see if certain practices can alter the timing and extent of hull degradation and shell split, and subsequently NOW egg deposition and infestation of kernels.

## Results and Discussion

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

In 2021 and 2022, high and low crop load experimental treatments were applied to 60 trees (var. Golden Hills) in a commercial pistachio block in west Fresno County (near Cantua Creek). Starting in late July, subsets of 10 cages for each of the two crop load treatments were inoculated with 5 mated (gravid) NOW females per cage, who were then allowed to oviposit for 1 week. Subsequent inoculations took place every month thereafter through October 15. At the time of inoculation, a subset of pistachios was sampled from each cage to record data on key chemical and textural properties.

*[NOW Egg Deposition – 2021/2022]* Both crop load (Year 1: $\chi^2$=45.3, n=25, $P$<0.001; Year 2: $\chi^2$=216.5, n=78, $P$<0.001) and inoculation date (Year 1: $\chi^2$=65.7, n=25, $P$<0.001; Year 2, $\chi^2$=1449.4, n=78, $P$<0.001) influenced NOW egg deposition onto the nuts (Figure 1), with more egg deposition onto the nuts on later sample dates and on high crop load clusters.

*[Texture Data – 2022]* Texture data indicated that the texture force of the hull, shell and kernel (Figure 2) were all influenced by sample date (hull - $\chi^2$=427.6, n=240, $P$<0.001; shell - $\chi^2$=26.6, n=240, $P$<0.001; kernel - $\chi^2$=83.7, n=240, $P$<0.001) but not by the crop load treatment (hull - $\chi^2$=1.7, n=240, $P$=0.20; shell - $\chi^2$=0.2, n=240, $P$=0.63; kernel - $\chi^2$=0.5, n=240, $P$=0.48).

*[Chemical Data – 2022]* While formal analysis is still underway, we can report that the production of volatile organic compounds generally tended to increase over time in each year of the study. More than 25

unique compounds were characterized, some of which either increased or decreased with each successive sample, resulting in a unique bouquet of volatiles each month.

Objective 2 – Optimize Caging Technique for NOW Egg Deposition Trials

This objective was completed in Year 1 of the study (2020). Window screen material was identified as the best cage material since it was the least preferred egg deposition substrate for NOW. For more details please see the Wilson/Burks 2020 Executive Summary for this project.

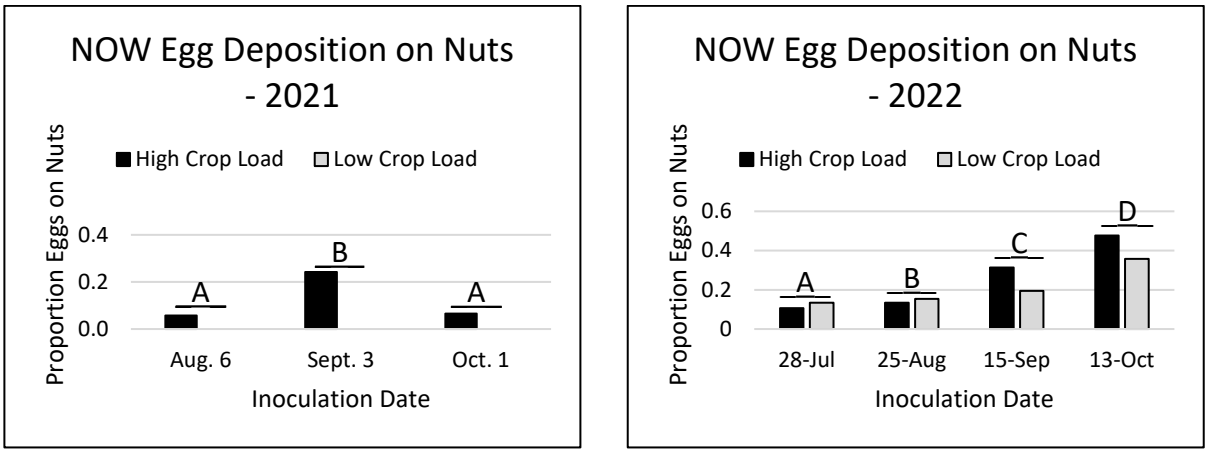



**Figure 1.** NOW egg deposition tended to be higher on 'High Crop Load' clusters and generally increased over time. For each year, sample dates that do not share letters are significantly different from one another.

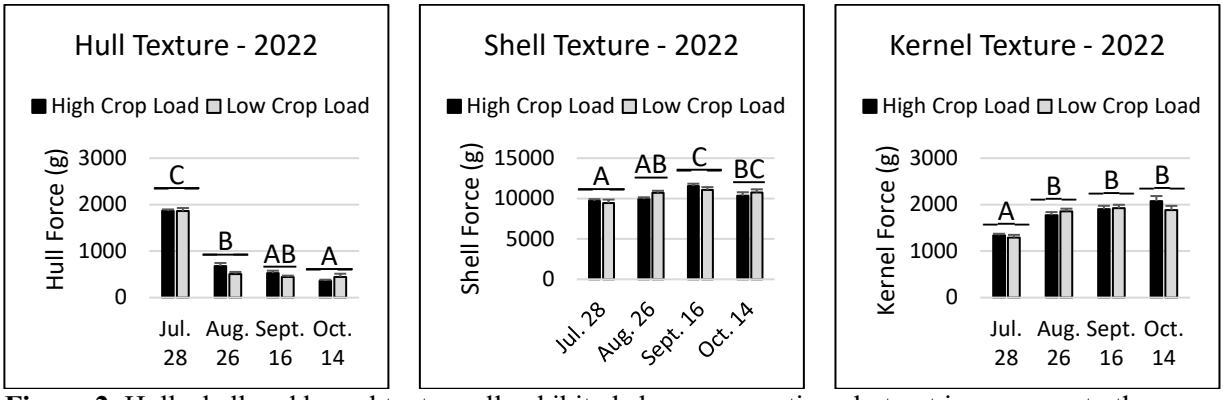



**Figure 2.** Hull, shell and kernel texture all exhibited changes over time, but not in response to the crop load treatment. For each measure, sample dates that do not share letters are significantly different from one another.

## Conclusion

Egg deposition by female NOW moths onto developing pistachio nuts increased over time, which is not surprising and has been observed previously (Beede et al. 1983, 1984). In contrast to these prior studies, this current project also took measurements of pistachio texture and chemical properties in parallel as a way of characterizing the specific changes that occurred in the developing nut. Egg deposition by NOW appears to be correlated with declines in hull firmness, as well as changes in the specific quantity and/or ratios of certain chemical compounds. Egg deposition was also increased in the high crop load treatment, although no changes in hull textural/chemical composition was observed as a result of the treatments. It may be that the greater availability of host material in the high crop load treatment stimulated increased egg deposition or simply provided increased available space for egg deposition.

# Spatiotemporal models to evaluate the potential value of sterile insect technique for control of navel orangeworm

**Authors:** **Houston Wilson**, Asst. Cooperative Extension Specialist, Dept. Entomology, UC Riverside; **Chuck Burks**, Research Entomologist, USDA-ARS, San Joaquin Valley Ag. Sci. Center, Parlier, CA; **Ran Wei**, Assoc. Professor, Center for Geospatial Sciences, School of Public Policy, UC Riverside; **Brittney Goodrich**, Asst. Cooperative Extension Specialist, Dept. Agriculture and Resource Economics, UC Davis; **Yujia Zhang**, Postdoctoral Scholar, Center for Geospatial Sciences, School of Public Policy, UC Riverside.

## Introduction

Recently, the pistachio industry made a significant investment in the development of sterile insect technique (SIT) for navel orangeworm (NOW). This program takes advantage of a pre-existing USDA mass-rearing and irradiation facility in Phoenix, AZ that was originally designed for a pink bollworm (*Pectinophora gossypiella*) SIT program. With SIT, large numbers of the target pest are mass-reared and sterilized (typically with gamma irradiation) and then released into crop fields or orchards. Pest population control occurs when sterile pests mate with wild pests, which negates the ability of the wild pests to reproduce. Since the 1950s, this tool has been used to control a wide range of agricultural pests that includes flies (Diptera), beetles (Coleoptera) and moths (Lepidoptera) in both annual and perennial cropping systems.

Development of SIT for any species/crop combination presents a unique set of challenges, from the radiation biology to the mass-rearing, transportation and release process. A key concept in SIT is the 'overflooding ratio', which refers to the ratio of sterile: wild pests per acre necessary to achieve acceptable control levels. Although the concept is straightforward, determining the appropriate timing and frequency to release sterile organisms is a function of multiple interacting factors. Even once the necessary overflooding ratio is understood, a mass-rearing process/facility must then be capable of producing an adequate number of sterile organisms to achieve this overflooding ratio over the total acreage desired – and to do so in a way that is economically feasible.

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

Here, we propose to develop spatiotemporal agent-based models to explore multiple scenarios for the use of SIT in tree nuts as a means of understanding the full potential of SIT for NOW. Models will estimate SIT program requirements, costs and potential impacts under various scenarios that incorporate a range of key variables, such as orchard size, overflooding ratios, dispersal rates, area requirements, mass-rearing production levels, and costs of sterile moth production, transportation, and release. This is not an exhaustive list. Each modeling scenario will assume different values for key variables, and in this way identify the most important program features that influence costs and efficacy (e.g. production vs.

transportation costs), highlight areas where more research is needed (e.g. dispersal rate, overflooding ratio), and most importantly estimate the total viable acreage for SIT and total number of moths needed to be successful. Findings from this effort are intended to generate a roadmap forward for the most logical and cost-effective development of SIT for NOW in California pistachios and almonds. Similar work has been conducted successfully with other sterile Lepidoptera programs, including painted apple moth in New Zealand (Wee et al. 2006) and sugarcane borer in South Africa (Potgieter et al. 2013, 2016).

## Results and Discussion

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

Co-PIs Wilson and Burks initially defined a series of key environmental, biological and production variables, as well as interactions between them, that would potentially drive regional NOW population development. Those variables have now been incorporated by co-PIs Wei and Zhang into an agent-based model (ABM) that can generate estimates of various outcomes, such as NOW population development over time and crop damage at harvest. A series of NOW management scenarios were subsequently defined to include different combinations of sanitation, pesticide applications, mating disruption and sterile insect releases. The ABM was then used to evaluate these different scenarios in multiple regions of California (10 total regions). During the initial model runs, it became apparent that the inherently large quantity of organisms per acre would require additional computing power. As such, co-PIs Wei and Zhang are now working with the UC Riverside High-Performance Computing Cluster (https://hpcc.ucr.edu/) to develop methods to run these models over large regions (10 x 10 km). In the interim, using a more basic computing setup, model outputs have been generated for smaller areas (0.01 x 0.01 km) as a proof of concept, although the utility of these outputs for analysis and decision-making is extremely limited.

In parallel, co-PI Goodrich has developed a template for economic analyses of the model outputs to determine feasibility of adoption. These analyses broadly fall into two categories, cost-benefit analysis and break-even analysis. The cost-benefit analysis utilizes data on fixed and variable costs of different elements of NOW management, which can be used to estimate the total costs of each ecological scenario relative to the benefits from any changes in NOW damage. The break-even analysis estimates the minimum/maximum values of key parameters for SIT to be economically feasible under the different ecological scenarios. Key parameters include the minimum almond price and/or damage reduction necessary for SIT to be economically feasible, as well as the maximum number of sterile moths that could potentially be released. Once the computing limitation of the ABM is solved (see above), these econometric models will be applied to the scenario outputs.

## Conclusions

This project is still underway, as we continue to work on overcoming the computational bottleneck required to execute these computationally intensive models. As such, the question of ecological/economic feasibility of SIT for NOW remains unclear. That said, project efforts to date have generated a spatially-explicit agent-based model for NOW, as well as templates for associated economic analyses. As mentioned, our current major barrier is computing efficiency. In Year 2, we plan to work on parallelization of the simulation algorithm using the high-performance computing resources on the UCR campus, as well as acquire field data that can be used for model validation and refinement. While this project was catalyzed by the need to evaluate economic feasibility of SIT and (if feasible) identify priority areas for release of sterile moths, we anticipate that the global NOW population model required to answer these questions will provide a platform for a series of additional useful projects, such as pest forecasting and estimating priority areas for other technologies such as mating disruption.

# Comparing efficacy of two registered atoxigenic strains biocontrol products to reduce aflatoxin contamination and expanding area-wide long-term mycotoxin management programs

**Authors:** **Ramon Jaime,** Project Scientist**; John Lake,** Lab Assistant; **Pummi Singh** Postdoctoral Scholar; **Apostolis Papagelis,** Field Assistant; **Agustina Ruiz-Posse,** Graduate student, Argentina; **Michael Luna,** Lab Assistant; **Giuseppe Fiore,** Graduate student, Italy; **Victor Gabri,** Staff Research Associate, **Ryan Puckett,** Staff Research Associate, **and Themis J. Michailides,** Professor. All authors at Department of Plant Pathology UC Davis & UC Kearney Agricultural Research and Extension Center.

## Introduction

Aflatoxins, the most toxic mycotoxin and highly regulated worldwide constitute a high economical threat to pistachio industry due to the risk of product rejection from the market by regulations. They are toxic secondary metabolites produced by fungi in the *Aspergillus* section *Flavi,* including *Aspergillus flavus* and *A. parasiticus*, the most common in pistachios in California. Aflatoxin contamination in pistachio is a problem that needs to be addressed because of the high threat it poses to human health. Also, the high value of pistachio makes any rejected load from the market a considerable loss for the grower by incurring on extra handling costs or even product destruction. With successful control of aflatoxins, pistachio nuts will be free of contamination, which will benefit both the consumers by consuming aflatoxin free nuts, and the growers by lowering the risk of income loss by crop rejections. Aflatoxins control in crops is difficult and unpredictable. Avoiding damage by insects, including Navel Orange Worm, helps to reduce the risk of aflatoxin contamination, but it does not completely eliminate it. Currently, the use of *Aspergillus flavus* atoxigenic strain technology is the only proven method to reduce aflatoxin contamination. The overall goal of aflatoxin management with this technology is to reduce the overall aflatoxin production potential of the aflatoxin producing fungi *Aspergillus* Section *Flavi* population in a crop system, which can be achieved by changing the population structure of the fungi in the soil of all crops susceptible to aflatoxin contamination from a population dominated by toxin producers to a population dominated by fungi that do not produce aflatoxins. Previous results show that besides the expected increase of the applied atoxigenic biocontrol (AF36) in treated orchards the untreated neighboring orchards also had an increase of AF36, indicating the capability of the fungus to spread over considerable distances, causing cross effects between treated and untreated orchards. However, toxigenic isolates from neighboring untreated and not cultivated areas will also move to treated areas reducing the effectiveness of the treatments. Therefore, implementing area-wide long-term aflatoxin control programs might be the best strategy to lower the risks of aflatoxin contamination in tree nut crops. Comparison of efficacy between AF36 Prevail® and Afla-Guard® will also be discussed.

## Results and Discussion

Previous research since the registration of the biocontrol *A. flavus* AF36 for use in California pistachios in 2012 indicate that the applications of AF36 have not been completely successful. Since aflatoxin contamination in crops is highly variable and is also affected by factors other than the structure of the population, the best indicator of the success of the biocontrol applications is measuring the percentage of displacement of toxigenic isolates by the applied atoxigenic isolate. A displacement of over 80% will be considered as successful. The average displacement in treated pistachios in California has historically been around 70%. We hypothesize that late applications not protecting from early infections by native toxigenic fungi and by other fungi migrating from neighboring not treated areas are the main causes for the biocontrol not reaching its full potential. Early applications of atoxigenic biocontrol product might increase the effectiveness of the treatments to reduce aflatoxin contamination. However, experiments from past seasons indicate that the biocontrol *A. flavus* AF36 Prevail® at the earlier applications does not sporulate satisfactorily to establish a founder population of atoxigenic strains. Results from last season indicate that sporulation of AF36 Prevail® in early May applications had poor sporulation (24% of grain

starting to sporulate two weeks after application). Sporulation improved in later applications reaching up to over 90% in applications made in July. Aflatoxin contamination of pistachio nuts indicate that harvest from 2021 has higher incidences of aflatoxin contamination than previous years, however, there were no significant differences in the percentage of samples with aflatoxin content above the permissible levels of 15 mg/kg, ranging from 2.7% to 6.6% in most times of application, with a higher incidence (15.6%) in the applications made late June for the normal harvest. Likewise, in the re-shake harvest most times of application had percentages of samples over 15 mg/kg ranging from 13.0% to 20.0%, and the applications made late June with 29.5%. Population data from post-harvest soil samples from the 2021 season indicate that the percentage of displacement, given by the percentage of AF36 and toxigenic isolates, was higher in the late applications (92.3% AF36 and 7.6% toxigenic strains) and lower for the not treated control (53.4% AF36 and 46.6% of toxigenic isolates). The rest of the times of application had similar percentages of displacement ranging from 71.3% to 74.8% of AF36 and from 25.2% to 28.7% of toxigenic isolates. In the 2022 season the new registered atoxigenic biocontrol Afla-Guard®, which in previous studies has had better sporulation in early applications, was also included in the times of application trials. Currently, both aflatoxin contamination data and population structure data for the 2022 season are underway and will be included in next-season's report.

The effect of area-wide applications of the biocontrol *A. flavus* AF36 was evaluated in an area where pistachio and almond are grown, and both are at risk of aflatoxin contamination. The area was divided in two parts, one where both pistachio and almond were treated, and other where only pistachios were treated. Soil samples from the orchards under this study were taken both before application and after harvest. Samples taken before application served as a base line of the population of the aflatoxin-producing *Aspergillus* fungi in both areas. Samples after harvest indicate the change of the population structure during the season as the displacement of toxigenic isolates by the applied biocontrol AF36. Comparing the population structure of *A. flavus* between the almond treated and not treated areas after harvest will indicate the influence of the treatments in an area-wide basis. The first applications were made in 2019, which indicated a favorable effect of the treatments in both crops with a higher percentage of AF36 isolates (94%) and lower percentage of toxigenic isolates (6%) in pistachios from the treated area, and the lowest percentage of AF36 (69%) and higher percentage of toxigenic isolates (22%) in the not-treated almond. Results from the 2021 season indicate a general declined of the applied AF36 biocontrol in all treatments. The treated orchards of pistachio and almond, regardless of the area, had similar percentages of displacement (ranging from 71.3% to 78.2% of the AF36 and from 21.7% to 28.7% of toxigenic isolates) than the almond orchards from the not treated areas with percentages of AF36 of 50.8% and toxigenic isolates of 49.2%. During the offseason of 2020 several almond orchards from both the treated and not treated area were removed. During the removal process large amounts of dust are created and could be deposited in the neighboring orchards, increasing the population of external isolates. These could explain the general decline of displacement in all orchards. Analysis of the AF36 and toxigenic isolates to determine the percentage of displacement in the 2022 data are underway. This analysis will indicate if the disturbance of soil caused by the removal of orchards had an effect on the population of *Aspergillus*.

## Conclusion

Considering that both the aflatoxin content and displacement data during the three years of study indicate no significant differences among the times of application, and that the earlier applications had an extremely poor sporulation, we consider that improving sporulation in the earlier applications might help to improve the efficacy of the atoxigenic strain biocontrol applications by establishing a founder population of atoxigenic strains. Once the atoxigenic strains are established in the orchard the overall aflatoxin production potential of the population will be reduce and consequently the risks of contamination. The use of products with better sporulation under low temperatures and soil moistures will certainly help to achieve the goals.

# Ochratoxin A contamination of California pistachios and identification of causal agents

**Authors: Pummi Singh**, Postdoctoral Scholar, Department of Plant Pathology UC Davis & UC Kearney Agricultural Research and Extension Center (KARE); **Ramon Jaime,** Project Scientist**,** UC Davis & UC KARE; **John Lake,** Lab Assistant, UC Davis & UC KARE; **Ryan D. Puckett,** Staff Research Associate, UC KARE; **Themis J. Michailides,** Principal Investigator, UC Davis & UC KARE**.**

## Introduction

Ochratoxin A (OTA) is a naturally produced toxin (mycotoxin) that contaminates a wide variety of foods and beverages. OTA is a class 2B carcinogen and a potent nephrotoxin. The European Union (EU) has stringent regulations for OTA in foods meant for human consumption. Pistachio nuts (*Pistacia vera*) are susceptible to OTA contamination when infected by fungi producing OTA. The United States (US) is the second largest producer and exporter of pistachios with the EU being an important import market. Due to frequent reports of high levels of OTA in imported pistachios, the EU will start regulating OTA at 5 µg/kg (ppb) from January 1st, 2023, forcing the US pistachio industry to meet these standards. Mycotoxin analysis of 609 pistachio library samples during 2018-20 detected 37% of the samples with > 5 ppb OTA and 3% of the samples exceeded 100 ppb OTA contamination. It has become imperative for the US pistachio industry to manage OTA to circumvent economic losses due to possible rejections of pistachio nuts by the EU.

The identification of causal agent/s is critical in devising management strategies. Fungi in the genera *Aspergillus* and *Penicillium* can produce OTA in a wide range of crops while decaying them. Within the genus *Aspergillus,* sections *Circumdati* and *Nigri* contain species known to produce high levels of OTA in liquid lab culture and dried vine fruits. These fungi have been reported in California (CA) pistachio orchards and could be important determinants of OTA contamination. The current report summarizes isolation and characterization of OTA-producing fungi collected from 14 orchards across California and effects of ambient temperatures supporting contamination. Levels of OTA detected in library samples in 2021 are also reported. The results provide insights into OTA contamination of pistachios in California and its mitigation.

## Results and Discussion

Pistachio library samples from 2021 (n = 200) averaged 16.4 ppb OTA ranging from Not Detectable (ND) to 1,137 ppb. Although the majority of the samples (86%) did not contain detectable levels of OTA, 14% of the samples exceeded 5 ppb levels. Furthermore, 12% and 5% of the samples were contaminated with >10 and >100 ppb OTA, respectively. These pistachio loads would be subjected to border rejections by the EU based on the regulatory limit of 5 ppb OTA in pistachios meant for direct consumption.

To gain an understanding of the occurrence of OTA-producing fungi in pistachio orchards across CA, orchards in the counties Butte (n = 5), Fresno/Madera (n =3) and Kern (n = 6) were sampled for soil (n=42), leaf (n=14) and pistachio nuts (at least 150 early splits (ES) and normal nuts each). *Aspergillus* isolates with black head (section *Nigri*) and yellow head (section *Circumdati*) conidiophores were isolated. Overall, a total of 313 isolates from leaves, 681 from soil and 973 from nuts were recovered. *Aspergillus* section *Nigri* dominated the potential OTA-producing community with incidences of 100%, 92% and 97% in leaves, soil, and nuts, respectively. Previous reports indicated that OTA producers within section *Nigri* are resistant to the fungicide boscalid. Because OTA production is either rare or absent in *A. niger,* this species was excluded from the collection by evaluating susceptibility on boscalid agar such that 216 boscalid-resistant section *Nigri* isolates remained for further analyses. *Aspergillus* species from section *Circumdati* were recovered in low frequencies compared to section *Nigri* from soils (52 isolates) and ES and normal nuts (33 isolates). These fungi could not be recovered from leaf isolations indicating

that soil and nuts are the preferred substrates for the proliferation of *Aspergillus* section *Circumdati*. Additionally, *Aspergillus* section *Circumdati* was recovered from Fresno/Madera and Kern counties whereas section *Nigri* was present in samples from all the three counties.

All isolates of section *Circumdati* (n=85) and 130 boscalid-resistant *Nigri* isolates were screened for OTA production in pistachios. OTA contamination by section *Circumdati* in inoculated pistachios ranged from ND to 82,124 ppb and averaged >10,000 ppb. Eighty-two percent of the isolates produced detectable levels of OTA with 78% exceeding 1000 ppb, 7 days post inoculation. It is noteworthy that the top 15 OTA producers were recovered from ES nuts indicating that ES nuts are especially susceptible to infection by high OTA producers. The pistachio industry should take special caution and ensure cultural practices that reduce the incidence and complete removal of ES nuts during processing and before packaging. Majority of section *Nigri* isolates did not produce detectable levels of OTA with the highest level measured as 46 ppb. This was a surprising result because these isolates were initially concluded to be *A. carbonarius* based on the boscalid resistance assay. The reference isolate of *A. carbonarius,* NRRL 369 demonstrated both boscalid resistance and OTA production in pistachios (4,120 ppb), indicating that the boscalid resistant isolates recovered from pistachios in the current study may be a different species within section *Nigri.* Despite the differences in OTA levels produced by fungi from sections *Circumdati* and *Nigri,* all isolates showed excellent growth on inoculated pistachios at the end of the 7 days incubation period.

The top five OTA producers from section *Circumdati* were further evaluated for OTA contamination of pistachios at 15, 20, 25, 30 and 35ºC with each treatment replicated thrice. OTA levels exceeded an average of 20,000 ppb at temperatures ranging from 15 to 30ºC and the highest contamination was detected at 25ºC (mean=43,485 ppb). While fungal growth was optimal between 15 to 30ºC, it was poor at 35ºC, and OTA levels dropped dramatically (mean=1,045 ppb). These results suggest that high levels of OTA contamination can occur under a wide range of temperatures. Pistachio orchards across CA experience several hours of temperatures ranging between 15 to30ºC, which may be contributing to high levels of OTA contamination and contamination may even proceed at 35ºC by some isolates.

Molecular analysis of section *Circumdati* (n=16) using partial sequences of β-tubulin and calmodulin genes resolved isolates into four distinct species: *A. ochraceous* (38%), *A. westerdijkiae* (19%), *A. melleus* (24%) and *A. bridgeri* (19%). While *A. bridgeri* did not produce detectable levels of OTA in pistachios, *A. westerdijkiae* produced the highest levels (mean=46,500 ppb), followed by *A. ochraceous* (mean=11,386 ppb) and *A. melleus* (mean=4,177 ppb). DNA sequences of section *Nigri* (n=33) resolved 94% of the isolates into a clade with *A. tubingensis*, a species that consists of low or no OTA producers with variability among isolates against boscalid resistance. The remaining isolates were identified as *A. niger.* While more isolates of both sections are being sequenced for precise identification, the results indicate that CA pistachio orchards harbor several distinct species of OTA producers, rendering OTA contamination of pistachios complex.

**Conclusion**

The results of the current study show OTA contamination of >5 ppb in CA pistachios. Considering EU regulations, OTA management in pistachios is imperative. Fungal characterization, along with their OTA producing ability, suggests that *A. westerdijkiae, A. ochraceous* and *A. melleus* are primarily responsible for OTA contamination of pistachios in central and southern CA where majority of pistachios are produced. The weather conditions in these regions are further conducive to optimal OTA production. Ochratoxin A mitigation in CA should be targeted towards the management of *A. westerdijkiae* and *A. ochraceous.* We will evaluate the potential of registered biopesticides for aflatoxin management in pistachio orchards and fungicides for efficacy against these fungi and OTA management.

# Assessing nitrogen uptake to develop best management practices and early leaf sampling protocols for pistachio cultivars 'Lost Hills' and 'Golden Hills'

**Authors: Douglas Amaral,** Cooperative Extension Advisor, UC ANR, **Phoebe Gordon,** Cooperative Extension Advisor, UC ANR, **Patrick Brown,** Professor, UC Davis, **Louise Ferguson,** Cooperative Extension Specialist, UC Davis, **Giulia Marino,** Cooperative Extension Specialist, UC Davis, **Elizabeth Fichtner,** Cooperative Extension Advisor, UC ANR, **Joy Hollingsworth,** Cooperative Extension Advisor, UC ANR.

## Introduction

This project aims to develop demand curves for nitrogen and other nutrients which will guide the quantity and time of fertilizer application allowing growers to match fertilizer supply with crop demand. It also aims to provide a sound and practical 'early-warning' and monitoring tool for 'Golden Hills' and 'Lost Hills' growers to optimize N management by developing an early leaf N prediction model. This tool will improve plant tissue sampling protocols to diagnose excessive, sufficient, and deficient nitrogen levels early in the season.

The study is being conducted in two high yielding commercial pistachio cultivars "Golden Hills" and "Lost Hills" orchards in the California San Joaquin Valley. Both varieties were grafted on UCB1 rootstock. We have been monitoring two replicated blocks of trees (3 trees per block, totaling 9 trees per orchard) for each cultivar for changes in nutrient concentrations in annual (leaves and fruits) and perennial organs (roots, trunk, scaffold, canopy branches and small branches) six times during the season at different phenological stages. Samples collected are being processed for analysis. Additional 15 orchards of each cultivar are being monitored for leaf nutrient content. Leaf samples were collected during spring and summer and are being processed for analyses. At the end of second season, an early leaf N prediction model will be developed and validated. In addition, to determine tree biomass at the beginning and end of season, trees that represent optimum leaf N concentrations and not showing any deficiency of other nutrients are being excavated annually.

**Figure 1.** Pistachio trees were excavated and separated into small and large roots, trunk, scaffold, canopy branches, small branches, and leaves to estimate total tree biomass and nutrient content.

## Results and Discussion

Variations in leaf nutrient status of the trees over the growing season are being used to estimate total nitrogen demand and to develop an early season sampling protocol. Seasonal nutrient content in leaves is shown in Figure 2. Data refer to the average of 9 trees per orchard during two growing seasons.

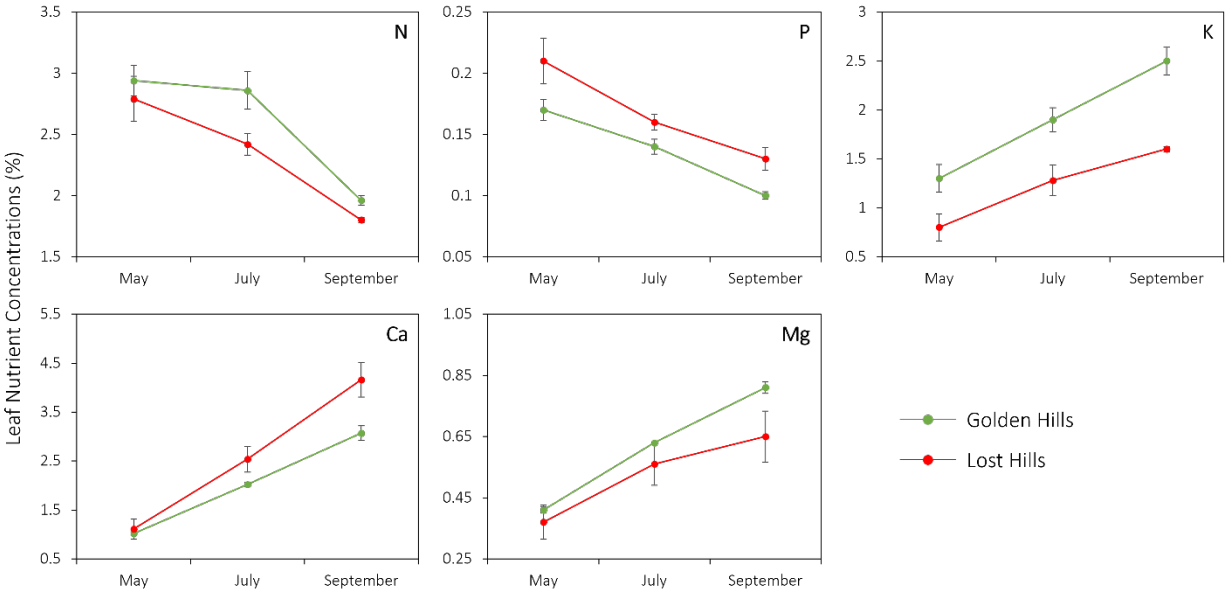



**Figure 2.** Changes in pistachio leaf (non-fruiting branches) nutrient concentrations over two growing seasons (averages: 2021 & 2022).

Preliminary data shows that the seasonal demand of nutrients in pistachio cultivars 'Golden Hills' and 'Lost Hills' is high early in the season from May through July. In contrast, from December to February, no net increase in nutrient was observed during this period. Knowing the dynamics of nutrient uptake during the season is a requirement to allow the management of the timing of nutrient supply with nutrient needs.

An additional one year of data collection is planned to complete this experiment to encompass three growing cycles and to fully interpret this experimental data set and to derive new sampling and management strategies. To complement the leaf tissue analysis and provide guidance for fertilization, we are currently developing yield and phenology based seasonal nutrient removal curves that qualify and quantify the time of nutrient uptake and total plant demand across different environmental conditions for major nutrients, including nitrogen (N), phosphorus (P) and potassium (K).

## Conclusion

It is important to note that the data shown in this report is a preliminary data from a 3-year project, then no conclusive data are shown. Our goal is to develop knowledge of the pattern of nutrient uptake and allocation during three seasons (2021-2024) in pistachio trees to develop a nutrient prediction model for pistachio cultivars "Golden Hills" and "Lost Hills" to guide fertilizer application based on crop phenology for the State of California.

# Determining non-bearing pistachio nitrogen and phosphorus needs

**Authors: Phoebe Gordon**, Orchard Systems Advisor, University of California Cooperative Extension, Madera and Merced Counties; **Douglas Amaral**, Orchard Systems Advisor, University of California Cooperative Extension, Kings and Tulare Counties

## Introduction

Nitrogen is the nutrient required in the largest quantities for non-bearing trees and applications are currently regulated through the Irrigated Lands Regulatory Program, however nitrogen demands for non-bearing pistachios has not been determined experimentally.

An additional nutrient of interest to researchers and the industry is phosphorus. While this nutrient has traditionally not thought to be necessary in the majority of production orchards in California, recent work done by Phoebe Gordon and Greg Browne in almonds has shown that phosphorus applied in the first year, soon after planting, is beneficial to almond growth (Gordon et al., 2019; additional unpublished data).

This research project seeks to determine nitrogen and phosphorus fertilization guidelines for non-bearing pistachio trees.

## Results and Discussion

Work this year has primarily been navigating the University of California's requirements for large purchases and contract labor. The irrigation system purchase was finally approved in November 2022, and we hope that it will be installed, and the trees will be planted in the winter of 2022/2023.

A donation of Golden Hills on Duarte Clone UCB1-D110 has been obtained from Duarte Nurseries and will be delivered upon completion of the irrigation system.

## Conclusion

No conclusions can be drawn from this trial this year.

# Evaluating commercially available plant water status sensing devices

**Authors:** **Phoebe Gordon**, Orchard Systems Advisor, Madera and Merced Counties; **Giulia Marino**, Assistant Professor of Cooperative Extension, UC Davis; **Mae Culumber**, Nut Crop Advisor, Fresno and Kings Counties; **Ken Shackel**, Professor of Plant Sciences, UC Davis; **Gary Banuelos**, Soil Scientist, USDA Agricultural Research Service; **Irvin Arroyo**, USDA Agricultural Research Service, **Louise Ferguson**, Professor of Cooperative Extension, UC Davis

## Introduction

Precise irrigation management involves monitoring the soil, irrigation system output and distribution uniformity, evapotransipirational losses, and plant water status. There are several commercially available devices that measure plant water stress: Saturas and Florapulse measure plant water potential, and Phytech, which is a dendrometer. Their performance in pistachios should be evaluated.

## Results and Discussion

This trial was embedded in a leaching trial conducted by the Banuelos lab at the Grassland Basin Authority near Firebaugh, California, in a pistachio orchard planted in 2009. The trees had previously been irrigated with saline drainage water, but recently were being irrigated with higher quality irrigation water. The Banuelos lab kept a subset of trees irrigated with saline drainage water (4.3 dS $m^{-1}$) to study the recovery of the trees being switched to a higher quality irrigation water (1.6 dS $m^{-1}$), though soil salinity was high in both plots (soil salinity was around 8 dS $m^{-1}$ in the saline plot and marginally less in the plot irrigated with higher quality irrigation water). Unfortunately, the amount of data we were able to collect this year was limited by several logistical factors, and the first irrigation was fairly late. Stomatal conductance and photosynthesis measures also indicated that the trees were stressed; there were no differences between treatments and values were below summertime values measured for rainfed trees in Sicily (Marino et al., 2018). After mid-September, the trees receiving the saline irrigation water began to defoliate and we could not collect SWP values from these trees; data collection continued on the trees receiving non-saline irrigation water.

The FloraPulse sensors were installed in three trees, one in the plot irrigated with the non-saline irrigation water, and two were installed in trees irrigated with saline irrigation water (Figure 1). The researchers had intended to install the sensors in more trees, however a misunderstanding in how long the sensor cables were prevented us from doing this. Average correlation between SWP and FloraPulse readings ranged from and $r^2$ of 0.76 and 0.83 (data not shown due to space limitations). Curiously, the FloraPulse sensors embedded in the "Saline" pistachio trees began to read erroneously high SWP (i.e., it was indicating the trees were not stressed) readings around late August/early September, even after irrigation ceased.

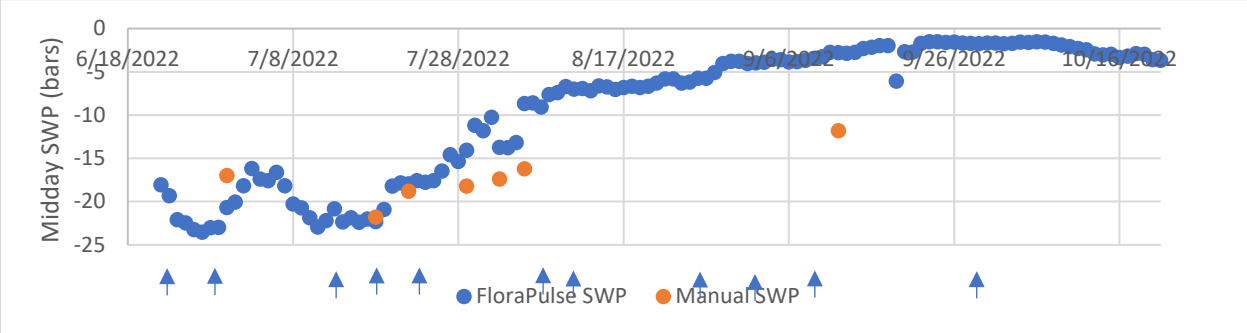



Figure 1: daily SWP measures from one of the two FloraPulse sensors installed in trees irrigated with saline irrigation water. Blue arrows indicate irrigation events.

The Saturas sensors were installed in six trees; three were placed in trees irrigated with high quality irrigation water and three were placed in trees irrigated with saline irrigation water (Figure 2). One sensor placed in the saline plot did not establish correctly at installation and the company installed a second sensor in a new tree, however we did not end up using the data from that sensor. Correlations between manual SWP measures and the Saturas sensors ranged from an $r^2$ of 0.22 to 0.27 in the saline-irrigated trees and 0.02 and 0.26 in the non-saline irrigated trees. One possible reason for the poor correlation is that the first manual SWP measurement differed significantly from the Saturas sensors; this was taken close to sensor installation, and it is possible that the sensor needed more time to equilibrate. After removing this first data point, agreement improved for some sensors but decreased for others; the saline sensors had an $r^2$ ranging from 0.43 to 0.76 and the control sensors ranged from an $r^2$ of 0.00 and 0.36. The sensor that showed a decrease in correlation between its output and the manual pressure chamber measurements was consistently showing a lower SWP than the pressure chamber.

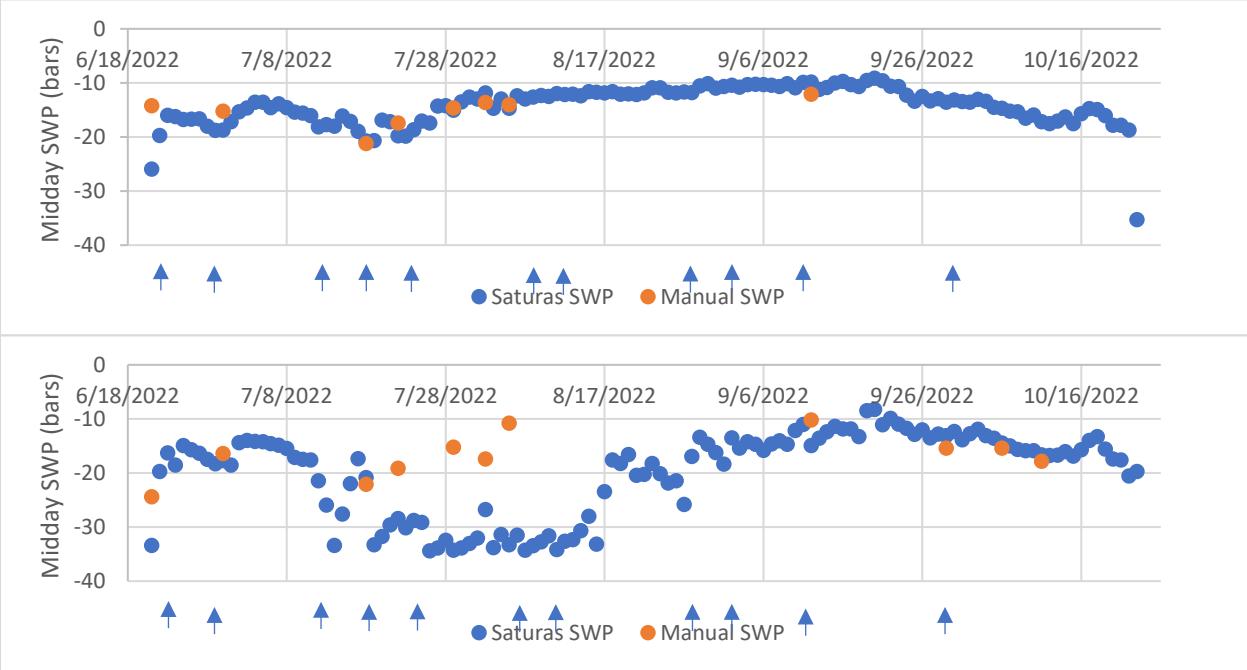



Figure 3: Saturas sensors with the highest agreement between manual SWP measurements (top), installed in a tree receiving 4.3 dS $m^{-1}$ water, and the sensor with the lowest agreement (bottom), installed in a tree receiving 1.6 dS $m^{-1}$ water. Blue arrows indicate irrigation events.

The Phytech sensors were installed much later than the other two due to COVID-19 related restrictions. As such, there are too few manual SWP measures to compare to the tree-stress measurements. The sensors indicated very little stress in the trees (Data not shown due to space limitations) which we think is due to the relatively late installation of the sensors. Trunk growth generally increased, though there was a later season cessation of trunk growth; this was also observed in Marino et al (2021).

## Conclusion

Due to the late installation of the sensors and extenuating circumstances that prevented us from collecting more manual SWP measurements we do not have enough data to make any conclusions. Another year of data collection is needed.

# Evaluation of salinity, boron, and soil hypoxia on pistachio tree growth, year 3

**Authors:** **Phoebe Gordon**, University of California Cooperative Extension; **Giulia Marino**, University of California, Davis; **Gary Banuelos**, USDA-ARS; **Louise Ferguson**, University of California, Davis; **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. 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

*Salinity and boron treatments*

Salinity and boron treatments were initiated in June of 2022. Treatments were ramped up over the course of several weeks in order to avoid acute responses to salinity and/or boron. Treatments are in Table 1.

Table 1: Boron and salinity treatments

| Boron (ppm) | Salinity (SAR) | Salinity (ECe; dS $m^{-1}$) |
|---|---|---|
| 1 | < 3 | 0.6 |
| 1 | 15 | 4 |
| 1 | 50 | 12 |
| 5 | <3 | 0.6 |
| 5 | 15 | 4 |
| 5 | 50 | 12 |
| 10 | <3 | 0.6 |
| 10 | 15 | 4 |
| 10 | 50 | 12 |

Leaf burn symptoms began to appear in September. Burn severity was assessed in October based on the average percentage of necrosis on the leaves (Figure 1). Salinity was the only factor that was significantly different, where leaf burn decreased as salinity levels increased. While boron was not statistically significant, there is a clear pattern for increasing leaf burn with increased boron concentrations. Work done by Ferguson et al (2002) found that pistachio trees irrigated with a 10-ppm boron solution exhibited leaf necrosis at low salinity levels, but less leaf burn at high salinity levels. Tree growth was examined via trunk diameter below the graft union and canopy area. Canopy area was estimated by photographing the south and the west side of each tree and estimating the canopy area by using a white box of known dimensions for scale, then averaging the two sides. There were no significant differences in trunk diameter or increase in trunk diameter. Canopy area decreased as salinity increased.

Stem water potential was measured six times in 2022. During the May reading, there were significant differences in the trees designated for the low (-11.3 bars) and medium (-12.4) salinity treatments, and the trees in the medium (-11.2) and high boron (-12.4) treatments, though treatments had not yet been imposed. We assume this is due to random chance, though we have no canopy measurements taken during this time period to confirm this is true. After treatments were imposed in early June, these differences appeared for the June and early July rating. In late July and September, stem water potential declined as salinity treatment Increased (Figure 3). Boron did not impact stem water potentials. By early October, there were no significant differences between treatments, likely due to the fact that irrigation was ceased in mid-September.

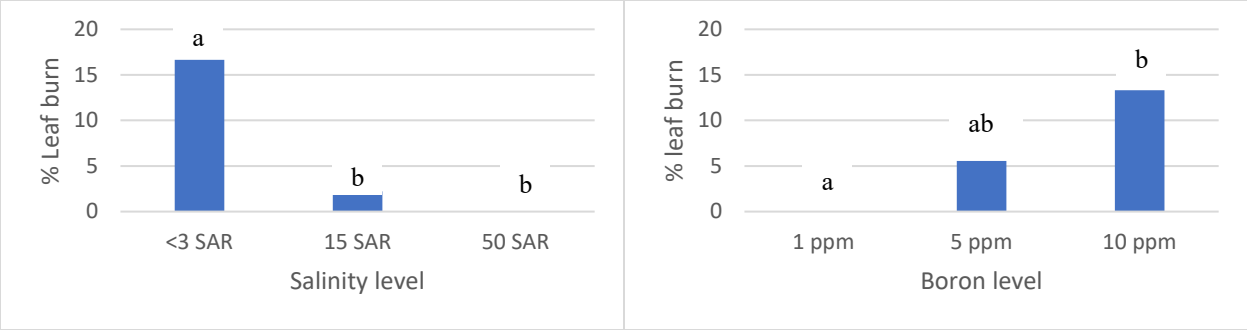



Figure 1: percentage of necrotic leaf area affected by salinity treatments (left) or boron treatments (right). In the salinity analysis, results were pooled over boron level. In the boron analysis, results were pooled over salinity level.

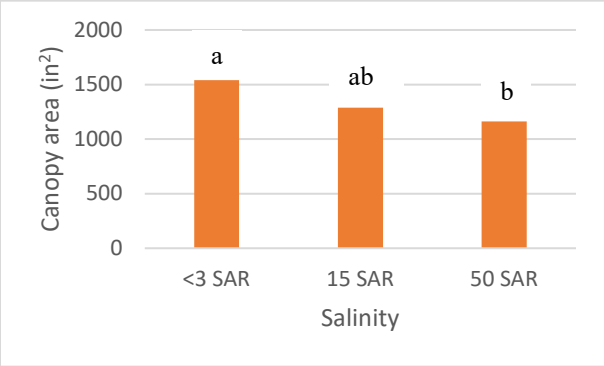



Figure 2: Canopy leaf area, as influenced by salinity treatment.

*Soil oxygen:*

We are still struggling to validate a method for this procedure, due to the dataloggers we constructed being unreliable in the field. We identified that the issue is due to the in-field solar charging of the batteries that power the panels overnight/ We are working on a solution to this. Additionally, we were using tomatoes as a proxy for pistachios, which attracted ground squirrels that destroyed multiple wires connecting the oxygen sensors to the dataloggers.

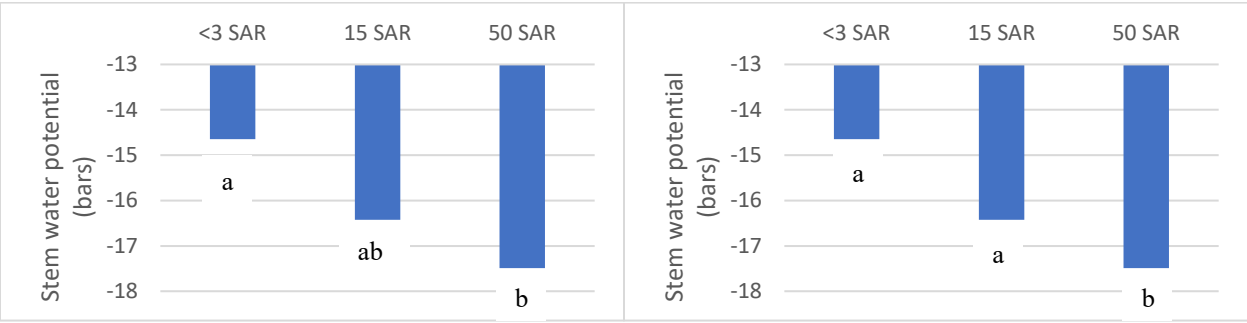



Figure 3: Stem water potential values on July 28 (left) and September 14 (right), as affected by salinity treatments. Values averaged over boron treatments

## Conclusion

At this point it seems as though salinity affects pistachio growth more than boron, though the trees have only been irrigated with their treatment solutions for four months.

# Evaluating new training systems for pistachio-1

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

## Results and Discussion

Trial #1 Kings County- By 2022, midday canopy PAR interception was similar among all treatments. Trees were mechanically shaken in 2022 with the conventional, modified central leader and unpruned treatments producing cumulative yields of 7677, 9104, and 9155 pounds per acre respectively. Nut removal was similar among treatments. The trees were only harvested once by the grower in 2022 but we did a hand harvest of remaining nuts two weeks after the mechanical harvest.

Trial #2 Kings County- This orchard was only flood irrigated one to three times each year. Similar to Kings County site #1, in 2022, midday canopy light interception was similar among all treatments. Trees were mechanically shaken in 2022 with cumulative yields for conventional, modified central leader and unpruned treatments of 3142, 3865 and 3509 pounds 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 grown in crowded conditions in the nursery. There was extensive cold damage from the nursery in these trees at planting. This did not impact the conventional or modified central leader trees since the damaged tips were pruned off during the dormant season. However, approximately 50% of the shoots on the unpruned trees were damaged and these shoots behaved like pruned shoots with the central leader often being lost. Approximately 10 conventionally trained trees broke loose of ties to the wooden stakes on extreme north wind days and bent over towards the ground as if they were made of rubber. Similar to both Kings County sites, canopy light interception was similar among all treatments in 2022. Cumulative yields for the 2021 and 2022 seasons for the conventional, modified central leader, and unpruned trees respectively were 54, 144 and 504 pounds per acre by 2022.

Westside Field Station Trials- Additional pruning trials (Golden Hills on UCB1 seedling and Plantinum rootstock) and a fall irrigation cutoff trial were planted in the spring of 2019 and trees were budded in early September 2019. Dataloggers for monitoring soil moisture and time lapse cameras were installed in 2020 and pruning treatments were initiated in the winter of 2021. In-season tipping to establish secondary and tertiary branches was imposed on conventional trees before mid-July. Irrigation cutoffs were initiated in the fall of 2020 and in 2021 and 2022 and tissues were sampled for carbohydrate analysis in fall and spring just prior to bud break. Trees did not have enough crop to harvest in 2022.

## Conclusion

Although these trials are in their infancy, the results to date look encouraging. Trees in all treatments grew well with midday canopy light interception similar among treatments at all sites in 2022. Although some unpruned trees had tops that were bending over (since they were often taller than the stakes), they appear to be straightening themselves out by re-sprouting branches that balance the lean similar to results we have seen in walnut. At Kings Site #1, cumulative yields were similar for the for the modified central leader and unpruned treatments while both were significantly higher than the conventional treatment by 2022. At the Kings Site #2, the modified central leader treatment had the highest cumulative yield followed by the unpruned and conventional treatments. This may have been due to alternate bearing effects since the unpruned had significantly higher yields in 2021. At the Yolo County site, cumulative yields were all significantly different with highest yields in the unpruned followed by the modified central leader and then the conventional treatment. Nut removal from mechanical shaking in 2022 was similar among treatments at both Kings County sites as well as the Yolo County site. Data collection will continue in all three of the original trials as well as the new trials at Westside Field Station in 2023. We expect the first harvest to occur at the Westside Field Station site in 2023.

# Evaluating new training systems for pistachio-2

**Authors:** **Mae Culumber**, UCCE Nut Crop Advisor for Fresno County, **Bruce Lampinen**, UCCE Nut Crops Specialist, UC Davis Dept. of Plant Sciences; **Elizabeth Fichtner** UCCE Orchard Systems Advisor for Tulare County; **Ramandeep Brar** UCCE Fresno Staff Research Associate; **Diana Camarena-Onofre** UCCE Fresno Staff Research Associate

## Introduction

As growers learn about research outcomes from the pistachio tree training project, there have been a number of inquiries into the feasibility of stopping pruning on trees already trained to a conventional or central leader structure. In walnuts, pruning cessation in the second leaf resulted in lower yields than trees left unheaded at planting and those that were unpruned after the first leaf. More scaffold breakage was observed in walnuts that were conventionally headed and pruned until after the second leaf. It is unknown how shifting from a conventional pruning system to reduced or non-pruning in the establishment years will influence pistachio tree structure and limb strength as the trees reach full bearing maturity. A small trial was initiated at the UC Westside research station to evaluate the growth response of pistachio when terminating pruning practices in modified central leader trained trees one to four years after the initial tree heading and subsequent pruning cuts. All trees in the trial were headed at ~60 inches in the winter between 2020-2021. The year 1 pruning termination treatment did not receive any additional tipping cuts in the second dormant season (2021-2022), year 2 pruning termination will occur in the 2022-2023 dormancy, year 3 during 2023-2024 dormancy, and year 4 the 2024-2025 dormant period. Data collection for each season will include annual measurements of tree growth (trunk cross-sectional area) of the rootstock and scion, tree height, midday stem water potential, mid-day canopy light interception, and yield when trees reach bearing age.

Young pistachios are thought to be susceptible to fall frost events because warm daytime temperatures and adequate soil moisture promote continued growth without entering dormancy. In a continued growing stage, trees maintain cellular hydration and low solute concentrations, conditions conducive to freezing damage of cellular membranes. To avoid damage, irrigation is often greatly reduced or ended for the season to reduce vigor and provide frost damage protection for the winter. Evapotranspiration (ETc) rates for tree crops can be high between September and the end of December in the southern San Joaquin Valley. This raises the question as to when to curtail irrigation for the season. There is some concern among growers and researchers that curbing irrigation too early could induce stress and prematurely slow tree growth. The optimal timing for cutoff has been difficult to predict as the soil textural characteristics can greatly influence water status in response to drought on young pistachio trees. In addition, growers often do not know the water status of the trees when they initiate cutoff. Trees modify cellular and molecular processes to adapt to abiotic sources of stress (e.g., training, frost, and drought). Young trees exposed to drought stress in fall have been shown to have more soluble sugars and proline osmolytes than well-watered trees. These solutes play a role in cellular osmotic adjustments that protect their cells from damage and help plants maintain water without significant changes to metabolism. Freeze damage sometimes does not become apparent in young pistachio trees until the following spring at leaf out. Kallsen and Sanden observed higher levels of starch in the rootstocks and scions of trees with freeze damage than those that were unaffected. The study concluded that the unaffected trees were able to mobilize starch reserves in the spring to produce new canopy growth, while damaged trees did not. More information is needed to understand the implications of fall irrigation management strategies on frost acclimation, and annual patterns of starch and soluble sugars. There are no current recommendations for optimal irrigation cutoff times in young pistachio. Information compiled over a decade of observations, was not developed based on replicated and randomized scientifically designed trials. There is much to prove and learn about the mechanisms of cold acclimation in pistachio. In 2019, a fall irrigation cutoff trial was installed within the pistachio training trial at UC Westside Field Station. The cutoff trial trees are

all are all Golden Hills on either Platinum or UCB1 seedling rootstocks. All trees are trained to a modified central leader tree structure. The fall irrigation cutoff trials have four irrigation cutoff dates with four replicates for each cutoff. The cutoff dates are initiated in early September (Cutoff 1), followed by cutoff 2, 3, and 4 separated by 2–3-week interval, with the final cutoff in the beginning of November. The irrigation application varied by about 4 inches of applied water from the first to the last cutoff. Rootstock and scion tissues were sampled for carbohydrate analysis once in fall, during winter, and spring just prior to bud break.

## Results and Discussion

Scion and rootstock tissue samples collected in the fall 2020 to spring 2022 have been analyzed for non-structural carbohydrates using a soluble sugar anthrone extraction method. Sugars did not vary significantly between the platinum and UCB1 rootstocks or by cutoff date in either the fall or spring months from fall 2020 to spring 2022. A significant difference between rootstock and scion sugar content resulted in spring 2022 but were no different in 2021. Samples will be further analyzed for starch content. The preliminary data indicates the timing of irrigation cutoff from the beginning of September until the first week of November did not influence the concentration of fall and spring solute concentrations in the rootstocks. Irrigation cutoff treatments were implemented again in the fall of 2022, and analysis is ongoing.

## Conclusion

The pistachio pruning termination and irrigation cutoff trials are still in the beginning stages. We will continue to implement and monitor tree growth responses to cessation of pruning over a period of years during the orchard establishment years. Preliminary data indicates little effect of the timing in late season irrigation cutoff in young trees ability to increase solute concentrations, which may presumably protect cellular membranes from the risk of frost damage. The clay loam soil at the trial site has considerable water holding capacity and the trees may not have been markedly influenced by the gradient of 4” applied between early and late season cutoff treatments over an approximate eight-week period. It is likely that the difference between the initial and final irrigation cutoff could have a more drastic impact in other areas with coarser textured soil with a reduced capacity for moisture retention.

# California pistachio weather models update

**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 interactive weather-related models are some of the most popular resources available, accounting for 7 of the top 10 visited pages on the FNRIC site. In 2021, due to cybersecurity issues, hosting these models on the current UC ANR platform became no longer feasible. These models, and the rest of our online resources are now hosted by the UC Davis Site Farm hosting service.

For pistachio growers and researchers, both the chill calculator and the pistachio bloom forecast models are very valuable resources. The chill calculator will still use up-to-date CIMIS data to provide chill hours based on location, as well as providing historical data where available. The pistachio bloom forecast model (originally developed by Katherine Jarvis-Shean and David da Silva) provides projected bloom dates based on CIMIS chill and heat and chill accumulation data.

## Results and Discussion

Funding was requested to move both the chill calculator and the pistachio bloom forecast models to the new FNRIC website. We worked with the IET Web Development Team to successfully rebuild both of these models to be more user-friendly and to meet modern accessibility guidelines. The models are available at https://fruitsandnuts.ucdavis.edu/weather-models.

## Conclusion

The new weather models are live as of Spring 2022. We have had no downtime and overall, very positive feedback from users. The primary limitation of all the FNRIC weather models is now the reduced number of available CIMIS stations. We hope to address this issue in the near future by adding other weather station data to our data aggregation.

# Saline irrigation in young pistachio 'Kerman' trees on UCB-1 and PG-1 rootstocks grown in field lysimeters

**Authors**: **Kaomine Vang**, CIT CSU Fresno; **Gary Banuelos**, Plant/Soil Scientist, USDA-ARS, Parlier; **Giulia Marino,** UCCE Kearny, Parlier; **Louise Ferguson**, UCCE Davis Monika **Sommerhalter,** Professor, CSU East Bay, Hayward; **Heraclio Reyes,** Technical Advisor, Reedley.

## Introduction

California is facing its fifth year of severe drought; a serious reality for growers in the San Joaquin Valley (SJV). Consequently, farmers are drilling wells to obtain groundwater for irrigation, but the water often contains excessive salts, boron (B) and selenium (Se) (Ayars et. Al. 1993). Na, Cl, and B concentrations can exponentially accumulate in the plant and negatively affect crop yields and plant health. For this reason, alternative salt and B-tolerant crops must be identified, if irrigated agriculture uses poor quality waters. One major crop under serious consideration for irrigating with these waters is pistachio (*Pistachio vera* L.) based upon pioneering research on saline irrigation in pistachios by Sanden and Ferguson. Their research indicated that irrigating with saline water may be key for sustaining the increased production of pistachios under drought conditions in California. Practical knowledge is still needed about pistachio rootstock tolerance to salts, although the hybrid UCB-1 rootstock demonstrates a better ability to exclude, sequester and recirculate selected ions (i.e., Na, B and Cl) than its more saline sensitive single species parent, PG-1. Both rootstocks are predominantly used in pistachio production in California. Utilizing lower-quality water on either rootstock requires obtaining new knowledge about recognizing tree-based stress responses to continuous saline irrigation. One major question that needs to be resolved when using saline waters on pistachios of either rootstock is at which age can pistachio trees receive irrigation with poor quality waters? Will salt and B tolerance and other physiological responses in young trees from either rootstock be expressed differently when irrigating with varied levels of salinity as soil chemical changes take place in the soil profile? To address these questions more effectively, our current research group has established a multi-year field lysimeter study supported by CPRB and CSU Fresno-ARI. We have drip-irrigated young trees with scion Kerman on both UCB-1 and PG-1 rootstocks with varied levels of salinity for over 4 years growing in field lysimeters. In these trees and soils, we have studied physiological responses, ion accumulation, soil chemistry changes, and real time measurements of soil EC and soil moisture at different depths.

## Results and Discussion

In this multi-year study**,** we have completed the fourth year of saline drip irrigation with the respective treatments: <1 dS/m and 6 mg B/l, 4 dS/m and 6 mg B/L, 8 dS/m and 6 mg B/L and 12 dS/m and 6 mg B/L on two rootstocks UCB-1 and PG-1 rootstocks budded to Kerman. Twenty-three 18-month-old trees from each rootstock were transplanted and budded later in 1.5 in deep x 0.7 m field installed tiles filled with oxalis salty clay loam soil collected from the westside of the SJV. The soil $Ec_e$ and soluble B ranged from 4-6 dS/m and from 4-6 mg B/L. Based on crop water coefficients used for younger nut trees and weather data collected from CIMIS weather station at UC Kearney, Parlier CA, trees were drip irrigated with the respective saline treatments. Meter Group Terros 12 soil sensors were installed at 30, 60 and 130 cm depths on selected tiles within a complete randomized block to measure 'real time' soil moisture, electrical conductivity, and temperature within the soil profile. Limited data were continually collected on a 15-minute interval and stored on Meter Group ZL6 data loggers (*Note*: *delayed shipment of soil sensors and parts, prevented us from completing soil sensor measurements; hence, the request for a no-cost extension on this project*). Soil samples were also collected at the beginning and ending of 3rd year growing season to 0-30, 30-60 and 60-90 cm depths, and analyzed for soil EC, and soluble Na, B and Cl from a saturated paste. Leaves were collected at end of season and analyzed for Na, B and Cl and

biochemical assays (pigment, total phenolics, proline and antioxidant activity) with ethanolic extracts from pistachio leaves.

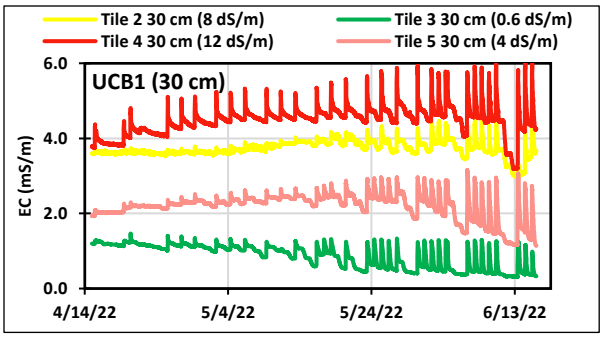
Overall, visual effects on tree growth or the showing of toxicity symptoms were not observed in leaves of either rootstock, except B toxicity symptoms (e.g., necrosis of leaf margins) were observed in PG-1 trees with treatment 1 dS/m and 6 mg B/. Overall, trees on UCB-1 rootstock showed significantly better and more consistent growth than PG-1 rootstock for saline treatments. Leaf photosynthesis and physiological parameters, including leaf area and leaf chlorophyll data (with LICOR measurements) and stem water potential showed no differences among treatments (irrigation salinity levels and rootstocks), however, similar seasonal differences were observed for both rootstocks, irrespective of treatments. Younger leaves had higher values for photopigments, proline, and phenolics than older leaves, but no significant differences among salinity treatments on both rootstocks were observed. Clearly, Na accumulated to levels in leaves that would not result in any high toxicity. Generally, Na levels were less than 100 mg/kg DM in older and younger leaves of UCB-1 and as high as 500 mg/kg DM in young younger and older leaves of PG-1 at high salinity treatment. Cl levels increased with higher saline treatments in young and old leaves and were highest (12,000 mg/kg DM) in older leaves of PG-1 rootstock compared to 5000 mg/kg DM in UCB-1. In contrast to Cl accumulation, B concentrations were decreased with increased saline irrigation (<400 mg/kg DM) in both younger and older leaves from both rootstocks and B concentrations were higher (> 840 mg/kg DM) at lower saline treatments, irrespective of rootstock. This antagonistic phenomenon is often observed between salinity and B uptake. Increased salinity (especially with Na salts) in the soil tightly adsorbs soluble ions, e.g., B, and reduces B uptake by plants. Nut clusters were observed on some UCB-1 trees (irrespective of treatments), however, only blanks were produced. Poor pollination was likely the reason for this result due to inconsistent pollen production from male trees.

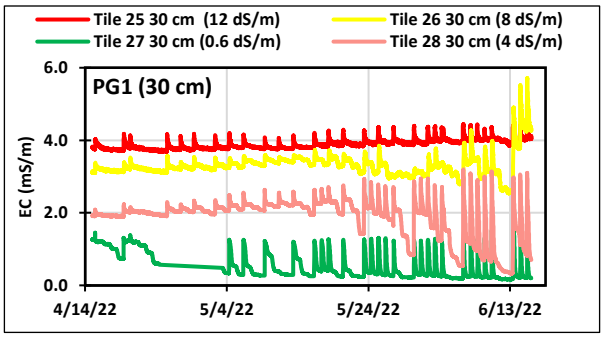
Soil analyses (from saturated pastes) show the soil EC (dS/m) increased with salinity treatments and ranged from 1 to 13 dS/m at 0-60 cm, while soluble soil B ranged from 7-10 mg B/L at the same depth. Real-timed data were partially successfully collected from sensors measuring soil moisture and soil salinity, respectively, in lysimeter soils to a depth of 130 cm). Overall, increases in bulk soil EC, measured by soil sensors increased with increased saline treatment to a depth of 30 cm (**See Figures**). No changes were observed at 130 cm, indication that minimal leaching occurred at that depth.

## Conclusion

Two- to five-year-old Kerman pistachio trees on either UCB-1 and PG-1 rootstock have tolerated a range of irrigation salinity levels up to 12 dS/m and 6 mg B/L for four years grown in field lysimeters filled with typical westside soil. Future investigations should evaluate potential toxic effects once nutting clusters consistently appear and influence the competition, movement, and uptake of ions within the trees.

**Figures Soil sensor data (bulk EC) during saline irrigation at different EC levels on UCB-1 and PG-1 rootstocks.**



# Saline irrigation strategies for pistachio: Year 1 of 3

**Authors: Louise Ferguson,** Professor of Extension, UC Davis**; Blake Sanden,** UCCE Farm Advisor Emeritus Kern County; UC Davis; **Emilio A. Laca,** Data Models**; Giulia Marino,** Asst. Professor of Extension; **Mae Culumber,** Farm Advisor, Fresno County; **Alireza Pourreza**, Asst. Professor of Extension, UC Davis; **Joseph Coelho, Ismael Patron, Jason Errecart, Ryan Doglione,** Maricopa Orchards; **Jordan Hazell, Tomas Teitelbaum** and **Anthony Miele**, Semios

## Introduction

The April 22, 2019, California Water Research Report noted 51% of Merced, 36% of Fresno, 89%of Tulare, 66% of Kings, and 55% of Kern counties' soils range from moderately, (4 dS/m), to extremely, (16 dS/m), saline. Of California's 371,386 acres of bearing pistachio trees, 312,901 are in these counties. Earlier research by Sanden, Ferguson and Marino demonstrated that pistachio can be grown profitably if soil ECe is maintained between ~ 4.5 – 6 dS/M. Currently, the most used salinity and boron management for orchards with serious salt accumulation problems is to apply 1 to 2 acre-feet for winter leaching with good quality water, with or without calcium or acid amendments, monitored by soil and water analyses before and after leaching.

As availability of good quality canal water for irrigation and winter leaching declines due to drought, growers are forced to rely on semi-saline groundwater. When relying on saline irrigation water it, is helpful to know both the available water content (AWC) and the soil salinity (ECe) profile in real time. Knowledge of these two parameters would enhance growers' ability to apply effective in-season leaching fractions if water were available.

Multiple capacitance sensors can continuously monitor available soil water content (AWC) and possibly salinity in real time. Therefore, we can now use real-time, in-season monitoring to investigate the best irrigation scheduling to maintain optimal soil profile AWC and salinity levels in the drip wetted rootzone. The efficacy of large volume sprinkler or flood leaching during dormancy is well documented. However, the currently available reclamation/ leaching fraction calculations were generated assuming one-dimensional 100% surface wetting that push water and salt straight down. The ability of single and double-line drip to perform 2-dimensional leaching needs to be investigated to manage root zone salinity and boron with in-season leaching fractions and small volume dormant leaching. To answer these questions, we are investigating in-season leaching fractions with and without dormant-season leaching in double- and single-line drip systems. We are also monitoring soil/water conditions and tree growth using *in-situ* irrigation sensors that track soil water content/salinity and trunk growth (SEMIOS system). We are also using remote/aerial to monitor Normalized Differential Vegetative Index (NDVI), water stress, canopy growth and chlorophyll content. Soil and tissue sampling and EM38 measurements of soil" magnetic conductance" complete the full data picture of the trial site.

## Results

In May 2022, in cooperation with Maricopa Orchards, Semios, Ceres Imaging and Delavalle Laboratories the following treatments were established with both single- and double-line drip plots:

1. Control: in-season scheduling to meet tree ET only
2. Control + pulsed dormant season leaching
3. Control + 15% in-season leaching fraction with every irrigation
4. Control + 15% in-season leaching fraction + pulsed dormant season leaching
5. Control for 3 years with a major dormant season leaching after 3 years

Each of the 10 treatments resulting from the two hose configurations and 5 irrigation treatments was replicated five times in five blocks. Three of the five blocks were instrumented with a Semios system to measure irrigation volume, soil water content, soil ion content and trunk shrinkage and growth.

Fig. 1 shows the experimental design with blocks replicated from N-S. This late August aerial image demonstrates the higher water stress (left panel) and lower vigor (right panel) in the two northern blocks relative to the three southern blocks showing the significant block effect of higher native soil ECe, Na and B in the northern versus southern blocks. This higher salinity stress in the northern versus southern blocks was corroborated by the by the 2022 baseline May soil and August leaf analyses, seasonal dendrometer and circumference growth measurements, and August harvest yield and quality. This N-S salinity gradient across the experimental blocks confers a distinct advantage for the experiment to determine the treatment effects on different initial levels of salinity. We will be able to compare the efficacy of the ten treatments under different soil salinity profiles.

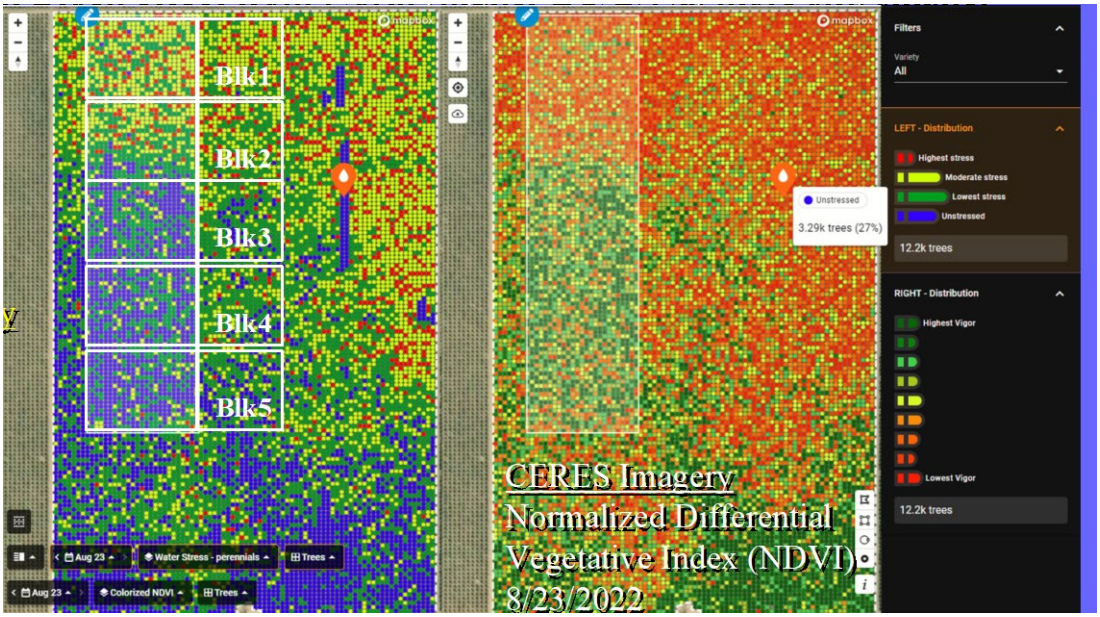



Fig. 1. August 2022 aerial image demonstrating a strong block effect higher soil ECe, Na and B on the individual tree water stress (left panel) and canopy growth (right panel); the northern two blocks demonstrate higher water stress and less canopy growth than the southern three blocks. This increasing gradient of soil salinity captured by the blocking was clearly corroborated in the soil chemistry profile with high ECe, Na and B, increased leaf Na and B, lower seasonal trunk growth and lower yield and nut quality.

## Conclusion and Practical Applications

As this trial was established in May 2022, after crop set, the treatments have not had sufficient time to express differences in most variables, but there is an indication that differences are developing. For example, trunk diameter increased more with the double irrigation lines. NDVI and chlorophyll at the end of the season were greater for trees with a 15% leaching fraction. Water stress was lower for trees with a 15% leaching fraction than the control and for the double than single irrigation lines. The baseline established in 2022 will serve as a control for each plot and tree, thus maximizing the power of tests to quantify treatment effects as they develop in 2023 and subsequent years. The practical application will be how to best irrigate in moderately saline soils with saline water supplies.

# Investigating the effects of winter cover cropping on radiation balance, soil-water dynamics, and water productivity of mature micro-irrigated pistachio orchards

**Authors: Daniele Zaccaria,** Associate Professor and Agricultural Water Management Specialist in Cooperative Extension, Department of Land, Air, and Water Resources - University of California, Davis.

## Introduction

Winter cover cropping is among the soil management practices being increasingly incentivized by federal and state agencies through climate-smart financial incentives to improve soil health and mitigate the effects of increasing climate variability and climate change. Several studies documented beneficial effects of cover crops on rhizosphere ecology, but little information is available on how winter cover cropping and its vegetation residues left onto the soil surface affect the radiation balance and actual crop water productivity. In the water-limited San Joaquin Valley, pistachio growers question whether winter cover cropping can lead to water productivity gains, i.e., more nut yield per unit of water.

To fill the existing knowledge gaps, early in 2022 a field trials was established in a mature, 3.0-ac micro-irrigated pistachio orchard at the UC Kearney Center in Parlier, CA. The trials aim to collect field datasets and develop, document, and disseminate comparative information on the effects that winter cover crops and inactive vegetation residues have on the partition of incoming radiation, on photosynthetically active radiation interception by trees' canopy, and on the differential soil-water dynamics, nut yield and quality, and water productivity for mature pistachio trees compared to trees grown with clean-cultivated orchard floor. The investigation focused on medium-tall winter cover crop "Blando Brome" (*Bromus hordeaceus*) that develops from November to May using rainfall and residual soil moisture. Growers usually terminate and mow the cover crop in mid or late April, leaving the dead light-colored vegetation residues on the ground of the tree row-middles over the entire pistachio growing season for mulching purposes.

## Results and Discussion

In January 2022, the trees were mechanically topped and edged in order to re-shape their canopy for productive purposes. The first half of the trees in the orchard were topped at a 45°-angle in order to provide an angled rooftop aspect, whereas the other half of trees were topped horizontally to provide a flat-top box aspect, as indicated in the Figure 1 below. The pruning residues were shredded and left on top of the soil in the row middles. Afterwards, basic N-P-K fertilizer in granular form was spread on the soil surface and slightly incorporated, prior to conducting shallow soil disking and smoothing out the soil surface. The "Blando Brome" cover crop was sawn in early February 2022 in alternate plots each consisting of 5 tree rows of 9 trees (Fig. 1), which were periodically irrigated with the existing micro-sprinkler system (fanjets) and fertilized to allow a good stand establishment and sufficient cover crop vegetative growth.

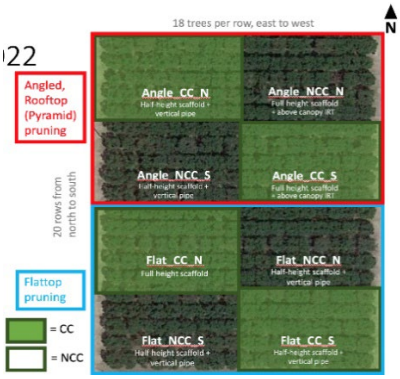



Figure 1. Experimental layout of the pistachio field trial at UC Kearney with four treatments replicated twice (Note: CC = Cover cropped; NCC = non-cover cropped; Angle = Roof-top pruning; Flat = Flat-top pruning)

The cover crop was mowed in late May 2022 after the plant seeded out, and the vegetation residues were left on the soil in the row middles. From early April (near bud-break), the UC research team started measuring in continuous the incoming and reflected radiation and their relative components (near infrared radiation, NIR and photosynthetically active radiation, PAR) with in-situ radiation sensors, both in the cover cropped plots (during periods of active green vegetation and dry inactive residues) and in the clean cultivated plots for developing comparative radiation balance information. The schematic of Figure 2 depicts the shortwave radiation and photosynthetically active radiation (PAR) measured at the canopy top (downwelling radiation) and after being reflected off the inter-row surface of the pistachio orchard (upwelling radiation).

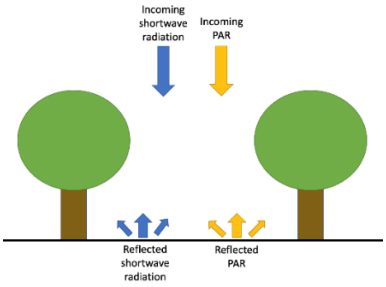



Figure 2. Main radiation parameters being measured at the UC Kearney pistachio study orchard in cover-cropped and clean-cultivated floor plots.

The tables presented below provide the reflectivity and albedo values for PAR and NIR components for the two pruning treatments for the cover-cropped and clean-cultivated plots. These values were calculated from data collected from April 28 to July 13, 2022. The data for the second part of the growing season is still being analyzed and interpreted.

**Table 1 – Roof-top pruned trees**

| **Reflectivity** | **Cover-cropped** | **Clean-cultivated plots** |
|---|---|---|
| PAR reflectivity | 0.032 | 0.028 |
| NIR reflectivity | 0.147 | 0.077 |
| Ratio of NIR/PAR | 4.53 | 2.73 |
| **Albedo** | **Cover-cropped** | **Clean-cultivated plots** |
| PAR albedo (estimated) | 0.169 | 0.127 |
| NIR albedo (estimated) | 0.767 | 0.346 |

**Table 2 – Flat-top pruned trees**

| **Reflectivity** | **Cover-cropped** | **Clean-cultivated plots** |
|---|---|---|
| PAR reflectivity | 0.035 | 0.056 |
| NIR reflectivity | 0.167 | 0.168 |
| Ratio of NIR/PAR | 4.72 | 3.01 |
| **Albedo** | **Cover-cropped** | **Clean-cultivated plots** |
| PAR albedo (estimated) | 0.141 | 0.131 |
| NIR albedo (estimated) | 0.665 | 0.395 |

From the tables, it can be seen that the ratio of NIR reflectivity to PAR reflectivity is greater in cover-cropped plots than clean-cultivated plots for both the roof-top and flat-top pruning treatments. The PAR albedo has slightly higher value for cover-cropped plots compared to clean-cultivated plots. The NIR albedo is markedly greater for cover-cropped plots compared to clean-cultivated plots for both roof-top and flat-top pruned trees. It is worth pointing that the downwelling radiation below the canopy was not measured directly but estimated from the incoming radiation above the canopy and the average leaf area index (LAI).

# Collaborative pistachio rootstock breeding

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

## Introduction

The California pistachio industry currently relies on seedling and clonal rootstocks from a very limited number of parents. These established rootstocks perform very well in most situations, but emerging soil-borne pathogens, variation in quantity and quality of available water, and climate change all create uncertainty about future production. Creating more diverse pistachio rootstock options is an efficient insurance policy that benefits growers, nurseries, and the entire pistachio industry. Understanding the genetic control of Verticillium tolerance/ resistance in existing rootstocks is a pre-requisite for confident adoption of new rootstocks to address challenges such as Phytophthora, salinity, and reduced winter chill. Our major goals for 2022 were to continue refining seedling assays for resistance/tolerance to Verticillium, Phytophthora, and salinity, as well as to generate, genotype, and propagate new seedling and clonal rootstock diversity.

## Results and Discussion

*Generation of seedling population diversity:* We have now accumulated a large stockpile of *Pistacia* rootstock diversity, both from controlled pollinations and collection of open-pollinated seed from the USDA germplasm repository. Controlled crosses have focused on the following: 1) crosses in both directions between *P. integerrima* and *P. atlantica* ("UCB-1 type" and "PG-II type" crosses), to further explore this heterotic combination; 2) crosses of UCB-1 individuals to *P. atlantica* or *P. vera*, for purposes of mapping Verticillium resistance; and 3) crosses of *P. integerrima* with species other than *P. atlantica*, including *P. vera* and interspecies hybrids, to broaden our search for rootstock hybrids with both vigor and tolerance to biotic and abiotic stresses. We have also collected open-pollinated from both *P. integerrima* and *P. atlantica* accessions to serve as negative and positive controls in Verticillium screens and made a limited number of *P. integerrima* X *P. integerrima* and *P. atlantica* X *P. atlantica* controlled crosses. *P. integerrima* flowers so much earlier than other Pistacia that open-pollinated seeds likely result from fertilization with *P. integerrima* pollen. Some emphasis has been placed on using early flowering parents for rootstock crosses, both because it simplifies crossing with *P. integerrima* and because the scion breeding program is also selecting in this direction. The USDA collection was hedged in February 2022 after missing a year in 2021. Seed yields in the collection were much lower in 2022 after a bumper crop in 2021.

*Introduction, maintenance, and distribution of micro-shoot cultures of clonal rootstocks:* This year we introduced 14 new UCB-1 selections into tissue culture. These consisted of 3 very early and 3 very late phenology selections, to test the hypothesis that early phenology drives vigor and increased stem diameter; and 8 precocious selections from a 5$^{th}$-leaf grower field in which ~50% of trees had no yield and <5% of trees had very high yield. A GWAS analysis of precocity scores from ~800 trees in this field showed several significant loci, indicating that genetic variation in the rootstock may affect precocity in the scion.

*Salinity and abiotic stress screening:* In 2021, ~750 trees from 5 families including UCB-1 were subjected to a greenhouse-based salinity screen. Previous results indicated that the UCB-1 population segregates for two alleles that control the accumulation of salts in leaves versus woody stem tissue. Leaf and stem tissue was destructively sampled from ~250 individuals in fall 2021 at the conclusion of the salt screen, and from another ~250 individuals in summer 2022 to determine the longevity of salt sequestration in woody tissue (plants had received no further salt treatment since the previous fall).

Pulverization of woody tissue and salt quantification is ongoing. A drought-stress experiment was performed on 470 potted pistachio plants (240 UCB-1, 230 *P. vera*) in summer 2022, following a field drought stress experiment that was unsuccessful in killing a single UCB-1 individual. This year 95% of UCB-1 individuals survived, compared with 51% of *P. vera* individuals.

*Phytophthora Resistance Evaluation*: The relative susceptibility/resistance of diverse UCB-1 seedlings to Phytophthora root and crown rot was evaluated. Experiment 1 consisted of 78, 3-year-old potted UCB-1 seedlings inoculated on October 21st 2021 and maintained in a greenhouse at the Kearney Agricultural Research and Extension Center (KARE). Experiment 2 consisted of 140 UCB-1 seedlings inoculated on June 6th, 2022 and maintained under natural conditions in a field at UC Davis. All seedlings were wound inoculated using mycelial plugs (6 mm) of *Phytophthora niederhauserii* isolate KARE446. After twelve and five months, respectively, the length of vascular discoloration produced in the inoculated stems was measured and compared among the various UCB-1 seedlings to assess differences in susceptibility/ resistance to Phytophthora root and crown rot. Results showed variable levels of resistance among UCB-1 seedlings. For experiment 1, the largest lesion length was 33 mm, and the lowest lesion length was 6mm (Fig. 1A). For experiment 2, the largest lesion length was 26.58mm and the lowest lesion length was 7.2 mm (Fig. 1B). Experiment 3 consisted of root inoculations with *P. niederhauserii* isolate KARE446 of potted UCB-1 seedlings and P20 seedlings on March 24th, 2022 and under greenhouse conditions. Experiment 4 included 91 UCB-1 seedlings that were root inoculated with *P. niederhauserii* and planted in the field at KARE in October 2021. Experiment 3 and 4 are incubating still and until symptoms expression in order to assess disease resistance levels among the inoculated seedlings.

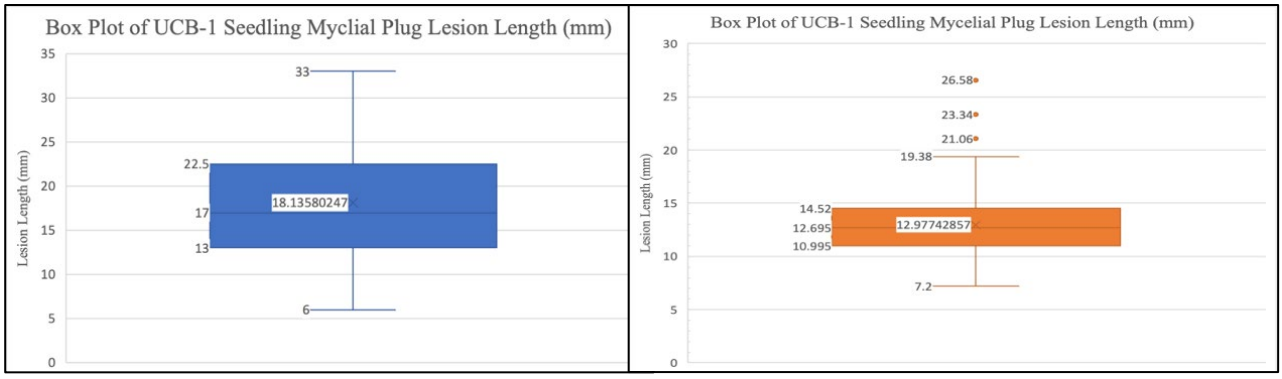



**Figure 1.** A (left). Experiment 1. Lesion length of inoculated UCB-1 seedling under greenhouse conditions with *P. niederhauserii* after 12 months. **B (right).** Lesion length of inoculated UCB-1 seedling planted under field conditions with *P. niederhauserii* after 5 months.

*Verticillium Resistance screening experiment.* Because the young plants (crosses and open pollinated) s were received from UC Davis late in season in jiffies, the inoculations were performed late and results are not available yet. Inoculation assays to speed up screening were performed just after seed germination and when the plants were about 2-2.5 inches using open pollinated UCB1 seedlings.

**Conclusions:** Based on mycelial plug experiments, UCB-1 seedlings show significant variation in Phytophthora resistance. Open pollinated *P. integerrima* seedlings showed less discoloration from *Verticillium* than UCB-1 or other populations tested.

# Pistachio improvement program

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

## Introduction

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

## Results and Discussion

*Germplasm blocks in Davis and Wolfskill:* Ample flowering was observed in spring 2022 in our two germplasm blocks, yet the only mature seed produced was in bagged breeding crosses, and many of these bags showed evidence of bird damage. In spring 2023 we plan to test the use of Bitrex (denatonium benzoate) mixed with latex paint and painted onto crossing bags as a bird deterrent. To reduce bird predation on open-pollinated clusters we will test the use of visual and auditory deterrents including distress calls and/or predator calls. Hedging of the USDA-NCGR Pistacia blocks A-D, as well as the departmental pistachio germplasm block (9 rows of pistachios left over from the Parfitt/Kallsen breeding program) was performed in February 2022 after a one-year hiatus.

*Seedling blocks at Wolfskill:* The 2022 seedling block was planted in April 2022 and consists of 1,290 trees from 56 families. Eight of these families are full-sib families from controlled crosses, and the remainder are half-sib families of open-pollinated seeds from selected females. Sixteen of these half-sib families are from mother trees in the USDA-NCGR collection, and the remainder are from trees in our germplasm blocks or the departmental pistachio block. In combination with the 2021 seedling block (n=630) and the 2020 seedling block (n=470) we now have ~2400 *P. vera* seedlings planted. The 2022 seedling block is not only much bigger, but also much more diverse than the previous two years. As in previous years, a sex marker was used to enrich for females and to plant at a ratio of 4:1 predicted females:males. A new sex marker was developed using improved genome sequence provided by the Monroe lab and deployed using KASP technology. This marker produced genotype calls that were 50% female, 40% male, and 10% unknown. Some families produced more unknown genotypes than others, and it is not currently known whether this result was due to genetic or technical variation.

*UCB-1 subclone experiment:* In previous years, *in vitro* microshoots of ten UCB-1 subclones were obtained from three commercial nurseries, verified to be genetically virtually identical using ~50K GBS markers, verified to be free of *Rhodococcus* bacteria, and planted into a field experiment on UC Davis campus with four 2-tree reps of each subclone. These plants have not yet been characterized further at the molecular level, but we are observing their phenotypes. The single clone exhibiting the "bushy" phenotype is now showing signs of apparent self-girdling, leading to die-back of the top of the main shoot and re-sprouting from lower nodes and suckers. We speculate that this could partially explain the lack of graft success on bushy rootstocks. This experiment remains open to visitation from interested researchers.

*In vitro germplasm maintenance and propagation:* We continue to maintain microshoot cultures of UCB-1 (multiple subclones of a commercial clone), Platinum, other UCB-1 selections, and additional diverse material. In 2022 we introduced 14 new UCB-1 seedling selections into tissue culture. Six of these were selected from a commercial seedling nursery based on phenology: we selected the three earliest leafing

and the three latest leafing seedlings from a set of many thousands of individuals. Based on the observation that a QTL allele for early leafing co-localizes with a QTL for stem diameter/vigor in mature orchards, we predict that the early and late leafing selections will show high and low vigor respectively. The other eight new clones were selected from a grower field of 5th-leaf Golden Hills on UCB-1 seedling rootstock on the basis of predicted 2022 nut production as scored in early June, before the second flush.

*Development of embryogenic cultures:* Last year, two immature *P. vera* kernels collected on June 18th 2021 generated embryogenic tissue. This year we were able to germinate and root plants from this material, demonstrating "end-to-end" recovery of plants from embryogenic tissue. However, this embryogenic tissue was zygotic rather than somatic in source, and therefore genetically different from the mother tree on which it was produced. A 2022 experiment testing different concentrations of cytokinins and auxins on somatic pistachio tissue has so far generated callus but not embryogenic tissue.

*Development of a chill requirement assay:* Budsticks from 500 trees were collected on each of three sampling dates: Dec 17, Jan 10, and Jan 31. Trees included 6 reps each of 5 cultivars with a range of leafing and flowering dates (Aegina, Gumdrop, Golden Hills, Lost Hills, Kerman), and the remaining 470 consisted of trees from the 2020 seedling block. Budsticks from each collection date were scored weekly for signs of terminal bud-break in the growth chamber for 4-6 weeks. Of budsticks from replicated cultivars from the Dec 17th collection date, only Aegina and Gumdrop showed any budbreak, and they took at least 3 weeks to do so. Budsticks of all 5 cultivars collected Jan 10 showed budbreak after 3 weeks in the growth chamber, and Gumdrop was the earliest to break bud, often pushing after only 2 weeks. For the third and final Jan 31 collection date, all cultivars except Kerman were breaking bud after 2 weeks in the growth chamber. An overview of data for all 500 trees is shown in Figure 1.

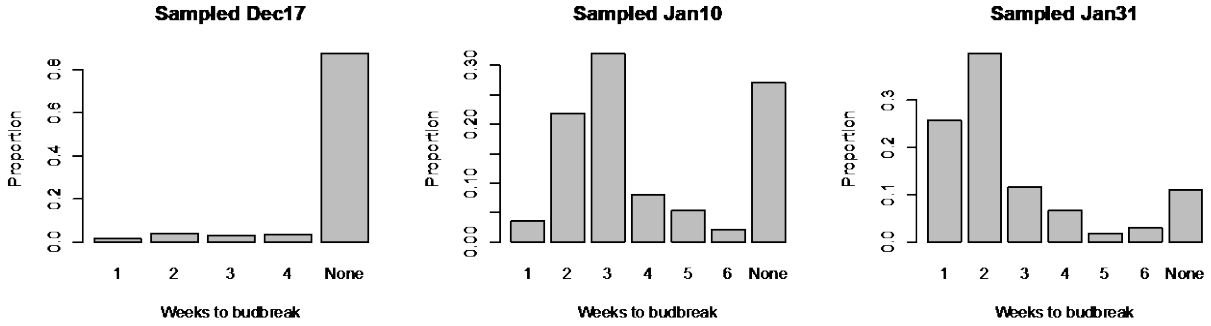



*Figure 1. Weeks to budbreak of sticks from 500 pistachio trees sampled on 3 dates. The proportion of trees showing eventual budbreak from a given time point reflects endo-dormancy, and the speed of this process (weeks to budbreak) reflects eco-dormancy. Relatively little additional information was obtained by extending the 2nd and 3rd sampling date experiments from 4 to 6 weeks.*

*Genetics of blanking and IKD:* The 2021 pilot experiment involving Illumina genotyping of 95 filled nuts (some with IKD) and 95 blanks had a low success rate: we obtained data for only 36 filled nuts and 28 blanks. We suspect that low DNA quality and quantity from kernel tissue is likely responsible for our low success rate. In addition, genotype data from all blank samples clustered together, probably reflecting the fact that most DNA from the blank samples was maternal in origin. This year we focused on obtaining better genotype data for the IKD experiment. By performing *in vitro* germination of embryos from IKD and non-IKD kernels, we have obtained leaf tissue, which we know will yield more robust genotyping results than kernel tissue.

## Conclusion

Our chill assay enables separate estimation of endo-dormancy and eco-dormancy requirements of pistachio germplasm.

# Dissecting the genetics/genomics of nutritional quality traits in pistachio

**Authors: Christine Diepenbrock**, Assistant Professor, Department of Plant Sciences, UC Davis; **Grey Monroe**, Assistant Professor, Department of Plant Sciences, UC Davis; **Pat J. Brown**, Associate Professor, Department of Plant Sciences, UC Davis.

## Introduction

This project aimed to develop methods for high-throughput quantification of compositional and other kernel traits (such as protein, starch, oil, moisture, total phenolics, and visual appearance/color) in pistachio, and to characterize these compositional traits in genotype-environment combinations of relevance to pistachio growers and dissect the genetic basis of these traits in a diversity collection and commercial cultivars. Near-infrared spectroscopy is a technology that enables quantification of compositional traits in plant and other tissues, through collection of spectra from 400 to 2500 nm with data collected every 0.5 nm (in the case of the instrument used in this project; note that ~400 to 700 nm would be the visible portion of the spectrum, enabling examination of kernel color and relevant plant pigments such as chlorophylls and phenolics). Wet-chemistry reference analyses (e.g., protein via combustion method, total starch via a premade Megazyme kit, and total phenolics using a different color-change reaction in a 96-well plate) are then used to develop calibrations for prediction of compositional traits in a larger set of samples based on their spectral patterns.

## Results and Discussion

Kernel samples were collected in collaboration with multiple UC researchers who work in pistachio. The experiments from which samples were collected were as follows: a breeding block, a trial with several agronomic treatments alongside a control treatment, an irrigation trial, and a pruning trial. These trials span a geographic range from the Sacramento Valley to Central San Joaquin Valley (both east and west sides), and include Golden Hills, Lost Hills, and Kerman, among several other commercially relevant cultivars. Samples from retail locations are also being included to capture the full supply/demand stream.

A total of 30+ samples have already been scanned on the NIRS and results generated for their compositional profiles (Table 1), with 100+ samples still to be analyzed within the project term. The large majority of samples involved in this project were collected in diverse field locations in Fall 2022, such that data collection (including scans on the NIRS instrument and wet-chemistry reference methods) and computational analysis of the collected data are still actively underway. Completion of scans on the NIRS instrument is scheduled to take place by end of January 2023, and final integration of wet-chemistry reference data—and adaptation of calibrations suitable for pistachio—is scheduled to take place ~1 month thereafter, due to the typical turnaround time by the relevant service provider for two of the involved traits. The team member who was set to carry out the primary data collection and analysis on this project is back from a leave period, such that analysis is ramped up and on track for on-time completion.

**Table 1**. Results on the NIRS instrument for samples collected in 2021 and 2022, as predicted using a pre-made calibration. Overall summary statistics per year are reported at this time until data are available on a larger number of 2022-collected samples for each genotype and/or location.

| Year of sample collection | Trait | Range (Average) |
|---|---|---|
| 2021 (n = 20) | Protein | 21.75 to 23.89% (22.22%) |
| | Fat | 37.73 to 44.43% (39.30%) |
| | Moisture | 2.16 to 3.08% (2.86%) |
| 2022 (n = 13 thus far) | Protein | 22.54 to 24.70% (23.80%) |
| | Fat | 39.90 to 46.29% (43.68%) |
| | Moisture | 1.87 to 2.75% (2.22%) |

Two notes regarding the results presented above: 1) Starch and total phenolics are not yet predicted within the pre-made calibration but will be predicted by the adapted calibration with the incorporation of wet-chemistry reference data for those traits. 2) Samples were freeze-dried prior to scanning on the NIRS instrument to ensure that they would grind into a powder more so than a paste, for maximized feasibility of analysis via NIRS. (Note that grinding tends to produce higher-quality results than analysis of whole kernels; however, analysis of whole kernels could be examined in the future if desirable for sake of throughput and/or if a non-destructive assay would be more helpful in certain use cases within the pistachio supply stream.)

Performance of the pre-made calibration looked reasonable based on global and neighborhood H values (key metrics within NIRS-based examinations, which represent distance of the analyzed samples from samples that were already in the database), among other metrics. Performance of the adapted calibration that we are developing as part of this project, which incorporates samples from the present study (both spectra from the NIRS instrument and wet-chemistry reference data), is expected to be even further improved. It appears that suitability of the adapted calibration for use on several cultivars and growing environments of relevance to California pistachio production can be expected. Note that updates to the calibration will continue to be needed on an annual basis for optimal performance (and are standard practice in the NIRS realm), using newly collected samples that are representative of the cultivars and growing environments surveyed in that year.

We would note that the collected samples which are in the queue for analysis in the next couple of months represent a larger number of environments and smaller number of genotypes than originally planned, due to a deficiency of nuts available from diverse material in the relevant experimental orchard. However, it seems that the analyses conducted are still of high relevance for pistachio production in California, given that they are 1) focused on commercially relevant cultivars and breeding material, 2) enable examinations both within and across agronomic treatments that are already being tested in the UC pistachio research community, and 3) represent a broader (than originally proposed) range of locations that are relevant for pistachio production. We are appreciative to the several researchers who made these samples available for our use and look forward to reporting our findings on the full set of involved samples. We plan to sample an even larger number of genotypes in the future, to still characterize the (likely even broader) range of variation for compositional traits in those samples and identify favorable parents/alleles for the compositional traits under study—while still maintaining the range of commercially relevant cultivars, agronomic treatments, and production environments as are represented in 2022-collected samples.

As one final methodological note, visual differences were preliminarily observed for two trait sets:

1) Visual differences were observed across samples both for kernel color and for the color of the powder/flour produced from grinding those kernels. Powder/flour color will be quantified as part of our NIRS workflow, and we will also capture visual appearance of the kernels before grinding.
2) Visual differences for powder vs. paste consistency of the samples were observed, even after freeze-drying. These observations will be noted in case they affect the resulting spectra in a way that is methodologically relevant, and/or in case this difference in consistency is related to one or more of the compositional traits under study (which it seems could feasibly be the case).

## Conclusion

Variation in compositional traits was evident in the samples analyzed thus far (Table 1), particularly for fat content (as a percentage of kernel composition), which will be examined further in the analyses still underway (on the same and likely also complementary analytical platforms/instrumentation). It appears that the pre-made NIRS calibration that we are using does have reasonable performance for both 2021- and 2022-collected samples, and the samples that will be used to further adapt and improve that calibration for use on California-grown pistachios are now in hand and under active analysis.

# Dissection of pistachio fruit development towards optimal hull split and insect resistance

**Authors:** **Georgia Drakakaki**, Professor; **Shuxiao Zhang**, Graduate student; **Alisa Chernikova, Shaina Eagle,** Undergraduate Students; **Minmin Wang**, Postdoctoral Fellow; Department of Plant Sciences, University of California, Davis, **Thomas Wilkop**, Director, MCB Light Microscopy Imaging Facility
Collaborators: Barbara Blanco, Louise Ferguson, Judy Jernstedt; Department of Plant Sciences, University of California, Davis, Sam Houston Wilson, Giulia Marino; Kearney Agricultural Research and Extension Center

## Introduction

The pericarp of the pistachio fruit is composed of the hull (the exo-mesocarp) and the shell (the endocarp), while the integrity of these two layers is highly important to the yield and quality of the pistachio nut. Consumers prefer split shell nuts for ease of consumption while an intact hull is necessary to minimize damage by pests and pathogens such as the Navel Orangeworm (NOW) and the fungus *Aspergillus*. Thus, we examined shell and hull split between different genotypes over two years, to better understand the basis of these traits.

In 2021 we examined Golden Hills and Kerman fruits at several time points during fruit ripening. These cultivars were selected because Golden Hills is a variety with higher shell split rate compared to Kerman (Parfitt et al. 2007). We found that Golden Hills has also a higher percentage of hull "tattering", allowing us to characterize the anatomy of hull breakdown.

In 2022 we expanded our sampling to include Lost Hills, a variety with similar shell split rate to Golden Hills (Parfitt et al. 2008). Further, based on a pilot experiment in 2021, we expected that lost hills will display higher hull cracking compared to Golden Hills. Our analyses of multiple morphological parameters from both years showed that shell split rate correlates with the kernel width. In addition, we identified that hull cracking and tattering are separate breakdown events at the cellular level. The identification of the traits associated with each event is currently ongoing work in our laboratory.

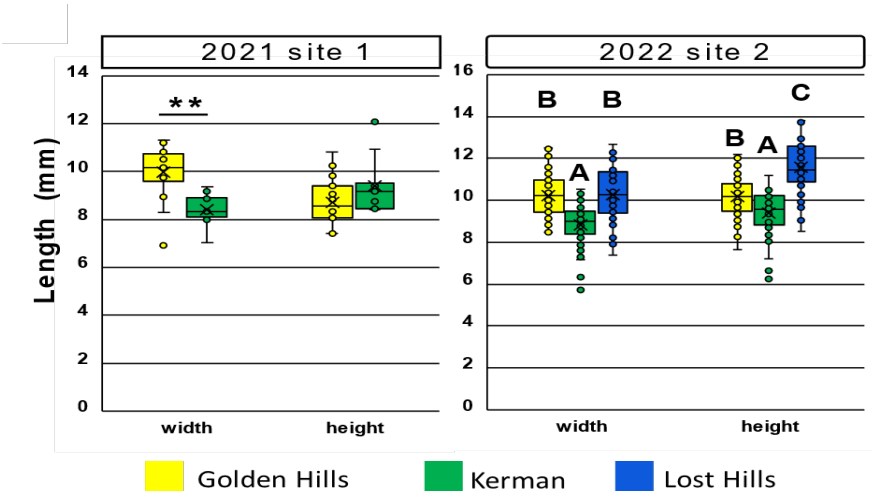



*Figure 1. Kernel width and height of Golden Hills, Kerman, and Lost Hills sampled in 2021 and 2022. ** = P < 0.01, two-tailed T-test, letters = LSM analysis alpha = 0.05, one-way ANOVA P < 0.01.*

## Results and discussion

*Shell split analysis*

Examination of fruit sections halfway between the tip and base of the fruit, showed that the width of the kernel, but not the height, significantly correlates with the shell split rates in Golden Hills, Kerman, and Lost Hills (**Fig. 1**). These measurements were taken over two years, in two different orchards, with each orchard utilizing a different rootstock (PGI for 2021, UCB1 for 2022), suggesting that this morphological feature is consistent across sites, management practices, and rootstocks.

In addition to kernel shape, quantification of several shell anatomical features showed that despite of lack of specialized cell types, reduction in shell thickness and shell cell size at the suture, combined with cell flattening, may lead to mechanical weak point that functions as a dehiscent zone.

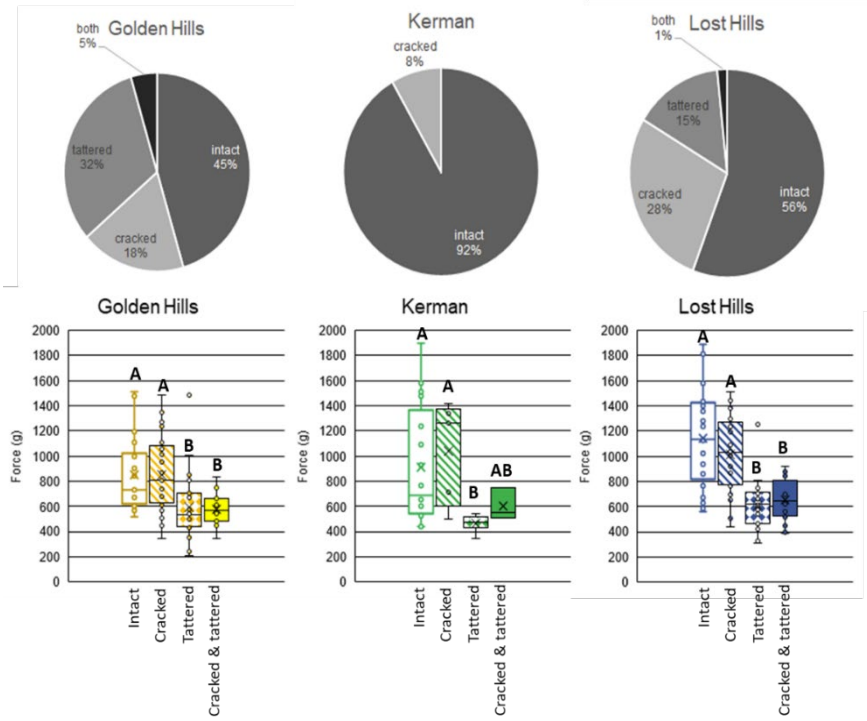



*Figure 2. Hull breakdown differences between Golden Hills, Kerman, and Lost Hills at final harvest. P < 0.01 Fisher's Exact Test. Different hull breakdown events show difference in hull texture. Letters = LSM analysis, alpha = 0.05, one-way ANOVA P < 0.01.*

*Hull split analysis*

Due to either unusual weather patterns, tree age, and / or difference in site & site management practices, we detected a significant percentage of cracked hull in Golden Hills fruits during 2022, which was not observed in 2021. Lost Hills still significantly show the highest percentage of cracked hull (**Fig. 2**).

Notably, the cracked hull showed similar texture to intact hull in all three cultivars tested in 2022, while hull tattering was associated with significantly softer hull. When hull cracking and hull tattering was identified on the same fruit, the hull texture more closely resembled a tattered hull. Work is currently in progress to assay the cell wall components, cell viability, and tissue water content to determine which factor may be the main driver behind this texture difference and its potential association with insect damage.

## Conclusion

We identified key morphological and anatomical features associated with shell split in pistachios that are consistent across multiple cultivars and years. Following our identification of the different anatomical events in hull breakdown, we can now classify them in two categories 1) hull cracking and 2) tattering, which appear to be driven by different events at the cellular level.

# Evaluation of pistachio scion and rootstock breeding selections

**Authors: Craig E. Kallsen**, UCCE Pistachio Farm advisor, Kern County; **Dan E. Parfitt**, Emeritus, Pomologist-AES, University of California, Davis; **Giulia Marino**, UCCE Orchard Specialist, UC Davis.

## Introduction

The original UC breeding program began with parent crosses made in 1989 by Dr. Dan Parfitt and Farm Advisor Joseph Maranto. Since this time, the program continued with the breeding and evaluation of novel scions, and as of 2009, experimental rootstocks. Private cooperating growers who donated long-term use of land, labor, equipment, and time made much of this research possible. The current focus of this program is evaluating the existing plant materials developed over the years that exist now in diverse locations. Breeding and associated research is continuing with the hiring of a walnut and pistachio breeder, Dr. Patrick J. Brown, Plant Science Dept. at UC Davis.

## Results and Discussion

In 2022, we continued evaluation of six advanced scion-selection trials and three trials composed of rootstocks with novel *Pistacia* heritage. The planting of these trials occurred from 2010 to 2019. Some of these trials have had novel plant material added to them, since planting, from existing seedling selection trials. The Jasmine trial is our longest currently monitored female scion advanced-selection trial. This trial was planted in 2010 in the "citrus belt" of Kern County where winter chilling if often borderline or inadequate. A female advanced selection identified as KB25-78 is noteworthy at Jasmine as it has produced 44.2% more cumulative edible yield ($7^{th}$ through $13^{th}$ leaf) than Lost Hills, which is the next highest-yielding cultivar in the trial. Data from KB25-78 are being collected in a second, younger trial established in 2015 at the UC Westside Research and Extension Center (WSREC). In this trial, the precocious Gumdrop was the only trial entry to produce enough yield to harvest in 2020 (847 lbs/acre). KB25-78 performed similarly to Golden Hills and Kerman in 2021 and 2022. Its comparatively larger tree size bodes well for good production in 2023. Unfortunately, because of the on-going drought, this trial has not been irrigated at full ET, which has adversely affected nut yield. For future further testing of KB25-78, over the past two years we have established three large new "observation trials" with cooperating growers comparing this selection with other existing commercial cultivars. A recent concern of the industry is the appearance of "internal kernel discoloration" (IKD) in Golden Hills and Lost Hills pistachio. This year, IKD was found in KB25-78 nut samples at less than 2% at both the Jasmine Trial and in the UC Westside REC trial. Internal kernel discoloration was not found in Kerman or Gumdrop in any of our trials.

In general, nut quality has been better in Gumdrop in our newer trials from 2020 – 2022 than it was in its debut in the Buttonwillow Trial (2012-2020). The Gumdrop trees appear to be performing well on Platinum rootstock in the West-of-Wasco Trial planted in 2014.

Normally, the male pollenizer Peters is not precocious, producing few flowers in four and five-year-old trees. Furthermore, Peters tends to bloom much later than Kerman in years with low winter chilling. In response to the lack of bloom synchrony between Kerman and Peters after the low-chill years of 2014 and 2015, three male pollenizer advanced-selection trials were initiated. The objective of these trials was to identify a pollenizer for Kerman that had a shorter juvenile stage and that would bloom dependably at the same time as Kerman under the climate existing in the San Joaquin Valley. Initial selection of many of the experimental male selections planted in these randomized and replicated trials occurred from the seedling-evaluation breeding trial planted at the WSREC in 2008. During the low chill years of 2014 and 2015, these selections demonstrated fewer bloom-related symptoms of inadequate chill such as increased length of the juvenile period, delayed spring bloom initiation, flower degeneration and extended and variable inflorescence opening across the tree canopy. In the spring of 2021, we completed our

evaluation of the experimental male pollenizers in these advanced trials, which included pollen germination. As a result, we selected a male pollenizer that in 2022 was accepted for release to the industry, probably in 2023. This selection, in combination with the previously released Famoso cultivar, will cover the bloom period of Kerman in both low chill and high chill years. This cultivar, as is the case with Famoso, has a shorter juvenile period than Peters. Plant breeding is a slow process, especially with pistachio, and when these trials began, we had not foreseen the degree with which new plantings of Golden Hills and Lost Hills would replace Kerman. However, Kerman remains a viable cultivar and its planting continues. We suggest that this new male pollenizer and Famoso be grafted into new and existing Kerman orchards in the San Joaquin Valley, especially in areas with deficient winter chilling, once budwood becomes available.

In 2018, we identified several selections from the seedling breeding trial, planted in 2012, that not only displayed harvest maturity equal to or in advance of 'Golden Hills', but also demonstrated a nut hull that appeared to be less susceptible to early tatter. Based on past and current work of other researchers, a hull less prone to pre-harvest tatter may make them less susceptible to navel orangeworm (NOW) damage. These selections were grafted into commercial and experimental rootstocks in replicated trials with Kerman and Golden Hills in 2019 at the WSREC and meaningful evaluation awaits their coming into bearing. These trials are in or close to an untreated and now mature seedling selection trial that will provide an abundant source of NOW for testing purposes once these new trials begin producing nuts.

We continued to evaluate seedling progeny selections from our breeding program that have displayed greater tolerance to an inadequate winter rest period by demonstrating fewer leaf-canopy and flowering symptoms, or that flower very early in the spring or have very novel parental genetics that could reduce their chilling requirement. We are testing these in the Coachella Valley, which is characterized as having a climate with much reduced winter chilling compared to the San Joaquin Valley. These crosses and advanced selections from our breeding program are being compared to cultivars that are commonly planted commercially in the San Joaquin Valley. Furthermore, we continued to introduce seedlings and advanced selections into the trial after its initial planting in 2017. To date in this trial, the seedling trees on their own roots, and grafted trees, both experimental and existing scion and rootstock cultivars, have demonstrated a diversity of vegetative growth under low-chill conditions. This trial will become more interesting, once, and if, the trees begin to bloom. Current observations from the trial suggest that none of the industry's common commercial cultivars will produce nuts in the Coachella Valley. This observation does not bode well for future pistachio production, as it is currently conducted in the San Joaquin Valley, which has been predicted to become more like the Coachella Valley over the next 50 years. We are evaluating hybrids between *Pistacia* species at this site, to determine if these might demonstrate economic yield production in an extremely low-chill environment. Most of these were not planted into the trial until 2019.

We have discussed our research into novel pistachio rootstocks in previous Executive and Full reports to the California Pistachio Research Board. Based on this previous experimentation, individual rootstock selections were made from two of our 'Endeavor' rootstock lines. The Endeavor rootstocks have genetics different from the existing commercial rootstocks currently in use by the industry. The novel characteristics of these experimental rootstocks included less suckering, boron exclusion in high salt environments and, in one line, tree dwarfing, which would be a useful characteristic in establishment of high tree density orchards. However, our research efforts to further test experimental rootstocks from our breeding program in more rigorously designed experimental trials has run into a propagation problem. To date, we have not been able to root what we think are superior clones from these lines. Efforts are underway to find a protocol so that cloning will be possible and that randomized and replicated scientific evaluations may proceed.

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

**Authors: Richard Michelmore**, Director, The Genome Center, 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; **Grey Monroe**, Assistant Professor, Department of Plant Sciences, UC Davis; **Bob Beede**, UC Farm Advisor, Emeritus, Kings County; **John Preece**, Research Leader, USDA, National Clonal Germplasm Repository (NCGP); **Maher Al Rwahnih**, Director, Foundation Plant Services, UC Davis.

## Introduction

There is significant variation in tree size, which determines productivity, in commercial orchards planted with UCB-1 seedling rootstocks. It has been unclear to extent to which this is due to genetic differences or environmental variation. Nurseries have tried to tackle this problem by rogueing young seedlings before they are planted in orchards; however, our data previously demonstrated that performance in the first year is a poor predictor of later tree size. Over the last six years, we have developed a comprehensive collection of genomic resources for *Pistacia* spp. We have sequenced and assembled the genomes of the three key *Pistacia* species: *P. atlantica*, *P. integerrima*, and *P. vera*, cvs. Kerman and Siirt (the last in collaboration with Salih Kafkas, Cukurova University, Turkey). In addition, we have collected a large amount of phenotypic and genetic data from both experimental and commercial orchards and now have DNA sequence-based marker data derived mainly from bark cambium for more than 3,000 trees. We developed a novel high-throughput, low-cost, genotyping and trait association approach that allows us to handle data from thousands of trees and dozens of traits simultaneously. Trunk caliper, tree height, and canopy volume were highly correlated indicating segregation of loci for general vigor. These data allowed us to identify two major loci that control vigor of UCB-1 rootstocks in commercial orchards and hence determine tree size. Therefore, the variation in tree size in commercial orchards has a large genetic component. This analysis has recently been published (Palmer *et al*. 2022) and the genome assemblies are publicly available from Genbank.

The current goal of this project is to provide molecular assays for these loci that will allow nurseries to select UCB-1 seedling rootstocks that will grow into more uniform, vigorous adult trees and productive orchards. We are also investigating the genetic basis sex and flowering time.

## Results and Discussion

Genotyping by sequencing data from experimental and commercial orchards and genome wide association studies (GWAS), combined with our chromosome-scale, high quality, genome assemblies for the parental *Pistacia atlantica* and *P. integerrima* trees resulted in two highly-informative molecular markers for vigor. In 2021, based on the genomic sequence information, we developed an inexpensive, quick, and easy qPCR protocol for single nucleotide polymorphism (SNP) marker analysis. We were able to predict the improved size distribution that extant orchards would have had if these markers had been used to rogue seedlings prior to planting in the orchards. In 2022, we improved the marker for the major locus, which was tightly correlated with the variation in tree vigor in the experimental orchard in Davis. This new molecular marker will be available for nurseries to rogue out trees that would exhibit low vigor later in an orchard.

In 2022, 99% of trees in the nine-year-old experimental orchard at Davis flowered and we recorded the fifth annual data set on the sex, flowering time, and seed development for the trees that had reached sexual maturity. Early blooming was correlated with larger trees, regardless of sex. Data on maturity collected in past years allowed us to identify a chromosome region determining shorter juvenility in UCB-1 in addition to the major locus determining vigor. It is unknown whether this locus affects juvenility in *P. vera* or whether segregation of this locus in rootstocks influences juvenility of the scion.

Therefore, we designed markers for the maturity-specific locus and analyzed rootstocks in a commercial orchard that had just started to flower; however, the results were unclear, possibly because the trees had been heavily pruned. We have located another orchard that will begin to flower next year that has not been heavily pruned and will test the genetic impact on flowering of the scion next year.

For the first time this year, we detected a hermaphrodite UCB-1 tree in the experimental orchard at Davis. Genotyping-by-sequencing of all the trees in the orchard allowed us to map the sex determining locus to a region on Chromosome 14. The hermaphroditic tree had a recombinant chromosome in this region. Therefore, we whole-genome-shotgun sequenced this tree and ~40 other trees that had cross-overs in this region. This has allowed us to refine the interval containing the sex locus. High-resolution analysis continues. We collected seeds and will sequence seedlings to see if the progeny are the result of selfing. Also, seedlings will be grown to see if some are hermaphrodites in the future.

Since March 2018, we have made annual 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 2022, we captured another round of multispectral drone imagery for nine of the sampled commercial orchards. We are currently processing the new data and compiling all the data into one large GIS evaluation. A segmentation model was built to delineate the crowns of each of the trees and zonal statistics for each tree were combined with structural field measurements via pre-assigned unique IDs. The drone-derived structural metrics include measurements of height, crown area, and perimeter length of each tree crown. Additionally, min, mean, median and maximum NDVI values were calculated from the multispectral datasets. Also, we correlated drone data from the experimental orchard with our vigor data and confirmed that the vigor marker can be associated with tree height, crown size, perimeter and NDVI. These data illustrate the potential benefits of timely and detailed UAV imagery to help managers estimate commercial yields more efficiently than conventional field measurements.

## Conclusions

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.

During this project, we have collected a large amount of phenotypic and genetic data from both experimental and ten commercial orchards and have now data for more than 3,000 trees. This year we continued to record sex, flowering time and seed development data in the experimental orchard in Davis. With collaborators, we also conducted another year of multispectral aerial imaging surveys of the sampled orchards. These data will accelerate and enhance our phenotyping efforts. We used genotyping by sequencing data from experimental and commercial orchards and GWAS, combined with chromosome-scale, high quality, genome assemblies for the parental *P. atlantica* and *P. integerrima* trees to identify two highly informative molecular markers for vigor. We developed and validated the predictive value of molecular maker assays using samples from the experimental and commercial orchards as well as samples provided by two nurseries. This year we designed and validated new markers that correlated with 100% of variation in experimental orchard in Davis. These markers enable selection of young UCB-1 seedlings that will result in rootstocks with predictable sizes and the culling of inferior genotypes prior to planting in orchards. They also allow the selection of vigorous genotypes for clonal propagation.

Palmer, W., Jacygrad, E., Sagayaradj, S., Cavanaugh, K., Han, R., Bertier, L., Beede, B., Kafkas, S., Golino, D., Preece, J., Michelmore, R.W, (2022). Genome assembly and association tests identify interacting loci associated with vigor, precocity, and sex in interspecific pistachio rootstocks. *G3, Genes|Genomes|Genetics,* jkac317, https://doi.org/10.1093/g3journal/jkac317.

# Pistachio pan-genome for accelerated breeding

**Authors: J. Grey Monroe,** Assistant Professor, Department of Plant Sciences, UC Davis; **Chaehee Lee**, Postdoctoral Researcher, Department of Plant Sciences, UC Davis

## Introduction

Genome sequencing has revolutionized the field of plant breeding, providing valuable insights into the genetic makeup of crops and enabling the development of new and improved varieties. This is particularly important for long-lived crops such as pistachios, which have a longer breeding cycle compared to annual crops. Our project seeks to accelerate the breeding process by creating comprehensive genomic resources for this important crop. By overcoming the limitations of existing genomic resources, our work will enable breeders to more quickly identify desirable traits and develop new varieties that are better adapted to changing climates and market demands.

Genome-enabled breeding approaches are crucial to expedite pistachio breeding to introduce a wide diversity of pistachio varieties well adapted to California's unique challenges. By sequencing the genomes of cultivars with different traits, researchers can identify genes and pathways that are associated with those traits. This information can then be used to identify potential targets for breeding efforts, enabling the development of superior plant varieties. Marker-assisted selection, genomic prediction, and discovery of agronomically important genes will be enabled using the pistachio genome and pan-genome resources.

With the aid of the advent of new genome sequencing technologies (e.g., PacBio HiFi, Iso-Seq, and Dovetail Genomics' Omni-C sequencing), the major aim of this project is to generate a high-quality pistachio reference genome and pan-genome of diverse cultivars to better understand genomic diversity and accelerate pistachio breeding for long-term productivity of pistachio in California. This work has been supported by invaluable investment from the California Pistachio Research Board.

## Results and Discussion

**Summary:** Our project has successfully refined the 'Kerman' reference genome to a high-quality, haplotype-resolved chromosome-scale genome. This genome has undergone final gene annotation, with 38,315 genes predicted, including details of gene annotation structure with and without isoform consideration. In addition, we have constructed the first platinum-quality Pistachio pan-genome, consisting of chromosome-scale reference-quality genomes for five additional Pistachio cultivars. More than 95% of each genome was anchored into 15 chromosomes, and final gene annotations were completed for all six genotypes. Our analysis detected strong presence and absence variation in gene content within the pistachio pan-genome. Finally, we characterized large structural variations, such as inversions, translocations, and duplications, within the pistachio pan-genome. Overall, these results provide valuable genomic resources for the pistachio breeding community.

**Kerman Genome:** We have completed the first ever chromosome-scale genome assembly in pistachio. Our newly assembled 'Kerman' genome was of exceptionally high quality with a size of 587 million base pairs (Mbp), a contig N50 size (common metric of sequence assembly contiguity quality) of 26 Mbp, and more than 98.7% BUSCO (Benchmarking Universal Single-Copy Orthologs) value. In order to improve the 'Kerman' genome assembly into the whole chromosome-scale reference genome, we first filtered non-nuclear (organelle) contigs out from the original 294 contigs. The resulting 102 contigs were scaffolded based on Omni-C chromatin interactions information (A total of 100 Gb of Omni-C reads with ~ 172x coverage) . We then successfully anchored 97 % of assembly into 15 chromosomes by scaffolding primary contigs with Omni-C assisted scaffolds as a reference using RagTag v2.1.0. The final 15 chromosomes resulted in a size of 561 Mbp with a contig N50 of 28.4 Mbp.

Transposable elements (TEs) are one of the major contributors to plant genetic variation and represent a valuable resource for plant breeding as many important agronomic traits can arise from dynamic activities of TEs in plant genomes. In 'Kerman' genome, the 64.94 % of the genome (376.5 Mbp) was characterized as TEs, which class I (retrotransposons) and class II (DNA transposons) TEs accounted for 48.9% and 9.6% of the genome, respectively. The 'Kerman' genome is highly repetitive compared to the other species, Mango genome (40.5%), in the same family, Anacardiaceae. With the extensive RNA-Seq (in collaboration with the Blanco-Ulate lab) and Iso-Seq data as extrinsic evidence and *ab initio* prediction in the repeat-masked genome, we identified 38,315 genes in the primary assembly and 36,560 and 37,098 genes in haplotype 1 and haplotype 2, respectively. The final annotation of the primary assembly had 98% BUSCO value, suggesting the very complete annotation.

**Pan genome:** In addition to 'Kerman' genome, the genome assemblies of five genetically diverse collections of pistachio cultivars ('Mateur', 'Sirora', Napoletana', 'Chaparrillo', and 'T41') were refined by re-assembly, filtration of non-nuclear contigs, and scaffolding into chromosome-level using 'Kerman' genome as a reference. These genotypes exhibit considerable trait diversity, especially with regards to winter chill requirements. Our new pan-genome analysis also reveals considerable genetic diversity in these lines. Syntenic analyses revealed substantial structural variation (inversion, translocation, and duplication) in six Pistachio genomes. The dynamics of TEs in six cultivars were shown with the size range from 64.94 ('Kerman') – 68.9% ('Chaparrillo'). The gene annotation for five genotypes was carried out with Iso-seq hints generated for each cultivar using our robust gene annotation pipeline used for 'Kerman' genome, resulting in the number of genes ranging from 37,500 to 41,500 ( 37,500 ('Mateur') – 41,500 ('Sirora'), which suggests the considerable presence-absence variation of genes in six genotypes. Further investigation of this diversity will be useful to discover genetic variants underlying traits such as winter chill in these cultivars.

## Conclusion

In summary, we refined the first reference genome of Pistachio 'Kerman' to a high-resolution, haplotype-resolved chromosome-level genome using state-of-the-art Omni-C data. Furthermore, using this genome as a reference, we were able to scaffold five additional genotypes into 15 chromosomes, allowing us to construct the first platinum-quality Pistachio pan-genome.

This work establishes a foundation for genome-enabled breeding in pistachio, and through a number of collaborations with other pistachio researchers will be used in 1) identifying and tracking genetic variations within and between populations, 2) identifying genes and pathways associated with important traits, and 3) improving the accuracy and efficiency of breeding programs.

Applying these new genomic resources in pistachios has the potential to drive significant economic value for the industry. By developing comprehensive pan-genomes for pistachios, we can enable more targeted and effective breeding efforts, leading to the development of superior pistachio varieties with improved yield, disease resistance, and environmental adaptability. Our pan-genomes will provide valuable genomic resources for the larger breeding community, enabling more efficient and effective breeding efforts well into the future.

# Effect of bloom time on pistachio hull integrity and nut quality at harvest

**Authors: Barbara Blanco-Ulate**, Plant Sciences Dept., UCD; **Giulia Marino**, Specialist, Plant Sciences Dept., UCD. **Collaborators: Robert Beede,** CE Kings County, UC ANR; **Phoebe Gordon**, CE Merced County, UC ANR; **Joseph Coelho**, Maricopa Orchards; **Ian Humrick**, Maricopa Orchards; **John Gebhardt**, Gebhardt Farm Management; **Muhammad Ismail Siddiqui**, Delano-2665; **Selina Wang**, Food Science and Technology Dept., UCD; **Louise Ferguson**, Plant Sciences Dept, UCD; **Georgia Drakakaki**, Plant Sciences Dept., UCD; **Houston Wilson**, Entomology Dept., UCR; **Charles Burks**, USDA-ARS

## Introduction

This was an odd year for many pistachio growers. In multiple production areas across the San Joaquin Valley, pistachio nuts displayed defects at harvest, including a higher proportion of blanks, poor release, low shell split, and shell stain, among others. This year's research focused on studying whether some of these defects are caused by delayed bloom due to warm winters with low chill, combined with high spring temperatures during early nut development[1]. Based on our data from 2021, together with this year's results, we observed that bloom time and the interaction with spring temperatures strongly affected nut development and quality at harvest. In the 2022 study, we expanded our work to 1) assess how bloom time and spring temperatures in different locations affect nut quality, 2) validate differences in nut attributes between pistachio varieties that differ in bloom time (Golden Hills vs. Kerman), and 3) test if bud breaking treatments (Dormex or oil) can anticipate bloom in low chill pistachio orchards.

## Results and Discussion

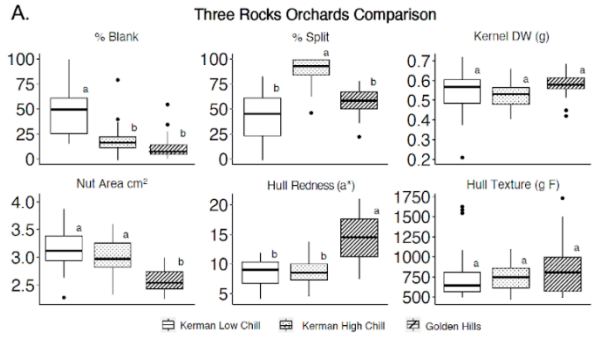
We selected commercial orchards in four locations across the San Joaquin Valley: Three Rocks, Madera, Coalinga, and Kern. Three Rocks orchard (cv. Kerman) was chosen due to the historical differences in chill accumulation (areas of low and normal chill). We also included a cv. Golden Hills orchard at this location. The Coalinga orchard (cv. Kerman) historically has a low winter chill, while the Madera (cv. Kerman) orchard has a high winter chill. Finally, two Kern orchards (cv. Kerman) were studied on saline soil because they present nut quality issues very similar to the ones related to a low chill. Temperature sensors and cameras were installed at each location to track chill accumulation in the winter, bloom time in spring, and accumulated heat during the spring and summer. From each orchard, we sampled eight trees with three clusters (30 nuts/cluster) per tree. We collected nuts at three sampling points per location, corresponding to the critical growth transition stages (i) complete nut filling (~1,800 growing degree days (GDD)), (ii) start of hull softening and shell split (~2,100 GDD), and (iii) harvest time (~2,400 GDD). Sampling times were determined based on the accumulated heat at each location. At the end of the season, single-nut data was obtained for 25,059 pistachios.

We tested the effectiveness of treatments used to help break dormancy. Treatments were applied early February in Three Rocks Kerman orchards and Coalinga orchards that experience low chill. Dormex was applied to both high-chill and low-chill areas in the Three Rocks orchard and compared with untreated trees in the same place. We found a difference in bloom time of approximately three days between treated and untreated areas, with little effect on nut development. In Coalinga, oil was applied to half of one orchard while Dormex was applied to the other. Bloom between the two orchards was only one day apart.

We assessed fruit quality by measuring blank nuts, nuts with shell split, and kernel dry weight. We found that larger proportions of blank nuts occurred in low-chill orchards such as Coalinga (46% blank) and the low chill area of Three Rocks (46% blank) as well as both Kern orchards (54% blank) (Fig. 1). Further, the low chill area of Three Rocks had significantly higher proportions of blanks than the normal chill area (Fig. 1). This trend is consistent with our results from last year in the same orchard of Three Rocks, strongly indicating that bloom time and spring temperatures influence nut yield. Shell split in filled nuts, determined at a time near commercial harvest, was lower in low-chill orchards than in normal chill areas.

This is best demonstrated in the Three Rocks orchard comparison (Fig. 1A), where low chill area nuts had significantly less shell split (40%) than normal chill area nuts (89%), as was observed in 2021 in this exact location. Similarly, the Coalinga oil-treated orchard had the lowest proportion of split nuts at harvest of all sites, with 33%, while the other treatment/locations had around 50% split (Fig. 1B). Even if there were differences across treatments, the overall low shell split across many orchards was indicative of the lower quality nuts observed in this growing season statewide compared to previous years. Golden Hills demonstrated low performance this year, with a 55% split compared to 78% in the exact location in 2021 (Fig 1A). While shell split and nut fill were affected, we did not observe a consistent pattern of differences in kernel size in the orchards. Kernels had significantly lower dry weight (DW) on average in the Madera and Kern orchard during the third time, corresponding to a smaller nut area in each, while Coalinga and Three Rocks low chill and high chill were similar (Fig 1). In previous growing seasons, we observed Golden Hills nuts to be larger and kernel weights higher than Kerman. These differences were not pronounced this year, and Golden Hills and Kerman nuts were significantly smaller than in 2021.

A.




B.

| Location | Treatment | % Blank | % Split | Avg. Kernel DW (g) |
|---|---|---|---|---|
| Kern | Heavy soil | 53.90% | 52.8% | 0.40 |
| | Sandy soil | 60.19% | 54.6% | 0.47 |
| Coalinga | Low Chill- Dormex® | 46.78% | 51.49% | 0.55 |
| | Low Chill- Oil | 42.19% | 33.40% | 0.55 |
| Madera | High Chill On-year | 36.88% | 49.09% | 0.35 |
| | High Chill Off-year | 27.47% | 56.14% | 0.39 |

**Figure 1.** ***Nut quality traits across orchards***. A) Comparison of low chill Kerman, high chill Kerman and Golden Hills at Three Rocks orchards at a sampling time close to commercial harvest. Letters represent significant differences by ANOVA followed by Tukey test. B) Table of key nut quality traits in other Kerman orchards (Kern, Madera, Coalinga). Values represent averages for percentage of blacks through all three time points, percentage of splits at the time close to harvest, and the kernel dry weight at the time close to harvest.

In the past two years of research, we have shown that hull texture and color are indicators of hull breakdown. Nuts must reach a balance between softening enough to detach from the tree without deteriorating and causing other quality issues (i.e., shell stain). Although hull texture was similar in Three Rocks orchard regardless of chill area, the hull texture was significantly higher for both areas in 2022 compared to 2021. This same trend occurred for the Golden Hills varieties, again indicating widespread issues with nut quality this growing season. Similarly, the other locations showed very firm hull texture and greener hull coloration at the final time, indicating ripening alterations that could lead to lower nut release at harvest (Fig. 1B).

## Conclusion

Here, we further demonstrated that late bloom negatively affects nut development, and that late-blooming trees produce poor-quality nuts. By surveying multiple orchards with low chill, we found high blanks and low shell split are common in orchards with higher winter and spring temperatures. These problems were exacerbated by the warmer conditions this year. Further, nuts in these locations displayed disrupted hull ripening that likely leads to low nut release, despite being filled. Our results from multiple orchards under different management and environmental conditions validate our previous year's smaller-scale study. Finally, we concluded that Dormex used in this study did not anticipate bloom significantly. We are still analyzing data from additional time points of our experiment, which will help us determine if differences in harvest quality can be predicted early in the growing season. Our results also stress the need to find management strategies to increase chill accumulation when winter temperatures are high or protect buds when there are unusually high or low spring temperatures.

# Early detection of pistachio hull breakdown by biomarkers in Kerman and Golden Hills

**Authors: Barbara Blanco-Ulate**, Associate Professor, Plant Sciences Dept., UCD. **Collaborators: Jaclyn Adaskaveg,** PhD Candidate, Plant Sciences Dept., UCD; **Grey Monroe**, Assistant Professor, Plant Sciences Dept., UCD; **Giulia Marino**, Specialist, Plant Sciences Dept., UCD

## Introduction

Pistachio hull breakdown occurs after ripening, leading to higher nut susceptibility to insect infestation and fungal decay. By studying the biological events preceding hull breakdown and how genetics and the environment influence them, we can identify reliable biomarkers that can help predict when hulls will start to deteriorate (**Fig. 1**). Biomarkers are molecular or physical signatures that can be measured in the field or laboratory to anticipate the occurrence of large physiological changes, like hull ripening and breakdown. Through previous CPRB-funded projects, we have determined a consistent pattern of physical traits that predict hull breakdown, such as hull texture and color, over multiple years and orchards. The early detection of hull breakdown traits using biomarkers will provide growers with evidence-based knowledge for implementing appropriate and timely management practices to avoid poor nut quality.

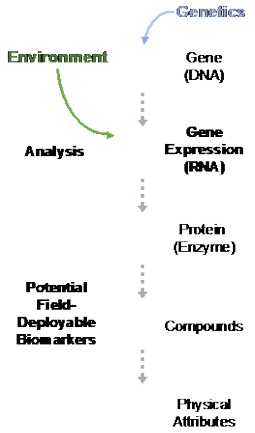



**Fig. 1.** Diagram of our proposed strategy to identify biomarkers using gene expression analyses

## Results and Discussion

To analyze genetic factors leading to hull breakdown and develop biomarkers we collected hull samples from c.v. Kerman trees in the 2019 growing season from an experimental orchard in Kearney. Nuts were collected over 15 weeks of development (**Table 1**). Three to four biological replicates were collected at each sampling date (consisting of nuts from three clusters of different trees). After sampling, hulls were separated, flash frozen, and stored at -80°C. RNA was extracted from each sample and sequenced using Illumina sequencing to obtain gene expression data. This data was mapped to the new high-quality pistachio genome, in collaboration with Dr. Grey Monroe.

**Table 1.** Sampling dates and corresponding growing degree days (GDD) for samples used for texture and color measurements and gene expression analysis.

| | Sampling Date | GDD |
|---|---|---|
| ***Hull Maturation*** | | |
| | Jun 18 | 865 |
| | Jun 25 | 993 |
| | Jul 2 | 1106 |
| | Jul 9 | 1223 |
| | Jul 16 | 1357 |
| | Jul 24 | 1508 |
| | Jul 30 | 1647 |
| | Aug 6 | 1759 |
| ***Hull Ripening*** | | |
| | Aug 13 | 1881 |
| | Aug 20 | 2007 |
| | Aug 27 | 2139 |
| | Sep 4 | 2284 |
| | Sep 10 | 2377 |
| ***Hull Breakdown*** | | |
| | Sep 24 | 2475 |
| | Oct 3 | 2564 |

We identified groups of genes that have relevant trends of expression at the start of hull ripening, either increased or decreased expression (**Fig. 2A**). Because hull texture and coloration changes are involved in hull ripening and breakdown, we correlated these traits with the gene expression trends of interest (**Fig. 2B**). For example, we compared the hull texture, measured by penetration force (kg Force) on a Texture Analyzer, with each gene expression trend, and found that Trends 3 and 4 were highly correlated with hull firmness. Hull coloration was quantified on the L*a*b* color scale and each color coordinate was compared to each trend. We found hull lightness (L*) and redness (a*) were correlated with genes that increased during ripening (Trend 3, 4, and 5).

We then found six genes with specific functions related to hull softening or color among the genes from Trend 3 and 4 (**Fig. 2C and 2D**). Three of those genes are related to texture and produce enzymes known to digest plant cell walls, pectate lyase (PL), polygalacturonase (PG), and β-glucosidase, respectively. PL and PG degrade pectin, which dictates many textural attributes in fruit tissues. β-glucosidase enzymes act in cellulose, breaking apart large molecules in the cell wall into smaller glucose molecules. High expression

of PG and PL is a reliable indicator of fruit ripening in other crops. The expression of PG and PL in pistachio was low until around 2000 GDD, corresponding to the start of ripening, where they exponentially increased. This induction also coincided with the increase in softening in the hull as indicated by the high Pearson's coefficient ($r^2 > 0.8$, **Fig. 2C**).

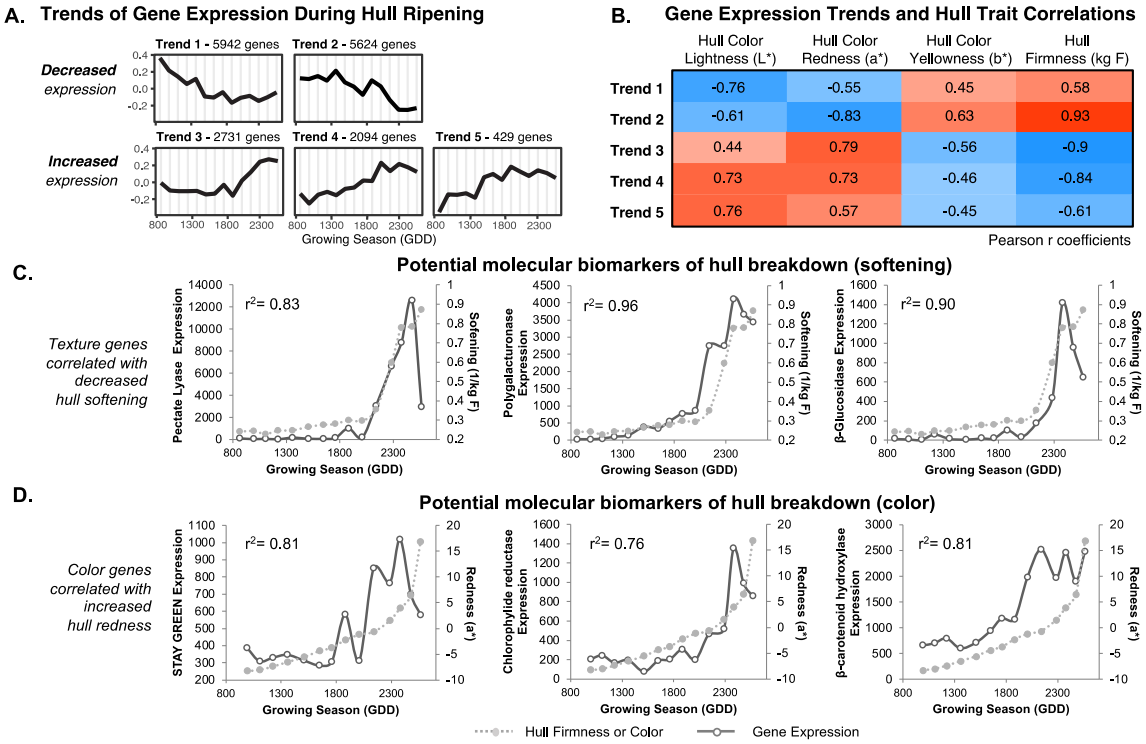



**Fig. 2** *Identifying biomarkers of hull ripening and breakdown with gene expression analysis.* **A.** Groups of genes with similar expression trends are represented by each line graph summarizing relative expression through hull development (800-2600 Growing Degree Days (GDD)). Two categories of trends were identified, genes that decrease during ripening and genes that increase during ripening. The number of genes in each trend are indicated. **B.** Gene expression trends correlated with hull degradation traits. All correlations are significant <0.003. Values indicate Pearson r coefficients. **C.** Genes identified from Trend 3 and Trend 4 that correlate with hull softening or color changes. Expression (normalized reads) is plotted on the left axis (solid line) of each graph, and hull texture and or color measurements are plotted on the right axis (dashed line) through time (GDD). Individual Pearson correlations between gene expression and traits are noted as $r^2$ values.

We also identified three genes that are involved in fruit color changes. These include two genes that degrade chlorophyll (STAY GREEN and chlorophyllide reductase), causing a decrease in the green color of the hulls, which happens before ripening in pistachios. The third gene, β-carotenoid hydroxylase, is a carotenoid biosynthesis gene that produces red-orange pigments. These genes increase earlier than the texture genes at 1800 GDD and follow the same pattern as the red/green color changes ($r^2$>0.75, **Fig. 2D**).

## Conclusion

From our analysis, we have selected six genes that are good candidates to serve as biomarkers of hull ripening and breakdown (**Fig. 2C and 2D**). We show that these genes have low expression during early nut development/maturation and begin to increase with the onset of ripening. Thus, as soon as the genes reach a specific threshold, they could be biomarkers to predict or monitor nut ripening to avoid hull breakdown. These biomarkers have the potential to be measured in the field by developing methods such as antigen test strips that quickly detect the presence of the proteins that these genes encode. We are still generating and analyzing the data from the Golden Hills nuts.

# Gene expression marker-enabled precise and reliable application of rest-breaking enhancing chemicals

**Authors: Li Tian,** Professor, Department of Plant Sciences, University of California, Davis; **Douglas Amaral**, Cooperative Extension Advisor, University of California; **Patrick Brown**, Distinguished Professor, Department of Plant Sciences, University of California, Davis; **Louise Ferguson**, Distinguished Professor of Extension, Department of Plant Sciences, University of California, Davis.

## Introduction

Pistachio (*Pistacia vera* L.) is a deciduous, woody perennial crop that is rapidly expanding its landscape in California, currently ranked among the top five commodities in the State. As a critical step in their growth and development cycle, buds of pistachios enter into dormancy/rest after the trees have lost their leaves in the Fall. Following exposure to a certain number of low-temperature hours ("winter chill"), buds are released from their dormancy state ("bud break") in the Spring, a key process that greatly affects pistachio yields. Bud break is comprised of a sequential process that starts with transition from endodormancy, which is regulated by developmental factors within the bud, to ecodormancy, which is regulated by environmental factors. To provide a baseline understanding of the physiological and biochemical processes underlying bud endodormancy break, we conducted transcriptome (global gene expression) analysis last year using buds that experienced different winter chills. From the transcriptome analysis, we identified genes that showed significantly changed transcript levels during bud endodormancy break and were also correlated with chilling accumulation. Moreover, results from the gene expression analysis were fully supported by our metabolite analysis data, which indicated that the precursors and products of the gene/protein activity were altered and consistent with the gene expression changes. These multiple lines of evidence collectively established the essential role of these candidate genes in bud endodormancy break and their use as gene expression markers. Our goals for the current project are to: 1) conduct field trials for horticultural oil application and bud sample collection (along with untreated controls), and 2) verify the effectiveness of the gene expression markers through molecular and biochemical analyses using the collected bud samples.

## Results and Discussion

We have been actively engaging three commercial pistachio orchards located in the Central Valley that span a historically diverse chilling accumulation to collaborate on this research. A generally low nighttime temperature (below 45$^{o}$F) has been observed at these locations since November 2022, which bodes well for achieving the amount of winter chill required to release flower buds from endodormancy in early 2023. We are carefully monitoring the temperature and other environmental conditions at each orchard location with our own climate installation and are using the model of Chill Portions for calculation of chilling accumulation. For trees without the application of horticultural oil, we will collect samples consisting of 25 pooled flower buds from throughout the orchard/block (5 buds per tree, 5 different trees) at weekly periods commencing from 9 weeks prior to the historic average bloom dates in each orchard. The horticultural oil applications will include a very early treatment (2 weeks before the anticipated optimal moment of application according to experience, typically mid-January) followed by one weekly treatment for the next 4 weeks. Final bud break and flowering will be carefully evaluated by monitoring commencement, full and last bloom in each orchard. Time of first/last and peak flowering of all trees in the experimental block along with cumulative chill portions will be recorded. All the of the collected buds will be immediately frozen using liquid nitrogen or dry ice and stored in a -80°C freezer until analysis.

## Conclusion

We are on track with the tasks planned for this project. The horticultural oil treatment and bud collection will be completed around February 2023. We will then use a subset of the bud samples collected from the field trials for real-time qPCR analysis. This will allow us to validate the markers we developed last year for associating gene expression patterns with the process of bud endodormancy break.

# Determining the severity of internal kernel discoloration incidence in pistachio cultivars

**Authors: Phoebe Gordon**, Orchard System Advisor, Madera/Merced Counties; **Shuxiao Zheng**, Ph.D. Student, UC Davis; **Minmin Wang**, Postdoctoral Scholar, UC Davis; **Georgia Drakakaki**, Professor of Plant Sciences, UC Davis; **Caleb Crawford**, Staff Research Associate, Kings/Tulare Counties; **Douglas Amaral**, Orchard System Advisor, Kings/Tulare Counties; **Elizabeth Fichtner**, Pomology Advisor, Tulare/Kings Counties

## Introduction

Internal kernel discoloration (IKD) is discoloration that presents as concentric rings of darkened tissue, resembling growth rings, starting from the base of the kernel. There is no indication that this affects pistachio kernel flavor, however there is a desire within the industry to understand the cause of this discoloration. Several preliminary sampling efforts have indicated that it is more prevalent in Golden Hills than Kerman, and it is unknown whether it occurs in Lost Hills or other pistachio cultivars. The incidence has been reported to be variable within an orchard. To the researchers' knowledge, no one has tried to formally quantify variability between cultivars on a statewide level. A two-year research trial by an affected grower demonstrated that the discoloration is not caused by hemipteran pests and that there may be an association with secondary pathogenic fungi, however it is unknown how strong this association is, or whether the association is correlative or causative. Additional work by Kent Daane and Judith Stahl also confirmed that IKD discoloration appears in fruit that has been protected from large bug feeding. There is a need to better understand this discoloration to guide further research efforts.

## Results and Discussion

Efforts in 2022 were focused on identifying fields to sample, developing a protocol for processing nuts in a way that keeps the kernel intact, and collecting preliminary data to perform a power analysis to inform future sampling work.

We were able to positively identify IKD in Golden Hills kernels by early August. We were able to find a small number of kernels with discoloration before that point, but it is unclear if this is IKD or some other issue. Kerman appears to have a different kind of discoloration that is very similar to plant bug feeding. It is unclear at this point in time if this is also IKD or late-season plant bug feeding. Conversations with others who have done exclusion studies have indicated that the Kerman-specific IKD appeared in kernels protected from plant bug feeding, however we are unaware of indicators that would enable us to distinguish between the two in fruit that was not protected from plant bugs.

We will be analyzing the data we collected this winter to determine the incidence of IKD in Golden Hills, Lost Hills, and Kerman, and to inform future sampling efforts.

A pilot analysis of samples by the Drakakaki lab indicated that the compounds that are enriched in the IKD region are mostly plant polyphenols. These compounds are naturally found in plants and are non-toxic to humans. We hypothesize that these polyphenols are a response to abiotic stress, however further studies are necessary to confirm this hypothesis.

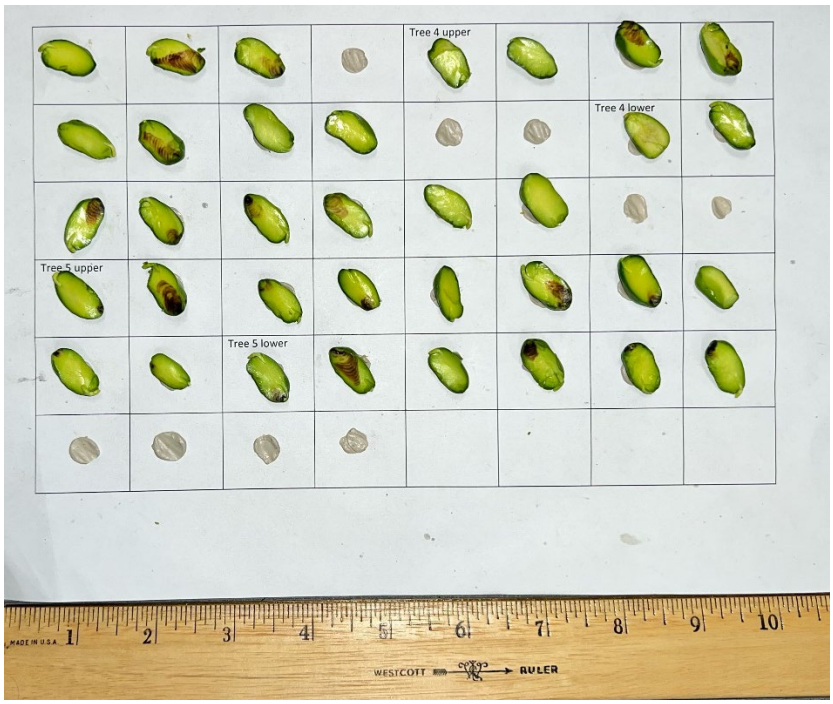



Figure 1: Golden Hills kernels with internal kernel discoloration.


Figure 2: Kerman kernels with possible IKD.

## Conclusion

Internal kernel discoloration appears early enough in the season that we can sample several weeks before harvest, simplifying sample collection and processing.

# Early to bed? Managing dormancy induction to enhance chill accumulation and endodormancy in pistachios

**Authors:** **Gurreet Brar**, Assistant Professor, Department of Plant Science, Jordan College of Agricultural Sciences and Technology, California State University Fresno Masood Khezri, Research Director, MR Research Institute, Fresno; **Louise Ferguson**, UCCE Specialist, University of California, Davis; **Faranak Hadavi**, Research Assistant, Department of Plant Science, Jordan College of Agricultural Sciences and Technology, California State University Fresno.

## Introduction

Dormancy induction is a phase in the phenology of temperate zone trees marked by bud set in late summer and is mediated by hormonal signals as well as several other metabolites in response to shortening photoperiod and reducing temperatures. Temperate zone trees have developed these safety mechanisms through evolution in order to prevent any damage to the tender tissues if the buds break during cold season. Therefore, during dormancy induction period, the buds go into a state of deep sleep called Endodormancy, to acquire sufficient exposure to cold temperatures, measure in chill accumulation, to be able to break flower buds and leaf out normally in the spring. Accumulating evidence has indicated that the plant hormone Abscisic Acid (ABA) biosynthesis is involved in controlling bud dormancy. ABA can primarily cease the shoot growth and promote the occurrence of the physiological events and important messenger preceding dormancy commencement. Ethylene is a stress hormone which is involved in leaf abscission in fall. ACC, a precursor for ethylene production has been shown to be effective in enhancing defoliation through enhancing ethylene biosynthesis. The current experiment was conducted as a preliminary study to evaluate PGR applications in enhancing dormancy induction and its effect on yield and nut quality in pistachios. Three treatments were: application of ABA @500 ppm; application of ABA @500 ppm+ ACC@500 ppm in late October; and an untreated control. The experiment was designed as a randomized complete block design with 4 replicate blocks. Each experimental unit (treatment unit) consisted of 12 female and 2 male trees.

## Results

The results are summarized in Tables 1 and 2. ABA treatment resulted in highest yield per tree and the best nut quality characteristics. Yield per tree was highest in ABA, followed by Control and then ABA+ACC. Total edible nuts by weight were also highest in ABA treatment. ABA treatment had lowest closed shell, undersized and blank nuts. After the first shake, the plots were evaluated for the amount of remaining nuts on the trees to determine the need for a second shake. All the treatments plots were then shaken a second time two weeks after the first shake. In the second shake crop, the weight of 100 nuts was highest in ABA treatment. However, the split nuts % was lowest in ABA treatment while ABA showed highest % of blank nuts in the second shake.

## Conclusions:

The results show that spraying ABA prior to leaf fall (in late October) has positive effect on yield and nut characteristic of pistachio trees. This was a preliminary trial, and a more detailed experiment was started in October 2022 where ZnSO4 and deficit irrigation have been added as treatments instead of ACC.

**Table 1:** Effect of ABA and ABA+ACC sprays on yield and yield components of Kerman pistachio variety. Different letters within the column indicate significant differences by Duncan's multiple range test at $P < 0.05$.

| Treatment | Yield/tree (lb.) | Total edible/lb. (g) | Closed Shell/lb. (g) | Undersize nuts/lb. (g) | Blank/lb. (g) |
|---|---|---|---|---|---|
| **ABA** | 17.4± 1.0 a | 432.5± 13.7 a | 14.7± 3.6 b | 5.8± 0.8 b | 30.7± 2.6 b |
| **ABA+ ACC** | 12.1± 1.1 b | 376.5± 18.1 b | 23.6± 2.9 a | 27.9± 4.7 a | 53.0± 1.4 a |
| **Control** | 15.1± 0.4 a | 417.0± 9.5 ab | 14.8± 2.4 b | 16.6± 1.2 a | 44.1± 5.0 a |

**Table 2:** Effect of ABA and ABA+ACC sprays on yield components of the second shake crop on the Kerman pistachio variety. No different letters within the column indicate no significant differences by Duncan's multiple range test at $P < 0.05$.

| Treatment | Weight of 100 Nuts (g) | Split nuts (%) | Non-Split nuts (%) | Blanks (%) | Malformed (%) |
|---|---|---|---|---|---|
| **ABA** | 236.5± 3.0 | 14.8± 4.6 | 85.3± 4.6 | 15.3± 6.5 | 20.5± 2.2 |
| **ABA+ACC** | 218.3 ± 4.2 | 18.0± 5.1 | 82.0± 5.1 | 13.5± 5.5 | 25.0±8.5 |
| **Control** | 223.3±5.6 | 21.8± 7.1 | 78.3± 7.1 | 11.0± 3.7 | 19.5± 5.7 |

# Do cover crops help lower canopy temperatures and enhance chill accumulation in pistachios?

**Authors: Gurreet Brar**, Assistant Professor, Department of Plant Science, Jordan College of Agricultural Sciences and Technology, California State University Fresno; **Masood Khezri**, Research Director, Malakan Agricultural Research Institute, Fresno**; Faranak Hadavi**, Research Assistant, Department of Plant Science, Jordan College of Agricultural Sciences and Technology, California State University Fresno.

## Introduction

The California pistachio industry has seen frequent warm winters in recent years and during warm days pistachio buds get significantly warmer. Research in other crops has shown that cover crops keep the soil surface cooler, while also cooling the canopy. In California specialty crops, cover cropping is not a common practice. A review of the literature showed that cover crop research relevant to California and across the world has focused on soil conservation benefits of cover crops, including building soil carbon (Tautges et al. 2019; Alvarez et al. 2017), enhancing nitrogen and nutrient content of soils (Poudel et al, 2001; Fu et al. 2015) and providing forage to bees during winter (Wilson et al., 2018). Literature also shows that cover crops reduce canopy temperatures significantly during winter months. Cover crops are also known to harbor ice-nucleating bacteria, thereby reducing ambient temperatures on the soil surface as well as the canopy level and keeping the orchard floor covered helps moderate soil temperature swings (Snyder and de Melo-Abreu, 2005). In mature pistachio orchards in California, the growers are not much concerned about frost damage as much as the lack of winter chill. However, the effect of cover cropping on winter chill accumulation, bud break advancement, and the blooming period of mature pistachio trees has not been investigated yet. Therefore, the objective of this study was to study the effect of winter cover cropping on the bloom advancement of female and male pistachio trees. The preliminary trial was carried out at Madera County (36°58'19.4"N, 120°10'43.2"W). The female and male trees were 'Kerman' and 'Randy' respectively all on 'UCB1' rootstock. The treatments were as follows: Treatment 1: Control: bare ground, no cover crop (CTRL); Treatment 2: Cover crop (CC). We used a commercial cover crop seed mix which included *Brassica sp*, Triticale, field peas and fava beans. The experiment was designed as a randomized complete block design (RCBD) with three replications. For this experiment, the site was visited regularly to determine the 50% bud swell stage and 80% full bloom stage.

## Results

According to our preliminary observations and bloom rating, the cover crop consistently advanced bloom in both male and female trees and cover-cropped plots advanced bud swell and full bloom stages around 5 to 6 days earlier than control plots (Tables 1 and 2). It seems cover crop reduces canopy and bud temperatures during winter months and enhance chill accumulation by the buds. This is because, during winter, bare soil warms during the day and radiates the heat back towards the canopy, thereby warming the canopy during the night. Cover crops create a buffer between the soil and air, therefore reducing the heat exchange.

**Table 1. The effect of cover crop on bud swell and bloom advancement of 'Kerman' pistachio trees**

| Treatments | Bud Swell (@50%) | Full Bloom (@80%) | Bud Swell Advancement over Control (day) | Bud Break to Full Bloom (day) |
|---|---|---|---|---|
| Control (No Cover Crop) | March 29th | April 10th | 0 | 12 |
| Cover Crop | March 23rd | April 5th | +5 | 13 |

**Table 2. The effect of cover crop on bud swell and bloom advancement of 'Randy' pistachio trees**

| Treatments | Bud Swell (@50%) | Full Bloom (@80%) | Bud Swell Advancement over Control (day) | Bud Break to Full Bloom (day) |
|---|---|---|---|---|
| Control (No Cover Crop) | March 25th | April 5th | 0 | 11 |
| Cover Crop | March 19th | March 29th | +6 | 11 |

**Conclusion:**

This field was considered to preliminary investigate the effect of cover cropping on the bud break and bloom advancement and the results of this study confirmed the advancement effect, although, in this trial, the cover crop treatment did not affect the bloom window. Cover cropping has proven to be an important soil conservation practice and can positively affect a wide range of soil health characteristics. Therefore, it is important to further evaluate its effectiveness on bloom synchronization of male and female pistachio trees as well. In this field, bud and soil temperature sensors were installed in early January 2022 to evaluate the sensor's accuracy and adjustments. This experiment will be continued in another field with female 'Kerman' and male 'Peters' and the chill portion accumulation will be evaluated from the bud temperature sensors in both male and female trees. The non-structural carbohydrates in the bud and bark of shoots as well as yield and yield components will also be compared between cover-cropped and control plots.

Figure 1a. Kerman bloom in (left) Control plot (no cover crop) and (right) with Cover crop.

Figure 1b. Randy male tree bloom in (left) Control plot (no cover crop) and (right) with Cover crop.

# Metabolomics analysis of pistachio bud and shoot samples collected during the dormant period

**Authors: Gurreet Brar**, Assistant Professor, Department of Plant Science, Jordan College of Agricultural Sciences and Technology, California State University Fresno; **Krish Krishnan**, Professor & Chair, Department of Chemistry & Biochemistry, College of Science and Mathematics, California State University Fresno; **Faranak Hadavi**, Research Assistant, Department of Plant Science, Jordan College of Agricultural Sciences and Technology, California State University Fresno; **Keeton Montgomery**, Research Assistant, Department of Chemistry & Biochemistry, College of Science and Mathematics, California State University Fresno.

**Introduction** The following is a summary of the work performed since the last executive summary submitted in June of 2022. The work entailed identifying metabolites and altered physiological pathways associated with pistachio cultivars treated with Rest Breaking Agents (RBAs), namely horticultural oils. Farmers have used RBAs for many years to induce bud breaking for pistachio plants that do not receive adequate chill portions during winter months. Although these horticultural oils have been used for many years, little is known regarding its mode of action or how it alters the plants metabolism. We aim to take a metabolomics approach to answer these questions. For our metabolomics work performed at Fresno State, we utilize proton nuclear magnetic resonance ($^1$H NMR) spectroscopy with a recently acquired 600 MHz JEOL NMR spectrometer funded by the National Science Foundation.

Thus far, the work has primarily focused on analyzing samples from previous temporal studies by Brar et al. assessing bud and bark pistachio tissues treated at different spray periods and locations. The previous executive summary submitted in June focused on comparing the Cantua sites for 2019 and 2021. This summary entails the work that has been performed since the last summary, and it will focus on the Madera 2021 time-dependent and budswell samples.

During 2022, 104 samples have been assessed, 64 from the 2021 Madera temporal study and 40 from the 2021 Madera budswell study. For the 2021 temporal study, there were 4 spray treatments, mid-January (S1), early February (S3), mid-February (S4), and late February (F5). Bud and Bark Tissue were collected ten days after treatment and were kept at -80 °C until metabolomics analysis. The 2021 Madera budswell study collected bud and bark tissue from trees from all spray treatments at the end of March. Like the temporal study, samples were kept at -80 °C until metabolomics analysis. Roughly 1000 mg of each tissue was finely ground utilizing a mortar and pestle with liquid nitrogen. 250 mg of each tissue was weighed and transferred to clean, pre-labeled 5 mL Eppendorf tubes. 3 mL of reagent-grade methanol was added to each tube, and the samples were sonicated for 15 minutes. Samples were centrifuged at 18.0 G for 30 minutes, and the supernatant was transferred to clean 5 mL Eppendorf tubes. The methanol evaporated in fume hood for 48 hours, and any remaining solvent was removed by lyophilizing the samples for 48 additional hours. 700 uL of a 0.2 mM imidazole, 90 mM $KH_2PO_4$, and 0.05 mM trimethylsilyl [2,2,3,3-d4] propionate (TSP) solution with the pH set to 6.8 was added to each Eppendorf tube. The Eppendorf tubes were vortexed for 20 seconds and then centrifuged for 30 minutes at 18.0 G. The supernatant was transferred to clean, pre-labeled 5 mm NMR tubes.

All $^1$H NMR experiments were performed in a 600 MHz JEOL NMR Spectrometer. All experiments were one-dimensional pre-saturation experiments performed at 30 °C. Each experiment consisted of 1024 transients with a 70° pulse width and a 1.81 s acquisition time. Raw NMR data was pre-processed using MestReNova, which entails phase correction, baseline correction, Sin-Square 90° apodization, normalization, and adjusting the spectrum size to 64K. The results were pre-analyzed by binning the data at a bin width of 0.004 bin width at a range of 0-10.0 ppm. Further processing of the binned spectral data

was achieved utilizing metaboanalyst.ca involving log10 transformation of the data, Pareto Scaling, and running the partial lease squares-discriminant analysis (PLS-DA).
In order to identify metabolites, further processing was accomplished utilizing ChenoMX NMR Suite Software 8.1 Processor application which involves calibrating the pH of the samples and calibrating the TSP peak. Metabolite identification and quantification were achieved utilizing ChenoMX NMR Suite Software 8.1 Profiler application with reference to available ChenoMX libraries and Human Metabolome Database (HMDB) libraries. Once metabolite data was acquired, it was processed utilizing metaboanalyst.ca involving log10 transformation of the data, Pareto Scaling, and running the PLS-DA

**Results**
*2021 Madera Temporal Study:* PLS-DA analysis was performed on metabolite data to compare control tissues to their respective spray treatments for each spray interval. Although there was some slight overlap between the control and treated bud and bark tissues, most of the controls had distinct clusters compared to the spray treatments suggesting different metabolic profiles. We noticed the same pattern when we analyzed the 2021 and 2019 Cantua temporal samples. These temporal studies may be suitable to determine how horticultural oils alter the metabolome of pistachio tissues.
Null hypothesis significance testing will need to pinpoint specific metabolites associated with RBA treatment.

*2021 Madera Budswell:* According to the PLS-DA plots of the binned data for both the bark and bud tissues, there was an abundance of cluster overlap with all treatments and the control, indicating the spectral bin data is relatively uniform throughout. This was also noted with both the bin and metabolite data for the 2019 Madera samples, the 2019 Cantua samples, and the 2021 Cantua Samples. Besides the last spray treatment, all the other spray treatments have already been treated for over a month when the budswell samples were collected. Therefore, it is reasonable to conclude that all of these tissues are in similar physiological states, and perhaps these budswell samples are not the best indicator to determine metabolic differences.

**Conclusions:**
This work has generated very important information so far. We found differences in metabolic profiles of pistachio tissues under various oil treatments during the dormant period. The results indicate that horticultural oil applications at different chill accumulation milestones alter the metabolome of pistachio tissues. The results also indicate that the samples taken at the budswell stage were not different in their metabolic profiles, suggesting that the pistachio tree metabolite levels are similar as they approach bloom. The sample processing for other sites is still going on and will be completed in 2023.

# Development of precision yield monitor for pistachio

**Authors:** **Stavros Vougioukas,** Professor, Department of Biological and Agricultural Engineering, University of California, Davis; Patrick **H. Brown**, Professor, Department of Plant Sciences, University of California, Davis.

**Introduction:** Pistachio orchards vary significantly in yield across years and within orchards. The extent of this phenomenon was illustrated in a series of trials performed from 2008-2012 by Rosenstock, Brown, and Hastings et al., who measured the yield of each of the 7-10,000 trees in a 60-acre field using a custom-made single tree harvester. The resulting yield map illustrated the extreme variability in yield, with 30% of the orchard yielding in excess of 6,000 lbs., while 30% yielded <2,500 lbs. This result clearly illustrates that large portions of the orchard were not achieving their full yield potential. Since all trees received identical management, this also suggests that there was also a > 250% variability in nitrogen and water use efficiency. This pattern of yield variability is undoubtedly common in Pistachio (though currently unmeasured). The starting point for any attempt to address this variability is the ability to measure it.

There are two basic methods by which individual tree yield may be measured: (1) by weight and (2) by volume. While the equipment built in 2008 achieved the goal of single tree harvest, the equipment was based upon a 30-year-old harvest technology and did not operate quickly enough to be utilized widely in the industry. In 2021, our team at UC Davis attached a custom-built weighing bin to an off-ground harvester and measured the weight of almonds caught by the harvester, along with the precise location of the machine. The yield weight of each tree was recorded, and the bin opened to deposit the almonds into windrows as the machine was driven to the next tree. This method was very accurate but slowed the harvest rate as it was necessary to wait until the conveyor belts had deposited all the almonds into the bin before they could be weighed.

Our goal in this project is to develop the technology to allow for high-resolution – down to the individual tree - yield determination on a commercial pistachio harvester operating close to full commercial harvest speed. Since pistachios are harvested off-ground and deposited into bankout wagons rather than windrows, a weighing bin is unsuitable for the pistachio harvest. In 2022 we tried the second (volumetric) approach, using a laser profiler to measure the volume of almonds pistachios inline on the conveyor belt.

**Results and Discussion:** The laser profiler, a model LMS 111 from the German company SICK, continuously scans the conveyor belt and produces a series of cross-sectional slices or height profiles of the material on the belt. The speed of the belt is measured using a wheel encoder in contact with the belt. A GPS-RTK unit was also mounted on the harvester to record the machine's location. A vibration sensor was mounted on the shaker head, so the exact time a tree was shaken was known. All the measurements were time-stamped.

Initially, when the conveyor belt is empty, the laser scanner records the belt's profile. This baseline profile is used as a reference. Under normal conditions (i.e., with the nuts flowing on the belt), the laser captures the profile of the nut stream in two dimensions. Then the instantaneous measurement of the profile is subtracted from the baseline. The result is a profile whose reference level is 0 (m). By combining the height profiles with the belt speed, the LMS 111 calculates the volume of material passing beneath the scanner. Once the volume flow was obtained, the shaking events recorded by the accelerometer were used – in combination with the GPS data, when those were available - to assign appropriate segments of the nut volume flow to individual trees. Last, the nut bulk density was measured to turn yield volume into mass.

Since the almond harvest slightly precedes the pistachio harvest in California, we tested the volume measurement method with almonds at two sites in August 2022. The laser profiler was mounted on an ENE harvester at the Westwind Farms orchard near Woodland, CA. A week later, the same system was used on a TOL harvester at the KG Ranch near Madera, CA.

The data acquisition hardware and software performed very well. However, we encountered a significant problem with the profiler in that the resolution of the height profile was inadequate when the almonds formed a single layer on the belt. The LMS 111 has a statistical error of approximately +/- 12 mm, which is about the height of a single layer of almonds. Numerous attempts were made to funnel the almonds with diverter plates into a narrower, deeper stream that could be more readily measured. However, this proved very difficult due to the large variability in the amount of nuts on the belt at a given time. If the tree had a high yield, the nuts would arrive too quickly, resulting in the stream clogging and overflowing the diverter plates. The accuracy of the system was reasonable if the stream on the belt was on the order of 10 cm thick. This was especially true with the ENE machine, which uses a 21-inch-wide conveyor belt. The TOL machine catches the nuts and splits them between two conveyor belts, each 36'' wide. Even with diverters in place, we couldn't create layers of nuts thick enough to get reliable measurements.

Given that a) pistachios are smaller than almonds and b) the growers we were collaborating with expected a lower-than-normal yield this past season, and c) the conveyor belt used had deep patterns acting as cleats, it was clear to us – after our experience with almonds - that the amount of pistachios on the cleated belt would be too small to get an accurate volumetric measurement. The decision was made to postpone data collection for the pistachio harvest until a more accurate system could be developed. We are currently re-designing the system.

**Conclusion:** In 2022, UC Davis developed an inline system to measure the yield as each tree is harvested. The system was tested in almond harvesting at two sites. The data acquisition hardware and software performed very well. Also, the developed system could localize trees accurately despite GPS outages by utilizing vibration data from the shaker. However, the laser scanner used, though suitable for outdoor conditions, had a statistical error of +/- 12 mm. Therefore, the estimation of volumes from segments of nut streams with low profile heights was unreliable. We are currently building an improved system. We are examining different types of laser profilers for the volumetric method to obtain the necessary resolution. We are also designing a lightweight weighing conveyor as a means to increase the speed with which the yield weight may be measured and considering active diverters or speed control to this conveyor to control the height of the stream of the nuts under the profiler.

# Efficacy of fungicides against Alternaria late blight and their effects on resistance levels

**Authors: Boris X. Camiletti**, Postdoctoral Scholar, Department of Plant Pathology, UC Davis and UC Kearney Agricultural Research and Extension Center (KARE); **Yong Luo**, Project Scientist, UC Davis, and KARE; **Victor M. Gabri,** Staff Research Associate, UC Davis and KARE; **Maria E. Campos,** Staff Research Associate, UC Davis and KARE; **Themis J. Michailides**, Principal Investigator, Department of Plant Pathology, UC Davis & KARE.

## Introduction

The principal measure to reduce Alternaria late blight (ALB) of pistachio is the application of multiple sprays per season. The prevalence of fungicide-resistant isolates in *Alternaria* populations is the major factor contributing to the unsuccessful control of ALB. The frequency of mutations can be used as an indicator of the resistance level in the orchards. Field trials were performed during the 2021 and 2022 seasons to evaluate the efficacy of several commercial fungicide products and their effects on the frequency of mutations associated with resistance to SDHI (H134R, S135R, and H277Y) and QoI (G143A) fungicides (Table 1). Trials were performed in an 8-year-old Kerman experimental plot located at Agricultural Research and Extension Center, in Parlier, CA. Treatments consisted of three applications per season approximately four weeks apart (3 Jun, 2 Jul – a critical time for spray – and 5 Aug). All fungicides were applied at the maximum rate as indicated on the label. Each treatment consisted of five-single trees replications. Non-sprayed trees were used as control. Sprays were applied with a handgun sprayer at 400 gallons per acre. Disease symptoms develop only very late in both seasons and for this reason evaluation was performed on 15 Oct 2021 and 2022. The severity was evaluated using a 0-to-5 rating scale, where 0 = no disease, 5 = several branches showing lesions on leaves, and 1,2, 3, and 4, are the intermediate levels of disease. In addition, 30 symptomatic leaflets per tree were collected. DNA extraction was performed using commercial kits. The qPCR assays developed by Camiletti et al. (2022) and Luo et al. (2007) were applied to determine the frequency of the mutations. The statistical analysis was performed using the R Studio software. A cumulative linked model was fitted to severity data using the function *clm* in package *ordinal*. Generalized fixed models were fitted to mutation frequency data using the function *glm* in package *stats.* Treatment marginal means were estimated with the *emmeans* function from the package *emmeans* and post hoc comparisons were conducted with Tukey's test ($P$=0.05).

## Results and Discussion

As expected, the non-treated control showed the highest severity scores. Except for Regalia, all fungicide treatments reduced the severity of ALB (Fig. 1). The fungicide treatment using Miravis Duo resulted in the lowest severity scores. Six fungicide products showed a similar performance to the above-mentioned treatment according to the statistical analysis. The remaining fungicides showed an intermediate efficacy in controlling the disease. The frequency of mutation H134R was higher on trees sprayed with Miravis Duo, Miravis Prime, Merivon, and Mibelya. Mutations H277Y and S135R showed very low frequency values and were not influenced by the fungicide treatment. Eight fungicide treatments increased the frequency of mutation G143A (Table 1).

## Conclusion

Fungicide products that provide the best control of ALB may increase the frequency of mutants associated with SDHI and QoI resistance. SDHI and QoI fungicides should be used in alternation or combination with other chemical classes to minimize the risk of resistance development.

## References

Camiletti et al. 2022. Frequency of Alternaria genotypes resistant to SDHI fungicides in California pistachio orchards determined by real-time PCR. Plant Disease. *In press*.
Luo et al. 2007. Pestic. Biochem. Physiol. 88:328–336.

**Table 1.** Fungicide treatments and frequency of mutations associated with resistance to SDHI and QoI fungicides.

| Fungicide treatment | Active ingredients (FRAC#) | SDHI [a] | | | | | | QoI[a] | |
|---|---|---|---|---|---|---|---|---|---|
| | | H134R | | H277Y | | S135R | | G143A | |
| Control | NA | 0.48 | a | 0.03 | a | 0.04 | a | 0.87 | a |
| Luna Sensation | Trifloxystrobin (11) + Fluopyram (7) | 0.50 | ab | 0.02 | a | 0.01 | a | 0.98 | b |
| Regalia | *Reynoutria sachalinensis* (P5) | 0.53 | ab | 0.03 | a | 0.00 | a | 0.94 | ab |
| Vacciplant | Laminarin (P4) | 0.53 | ab | 0.04 | a | 0.00 | a | 0.95 | ab |
| Luna Sensation + Serenade | Trifloxystrobin (11) + Fluopyram (7) + *B. subtilis* (BM02) | 0.53 | abc | 0.02 | a | 0.01 | a | 0.98 | b |
| Adament | Tebuconazole (3) + Trifloxystrobin (11) | 0.54 | ab | 0.03 | a | 0.00 | a | 0.99 | b |
| Cevya | Mefentrifluconazole (3) | 0.57 | abc | 0.08 | a | 0.05 | a | 0.99 | ab |
| Luna Experience | Fluopyram (7) + Tebuconazole (3) | 0.57 | abc | 0.03 | a | 0.01 | a | 0.95 | ab |
| Luna Experience + Serenade | Fluopyram (7) + Tebuconazole (3) + *B. subtilis* (BM02) | 0.58 | abc | 0.03 | a | 0.01 | a | 0.98 | b |
| Miravis Duo | Difenoconazole (3) + Pydiflumetofen (7) | 0.65 | bc | 0.05 | a | 0.01 | a | 0.99 | b |
| Miravis Prime | Fludioxonil (12) + Pydiflumetofen (7) | 0.66 | bc | 0.04 | a | 0.00 | a | 0.98 | b |
| Merivon | Fluxapyroxad (7) + Pyraclostrobin (11) | 0.68 | bc | 0.05 | a | 0.01 | a | 0.99 | b |
| Mibelya | Fluxapyroxad (7) + Mefentrifluconazole (3) | 0.72 | c | 0.04 | a | 0.00 | a | 0.99 | b |

[a] Different letter in the same column indicates significant differences among treatments according to Tukey's test ($P$=0.05).

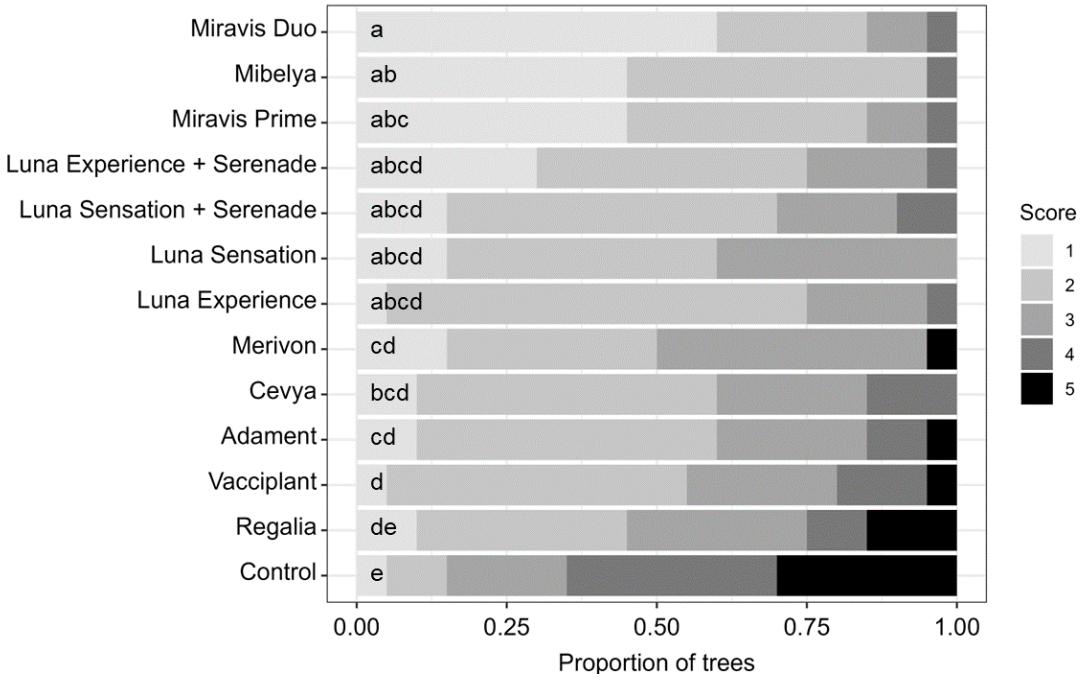



**Fig. 1**. Effect of fungicides on ALB severity in an experimental pistachio orchard in Parlier, CA. A higher score indicates higher severity. Different letter indicates significant differences (Tukey's test; $P$=0.05).

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

**Authors: Andreas Westphal**, UC Riverside Professor of CE Nematology, Department of Nematology, University of California Riverside, Kearney Agricultural Research and Extension Center, 9240 S. Riverbend Ave, Parlier, CA 93648.

## Introduction

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

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

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

In this project, host suitability to *Pratylenchus vulnus*, *Meloidogyne incognita,* and *Mesocriconema xenoplax* of currently available pistachio rootstocks foremost clones of UCB1 is determined. Genotypes generated by Dr. Mallikarjuna Kuma Aradhya (USDA-ARS Davis) are tested along with *Prunus* or *Juglans* rootstocks with known susceptibility. In attempts to broaden the genetic basis of pistachio rootstock Dr. P.J. Brown generates novel genotypes in breeding families that are also tested in the nematology program.

## Results and Discussion

Field screening for RLN and RKN host status After several years of observing low population densities in pistachio roots compared to other nut crop hosts, numbers seemed to relatively increase in the 2017 planting when sampled in January 2022. At this sampling time, root lesion nematode numbers in several of the UCB1 clones were similar to the susceptible comparative nut crops, peach rootstock 'Nemaguard' and walnut rootstock 'VX211' (nematode-tolerant, but not resistant). In younger screening plantings, the root lesion nematode numbers appeared still lower than in the other nut crop rootstocks. Summarizing these data sets, we hypothesized that root lesion nematode does reproduce on California rootstocks but that it takes several years to reach high reproductive capacity on these hosts.

Screening the novel rootstock breeding families from Dr. P.J. Brown's program revealed variability within each family in plant vigor, and frequent but variable population densities of root lesion nematode sustained in the root zones. Root-knot nematodes in the root zones of these trees were low, close to the detection level.

Microplot screen for susceptibility to ring nematode These are ongoing studies. In the final evaluation at the end of 2021 of the 2017 planting, pistachio rootstocks had grown differently vigorous. Plant growth differences were reported previously (2021). Ring nematode population densities appeared to decline to almost non-detectable from year to year for undetermined reasons. Bioassay examinations are underway to elucidate the cause of this decline. Two more ring nematode screens are to be finally harvested within the next reporting period.

Microplot experiments to determine root lesion nematode damage potential Two microplot experiments (experiment 1: planted in 2018, experiment 2: planted in 2021) are in place examining the quantitative response of two different pistachio rootstocks (one the most "susceptible", the other one the most "resistant" line) to increasing population densities of the root lesion nematode. Each of the trials is half planted to sandy loam soil and half in sand. In experiment 1, the key observation was that the resistant cultivar grew more vigorously and had fewer root lesion nematodes in its rootzone. Quantitative growth responses to the nematode infestation level were difficult to detect. In experiment 2, infestation levels were much more distinct. In sand and sandy loam soil, both rootstock cultivars appeared thinner at the highest nematode infestation level. These data suggest that damage by root lesion nematodes at very high population densities on young pistachio can occur. Further exploration of these findings is necessary.

Microplot experiments with dagger nematodes in 2021, one pistachio orchard was identified where *Xiphinema index* was associated with weakly growing pistachio trees compared to other trees in the same orchard. Soil from the root zones of these weak trees contained *X. index.* Such soil was collected and transported to KARE in 2021 and 2022 for microplot experimentation. In both years, soils were either left untreated or autoclaved before they were added to the microplot soil. Half of each soil category was treated with an experimental nematicide, the other one left untreated. One series of plots was left non-amended. All plots were planted to pistachio 'Golden Hills' grafted on UCB1. After one year of the first experiment, none of the trends were significant, but trees grown in plots amended with autoclaved soil grew thicker during the first growing season. Soil samples revealed the presence of low population densities of *Xiphinema index*. This nematode has a long-life cycle that results in long periods before population increases can be expected. Plots will be continually monitored.

**Conclusion**

A distinct role of plant-parasitic nematodes in the vigor and performance of pistachio rootstocks cannot not be defined. Very high populations comparable to numbers that can be left behind after a walnut or almond orchard can damage pistachio rootstocks in experimental context. The build-up of root lesion nematode on pistachio roots to levels comparable to susceptible *Prunus* or *Juglan*s took five years. It is too early in the research process to generally suggest soil fumigation when root lesion nematodes are detected but at very high population densities of root lesion nematode such should be given consideration. With the current cropping pattern of pistachio following walnut, it seems prudent to take soil samples to learn about infestation levels if new pistachios are to be planted.

# Evaluating the efficacy of phosphites, mefenoxam and new Oomycota fungicides for managing phytophthora crown and root rot of pistachio

**Authors: Alejandro Hernandez Rosas**, Graduate Student, KARE-UC Davis; **Rosa Jaime-Frias**, Laboratory Assistant, KARE-UC Davis; **Florent Trouillas**, Associate C.E. Specialist in Plant Pathology, KARE-UC Davis.

## Introduction

Phytophthora root and crown rot of pistachio is widespread in California and represents a serious threat to pistachio trees. Each year, our laboratory identifies new pistachio orchards with severe cases of Phytophthora crown and root rot. Newly reported Oomycetes pathogens associated with this disease include *Phytophthora niederhauserii*, *P.* taxon walnut and *P. mediterranea*. One aspect to disease management is the use of fungicides. Traditional fungicides used for control of Phytophthora root and crown rot in nut crops have included potassium phosphite or other phosphonate compounds (e.g., Kphite®7LP), and mefenoxam (Ridomil Gold®). New fungicides belonging to different FRAC groups with different modes of action specifically targeting oomycetes have since become available, although not all are registered for pistachio yet. These fungicides include oxathiapiprolin, fluopicolide, ethaboxam, and mandipropamid. No data is currently available on the efficacy of these products against *Phytophthora* species affecting pistachio trees and no information is available about best application timing.

The goal of our research is to assess the efficacy and best application timing of these fungicides for use in managing Phytophthora crown and root rot. This year we worked on establishing the baseline sensitivities of these fungicides by evaluating the half maximal effective concentration ($EC_{50}$) towards *Phytophthora* isolates obtained from diseased pistachio trees in multiple counties. These baseline sensitivity data will serve as reference points for monitoring fungicide effectiveness over time and assess potential resistant development in *Phytophthora* populations to any one of these fungicides. Baseline sensitivities were assessed using two methods: a spiral gradient dilution method (Fig. 1) and an agar dilution method. $EC_{50}$ values using spiral the gradient dilution method were assessed for 30 isolates of *P. niederhauserii* and 3 isolates of *P. mediterranea* for mefenoxam, oxathiapiprolin, mandipropamid, ethaboxam, and fluopicolide (Table 1). $EC_{50}$ values using the agar dilution method were obtained for 7 isolates of *P. niederhauserii*, 7 isolates of *P.* taxon walnut and 7 isolates of *P. mediterranea* (Table 1). Sensitivity evaluations of additional *Phytophthora* isolates to these fungicides are still ongoing. Further studies of the efficacy of fungicides are underway in greenhouse and field experiments to determine the most effective products for the management of Phytophthora crown and root rot of pistachio.

## Results and Discussion

**Spiral Gradient Dilution Method.** All *Phytophthora* spp. isolates tested were most sensitive to oxathiapiprolin with $EC_{50}$ values for mycelial growth inhibition ranging from 0.001-0.0004 µg/ml and averaged values of 0.002 and 0.0003 µg/ml for *P. mediterranea* and *P. niederhauserii*, respectively. For *P. mediterranea*, the highest $EC_{50}$ value for oxathiapiprolin was approximately 13 times lower than the lowest $EC_{50}$ value of mandipropamid ranging from 0.0053-0.0072 µg/ml and with an average of 0.0063 µg/ml. Oxathiapiprolin $EC_{50}$ values for *P. niederhauserii* were approximately 100 times lower than the next lowest $EC_{50}$ value of mandipropamid ranging from 0.0050-0.0157 and with an average of 0.0101 µg/ml. In summary mean effective concentration values to inhibit mycelial growth by 50% for mefenoxam, oxathiapiprolin, mandipropamid, ethaboxam, and fluopicolide were 0.041, 0.0002, 0.0063, 0.073, and 0.143 µg/ml, respectively, for *P. mediterranea*; 0.131, 0.0003, 0.0101, 0.085, and 0.102 µg/ml, respectively for *P. niederhauserii* (Table 1). Differences in the range of sensitivities for mefenoxam, oxathiapiprolin, mandipropamid, ethaboxam, and fluopicolide against *P. niederhauserii* were 10.4-, 3.1-, 3.1-, 10.2-, and 5.4-fold, respectively. Differences in the range of sensitivities for mefenoxam,

oxathiapiprolin, mandipropamid, ethaboxam, and fluopicolide against *P. mediterranea* were 2.1-, 2.3-, 1.35-, 1.31-, and 1.31-fold, respectively.

**Agar Dilution Method.** $EC_{50}$ values of potassium phosphite were evaluated by the agar dilution method using concentrations of 0, 10, 40, and 120 µg/ml (Table 1). Out of the 7 isolates per species tested so far, 3 isolates of *P. mediterranea*, 2 of *P. niederhauserii*, and 3 isolates of *P.* taxon walnut had an $EC_{50}$ value greater than 120 µg/ml. These isolates will need to be run again using higher concentrations of potassium phosphite in order to determine their true $EC_{50}$. For those isolates whose $EC_{50}$ value fell within 0-120 µg/ml the $EC_{50}$ values are illustrated in Table 1. *Phytophthora mediterranea* had an average $EC_{50}$ of 66.10 µg/ml and a range of 59.25-73.45 µg/ml. *Phytophthora niederhauserii* had an average $EC_{50}$ of 34.60 µg/ml and a range of 14.88-62.89 µg/ml. *Phytophthora* taxon walnut had an average $EC_{50}$ of 68.61 µg/ml and a range of 67.46-70.95 µg/ml.

## Conclusion

All *Phytophthora* isolates tested in this study were sensitive to conventional and new fungicides. $EC_{50}$ values for mycelial growth inhibition for the new fungicides were generally lower than the currently registered mefenoxam and potassium phosphite fungicides. Baseline sensitivities and the overall high efficacy of fungicides in preventing mycelial growth in-vitro make them good candidates for future field and greenhouse experiments. Data from field and greenhouse assays will be used to support the registration of most effective products for use in pistachio. The availability of multiple fungicides in different FRAC groups (with different modes of action) will allow for rotation programs to prevent resistance development among Phytophthora species and thus promote a more sustainable management strategy for Phytophthora crown and root rot. Research continues in our laboratory to provide the California pistachio industry with knowledge of product efficacy and best application timing.

| EC50 Values for Mycelial Growth (ug/ml) | | | | | | |
|---|---|---|---|---|---|---|
| Species | Mefenoxam | Oxathiapiprolin | Mandipropamid | Ethaboxam | Fluopicolide | potassium phosphite |
| *P. mediterranea* | 0.030-0.063 (0.041) | 0.0001-0.0003 (0.0002) | 0.0053-0.0072 (0.0063) | 0.062-0.081 (0.073) | 0.128-0.167 (0.143) | 59.25-73.45 (66.10) |
| *P. niederhauserii* | 0.026-0.269 (0.131) | 0.0001-0.0004 (0.0003) | 0.0050-0.0157 (0.0101) | 0.020-.204 (0.085) | 0.049-0.263 (0.102) | 14.88-62.89 (34.60) |
| *P.* taxon walnut | - | - | - | - | - | 67.46-70.95 (68.61) |

**Table 1.** Range of $EC_{50}$ values for mycelial growth inhibition of *P. mediterranea and P. niederhauserii, P.* taxon walnut. Mean values are in parenthesis.

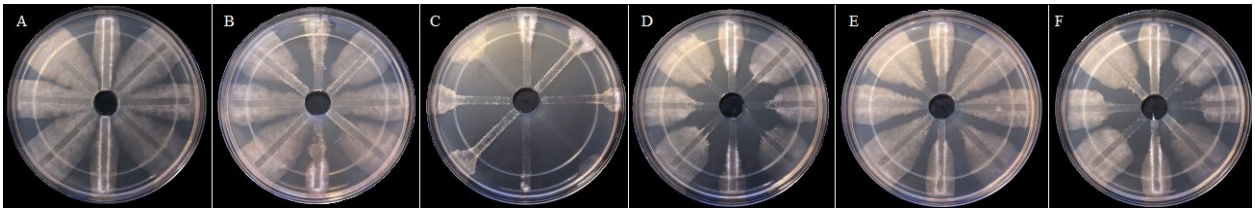



**Figure 1.** Spiral Gradient Dilution Method used with various *P. niederhauserii* isolates and after 2 days at 30 °C. Counterclockwise are isolates KARE2250, KARE2196, KARE2349, and KARE2353. (A.) Water Control plate. (B.) Mefenoxam at 10 µg/ml. (C.) Oxathiapiprolin at 5 µg/ml. (D.) Mandipropamid at 10µg/ml. (E.) Ethaboxam at 50 µg/ml (F.) Fluopicolide at 100 µg/ml.