Executive Summaries
2023

California Pistachio Research Board
499 W. Shaw Ave., Suite 140
Fresno, CA 93704

MANAGER'S ANNUAL REPORT – 2023

Bob Klein, Manager

Annual reports are a chance to put the past year in perspective by comparing it to previous years. In that sense, 2023 was in many respects an anomalous year. After years of drought and concern over declining chill, rainfall and snowpack far exceeded "normal" levels, leading to flooding and the rebirth of Tulare Lake. At its 2023 peak, Tulare Lake covered about 110,000 acres and was 5-7 feet deep. While much smaller than its historical size of over 500,000 acres and 40 feet deep, 2023 was the first significant flooding since 1983. Only recently have tree crops, principally pistachios, been planted in the Tulare Lake basin and about 5000 acres of pistachios were killed and some additional acreage damaged in flooding of Tulare Lake tributaries.

The increased rainfall and snowmelt contributed to ample water for groundwater recharge and exposed a need for better infrastructure to move excess water to areas suitable for percolation. Rainfall filled the soil profiles around the Central Valley, reducing the need for irrigation until late May and early June and provided significant leaching of salts that had accumulated in the soil profile. However, wet soils limited access to fields made orchard sanitation (mummy removal) difficult for pistachios and almonds. This led to increased navel orangeworm populations later in the season, particularly in almonds which then migrated into late-harvest pistachios.

Chill accumulation was phenomenal. It doesn't matter which chill model is used or where it was measured, 2023 had the best chill seen in the last 20 years and possibly ever in the 40-year history of the California pistachio industry. Cool weather persisted in the spring, leading to a late bloom and concerns about whether the growing season would be adequate to bring the crop to maturity.

Harvest did begin late, about 7-10 days later than normal, but yields were better than many expected. There were few if any issues with nut removal; reshakes were more limited than in recent years due to better nut removal but also lower pistachio prices. Early season predictions for a 1.2-billion-pound crop were exceeded and the crop came in at 1.5 billion pounds from an estimated 461,079 bearing acres. Crop quality, especially in the first half of the harvest, was exceptionally good with low insect damage, large nut sizes, and very low aflatoxin contamination.

Since about 2011, nearly all new pistachio plantings have been made with the Golden Hills cultivar and, to a lesser extent, Lost Hills. Kerman has largely been limited to replants. Although we do not collect statistics on the harvest proportions of each cultivar, 2023 is likely the year that the total Golden Hills crop exceeded that of Kerman. With additional Golden Hills acreage maturing and with greater yield potential, Golden Hills has become and will remain the largest pistachio producer. An acreage survey by LandIQ estimated plantings from 2011-2018 totaled 233,137 acres. If 90% of these plantings are Golden Hill, there are about 210,000 acres of Golden Hill versus about 220,000 acres of Kerman. There are 144,363 acres of nonbearing pistachios (planted in 2019 or later); the number of current nonbearing acres exceeds the bearing acres in 2010. The total (bearing plus nonbearing) acres is now approximately 605,442 acres. When all these acres come into bearing, the total crop will exceed 2 billion pounds.

There are several headwinds the industry will need to face in the upcoming years. Many of the state water regulations will come into play in the very near future and acreage in overdrawn groundwater basins without surface water allocations may need to be removed. Acreage that currently utilizes saline irrigation supplies but lacks an adequate leaching fraction may also need removal. The California Department of Food and Agriculture (CDFA) is a strategic vision statement that emphasizes sustainability and regenerative agriculture but has yet to define these terms.

In general, current concepts find sustainability and pesticide use incompatible, hence the state's efforts to eliminate pesticides by 2050. Regenerative agriculture focuses on soil health and generally finds synthetic fertilizer applications incompatible with soil health. The Central Valley of California has been described as the most productive farmland as well as the largest and most extensively engineered landscape in the world. It is difficult to imagine agriculture in the Central Valley without adequate water, pesticides, and synthetic fertilizers. We will also be facing invasive diseases and pests, particularly brown marmorated stinkbug, spotted lantern fly, Carpophilus beetle, and exotic fruit flies. Not all and maybe none will develop into significant pest problems but the number of invasive pests raises concerns about border inspection efficacy and efficiency.

I will soon be ending my time as the Manager of the Administrative Committee for Pistachios and the California Pistachio Research Board. I would like to thank all those I have worked with over the past two-plus decades and have made this position a professionally and intellectually rewarding experience.

Thank you all and best wishes for a prosperous future!

Table of Contents

California Pistachio Nitrogen and Potassium Prediction Model

Authors: **Julia Stover-Blackburn**, Academic Coordinator, Department of Plant Sciences, UC Davis; **Kevin Taniguchi**, Instructional Designer, 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, agricultural models, and relevant links for specific crops. In addition to the website, FNRIC works to coordinate UC and industry communication through conferences, meetings, and courses.

One of the agricultural models that has long been hosted on the FNRIC website is a Nitrogen and Potassium Prediction Model for Pistachio, originally developed by Dr. Patrick Brown. This model was only accessible as a series of PDFs and excel files and was neither user-friendly, nor did it meet accessibility guidelines.

Results and Discussion

Through 2022, FNRIC began working with the IET Web Development Team to move this Nitrogen and Potassium Prediction Model to the updated FNRIC site. The beta version of the model is available at https://fruitsandnuts.ucdavis.edu/nitrogen-prediction and is both significantly more user friendly than the older version of the model and it meets modern accessibility guidelines. Funding was requested to complete this upgrade, incorporate user feedback into the final version of the model, and present the new tool at the Pistachio Workgroup Meeting and other appropriate events.

Conclusion

The new model is live as of Spring 2023. We have had no downtime and overall very positive feedback from users. The Pistachio Workgroup Meeting was not held in 2023, but we have reached out directly to a number of pistachio advisors to ensure that the primary anticipated users are aware of the new tool.

California Pistachio Research Board Event Facilitation

Authors: **Julia Stover-Blackburn**, Academic Coordinator, Department of Plant Sciences, UC Davis; **Kevin Taniguchi**, Instructional Designer, 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 2023, FNRIC was primarily responsible for coordinating abstract submission and agenda development for the VIII International Symposium on Almond and Pistachio and managing the CPRB proposal review process.

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.

Optimizing phosphine for the future

Authors: **Raman Bansal**, Research Entomologist, USDA-ARS, San Joaquin Valley Agricultural Sciences Center, Parlier, CA; **Spencer Walse**, Research Chemist, USDA-ARS, San Joaquin Valley Agricultural Sciences Center, Parlier, CA.

Introduction

The red flour beetle (RFB), *Tribolium castaneum* is a serious, cosmopolitan pest of stored pistachios and other commodities. Currently, fumigation with phosphine is the major means of control of this species world-wide. The extensive use of phosphine as a fumigant has led to the emergence of insecticide resistance in RFB in many parts of the world, severely compromising the ability of phosphine to effectively safeguard stored products from this pest. The long-term goal of this project is to identify operationally effective procedures and label instructions, which are ultimately required if fumigant phosphine is to remain an effective tool. Pest responses to phosphine exposure at suboptimal concentrations have been studied in detail, however, research focus needs to be shifted to understand pest tolerance to exposure at optimal concentrations of phosphine. In the current research, our objective is to determine molecular response of RFB to optimal concentrations of phosphine.

Results and Discussion

Phosphine resistance is a genetically controlled and heritable trait, therefore, to determine RFB's molecular response, we adopted genome-wide approach to analyze gene expression using high-throughput sequencing. Separate groups of phosphine susceptible and resistant RFB adults (n = 100) were exposed to steady state phosphine dose of 800 ppmv in sealed gas-tight glass jars. Replicated treatments were conducted at 24°C for 30 min to cause 50% mortality (LT50s). Survivor RFBs following the treatment were processed for RNA-Seq analysis. More than 40 million paired-end reads were obtained for each replication (Table 1). For expression measurements, nearly 90% of total reads from each replication mapped to reference genome, with nearly all of those reads mapping uniquely.

Table 1. Statistics on RNA-Seq yield and read mapping

Treatment	Replicates	Total reads	High quality paired reads	Mapped reads (%)	Uniquely mapped reads (%)
Phosphine-susceptible red flour beetle	R1	42,327,238	42,031,646	89.67	88.42
	R2	63,512,516	63,112,336	88.46	87.00
	R3	40,890,130	40,601,622	89.16	87.83
	R4	45,474,998	45,160,612	89.90	88.58
Phosphine-resistant red flour beetle	R1	45,545,564	45,254,740	90.08	88.97
	R2	46,337,130	46,029,226	89.74	88.53
	R3	40,942,110	40,619,626	84.12	83.10
	R4	43,525,772	43,208,248	87.40	86.25

Visualization with principal components analysis (PCA) score plots revealed clear separation between the phosphine susceptible and resistant RFB samples along PC1, which explained 46.5% of the variance (Fig. 1). The phosphine susceptible samples clustered on the left side of PC1, while the phosphine resistant samples clustered on the right.

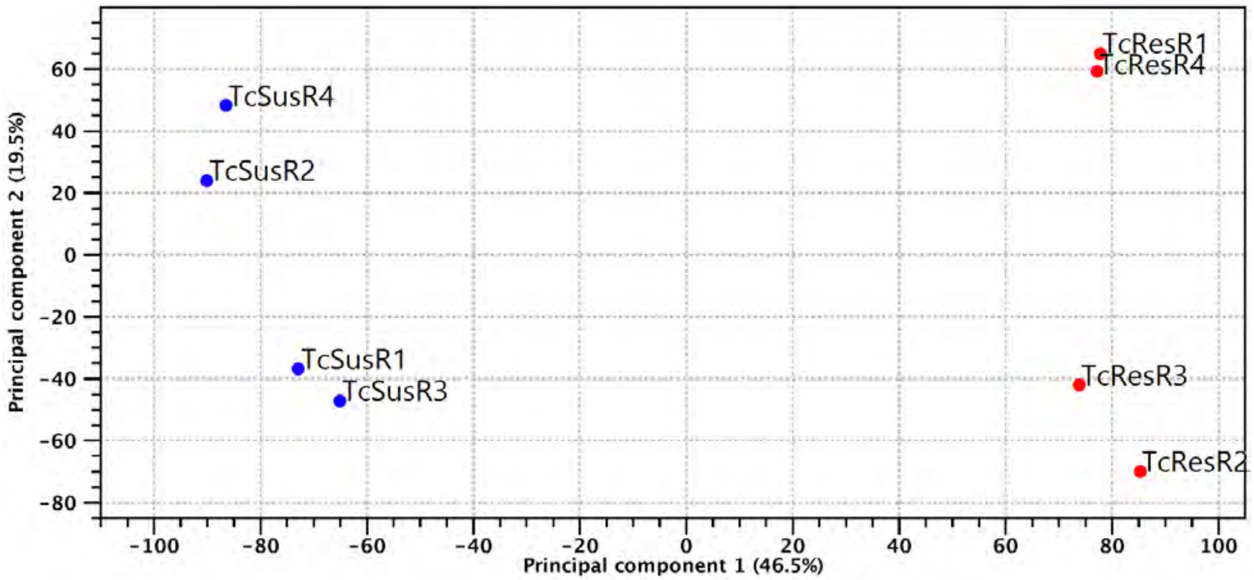


Figure 1. Principal component analysis (PCA) score plot of gene expression data. PCA was conducted on the full dataset of gene expression measurements on 8 samples. The first two principal components (PC1 and PC2) are displayed, which explain a total of 56% of the variance. Each dot represents one sample colored by group i.e., blue = phosphine susceptible TcSucR1-R4, red = phosphine resistant TcResR1-R4).

Statistical analysis revealed that 139 genes (out of total 14,491) were differentially expressed in phosphine resistant RFB compared to susceptible one (FDR $P < 0.05$), and all differentially expressed genes were upregulated in the resistant strain (Fig. 2).

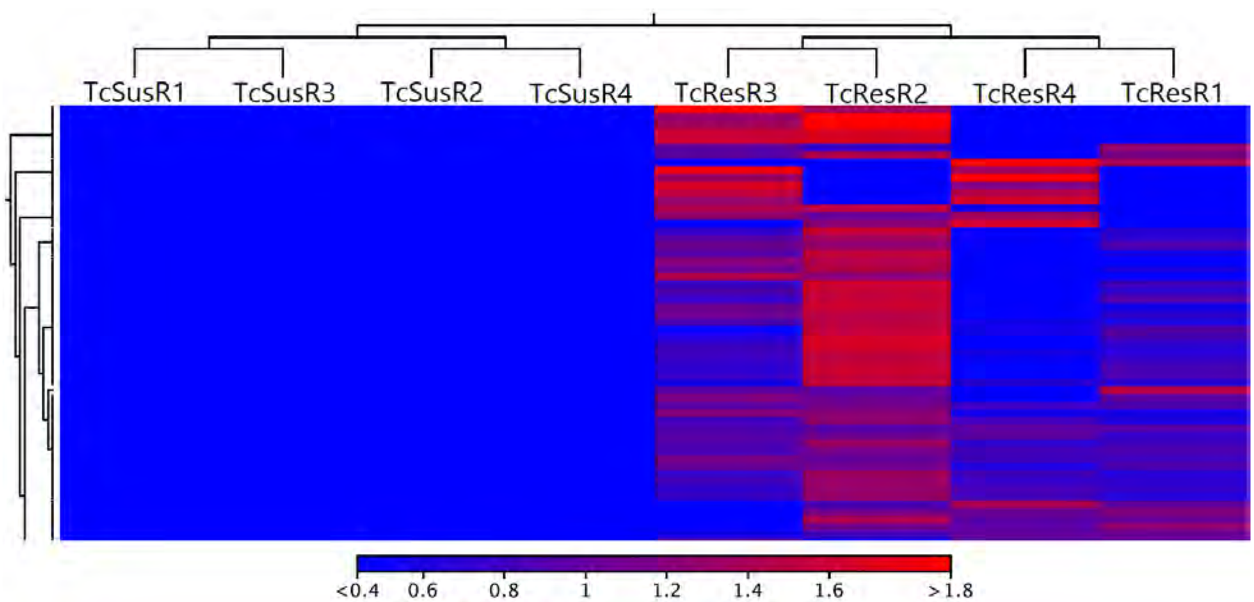


Figure 2. Hierarchical clustering heat map of differentially expressed genes between the phosphine susceptible (TcSucR1-R4) and phosphine resistant (TcResR1-R4) RFB samples. Gene expression values were clustered using Euclidean distance and complete linkage. Each row represents a gene and each column an individual sample.

<u>Conclusion</u> The results show that phosphine susceptible and resistant RFB respond differently at molecular level when exposed to optimal levels of phosphine. Investigations into identity and molecular function of differentially expressed genes are currently underway.

Improving effectiveness and sustainability of Gill's mealybug management program in pistachio

Authors: **Raman Bansal**, Research Entomologist, USDA-ARS, San Joaquin Valley Agricultural Sciences Center, Parlier, CA. **Cooperator: David R Haviland**, Farm Advisor, University of California Cooperative Extension, Kern County, Bakersfield, CA.

Introduction

Gill's mealybug (GMB) has emerged as one of the major pests of pistachio in California. It causes economic impact by reducing the pistachio crop value associated with yield in terms of total dry weight and nut quality. Besides causing direct economic damage, GMB also poses handling challenges for processing industry. Pistachio growers rely solely on the use of insecticides for controlling GMB. However, only a limited number of chemicals namely acetamiprid, spirotetramat, and buprofezin showed effectiveness against GMB and thus were recommended in early 2000s.

Several pistachio growers, consultants, and pest control advisors from the Central Valley in California have reported failure of recommended insecticides to control GMB since around 2019. Since then, the prevalence of these reports has increased, as has the geographic range from which they have been reported. In the worst-case scenario, in addition to the typical late May insecticide application at the time of emergence of the first in-season generation, growers have resorted to additional insecticide applications after harvest, in late March when overwintering nymphs first appear in the tree canopy, or in late July during crawler emergence of the second in-season generation. Failures of traditional one-spray programs used since the early 2000s could potentially be attributed to insecticide resistance. We lacked the ability to determine if the lack of efficacy is related to insecticide resistance. The purpose of this research was to establish a method for routine monitoring of GMB susceptibility to acetamiprid and spirotetramat. We also aimed to evaluate the toxicity of insecticidal compounds with the potential to broaden the pool of available chemicals.

Results and Discussion

Objective 1 – Establish a baseline for susceptibility of Gill's mealybug populations in California to acetamiprid and spirotetramat: To establish baseline susceptibility, the GMB were collected from infestations in pistachio orchards at five locations in in the Central Valley: Coalinga, Kettleman City, Lost Hills, Hanford, and Riverdale. The pistachios at the Coalinga location were grown organic without use of synthetic chemicals whereas other locations had histories of receiving conventional insecticide treatments to manage GMB. To determine GMB populations' response to acetamiprid and spirotetramat, leaf-dip bioassays were performed. Second instar nymphs were exposed to bean leaves dipped in serial concentrations of the insecticide. Further, dyna-amic was used adjuvant for spirotetramat exposure. Concentration–response was assessed 1- and 2-days after initial exposure with acetamiprid, and 2- and 5-days in case of spirotetramat. The LC_{50} values of acetamiprid for all field

Table 1. Susceptibility of second instar GMB collected from five California locations to acetamiprid

Location	N[1]	LC_{50} [μg(AI)ml^{-1}][2] (95% FL)[3] at 1-d	LC_{50} [μg(AI)ml^{-1}] (95% FL)[3] at 2-d
Coalinga	1176	1.077 (0.872 - 1.307)[a]	0.511 (0.382 - 0.650)[a]
Riverdale	1168	0.546 (0.414 - 0.683)[b]	0.400 (0.296 - 0.511)[a]
Kettleman City	987	0.605 (0.435 - 0.788)[b]	0.367 (0.254 - 0.496)[a]
Lost Hills	1202	0.686 (0.547 - 0.841)[b]	0.533 (0.401 - 0.677)[a]
Hanford	1201	2.459 (2.096 - 2.881)[c]	2.398 (2.060 - 2.753)[b]

[1]Number of insects bioassayed; [2][µg(AI)ml-1]-Microgram of active ingredient per ml of water; [3]LC_{50}s were considered significant when 95% fiducial limits (FL) were non-overlapping.
populations at 1-d ranged from 0.546 to 2.459 µg(AI)ml^{-1} while the corresponding LC_{50} values at 2-d ranged from 0.367 to 2.398 µg(AI)ml^{-1} (Table 1).

Significantly higher concentrations of acetamiprid were needed to produce similar mortality within the Hanford population compared to all others at both response times (both $t = 0.000$, degree of freedom = 2, $P < 0.05$ for all comparisons) (Table 2). Based on LC_{50} value comparisons, resistance ratios of the Hanford population at 24 h ranged from 2.3 to 4.5-fold [with reference to Coalinga=2.3 (1.8–3.0); Kettleman City = 4.1 (3.0–5.5); Lost Hills = 3.6 (2.7–4.7); and Riverdale = 4.5 (3.4–5.9)]. Corresponding resistance ratios of the Hanford population at 48 h ranged from 4.5 to 6.5-fold [with reference to Coalinga = 4.7 (3.6–6.2); Kettleman City = 6.5 (4.8–9.0); Lost Hills = 4.5 (3.4–6.0); and Riverdale = 6.0 (4.6–7.9)].

Table 2. Resistance ratios of second instar GMB collected from Hanford, California to acetamiprid

Reference population	RR_{50} (95% FL) †		RR_{90} (95% FL)†	
	1-d	2-d	1-d	2-d
Coalinga	2.3 (1.8-3.0)	4.7 (3.6-6.2)	1.2 (0.7-2.1)	2.2 (1.4-3.4)
Riverdale	4.5 (3.4-5.9)	6.0 (4.6-7.9)	3.6 (2.3-5.7)	3.7 (2.5-5.6)
Kettleman City	4.1 (3.0-5.5)	6.5 (4.8-8.9)	2.4 (1.5-4.1)	2.5 (1.6-3.9)
Lost Hills	3.6 (2.7-4.7)	4.5 (3.4-6.0)	1.5 (0.9-2.6)	1.8 (1.1-2.8)

†Resistance ratios RR_{50} and RR_{90} were obtained at the LC_{50} and LC_{90} levels, respectively, and were calculated by dividing the LC value [and 95% fiducial limits (FL)] of the Hanford population by the corresponding value of the reference population at 1- and 2-d response times.

Similarly, the LC_{50} value of spirotetramat for GMB population from organic pistachio was 13980.094 (3806.600 to 516522.932) and 108.237 (57.509 to 170.033) µg(AI)ml^{-1} at 2- and 5-d response times, respectively. Research to establish GMB susceptibility to spirotetramat is still underway.

Objective 2 – Determine toxicity of various insecticides to Gill's mealybug: Various insecticide formulations (Neem oil, Baythroid, Valent, Sivanto, Belay, Sequoia, Grandevo, Diflubenzuron, Sieze) were evaluated for efficacy to control GMB in replicated greenhouse trials. Preliminary analysis suggested that Sequoia and Belay performed on par with positive controls (Centaur, Movento, and Assail) and were significantly different from negative controls (Untreated, Water treated, Dyne-amic only).

Conclusion

The GMB population collected from the Hanford area indicated up to 6.5-fold resistance to acetamiprid as it demonstrated significantly decreased mortality to acetamiprid compared to other populations. The resistance identified in this study, although relatively low, indicates that there has been repeated pressure on this population to select for acetamiprid resistance and resistance level can further amplify if effective management steps are not taken. Given the current dry pipeline for novel chemistries and newly registered products, the emergence of insecticide resistance in GMB presents a significant challenge to pistachio production. Nonetheless, GMB control in pistachio is expected to continue to rely primarily on foliar applications of just a few chemicals due to lack of nonchemical alternatives. Therefore, efficacy of these chemicals should be preserved through use of insecticide resistance management (IRM) tactics. Monitoring of pest susceptibility, which changes over time due to heavy reliance on insecticides, is a critical IRM component. Baseline susceptibility of GMB established in this study can be used to monitor shifts in susceptibilities to reveal any changes in toxicological responses of this pest over time. Further, promising candidates from greenhouse trials are being tested in the field to provide different mode of action chemistries to control GMB.

Attractants for Leaffooted Bugs in Pistachios

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

Introduction

The leaffooted bug species, *Leptoglossus zonatus*, is a large insect found on over sixty plants including pistachio, and bug feeding can result in crop loss (Joyce et al. 2017b). There is a need to develop traps or monitoring devices to detect the presence of this insect in orchards prior to observing damage. There are few insecticides known to be effective to control these large bugs in conventional or organic orchards. Chlorpyrifos is now eliminated as a control option in California, and pyrethroids are regulated to prevent environmental contamination. The overall goal of this proposed work is to further our understanding of potential attractants for leaffooted bugs in order to aid in monitoring of this pest.

L. zonatus moves into pistachio and may be attracted either to host plant odors or pheromones. Previous studies of potential attractants for *L. zonatus* include investigating host plant associated products such as pistachio or almond meal, while others have investigated insect-produced odors such as pheromones. We investigated adult *L. zonatus* flight behavior to attractants in a wind tunnel and found that mating pairs of *L. zonatus* were attractive to both *L. zonatus* males and females. This suggested that volatiles from mating pairs might be developed as an attractant for monitoring leaffooted bugs. Panel traps have been investigated by Wilson et al. (2020) who found they could be used for monitoring leaffooted bugs. Others are investigating male produced compounds from leaffooted bugs as attractants and progress has been made. A commercial attractant is not yet available for leaffooted bugs, but will be a welcome addition to the IPM toolbox once available.

Another factor which could influence the monitoring of *L. zonatus* using pheromones is that there are two genetically distinct strains of this species in California. *Leptoglossus zonatus* in California has been collected from Butte County to the Bakersfield area; two genetically distinct populations exist (Joyce et al. 2017). One strain is primarily found in California, while the second strain is found from California all the way to Brazil; this strain will be called the 'widespread' strain. We found that the widespread strain is the dominant strain found from California to Brazil (Joyce et al. 2021). It will also be important to know if the two strains of leaffooted bug cross attract, if they could be attracted to the same pheromone, and if they produce the same volatile compounds.

The objectives of this proposal include comparing biology (fecundity, longevity, mating behavior) of two strains of the leaffooted bug *L. zonatus*, examining the relative attraction of adult *L. zonatus* to food and pheromone sources, and to relate trap catches of *L. zonatus* to bug damage on pistachios. This is a summary of work from year 1-2 of the project.

Results and Discussion

Biology and Cross Mating of Strains: The biology was compared between the two strains of *L. zonatus*. We found that the average number of eggs per mating pair in the two strains was not significantly different. To examine mating behavior, we examined cross attraction of the two strains of *Leptoglossus zonatus*, using insects from lab colonies. If there was no preference for male or female mates of either strain, then there would be equal mating between the two strains. Fifty mating pairs were selected from lab colonies, and the mitochondrial DNA COI barcode was sequenced for each male and female pair. We found that there was *some* cross mating between the two strains, in about *one third of the trials*. The fertility from mating pairs of the two strains was not found to differ, but there may be preference in attraction between the two strains.

Colonies of the two *L. zonatus* strains were started and maintained in the laboratory. We have been calling the two strains the 'California' strain and the 'Widespread' strain. Previous work found that in the San Joaquin Valley, both strains were present. To expedite the identification of the two strains, we identified and tested several DNA probes which can be used with real time qPCR. Hundreds of mating pairs of *L. zonatus* were screened to determine which strains were mating, and their offspring were used to start and to maintain the two colonies of the 'California' and the 'Widespread' strain of this species. We have now isolated and maintained the two different strains of *L. zonatus* so they can be used in cross attraction studies in the wind tunnel, and so that volatiles can be collected from the two strains for comparison.

The second objective was to determine if adult *L. zonatus* have a preference for attractants (odors) associated with food odors (pistachio) compared to pheromones (odors associated with mating pairs). The purpose was to identify which of these may be more productive for finding attractants for this insect. In previous work, we found that pheromones associated with mating pairs were more attractive than odors produced by males alone, or females alone. We also previously examined the relative attraction of food odors vs. pheromones, by examining flight of adult leaffooted bugs to odor sources in a wind tunnel. First, we examined attraction of mated and unmated males to attractants in the following experiments; 1) mating pairs or a control, 2) pistachios or a control, and 3) pomegranate compared to a control. In each experiment, the male was released and observed. Attraction was higher to mating pairs than pistachio odors.

We are investigating cross-attraction of adults of the two *L. zonatus* strains in wind-tunnel trials. This provides insight into whether a pheromone produced from one strain will attract both strains in the field. Volatiles are also being collected from the two strains. The project has been extended and the work will continue into next year.

Conclusion

The leaffooted bug species, *L. zonatus*, has become more abundant in the last decade in California. There are two strains of this insect, one known primarily from California, and the second strain which is widespread from California to Brazil. To date, our work found the fertility of mating pairs of the two strains was similar. In addition, the two strains can cross attract in the lab when in close proximity. When comparing attraction to insect vs. food odors, we found that mating pairs were more attractive to adult *L. zonatus* than pistachio odors. This work continues to examine whether there is cross attraction in the wind tunnel 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.

Literature Cited

Beck, J.J.; Gee, W.S.; Cheng, L.W.; Higbee, B.S.; Wilson, H.; Daane, K.M. Investigating Host Plant-Based Semiochemicals for Attracting the Leaffooted Bug (Hemiptera: Coreidae), An Insect Pest of California Agriculture; American Chemical Society: Washington, DC, USA, 2018.

Daane, K. M., Yokota, G.Y., Bentley, W.J., and D.R. Haviland. 2008. Winter/Spring Sampling for Leaffooted bug in nut crops. Reference handout 2008-LFB-1, March pg. 1-4.

Joyce AL, Barman A, Doll D, Duncan R, Higbee B. 2017a. Understanding aggregation behavior of the leaffooted bug, *Leptoglossus zonatus*. Annual Report to the Almond Board of California for 2016-2017. August 1, 2017.

Joyce AL, Higbee BS, Haviland DR, Brailovsky H. 2017b. Genetic variability of two leaffooted bugs, *Leptoglossus clypealis* and *L. zonatus* (Hemiptera: Coreidae) in the Central Valley of California. *Journal of Economic Entomology* DOI: 10.1093/jee/tox222

Joyce AL, H. Parolini, H Brailovsky. 2021. Distribution of Two Strains of *Leptoglossus zonatus* (Dallas) (Hemiptera: Coreidae)in the Western Hemisphere: Is *L.zonatus* a Potential Invasive Species in California? Insects 12, 1094. https://doi.org/ 10.3390/insects12121094

Millar J, Wilson, H., Daane K. 2018. Another look at pheromonal and related attractants for leaffooted bugs infesting California nut crops. California Pistachio Research Board Executive Report Dec.pg. 11-12.

Wilson H, JJ Macaro, K Daane. 2020. Optimizing trap characteristics to monitor the leaffooted bug *Leptoglossus zonatus* (Heteroptera: Coreidae) in Orchards. Insects 2020 11, 358.

The effects of insecticide treatments on multiple generations of Gill's mealybug, *Ferrisia gilli*, in pistachios

Authors: David Haviland, UCCE Entomology and Pest Management Farm Advisor, Kern County; **Stephanie Rill,** UCCE Entomology and Pest Management Research Associate, Kern County

Introduction

Gill's mealybug is a significant pest of pistachios throughout California that is associated with reductions in crop yield and quality. During 2023 we conducted a trial in a commercial pistachio orchard near Lost Hills, Kern Co., CA to evaluate the effects of insecticide treatments on mealybug density from late March until harvest. The trial looked at the effects of one or more insecticide applications at the end of March, end of May, and/or end of July. Late March applications targeted small overwintering mealybug nymphs as they moved to the buds as dormancy ended. The late May treatments (traditional timing) targeted first instar nymphs (crawlers) of the first in-season generation as they emerged from adults from the overwintering generation. The late July treatments targeted crawlers of the second in-season generation of mealybugs that, if allowed to survive, would become adults during harvest.

Results

Late March treatments

Prior to treatment on 29 March there were no significant differences in mealybug densities and averaged approximately 0.3 mealybugs per 10 clusters (data not shown). Evaluations of plots treated with an insecticide in the spring (30 March) showed no impact from applications of Assail, Sequoia or Sivanto. An application of Centaur resulted in an approximate 50% reduction in the number of mealybugs compared to the untreated check and all other treatments during the overwintering and first and second in-season mealybug generations. When each of the four insecticides applied in March was combined with an application of Movento on 30 May, the two-spray program was not much more effective than the one-spray program due to the relative lack of efficacy of Movento throughout this trial. This contrasts with previous trials where Movento in late May to early June has repeatedly been the industry standard and most effective product.

Late May treatments

Evaluations of plots treated with an insecticide at the traditional timing in late May found significant reductions in mealybug density in plots treated with Centaur, Assail, and MBI-306. Centaur is an industry standard growth regulator that can only be used at this timing if it can be confirmed that the crop is not destined for the European Union due to stringent restrictions on residues. Assail is also an industry standard product representing the neonicotinoid class of chemistry. MBI-306 is an experimental microbial pesticide with the active ingredient *Burkholderia rinojensis* strain A396. No other insecticide resulted in statistically significant reductions in mealybug density, including the industry standard Movento that historically has been the top insecticide across multiple trials over multiple years.

Late July treatments and programs

Comparisons of Assail, Fujimite, Sivanto and AzaDirect when applied as stand-alone treatments on 30 May compared to 27 July showed no significant differences within each insecticide comparison. Both timings were equally effective or not effective at all. Among the products, Assail was the most effective at either timing while none of the other three products significantly reduced mealybug density or cluster damage ratings compared to the untreated check.

All three late-season combination programs (May + July) represented some of the best treatments in the trial. This included Movento followed by Assail, Fujimite followed by Assail, and Assail followed by Fujimite. However, none of these programs significantly reduced damage compared to what was achieved using Assail by itself. In other words, a single application of Assail, applied either in May or July, was as good as any of the two-spray programs that rotated Assail with other products.

Conclusion and Practical Implications

The most significant result of this trial was that Movento was not effective in any of the six treatments where it was included. This is in stark contrast to previous trials where it has repeatedly been the best treatment. The exact reason for the lack of efficacy is unknown, though resistance is suspected and being investigated. To the contrary, the industry standard product Assail was effective as a solo treatment or in combination with other products when applied in late May or late July, such that it can be used as an alternative for Movento in orchards where reduced efficacy has been observed.

The other significant finding was validation that late March is a viable application timing for Centaur. This result is highly valuable to growers that desire to use Centaur due to its history of effectiveness, but that cannot use it in late May or late July due to risks of not satisfying ultra-strict default tolerance levels for the European Union. Other products that resulted in a significant reduction in mealybug density at the traditional timing in late May include Sivanto (foliar), Sefina and MBI-306, though none of these products was statistically equivalent to the best treatments.

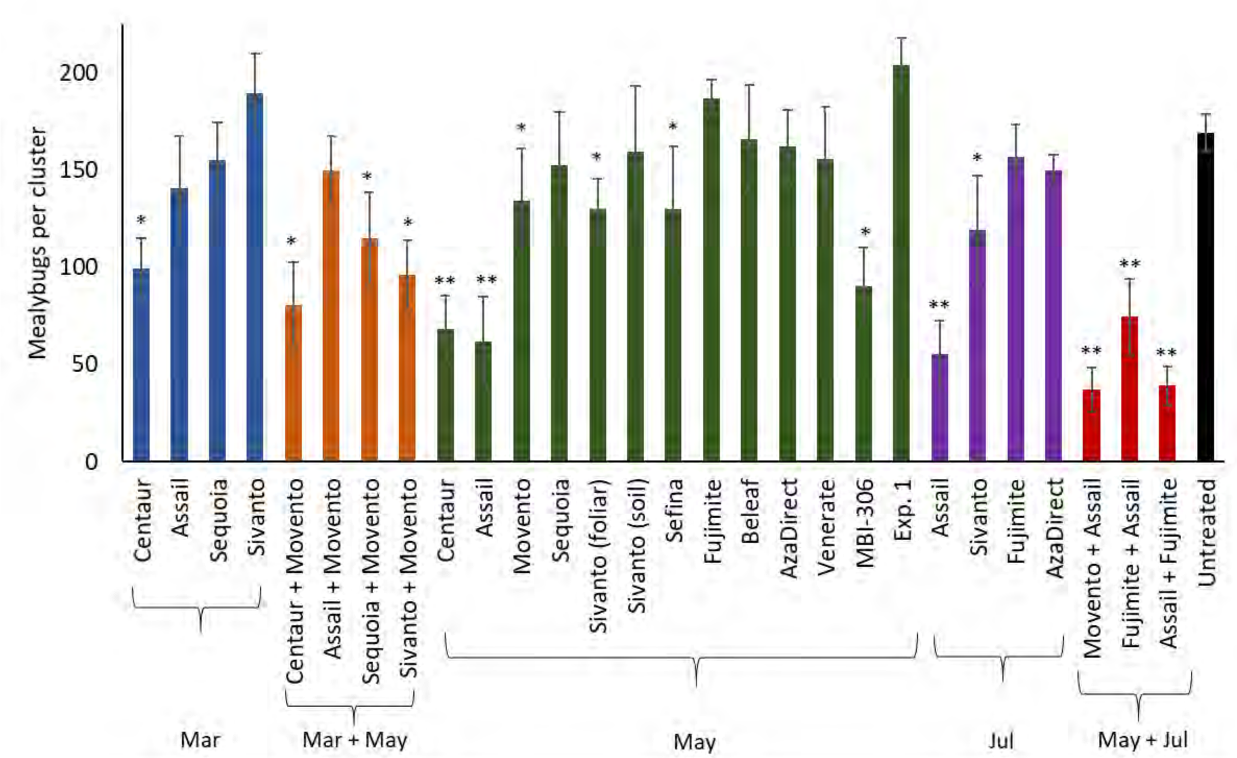


Fig. 1. Average mealybugs per cluster during evaluations in August and September. Single asterisk means significantly lower than the untreated check. Double asterisk means statistically equivalent to the top treatment.

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

Product names are for specific information purposes and their mention does not constitute an endorsement by the USDA. The goal of these studies is to improve control of navel orangeworm (NOW) by reducing water use and improving coverage. In an experiment conducted in 2023 at Agri-World Coop, we examined the efficacy of an application rate of 70 gallons per acre (70 gal/ac) combined with Intrepid 2F (24 oz/ac; Corteva) plus Omni Brand Bifenthrin 2EC (12.8 oz/ac) with Kinetic, an organosilicone adjuvant used at a rate of 12.8 oz per 100 gal (Helena Agri-Enterprises). The results were compared to a grower standard application rate of 100 gal/ac with the same insecticide combination and the adjuvant used was Cohere (12.8 oz/100 gal; Helena Agri-Enterprises). The trial was conducted in August in Madera County. We measured the duration of control at a height of 14 feet (Hook) using a filter paper contact toxicity bioassay. Filter paper was also placed at 5 feet (Ground) and collected seven days after application to establish maximum potential percent kill in case there was loss in the upper canopy. A commercial ground application was made using an Air-O-Fan GB36 PTO air blast sprayer (500-gallon tank) at 2 MPH. Filter papers were collected on days 7 and 14 after application. 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 15-18 days later.

Results and Discussion

Table 1. Year 2023 duration of control for Intrepid and Bifenthrin 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 7	57.78%		450
Cohere 100 gpa **Ground** Day 7	91.10%	78.95%	450
Kinetic 70 gpa **Ground** Day 7	97.80%	94.79%	500
Cohere 100 gpa **Hook** Day 7	98.00%	95.26%	1,960
Kinetic 70 gpa **Hook** Day 7	88.75%	73.36%	2,000
Control Day 14	45.20%		750
Cohere 100 gpa **Hook** Day 14	94.30%	89.60%	2,000
Kinetic 70 gpa **Hook** Day 14	84.80%	72.26%	2,000

In contrast to previous years, the mortality for the two treatments differed in the upper canopy, with 70 gpa having lower contact toxicity than 100 gpa. This did not occur for the ground filter paper (5 feet) where the kill for 70 gpa was higher. There may have been differences in the calibration of the sprayers used in the study, with the Grower Standard aiming higher in the canopy. However, for the 70 gpa treatment the contact mortality was still satisfactory 14 days after application at 84.8%. For both adjuvants, there was a slight decrease in contact toxicity when days 7 and 14 days are compared. Although small, this reduction is still greater than what was observed in previous studies at 14 days and demonstrates the inherent variability inherent in these trials. In a separate trial conducted at the FMC Research Farm in Madera County in 2023, the maximum rate of Intrepid 2F was applied at 150 gal/ac with Dyne-Amic (Helena) as the adjuvant at 8 oz/100 gallons. The hooks in the canopy (14 feet) were sampled on days 7, 14, 21 and 28 after application and the contact mortality was 98.2%; 97.6%; 96.8%, and 96.5% respectively. There was no meaningful decrease over a 28-day period. Consequently, the decrease in the Agri-World treatments at 14 days after application was surprising, considering the insecticides that were used. There will always be some variation in these studies, but the combination of organosilicone adjuvants and Air-O-Fan spray rigs equipped with multi nozzles were still successful at 70-80 gal/ac when other organosilicone adjuvants were used.

A second study evaluating an electrostatic sprayer (OnTarget Spray Systems, Angel, OR) was conducted at Agri-world. The application volume was 40 gal/ac, ground speed was 3 mph, the insecticide used was Shenzi (UPL-their chlorantraniliprole) at 2.25 oz/ac, and Vintre was the adjuvant at 16 oz/100 gal (Oro-Agri, Fresno). The hooks were placed at 12-14 ft in the canopy as described above and the filter paper evaluated at 7 days after application. The next day after the filter papers were collected, news filter paper were placed on the same hooks and sprayed using the grower standard Intrepid 2F (24 oz/ac; Corteva) plus Omni Brand Bifenthrin 2EC (12.8 oz/ac) with Cohere (12.8 oz/100 gal; Helena Agri-Enterprises) as adjuvant. These filter papers were also collected 7 days after application..

Table 2. Year 2023 duration of control for Shenzi (2.25 oz/ac) applied at 40 gpa with Vintre compared to the grower standard of Intrepid 2F + Bifenture +Cohere, applied at 100 gpa, August.

Treatment	**Mortality**	**Reduction**	**Eggs**
Control Day 7	56.050%		500
Electrostatic 40 gpa hook Day 7	97.53%	94.39%	1,500
Grower Standard 100 gpa hook Day 7	97.31%	93.83%	2,900

The electrostatic sprayer performed comparably to the Grower Standard when assessed 7 days after application. Given my previous experience with application research, I predict that contact toxicity would have been similar at 14 days after application or even longer.

Conclusion

The purpose of these studies is to evaluate application methods that can both reduce water volume and improve spray coverage. If we can successfully reduce application rates it will increase spray efficiency and save time, which in turn means saving money. Currently, air application is the only method that can rapidly treat many acres with reduced water (10-20 gpa), but the coverage is reduced in the lower canopy; in my studies the best ground applications produced up to 98% mortality in the upper canopy (14 feet) while the best air applications produced 85% mortality at the same height. These coverage studies funded by the Pistachio Research Board over the past four years demonstrated that when organosilicone adjuvants were used in conjunction with Air-O-Fan multinozzle sprayers at 2 mph, application volume could be reduced to 70 gpa. This decreased volume allowed an extra row to be sprayed per tank-load (500 gallon tank), assuming that each row is an acre, with an increase in efficiency of 20%. Electrostatic sprayers are a technological alternative to air blast sprayers and use a lower water volume (50 gallons maximum) and higher speeds (3-3.5 mph), enabling more acres to be treated in the same time period as an airblast sprayer. My future research will evaluate their coverage, focusing on coverage consistency and assessing if there are classes of adjuvant the provide superior coverage.

Producing Sterile Navel Orangeworm on Demand for Improvement of Pest Management

<u>Authors</u>: Houston Wilson, Assoc. Cooperative Extension Specialist, Dept. Entomology, UC Riverside; **Chuck Burks**, Research Entomologist, USDA-ARS; **Raman Bansal**, Research Entomologist, USDA-ARS. **Dylan Tussey**, Postdoctoral Scholar, Dept. Entomology, UC Riverside; **Jean Liu**, Postdoctoral Scholar, Dept. Entomology, UC Riverside; **Nathalie Baena-Bejarano**, Postdoctoral Scholar, Dept. Entomology, UC Riverside; **Sarah Meierotto**, Staff Research Assoc., Dept. Entomology, UC Riverside.

<u>Introduction</u>

The pistachio industry has invested in the development of sterile insect technique (SIT) for navel orangeworm (NOW), leveraging 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 six years, co-PIs Wilson and Burks have led scientific efforts to evaluate quality and performance of sterile NOW produced by this facility. Initial studies documented poor performance, and subsequent efforts focused on identifying and fixing key bottlenecks in the production, transportation and release processes. Research in 2023 was focused on evaluating field performance of a new strain of sterile NOW, the use of x-ray sterilization, new external markers for NOW, radiation damage to spermatophores, and pheromones for sex separation, along with more basic research on NOW biology/ecology necessary to address other production limitations.

<u>Results and Discussion</u>

Objective 1 – Verify and optimize x-ray sterilization of NOW: *<u>[X-ray Sterilizing Dose and Inherited Sterility]</u>* NOW were irradiated with increasing doses of x-ray radiation and then paired with non-irradiated moths to determine dose-sterility response. Results showed that egg survival decreased at irradiation levels >200 Gy (females) and >300 Gy (males), although some residual fertility was observed. Data suggest that both the fully sterilizing dose and inherited sterility in NOW require higher doses than previously thought. *<u>[Performance of X-ray Irradiated NOW]</u>* Results suggest limited impacts of x-ray irradiation on moth flight ability, longevity, mating frequency and egg production, with only some negative impacts observed on females at very high rates (i.e. >300 Gy).

Objective 2 – Improve Methods to Monitor Sterile Male Activity in the Field: *<u>[Develop a Marker for NOW Spermatophores (Bansal/Tussey)]</u>* Mating tables are used to measure mating success of sterile NOW in the field but are time/labor intensive. It may be more ease to determine mating success by examining irradiation damage in spermatophores within mated females recovered in ovibait traps. As such, males were irradiated and spermatophores examined up to 96 hours afterwards for differential gene expression relative to non-irradiated controls. Preliminary data found ~300 genes differentially upregulated in irradiated moths, including typical DNA repair genes such as γH2AX, which may prove to be a good marker. *<u>[Evaluate a New Liquid External Marker]</u>* Fluorescent powders are currently used to mark NOW adults for field studies, but may cause negative impacts on performance. Here, we evaluated the use of a vaporized liquid marker (i.e. "SmartWater"). NOW were marked with different colors (red, green, blue, yellow) and evaluated for uptake and performance. Uptake of the green and yellow dyes was best. Performance of males, but not females, was slightly diminished but overall mortality not affected. Marker retention was studied by placing marked moths in cages in a pistachio orchard and observing over time. While some marker degradation was observed after 1 day (~3% loss), a more significant drop-off occurred at 7 days (~22% loss) and 14 days (~30% loss).

Objective 3 – Evaluate Performance of a New Strain of NOW Developed for Mass-Rearing: *<u>[Field Recapture]</u>* Paired cohorts of the original "PST" and the improved "MCS" strain moths were released in alternating weeks between two small almond and pistachio blocks. Recovery was monitored with traps separately baited with pheromone, ovibait and phenyl proprionate (PPO) lures over a 2-week period following each release. For the second year in a row, recovery of MCS strain moths was ~2x higher than

PST moths in pheromone and ovibait traps, but there were no differences between PPO traps. *[Mating Success]* Mating tables were set out in the blocks for the first three nights after each release event. Each table was separately loaded with an unmated female from the mass-reared MCS, mass-reared PST, or locally-reared PST strain. While the locally-reared females performed best, mass-reared MCS and PST females did successfully attract wild males. Males of both mass-reared strains were recovered equally across all strains of sentinel female, and their presence in the tables tended to increase on each successive night, suggesting they may need to adjust to local conditions initially following release.

Objective 4 – Evaluate Dispersal of Sterile NOW in a Large Block Setting: We measured dispersal of sterile NOW across three 640-acre pistachio blocks. At each site, sterile NOW were released weekly over a 160-acre section and the monitored with a grid of traps over the entire 640-acre block. Data from this study are still being processed, so there are no findings to report at this time.

Objective 5 – Evaluate Impacts of Sterile NOW on Wild NOW Populations and Crop Damage in a Large Block Setting: For a third year, we compared NOW populations and crop damage in three pairs of 160-acre almond blocks with and without weekly release of sterile NOW. While data on NOW abundance are still being processed, crop damage both to nonpareil and pollenizers did not appear to be influenced by the addition of sterile NOW. That said, release of sterile moths was not initiated until ~July 15 due to production limitations and mechanical issues with the release aircraft.

Objective 6 – Effects of environmental variables (cold, motion) on NOW field performance: *[Pheromones to Prevent Mating after Adult Emergence]* Wwe used an olfactometer to evaluate our ability to separate male and female moths using pheromone. As expected, males were attracted to pheromone lures, although not all males responded and a small proportion of females moved towards the pheromone. We then tested male response to different concentrations of a 4-component pheromone blend, finding the highest responses at 10,000 and 100,000 ng. *[Effect of Moth Size on Mate Selection]* While mass-reared moths can locate and mate with wild NOW, there may be bias due to size differences relative to wild moths. Preliminary data suggest that NOW females tend to select males with shorter heads and longer forewings, whereas males selected females with shorter forewings. More trials are needed to identify if these preferences are consistent. *[Timing of NOW Emergence and Flight Readiness]* To improve the moth collection process, we studied the timing of NOW adult emergence from pupae as well as time needed after to fly. Eclosion was highest between 18:00 - 21:00 hrs and moths were first capable of upward flight at ~30 minutes after emergence. *[Extending Adult Longevity and Vigor]* A small-scale cold collection system found similarly satisfactory collection performance regardless of whether NOW adults left the emergence chamber through an exit port at the bottom or top of the chamber. A local capacity to collect moths under more gentle conditions will provide better baseline data to document their capacity for field performance.

Objective 7 – Use sterile NOW to compare recapture in mating disruption and non-mating disruption fields: *[Timing of NOW Release]* NOW adults (MCS strain) shipped overnight from Phoenix were used to compare releases in either the morning or evening. Preliminary data indicate that recapture was far greater for moths released shortly before sunset compared to those released shortly before sunrise.

Conclusion

Studies over the past six years have shown that the mass-rearing process negatively impacts NOW. The new MCS strain was developed to better tolerate mass-rearing, and over the past two years we have shown that it performs better than the previous PST strain. We have also developed new markers to improve our ability to study mass-reared/sterile NOW under field conditions, and our work on emergence/flight timing will help improve collecting protocols within the mass-rearing facility. The use of x-ray and ebeam irradiation are promising alternatives to gamma radiation, but more work is needed to refine them. Field studies on sterile NOW impact and dispersal were hindered by production limitations and mechanical problems with the release aircraft. All of the above studies are either still underway or will be repeated in 2024.

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.

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

Authors: Houston Wilson, Assoc. Cooperative Extension Specialist, Dept. of Entomology, UC Riverside; **Jocelyn Millar**, Professor, 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 or brown spot 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, attractant-baited trapping systems 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 *L. zonatus* pheromone and were able to demonstrate its ability to attract LFB adults in the field. Since then, work in 2022 and 2023 has focused on efforts to (1) improve the efficiency of the synthesis process and (2) compare different blends and ratios of possible pheromone compounds – all aimed towards development of an operational pheromone lure for LFB. We have also started to work with a private company (Sterling International, Spokane, WA) to explore production of a pheromone lure at scale.

Results and Discussion

[Further Improved Synthesis of Leptotriene] In past years, we showed that sexually mature adult male *L. zonatus* produce ~10 volatile compounds, including "leptotriene", a compound entirely new to science. Leptotriene is a 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. 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, and the initial lab and field trials produced promising results (see Executive Summary for Millar et al. 2021). Work in 2022 and 2023 focused on careful optimization of the synthesis conditions, which resulted in a ~five-fold increase in the yield, providing more material for bioassays. In 2023, we also explored the use of "technical grade" leptotriene, which may present opportunities for further cost savings because its preparation is less time- and materials-intensive. Additionally, we collected volatiles from two other *Leptoglossus* species to determine the extent of overlap of their pheromones with *L. zonatus*.

[Field Trials with Candidate Lures] For a second year we conducted field trials to evaluate leptotriene, both alone and in combination with other components, as well as compare pure and technical grade leptotriene. Lures were formulated at UC Riverside and shipped to Kearney Ag. Center for testing. In all orchard trials, lures were deployed in black hanging-panel traps coated with fluon, with 5 replicates per treatment at each site in each trial. Lures were replaced every 2-4 weeks and traps were serviced weekly. The first trial measured LFB response to the compounds in three almond orchards during the typical spring colonization period (4/3/23 – 5/18/23). Treatments included leptotriene, leptotriene + aldehydes, leptotriene + bergamotene, leptotriene + aldehydes + bergamotene, and a solvent only (hexane) control. This is the third iteration of this experiment, which was previously conducted in almonds (spring) and pomegranates (fall) in 2022. Data presented here are a summary across these three trials. Results suggest that regardless of crop type or time of year, leptotriene was more attractive than the hexane control, and the addition of aldehydes and bergamotene in combination led to even greater attractancy (Figure 1). The second trial evaluated technical grade to pure leptotriene in an almond, pistachio and pomegranate block at the Kearney Ag. Center from 7/5/23 to 11/7/23. Results suggest that the technical grade leptotriene is as attractive as pure leptotriene (Figure 2). The third trial compared the use of rubber septa to vial lures in a pomegranate orchard at the Kearney Ag. Center from 9/23/23 – 11/28/23. Results found that both septa and vials worked well as a carrier for leptotriene (data not shown).

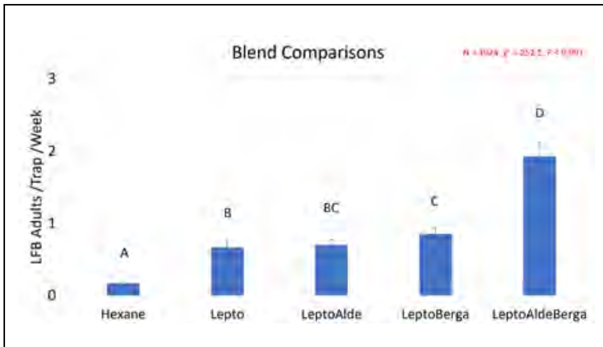


Figure 1. Leptotriene was more attractive than the hexane control, and this effect was enhanced with the combined addition of aldehydes and bergamotene.

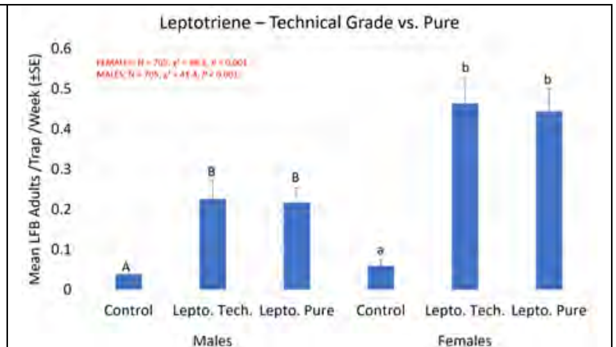


Figure 2. Technical grade leptoriene, which is cheaper and quicker to produce, was equally attractive to pure leptotriene.

[Commercial Synthesis and Traps] Field trials with trial commercial lures and traps were conducted in spring 2023 in three almond orchards as described above. The first experiment compared different candidate commercial lures deployed in experimental pyramid traps. Surprisingly, none of the commercial iterations of the lure were attractive (data not shown). The second experiment compared the pyramid to the panel trap using a leptotriene lure generated by the Millar Lab. Results demonstrated that the panel trap outperformed the pyramid trap (data not shown).

Conclusions

Having identified and synthesized all possible components of the *L. zonatus* pheromone, we spent the past two years increasing the efficiency of production and optimizing the lure blend, and started to work with pheromone companies to see if an affordable lure can be produced at scale. Confirming what we saw towards the end of 2021, our assays in 2022-2023 showed that 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 demonstrated the potential for this new monitoring system in orchards. In 2024, we plan to continue evaluating new iterations of the lures, revisit the selection of an optimal trap type, and continue to work with commercial entities to develop an operational LFB trapping system for transfer to growers and pest control advisors.

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

Authors: **Houston Wilson**, Assoc. 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.; **Sarah Meierotto**, Staff Research Association, Dept. Entomology, UC Riverside. **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 (Pyralidae: *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 season, degradation of the hull and/or shell split provides access to NOW larvae. NOW females can likely detect when such changes in the nut begin to take place, which subsequently triggers them to increase egg deposition on degraded nuts. 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 degradation 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.

This NOW project was developed in parallel with another CPRB-funded project (i.e. Blanco-Ulate, Marino, Ferguson) that evaluates the relationship between the accumulation of heat units and pistachio nut physiology. The goals of our NOW project are to (i) improve our understanding of the textural and chemical changes associated with hull degradation, (ii) document how NOW responds to these changes, and (iii) develop methods to better predict the timing and extent of this hull degradation process.

In our previous studies (2020-2022) we evaluated the influence of two different crop load treatments on textural and chemical changes to Golden Hills variety nuts over time, as well as NOW response to these changes. This initial effort also included work to develop and refine a suitable method for caging clusters and inoculating them with mated NOW females. In this current project (2023) our aim was to evaluate differences in hull degradation between Kerman and Golden Hills, including a comparison of volatile emissions during the day and night, which is likely important because NOW are nocturnal.

Results and Discussion

Objective 1 – Compare Changes in Hull Integrity and NOW Egg Deposition on "Golden Hills" and "Kerman" Variety Pistachios:

[Methods] Experimental work took place in two mature commercial pistachio blocks in west Fresno County (near Cantua Creek). One block was Golden Hills (planted 2010) and the other Kerman (planted 2000). On 5/17/23, insect-exclusion cages were placed over 60 clusters in each block using methods developed by our previous CPRB project. Briefly, window-screen material was used to construct small cylindrical cages (60 cm L x 20 cm W) that were then placed over single clusters and sealed with a metal twist-tie. A subset of 10 caged clusters in each block were then each inoculated with five mated NOW females on 7/13/23, 8/17/23 and 9/14/23. On each date, prior to inoculation, ten nuts were removed from each cluster and sent to collaborator Blanco-Ulate and co-PI Wang for textural and chemical analyses, respectively. An additional sample of nuts was collected from each cage that night around 02:00 hrs for additional chemical analyses, since there may be unique volatiles given off at night that NOW (which is nocturnal) may respond

to. After collecting the nuts, mated NOW females were then introduced into the cages and allowed to deposit eggs over a 1-week period, after which the cage was removed from the orchard to determine total egg deposition onto the cage material, leaves, twigs and nuts.
[NOW Egg Deposition] Egg deposition onto the nuts tended to increase over time on Golden Hills (n=29, χ^2=468.0, P <0.001) but not Kerman (n=30, χ^2=3.5, P=0.18) (Fig. 1)
[Nut Texture Data] Samples taken on later dates tended to have softer hulls (Kerman: $F_{2,86}$ = 96.9, P <0.001; Golden Hills: $F_{2,87}$ = 32.8, P < 0.001), which is not unexpected. Hull texture tended to be lower on Golden Hills variety, with differences between the two varieties observed on each sample date (July: $F_{1,58}$ = 17.0, P < 0.001; August: $F_{1,57}$ = 6.1, P = 0.02; September: $F_{1,58}$ = 14.1, P = 0.04) (Fig. 2).
[Nut Chemical Data] Formal analysis of volatiles produced by the nuts is still underway, but we can report that the production of volatile organic compounds generally tends to increase over time and some volatiles appear to be differentially expressed during the day and night. More than 25 unique compounds have been characterized to date, some of which either increased or decreased with each successive sample, or at night, resulting in a unique and shifting bouquet of volatiles over time and within the day.

Figure 1. NOW Egg deposition increased over time on Golden Hills but not Kerman variety.

Figure 2. Hull texture tended to decline over time in both varieties, but was generally higher on Kerman relative to Golden Hills.

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, which seem to vary between the day and night. While we will repeat this study in 2024 to confirm our findings, additional efforts are now being made to determine NOW response to specific volatiles using an electroantennogram, as well as explore the use of new sensors to monitor key volatiles in the orchards.

Impacts of Sheep Grazing on Pistachio Orchard Sanitation

Authors: Houston Wilson, Assoc. Cooperative Extension Specialist, Dept. of Entomology, UC Riverside; **Joel Siegel**, Research Entomologist, USDA Agricultural Research Service; **Joseph Coehlo**, Agronomy Manager, Maricopa Orchards; **Johnny Etchamendy**, Owner, Mendi Ag Services LLC.

Introduction

Navel orangeworm (*Amyelois transitella*) (NOW) is the key pest of pistachio. Adults oviposit on nuts and the larvae develop on the kernel, which negatively impacts nut yield and quality, and is associated with the presence of *Aspergillus* fungi, which can lead to increased levels of aflatoxin, a known human carcinogen that is heavily regulated in key markets. Winter sanitation is the foundation of NOW management in pistachio. This involves removing remnant "mummy" pistachio nuts from the tree canopy and berms, aggregating these nuts in the row middles and then destroying them with either mowing or tillage. The ability to adequately sanitize tree nuts is a function of weather conditions crossed with the availability and cost of labor and equipment. Furthermore, of all the tree nuts, pistachios are arguably the most difficult to sanitize, due to their small size and durability relative to almonds and walnuts.

Recently, the use of animal integration in orchards has become part of a broader effort to promote orchard sustainability. In fact, some growers are experimenting with the use of sheep grazing during the winter/spring period to promote soil health and control resident weedy vegetation. In tree nut orchards, winter/spring grazing may also contribute to crop sanitation, as the animals may potentially degrade, destroy or directly consume mummy nuts on the orchard floor. As such, we proposed an initial small-scale study to evaluate the impacts of sheep grazing on remnant mummy nut densities in pistachio orchards.

Results and Discussion

[Methods] This experiment took place in a 640-acre block of mature pistachios (Golden Hills, planted 2011) in west Fresno County (near Cantua Creek). The orchard was subdivided into 44 plots (~14 acres/plot with 25 rows x 75 trees/row) and then 10 plots were randomly selected for the study. Prior to grazing in each plot, we measured the total abundance of remnant mummy pistachios and groundcover biomass at 10 randomly selected sample points within the plot. Mummy nut abundance was quantified using a 50 x 50 cm quadrat that, at each sample point, was placed (i) adjacent to the tree, (ii) in the row middle adjacent to the tree, (iii) on the berm in the middle of two trees and (iv) in the row middle between two trees. At each position, all mummy nuts were collected and then brought to the lab to determine if the nut was old or new as well as whether it was filled or empty (i.e. a blank). At the same time, at each sample point, a 50 x 50 cm quadrat was also used to measure groundcover biomass by sampling (i) on the berm between two trees and (ii) in the row middle between two trees. For each biomass sample, all vegetation was removed and brought to the lab to be dried and weighed. Following this initial sampling, the experimental plot would then be grazed with approximately 900 head of sheep (~64 sheep/acre) ("Dorper" breed) for a 3-day period. After grazing, the same measurements of mummy abundance and biomass would be collected again from the plot using another set of 10 randomly selected trees.
[Abundance of Remnant Mummy Nuts] Sheep grazing reduced the overall number of remnant mummy pistachio nuts in the plots (n=633, χ^2=398.3, $P < 0.001$) (Fig. 1). The sheep did not appear to differentiate between filled and empty nuts, which were both equally impacted by grazing (filled: χ^2=471.7, $P < 0.001$; empty: χ^2=73.7, $P < 0.001$). Similarly, both old and new nuts were equally consumed by the sheep (new: χ^2=18.9, $P < 0.001$; old: χ^2=685.6, $P < 0.001$). There were some interaction effects though, with negative impacts on mummy abundance observed for both empty (χ^2=143.9, $P < 0.001$) and filled (χ^2=902.5, $P < 0.001$) old nuts, as well as empty new nuts (χ^2=23.8, $P < 0.001$) but not filled new nuts (χ^2=2.1, $P = 0.15$). While the latter is surprising, given that filled new crop nuts likely have the highest nutritional value, it may simply be due to the extremely low density of these nut types in the experimental orchard (Fig. 2).

On average there was about a 25% reduction in mummy densities following this grazing regime. Impacts tended to be highest on the most abundant nut types in the plots (Fig. 3), likely because they were easier for the sheep to find and consume.
[Groundcover Biomass] Grazing also reduced groundcover biomass (n=187, χ^2=40.1, $P < 0.001$) (Fig. 4), suggesting that sheep grazing could contribute to both crop sanitation and weed control.

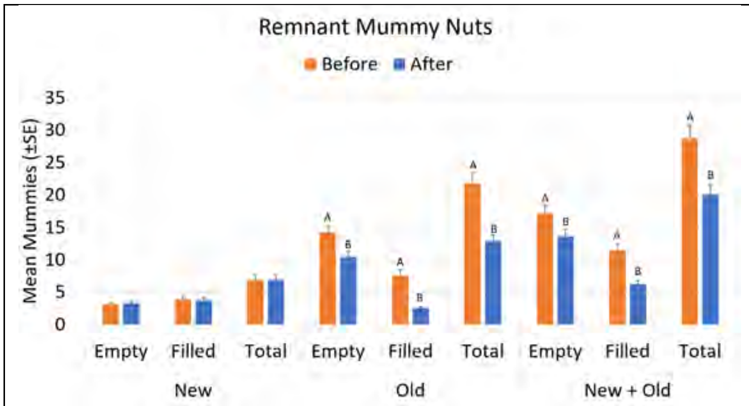


Figure 1. Grazing led to reductions in various types of remnant mummy pistachio nuts.

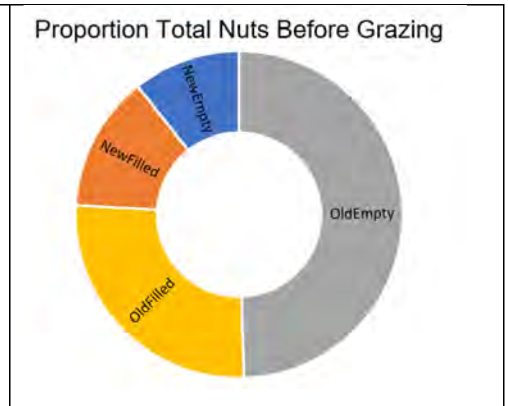


Figure 2. Plots mostly contained old blanks, followed by old filled nuts.

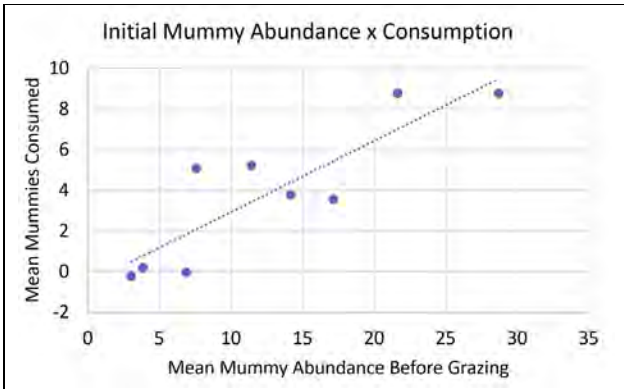


Figure 3. Consumption tended to be highest on the nut types that were more abundant prior to grazing.

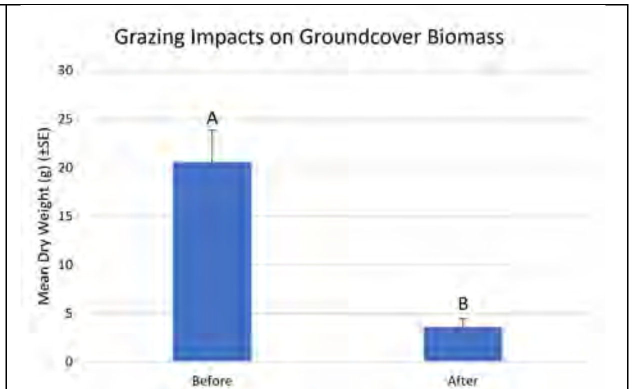


Figure 4. Grazing had a strong negative impact on groundcover biomass.

Conclusions

Orchard sanitation is the foundation of NOW management in tree nuts. A recent survey of NOW management in California (Gordon et al. 2023) found that the main impediment to sanitation was inclement orchard conditions, as well as cost and availability of equipment and labor. Here, we evaluated the use of sheep grazing as an alternative approach to crop sanitation. Sheep can enter an orchard and graze under conditions that would typically prevent the use of heavy equipment, and their grazing appears to reduce the abundance of both mummies and weeds. There are likely benefits to soil health as well from their manure and hoof activity. Of course, there are potential risks around food safety, which is currently be explored into another project (funded by USDA) with Wilson as co-PI. For this current CPRB-funded project, it will be important in Year 2 to further explore different stocking rates and grazing times, as well as to develop an economic analysis to compare grazing with more conventional practices for sanitation, weed control and crop fertility.

Causal agents of and factors influencing ochratoxin A contamination in California pistachios

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; **Apostolos Papagelis,** Lab Assistant, UC Davis & UC KARE; **Joel Siegel,** USDA/ARS, San Joaquin Valley Agricultural Research Service, Parlier; **Themis J. Michailides,** Principal Investigator, UC Davis & UC KARE, Parlier

Introduction

Ochratoxin A (OTA) is a naturally occurring mycotoxin produced by *Aspergillus* and *Penicillium* species on a wide range of foods and beverages. OTA is an IARC class 2B carcinogen and a possible nephrotoxin. Due to several reports indicating high levels of OTA in pistachio nuts in the past decade, the European Union (EU) began regulating OTA at 5 µg/kg (ppb) in ready-to-eat pistachios from January 1st, 2023. Pistachio is a high-value crop with the US being the world's largest producer and exporter of these nuts. California (CA) contributes nearly 99% to the total pistachio production in the US. Pistachios are susceptible to infection by *Aspergillus* species and subsequent contamination by OTA. OTA analysis of CA pistachios (n=809) during 2018-2021 detected 18% of the samples with >5 ppb OTA. The current report summarizes the identification of etiologic agents of OTA contamination of pistachios in CA, along with highlighting the role of early splitting of pistachios, navel orangeworm (NOW) damage, and temperatures prevalent during the growing season towards contamination. Levels of OTA detected in library samples obtained from the year 2022 are also reported. The results provide insights into the sources of OTA contamination of pistachios in CA and its mitigation.

Results and Discussion

Pistachio library samples from 2022 (n = 281) averaged 8.1 ppb OTA ranging from <2 to 282.3 ppb. Twenty-two percent of the samples exceeded of 5 ppb and 1.4% exceeded 100 ppb OTA. Library samples from first-shake harvest (n=162) were contaminated with lower levels of OTA than those from re-shake harvests (n=119) (6.56 versus 10.19 ppb, respectively); however, these differences were not statistically significant (p>0.05). The highest levels of OTA contamination were recorded in re-shake samples for the 2022 growing season and previous seasons (2018-21) analyzed. This indicates that pistachios that are not harvested during the first shake harvest are exposed to extended periods of infection and contamination in the orchard and have a higher risk of OTA contamination.

Based on fungal isolations from soil, leaf, early split, and healthy pistachio nuts collected from orchards in Butte (n=5), Fresno/Madera (n=3), and Kern (n=6) counties in the year 2021, we had identified yellow aspergilli from *Aspergillus* section *Circumdati* as the causal agents of OTA contamination of pistachios in CA. Although boscalid-resistant isolates of *Aspergillus* section *Nigri* were more prevalent compared to section *Circumdati,* isolates of the former produced an average of 3.8 ppb OTA in inoculated pistachios while, OTA contamination by section *Circumdati* averaged 11,729 ppb.

Molecular analysis of section *Circumdati* (n=85) using partial sequences of β-tubulin (0.5 kb) and calmodulin (0.6 kb) genes resolved isolates *A. ochraceous* (33%), *A. melleus* (28%), *A. bridgeri* (21%) and *A. westerdijkiae* (19%), whereas all boscalid-resistant black aspergilli were identified as *A. tubingensis* (low OTA producer). *Aspergillus westerdijkiae,* which was mainly recovered from early split nuts, produced the highest levels of OTA in inoculated pistachios (mean=47,401 ppb), followed by *A. ochraceous* (mean=9,601 ppb) and *A. melleus* (mean=3,327 ppb). *Aspergillus ochraceus* was isolated from early split, healthy nuts, and soil, whereas *A. melleus* was solely isolated from soil. *Aspergillus bridgeri,* recovered from early split nuts and soil, did not produce detectable levels of OTA in pistachios. Isolates of *A. ochraceus* originating from early split nuts produced significantly higher levels of OTA than those from soil (15,491 vs 8,289 ppb; $P<0.05$). Overall, OTA production and molecular analyses demonstrated that *Aspergillus* section *Nigri* plays little or no role in OTA contamination of pistachios in CA, whereas the yellow aspergilli, particularly *A. westerdijkiae,* and *A. ochraceus* are important causal agents of OTA contamination. Additionally, isolates from early split pistachios produce higher levels of OTA than those present in the soil. Based on the results from the 2021 season, early split nuts were sampled from 19 orchards in 2022 season from Kern County. A total of 240 yellow aspergilli isolates were recovered from early split nuts collected from eight orchards. Evaluation of 150 representative isolates showed that isolates produced an average of 26,112 ppb (1,683-61,088 ppb) OTA, with 82% isolates producing >10,000 ppb OTA. Yellow aspergilli that produced <5,000 ppb OTA in inoculated pistachios may be potential *A. melleus*, which was not detected from early split nuts in the 2021 season. Adequate irrigation can reduce early splitting in pistachios and is highly recommended.

NOW is an important pest in pistachio orchards. We hypothesized that pistachio nuts infested by NOW support higher levels of OTA. We conducted field experiments in summer 2023 between 19th July and 31st August by first inoculating individual pistachio clusters with ~50 NOW eggs, followed by fungal inoculation seven days later for a total of four time points. Pistachio clusters inoculated solely with fungal inoculum served as controls. The highest OTA-producing isolates of *A. westerdijkiae* and *A. ochraceus* were used in the experiment. Each treatment was replicated four times and pistachios were harvested on 28th September (four weeks after the final NOW inoculation). OTA contamination could only be detected for inoculations done on and after 17th August (3rd time point). NOW inoculated pistachio clusters had higher OTA levels than those inoculated only with the fungus (51 vs 35 ppb and 153 vs 137 ppb for *A. ochraceus* and *A. westerdijkiae, respectively* at 3rd time point and 79 vs 52 ppb and 190 vs 178 ppb for *A. ochraceus* and *A. westerdijkiae, respectively* at 4th time point).

Environmental conditions optimal for OTA production in pistachios were identified by evaluating the OTA-producing ability of *A. westerdijkiae, A. ochraceus*, and *A. melleus* (n=3 each) at 15, 20, 25, 30, and 35ºC in triplicates. The highest level of OTA contamination in inoculated pistachios was detected at 25ºC (mean=15,731 ppb), followed by 30ºC (mean=12,321 ppb) and 20ºC (mean=7,199 ppb). While *A. ochraceus* and *A. melleus* failed to produce OTA at 15 and 35ºC, *A. westerdijkiae* produced an average of 2,689 ppb and 350 ppb OTA at these temperatures, respectively. Furthermore, *A. westerdijkiae* produced the greatest levels of OTA at each of the five temperatures evaluated, highlighting the ability of this species to contaminate pistachios with OTA under a wide range of temperatures frequently occurring during the pistachio growing season in CA.

Conclusion

The results of the current study show that CA-grown pistachios are frequently contaminated with OTA at levels exceeding 5 and even 100 ppb every year. Pistachio harvest times directly influence overall OTA levels, with a higher percentage of re-shake samples exceeding 5 ppb. Fungal characterization, along with their OTA-producing ability under a range of temperatures, suggests that *A. westerdijkiae* is the most important causal agent of contamination. Contamination may further be exacerbated by *A. ochraceous* and *A. melleus* at 20 to 30ºC. Early split nuts are inhabited by highly ochratoxigenic fungi, thus serving as potential source of increased contamination. NOW infestation at later stages of pistachio development may also contribute to increased OTA levels. OTA mitigation across major pistachio-producing regions of CA should be targeted toward the management of early split nuts and control of *A. westerdijkiae.* We are evaluating the potential of the registered biopesticides for aflatoxin management in pistachio orchards along with the non-ochratoxigenic *A. bridgeri* in limiting OTA contamination in pistachios.

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

Authors: **Ramon Jaime,** Project Scientist**; John Lake,** Lab Assistant; **Pummi Singh** Postdoctoral Scholar; **Apostolis Papagelis,** Field Assistant; **Ramon Jaime-Frias,** Lab Assistant; **Victor Gabri,** Staff Research Associate, **and Themis J. Michailides,** Principal Investigator and Professor. All authors at Department of Plant Pathology UC Davis & UC Kearney Agricultural Research and Extension Center.

Introduction

Aflatoxins is a high economical threat to pistachio industry of California due to the risk of product rejection from the market on the basis of strict regulations. They are toxic secondary metabolites produced by fungi in the *Aspergillus* section *Flavi*, including *Aspergillus flavus* and *A. parasiticus*, the most common found in pistachios in California. Aflatoxin contamination needs to be addressed because of the high threat it poses to human health and the considerable economical loss for the grower when contaminated loads are rejected from the market 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, (Navel Orange Worm), reduces risk of aflatoxin, but does not eliminate it. The use of *A. flavus* atoxigenic strain technology is a proven method to reduce aflatoxins in crops. The overall goal of aflatoxin management with this technology is to reduce the overall aflatoxin production potential by the *Aspergillus* Section *Flavi* population in crops with higher rates of atoxigenic isolates.

Results and Discussion

Research on the efficacy of the atoxigenic *A. flavus* strain biocontrol technology to reduce aflatoxin contamination of pistachio in California since the registration of *A. flavus* AF36 in 2012 indicates 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 determining the percentage of displacement of toxigenic isolates by the applied atoxigenic strain(s). A displacement of over 80% will be considered successful. The average displacement in treated pistachios in California has historically been around 70%. Late applications not protecting from early infections by native toxigenic fungi and by other fungi migrating from neighboring not treated areas could be the main causes for the biocontrol not reaching its full potential. Early applications of atoxigenic biocontrol products might increase the efficacy of treatments. Results from the 2022 season indicate that sporulation of AF36 Prevail® is poor (2 grains/m^2) at early applications, improving to 20 grains/m^2 at standard and late applications. On the other hand, Afla-Guard had higher sporulating grains per m^2 at early application (20 grains), but lower at standard (15 grains) and late (10 grains) applications. Aflatoxin contamination of pistachio nuts indicates that harvest from 2022 had lower aflatoxin contamination than the previous year of 2021, with no significant differences in the content or the percentage of samples with aflatoxin above 15 mg/kg. For normal harvest aflatoxin content ranged from 0 to 1.2 mg/kg and the percentage of samples above 15 mg/kg ranged from 0% to 5.0 %, depending on the time of application and biocontrol product used. The higher contamination occurred in the earlier applications with both products (Afla-Guard 1.2 ppb and 5.0 % >15 mg/kg; AF36 Prevail 0.9 ppb and 3.1 % >15 mg/kg), standard application with Afla-Guard (0.8 ppb and 3.1 % > 15 mg/kg), and the control (0.6 ppb and 1.4% > 15 mg/kg). Late applications did not have aflatoxin contamination regardless of biocontrol product applied. Likewise, in the re-shake harvest aflatoxin content ranged from 0.1 to 5.7 mg/kg and the percentage of samples above 15 mg/kg ranged from 0% to 10.0 %, depending on the time of application and biocontrol product used. The lowest aflatoxin contamination in the re-shake harvest occurred in the earlier applications with Afla-guard (0.1 ppb and 0 % >15 mg/kg), and the standard application with AF36 Prevail (0.4 ppb and 0 % >15 mg/kg), while the highest aflatoxin contamination occurred in the control (5.1 ppb and 15.4 % >15 mg/kg) and the earlier applications with AF36 Prevail (5.7 ppb and 10 % >15 mg/kg).

Population data from post-harvest soil samples from the 2022 season indicate that the percentage of displacement, was significantly different, depending in the time of application and the atoxigenic biocontrol product used. Preliminary data indicate that the lowest average incidence of toxigenic isolates occurred in orchards applied with AF36 Prevail at late and standard applications, and Afla-Guard at early applications, while the highest occurred in the control orchards with an average incidence of toxigenic isolates (Table 1). Orchards treated with AF36 Prevail as expected had average incidences of AF36 above 60% with higher incidence at the late application and lower at the early application. The incidence of Afla-Guard in these orchards was minimum, depending on the time of application. Orchards treated with Afla-Guard had the highest average incidence of Afla-Guard in those applied at the early application with unexpected low incidences in the standard and the late applications. Since most orchards were annually treated with AF36, the average incidence of AF36 in orchards treated with Afla-Guard was high compared to Afla-Guard in orchards treated with AF36 Prevail. The highest incidences of AF36 in orchards treated with Afla-Guard was in the late and standard applications, with a lower incidence in the early application. In young orchards not previously treated with atoxigenic biocontrol products, the incidence of AF36 in orchards treated with Afla-Guard was considerable higher (19%), compared to the incidence of Afla-Guard in orchards treated with AF36 Prevail (1%). This indicates that the movement of AF36 from treated orchards to a neighboring orchard is higher than the movement of Afla-Guard. However, AF36 has been treated for several years in the area it might be established in the orchard's soils and have a higher influence in not treated orchards. Currently, both aflatoxin contamination data and population structure data for the 2023 season are underway and will be included in next-season's report.

Table 1. Percent of isolates belonging to the applied atoxigenic biocontrol strains AF36 or Aflaguard, and to toxigenic or other atoxigenic strains in pistachio orchards treated with the biocontrols AF36 Prevail and Aflaguard at different times of treatment. Not treated orchards are also included as controls. Season 2022.

Time of Treatment	Biocontrol Product	Pre-Treatment					Post-harvest				
			Percent of isolates of:					Percent of isolates of:			
		N	AF36	Aflaguard	Toxigenic	Other	N	AF36	Aflaguard	Toxigenic	Other
Early	Mean	154	36.4 b	14.9 a	27.7 c	21.0 a	249	44.2 a	18.9 a	33.7 b	3.2 a
	AF36 Prevail	82	51.2*	1.2*	23.2	24.4	132	60.6*	1.5*	34.8	3.0
	Afla-guard	72	19.4*	30.6 *	31.9	18.1	117	25.6*	38.5*	32.5	3.4
Standard	Mean	172	51.7 a	4.1 b	26.2 c	18.0 a	339	44.0 a	19.5 a	32.2 b	4.4 a
	AF36 Prevail	105	50.8	2.9	25.7	20.6	172	61.6*	1.2*	31.4*	5.8
	Afla-guard	67	53.7	6.0	26.9	13.4	167	25.7*	38.3*	32.9*	3.0
Late	Mean	259	51.7 a	0.4 b	42.9 b	5.0 b	336	53.0 a	8.0 b	34.5 b	4.5 a
	AF36 Prevail	159	47.8	0.6	47.8*	3.8	193	67.9*	0.5*	28.0*	3.6
	Afla-guard	100	58.0	0.0	35.0*	7.0	143	32.9*	18.2*	43.4*	5.6
Mean	Control	73	17.8 c,y	0.0 b,y	65.7 a,z	16.5 a,z	72	19.4 b,y	4.2 b,y	76.4 a,z	0.0 a,z
	AF36 Prevail	346	49.4 z	1.4 y	35.3 y	13.9 z	497	63.8 z	1.0 y	31.0 y	4.4 z
	Afla-guard	239	45.2 z	10.9 z	32.0 y	11.9 z	427	28.1 y	31.6 z	36.3 z	5.0 z

Means with the same letter are not significantly different by Tukey HSD test (a=0.5). Letters a, b, c indicate differences among times of application means and letters x, y, z indicate significant differences among Biocontrol Products means. Asterisks (*) indicate significant differences between biocontrol products within each time of application.

Conclusion

The use of products sporulating better under low temperatures and soil moistures at earlier applications might help increase the efficacy of the atoxigenic *A. flavus* strain biocontrol technology. Displacement data of toxigenic strains by the biocontrol products AF36 Prevail and Afla-Guard at different application times indicate that Afla-Guard might be more efficient at earlier applications, while AF36 Prevail might be more efficient in the later applications. The high incidence of AF36 in orchards treated with Afla-Guard indicates that the treated biocontrol can reside in the applied orchard for at least one more year.

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 yield was collected each season to help estimate total tree biomass and nutrient demand.

Results and Discussion

Preliminary data shows that in field trials established in two orchards with 10-year-old ‘Lost Hills’ and 12-year-old ‘Golden Hills’ pistachio trees located in the San Joaquin Valley, the average of 29 lbs of N are removed from the orchard per 1000 lbs of marketable yield (CPC). This value includes all nutrients removed in hulls, shells and kernels, blank nuts and other non-marketable yield per 1000 lbs. As an example, in an orchard with a marketable yield of 4000 lbs/acre, 116 lbs N/acre are removed from the orchard.

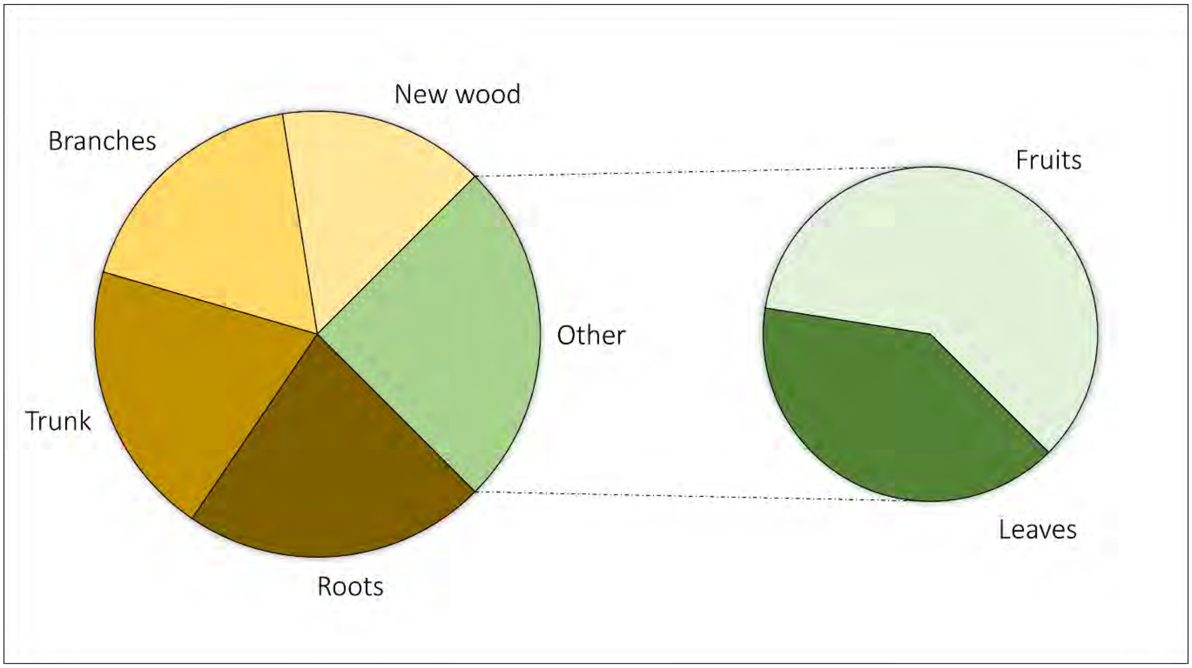


Figure 2. Nitrogen partitioning in mature pistachio trees.

In addition, N is needed to support tree growth requirements. On average, N uptake exceeded removal with fruits and abscised leaves (Figure 2). In an orchard with 120 trees per acre, this corresponds to about 20-24 lbs N/acre. However, most N accumulates in perennial tissues during 'off' years, while the N stored in perennial tissues decreases during 'on' years. The N stored during ‘off’ seasons is remobilized the following 'on' season mainly between early leaf out and early hull split.

At least 90% of nutrients should be applied during the active tree growth period starting in early spring (at 1/2- to 2/3-leaf expansion) and continuing through early hull split. Based on field trials, we recommend applying 25% of the annual demand during leaf-out, 35% during fruit growth, 30% during kernel fill and 10% during fruit maturity or early post-harvest as long as leaves are still healthy and trees are showing symptoms of deficiency. Four or more fertilization events are ideal and should be initiated during leaf-out. Frequent fertigation with smaller amounts of N ensures adequate soil N concentrations for plant uptake while reducing the periods of high N concentration that may be subject to leaching loss in subsequent irrigation or rainfall events.

Conclusion

All decisions of fertilization are influenced by local environment and must be adjusted accordingly. Here, it is important to note that the data shown in this report is a preliminary data, 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.

Saline Irrigation Strategies for Pistachio: Year 2 of 3

Authors: Louise Ferguson, Professor of Extension, UC Davis**; Mukesh Mehata,** Post-doctoral scholar, 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

According to the April 22, 2019, California Water Research Report, 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. These five counties cover about 85% of California's bearing pistachios acreage. Previous research by Sanden, Ferguson, and Marino revealed that pistachio can be produced profitably under moderately saline conditions with rootzone soil salinity (EC_e) between 4.5 and 6 dS/m and irrigation water up to 4.5 dS/m.

For orchards with substantial salt buildup problems, the most common salinity and boron management strategy is to apply 1 to 2 acre-feet of good quality water for winter leaching by flood or sprinkler irrigation. But the challenge is the availability of good quality surface/canal water, which is declining due to continued drought in California. As the availability of good quality canal water for in-season irrigation and dormant season leaching declines, growers are relying more on semi-saline pumped groundwater, particularly in the lower Westside San Joaquin Valley. It would be beneficial to understand if the volume of dormant season leaching could be reduced, if in-season methods of leaching could be effective, and how to use moderately saline water most efficiently for both dormant and in-season leaching.
Currently, advanced monitoring technologies with multiple capacitance sensors can consistently track the available soil water content (AWC) and potentially, salinity, in real-time. This allows real-time monitoring of the wetted root zone through growing season, and the potential to irrigate more effectively to maintain optimal AWC and salinity. In the wetted root zone. The efficacy of high-volume sprinkler or flood leaching during dormancy is well documented. However, the existing calculations for reclamation and leaching fractions were developed under the assumption of one-dimensional 100% surface wetting, where water and salt move directly downward.
The relative capacity of single and double-line drip systems to achieve two-dimensional leaching needs to be investigated. It is not known which, a single or double-line drip system, delivering the same amount of water in the same amount of time, is more effective in reducing soil salinity and boron levels in the wetted root zone. Similarly, in-season leaching fractions and small volume dormant leaching have not been investigated. To answer these questions, we are investigating in-season leaching fractions with and without dormant-season in-row leaching with double- and single-line drip systems. To fully characterize the effects of the different irrigation regimes we are monitoring the soil water content and salinity, and the trunk daily shrink and swell, with *in-situ* soil sensors and trunk dendrometers, respectively. (Semios®). We are using remote sensing and aerial techniques to monitor the Normalized Differential Vegetative Index (NDVI), water stress, canopy growth, and chlorophyll content. This real time soil and tree monitoring is complimented with soil and tissue sampling, and harvest yield and grading, to determine the effects of the treatments on the soil salinity, tree nutrient status and yield and quality.

Results and Discussion

Two years of this three-year study have been completed. The five irrigation treatments with both single- and double-line drip plots are: (1) Control: in-season irrigation scheduling to meet tree ET; (2) Control + pulsed dormant season leaching; (3) Control + a 15% in-season leaching fraction with every irrigation; (4) Control + a 15% in-season leaching fraction + pulsed dormant season leaching; (5) Control + 3-4 years followed by a major dormant season leaching. These treatments were replicated in five blocks with each block divided into single- and double-line hose configurations. Three of the five blocks were instrumented with a Semios® system that measured irrigation volume, soil water content, soil ion content and trunk shrinkage and growth. The orchard was planted in 2015 with field budded Golden Hills on cloned UCBI rootstocks spaced at 18 x 20 feet. The soil is a silty clay loam saline-sodic Cerrini complex with salinity ranging from 3 to 15 dS/m, pH 7.5- 8.2, boron levels of 3-12 ppm and soil sodium levels ranging from 16 to 130 meq/l (370-3000 ppm). In 2022, year one the irrigation water was 2-3 dS/m. In year two, due to a pump failure, better quality surface water, 0.2 dS/m, was used.

Generally, all leaching treatments (in-season leaching, dormant leaching and the combination of both, applied with both single and double hose lines) significantly decreased soil EC_e relative to the control, reducing EC_e 13-22% relative to their respective controls. All the leaching treatments also effectively prevented boron from accumulating in the soil relative to the control. No clear effects of line configuration, double versus single line, were detected. These findings indicate that treatments are effectively reducing salinity or at least preventing the increases observed in the control. Leaf analysis demonstrated in-season leaching (with/without dormant leaching) significantly increased leaf nitrogen and phosphorus, and reduced boron, leaf levels. Visible leaf "boron toxicity" and boron leaf levels were positively correlated and decreased by in-season leaching treatments. Aerial image analysis demonstrated in-season leaching treatments increased NDVI and chlorophyll content, and lowered tree water stress with the double line hose. Average tree height, canopy circumference, and canopy volume were also larger for in-season leaching treatments with double line. No statistical differences were found among irrigation treatments in yield or quality. Irrigation with a double hose produced significantly higher yields.

Overall, the second-year data produced clear differences among treatments and hose lines. The double line hose more effectively decreased root zone salinity, resulting in better growth, and yield. The in-season leaching was most effective at decreasing salinity in the wetted root zone.

This study also investigated if pistachio yield is impacted by soil salinity, soil boron, leaf boron or percentage of leaf damage. Initial findings indicate that soil EC_e and soil boron were moderately negatively correlated with yield. Also, that as soil salinity rose leaf boron levels decreased. However, leaf boron levels and percentage leaf damage also had a poor correlation with yield. This finding suggests that boron leaf levels and leaf damage may not reduce yield.

Conclusion and Practical Applications

The primary goal of this study is to determine the best irrigation management strategy that help more effectively decreases root zone salinity and boron, resulting in better growth, and yield. After the second season, results demonstrate in-season leaching with double-line drip system has potential to decrease or prevent increases in salinity in moderately saline-sodic soils. As a practical application, we hope to ultimately produce a user-friendly tool for growers to determine the most efficient way to maintain soil salinity between 4.5-6.0 dS/m in the San Joaquin Valley under drip irrigation using moderately saline water supplies, < 4.5dS/m.

Determining non-bearing pistachio nitrogen and phosphorus needs, year 2

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 most commonly required macronutrient 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 is phosphorus. While this nutrient has traditionally not thought to be necessary in 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., accepted for publication in HortTechnology).

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

Results and Discussion

The research orchard was planted in April 2023 to Golden Hills on Duarte clone UCBI-D110, with pollinators Randy and Tejon. The trees will be pruned with a modified central leader system. The experiment was put in a randomized complete block design.

We used four rates of nitrogen and two rates of phosphorus (Table 1). Because we had extra space, we decided to add on an unplanned additional treatment: two years of phosphorus fertilization. The trees in the remaining sections that were not under experimental consideration in 2023 received 2 oz of nitrogen per tree in the same fertilization schedule as treatment 3N. Two ounces of nitrogen was selected, as per the fertilization guidelines in the 2020 Sample Costs to Produce Pistachios suggest 1.8 oz of nitrogen per tree in the first year.

Table 1: Treatments and specific fertilization rates for year 1 of the fertilization trial.

Treatment	Phosphorus application rate (oz P_2O_5/tree)	Nitrogen application rate (oz N/tree)
1N	0	0.75
2N	0	1
3N1P	3	2
3N3P	6	2
3N0P	0	2
4N	0	4
3N1P, 2 years	3 in year 1, 3 in year 2	2
3N2P, 2 years	6 in year 1, 6 in year 2	2

Fertilization began in the first week of May. Trees were given 0.25 oz of nitrogen by hand. The fertilizer was delivered near the base of the tree due to the limited rootzone. After we were sure the trees had established, we moved the fertilization zone slightly further away, but still within a foot of the tree. We used granulated urea fertilizer (45-0-0). After fertilization, the irrigation was turned on and run for no more than two hours to push the fertilizer into the root zone. The treatments that received 0.75 and 1 ounce of nitrogen were spread out between May and July, whereas the 2 and 4 oz N/tree treatments were fertilized weekly; the 2 oz treatment rate was terminated earlier than the 4 oz rate.

The soil was sampled before planting in March 2023, and in late November-early December 2023. At the time of writing this report, we have not received all the results and they will be published in the annual report. Leaves were collected for analysis in July, and calipers 20 cm above the soil line and 90 cm above the soil line were measured in April, after planting, and in November.

There were no significant differences in caliper, either actual caliper (data not shown) or caliper growth (Figure 1), between nitrogen treatments in November. The leaf tissue analysis results also showed no significant differences between treatments. The average leaf tissue nitrogen content was 2.87% and ranged from 2.62 to 3.08%, confirming that the suggested range of 2.6-2.9 leaf tissue nitrogen for rapidly growing pistachio trees is correct.

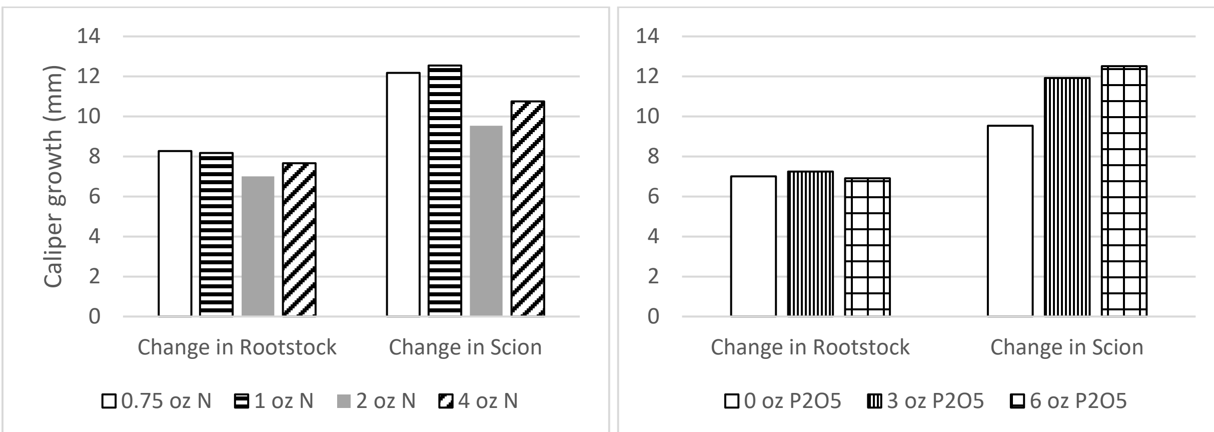


Figure 1: Change in caliper 20 cm above the soil (Rootstock caliper) and 90 cm above the soil (Scion caliper) after one year of growth in the nitrogen rate trial (left) and the phosphorus rate trial (right)

Similarly to nitrogen, the pistachio trees did not respond to phosphorus fertilization in caliper growth (Figure 1). While there were no significant differences between leaf tissue analyses when analyzing the results as an ANOVA, there was a strong trend ($p=0.1$) for increased leaf phosphorus levels with increased phosphorus fertilization. When analyzed as a linear regression, the model was significant ($p=0.041$), however the correlation was moderate (R^2 of 0.595, in other words only 60% of the variation could be explained by phosphorus treatments). This is likely because there was a wide variation in leaf tissue results, particularly in the 6 oz of P2O5 treatment. We can demonstrate that the pistachio trees accumulated more phosphorus in their leaves when fertilized with phosphorus but it did not result in greater growth.

The average leaf phosphorus concentration in July was 0.45%, and they ranged from 0.29 to 0.70. These values are far higher than the reported sufficiency range of 0.14 – 0.17%.

Conclusion

It is possible that the current suggested nitrogen rates are too high for non-bearing pistachio trees. This needs to be confirmed in more locations and in different soil types. Fertilization with phosphorus does not seem to improve tree growth in the first year of planting.

Acknowledgements

Thank you to Duarte Nursery for donating the trees, and Jeb Headrick for donating tree stakes. Thank you to Richard Saldate, Baudelio Perez, Kelsey Galvan, and Rito Medina for their work on this project.

Evaluating new training systems for pistachio (2 of 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), the year 2 termination treatment occurred in the 2022-2023 dormancy, and year 3 and 4 termination will be implemented in 2023-2024 dormancy, and 2024-2025 dormant periods respectively. Data collection for each season includes 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 scientifically replicated and randomized designed trials. There is much to 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 has four irrigation cutoff dates with four replicates for each cutoff. The cutoff dates are initiated in mid-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

Research Results: 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. Samples are being 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 in 2020 and 2021 did not influence the concentration of solutes in the scion of trees with either Platinum or UCB1 rootstocks in spring 2021 and 2022. Overnight temperatures did not go below freezing in the fall of 2020 or 2021, the time periods proceeding the bud-break carbohydrate analysis. Freezing temperatures were observed for several consecutive nights in November of 2022. Analysis of fall 2022 and spring 2023 carbohydrate content for different cutoff treatments is ongoing. Measurements of plant water status also found no differences in tree stress levels in response to irrigation cutoff. Tree growth parameters (trunk size, shoot growth, and abundance of preformed leaves) are also undergoing analysis. More information is needed to understand the implications of fall irrigation management strategies on frost acclimation, annual patterns of starch and soluble sugars, and water use needs for young trees late in the year. 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. 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.

Evaluating new training systems for pistachio

Authors: Bruce Lampinen, Professor of Cooperative Extension, 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**,** and **Loreto Contador**, UC Davis, Dept. of Plant Sciences and **Heraclio Reyes**, nurseryman

Introduction

Three pruning trials to investigate alternative training systems for pistachio were established 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 in 2017. 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' on 'PG1' seedling rootstock block. 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.

All three trials were 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 untrained treatment. The conventional training method involves heading the trees at approximately 43 inches and then doing in-season tipping as well as 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. In the modified central leader treatment, trees were headed at approximately 62" and any shoots that elongated more than 18" were dormant headed at 18". No in season tipping was done on this treatment. The untrained treatment was untouched except for removing any branches that were too low or in the way of tractor traffic (but these were removed from all treatments). Irrigation was supplied with double line drip with in-line emitters except at the Kings Site #2 which was flood irrigated. Dataloggers with Watermark and temperature sensors were installed in one replication of each treatment at all three pruning sites. Timelapse cameras were installed in each treatment at each trial and were set to take one photo per day.

Westside Field Station Trials- Additional pruning trials (Kerman and Golden Hills on UCB1 seedling and Platinum 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. Trees did not have enough crop to harvest in 2023.

Results and Discussion

Trial #1 Kings County- In 2023 this trial was in it's 8th leaf. Midday canopy light interception was similar among all treatments in 2023. Trees were mechanically shaken in 2023. A second hand-harvest was done two weeks later. The conventional, modified central leader and untrained treatments produced cumulative yields of 7359, 8862 and 8407 pounds per acre respectively. Percent removal and percent blanks were similar among all treatments in 2023.

Trial #2 Kings County- This orchard was only flood irrigated one to three times each year. Similar to Kings County site #1, in 2023, midday canopy light interception was similar among all treatments. Trees were mechanically shaken in 2023. A second hand harvest was done two weeks later and the cumulative yields for conventional, modified central leader and untrained treatments were 5436, 6116 and 6034 pounds per acre respectively. Percent removal was similar among treatments. Percent blanks were highest in the conventional, followed by the modified central leader and least in the untrained treatment. Since this trial has not been growing vigorously, very little pruning has been done on any treatments for the past two years due to little elongation growth longer than 18”.

Trial #3 Yolo County- This trial was planted in 2018 and utilized nursery grafted trees. There were more problems with leaning trees at this site than at either of the other trials described above. This has been previously observed by others with nursery grafted trees. 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 first dormant season. However, approximately 50% of the shoots on the untrained 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. Midday canopy light interception was similar for the modified central leader and untrained treatments but significantly less for the conventional treatment by 2023. Cumulative yields at the Yolo County trial for the 2021-2023 seasons for the conventional, modified central leader, and untrained trees respectively were 1178, 1792 and 1985 pounds per acre by 2023. Percent crop removal on the first mechanical shake were similar among treatments. Percent blanks were highest in the conventional treatment and similar between the modified central leader and untrained treatments.

<u>Conclusion</u>

Trees in all treatments grew well with similar levels of midday canopy light interception among treatments at all sites in 2023. Although some untrained trees had tops that were bending over (since they were often taller than the stakes), they have largely straightened 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 untrained treatments while both were significantly higher than the conventional treatment by 2023. At the Kings Site #2, the modified central leader treatment had the highest cumulative yield followed by the untrained and conventional treatments. At the Yolo County site, cumulative yields were all significantly different with highest yields in the untrained followed by the modified central leader and then the conventional treatment. Nut removal from mechanical shaking in 2023 was similar among treatments at both Kings County sites as well as the Yolo County site. The total number of pruning cuts per 80 acres over the first 6 years for the conventional, modified central leader and untrained training systems were 1,413,968 cuts, 832,944 cuts, and 0 cuts respectively.

Data collection will continue in all three of the original trials as well as the new trials at Westside Field Station in 2024. We expect the first harvest to occur at the Westside Field Station site in 2024.

Continue investigating the effects of winter cover cropping on radiation balance, soil-water dynamics, and water productivity of mature micro-irrigated pistachio orchards over the crop season 2023

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 in perennial fruit and nut crops continues being encouraged by federal and state agencies through climate-smart financial incentive programs as a floor management practice aimed to mitigate the adverse impacts due to increasing climate variability and climate change.

Early in 2022, the UC Davis project team established a replicated field trials in a mature, 3.0-ac micro-irrigated pistachio orchard located at the UC Kearney Agricultural Research and Extension Center in Parlier, CA, with the 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 radiation dynamics for trees grown with cover-cropped versus clean-cultivated floor (Figure 1).

Figure 1 – Trees grown with cover-cropped and clan-cultivated floor.

At the time the experiment was established, the project team found several studies in the scientific literature documenting beneficial effects of cover crops on rhizosphere ecology, but little information was available on how winter cover cropping and its vegetation residues left onto the soil surface affect the radiation balance and actual water productivity in mature orchards. The present study focuses on medium-tall winter cover crop "Blando Brome" (*Bromus hordeaceus*) and on the effects that active cover crop vegetation growing on the ground and inactive residues mowed and left onto the ground have on the partition of incoming shortwave radiation between photosynthetically active radiation (PAR) and near-infrared radiation (NIR), on the percentages of PAR and NIR that are reflected by the orchard floor, and on the fractions of the reflected PAR and NIR that are finally intercepted by trees' canopy.

These field datasets could help answering a main question raised by pistachio growers about whether winter cover cropping can lead to water productivity gains, i.e., more nut yield per unit of water in the water-limited San Joaquin Valley of California.

Results and Discussion

During the crop season 2023, the UC research team continued the experimental activities and collected comparative field datasets of different biophysical parameters (incoming and reflected radiation, partition

of the radiation between NIR and PAR, midday PAR light interception by the tree canopies, soil moisture, applied water, canopy and soil temperature, etc.) at the cover-cropped and clean-cultivated plots.
The cover crop was terminated and mowed in early May 2023 and the dead vegetation residues were left onto the soil surface for the remainder of the 2023 pistachio growing season. The clean-cultivated control plots were kept weed free throughout the winter, spring, and summer periods through use of herbicides and mechanical weed control. A new double-drip irrigation system was installed on the entire orchard in order to keep the row middles dry over the course of late spring and summer even during irrigation events.
The tables presented below provide the average albedo values (ratio between reflected radiation and incoming radiation) for the total shortwave radiation, PAR, and NIR measured at the cover-cropped plots in April, May, and June 2023.

Table 1 – Average albedo values for total shortwave radiation and for the PAR and NIR components

Total Shortwave Radiation Albedo Averages

Date	Cover Crop	Non-Cover Crop
4-13-2023 to 4-18-2023	0.22	0.22
4-28-2023 to 5-10-2023	0.32	0.22
6-7-2023 to 6-19-2023	0.38	0.23

PAR Albedo Averages

Date	Cover Crop	Non-Cover Crop
4-13-2023 to 4-18-2023	0.05	0.13
4-28-2023 to 5-10-2023	0.06	0.09
6-7-2023 to 6-19-2023	0.11	0.09

NIR Albedo Averages

Date	Cover Crop	Non-Cover Crop
4-13-2023 to 4-18-2023	0.38	0.30
4-28-2023 to 5-10-2023	0.59	0.35
6-7-2023 to 6-19-2023	0.65	0.37

The field datasets collected during the crop season 2023 revealed that the average albedo for the PAR and NIR components of the shortwave radiation are markedly greater for cover-cropped plots compared to clean-cultivated plots, especially during periods when the trees have leaves on. Also, the field data showed that a bit more NIR than PAR was reflected by the cover-cropped orchard floor, which likely led to increased tree transpiration, if soil moisture was available, for dissipating the higher energy intercepted. The relatively higher reflection of NIR than PAR may have been due to the row middles surface being wetted by several rain events, instead of being dry. As a matter of fact, winter and spring 2023 were among the wettest on record, as a result of a number of rainfall events of high intensity and relatively long duration. As such, the radiation dynamics and partition between PAR vs. NIR components were possibly affected by the nearly-constant wetness of the topsoil layer both in the cover-cropped and clean-cultivated plots during spring and early summer.

Measuring evapotranspiration (ET) and crop coefficients (Kc) of well-watered, young pistachio orchards grown on winter cover cropped versus clean-cultivated ground for use in water resource planning and irrigation scheduling

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

In the water-limited Central Valley of California, resource-efficient water resource planning and irrigation management rely on accurate determination of crop evapotranspiration. In the last decade, the pistachio acreage planted in the Sacramento Valley has noticeably increased from about 2,000 acres to 12-15,000 acres. Scientists from the California Pistachio Research Board indicated that pistachio acreage is expanding to the north, but most orchards are smaller than those found in southern growing areas, and most of the growers are new to pistachio production, thus need accurate information and assistance. The main drivers for pistachio acreage expansion in the north Sacramento Valley are: 1) larger availability of water, and better water quality; 2) relatively high boron content in water and soils; 3) more favorable chill conditions.

The larger water availability and the presence of heavier soils encourage growers to adopt winter cover cropping in the new plantings as a common orchard floor management practice to avoid soil compaction and hypoxia. Winter cover crops are usually established from mid to late November to early January to provide soil cover during the winter months (December-January through March). Growers then mow the vegetation cover in spring, leaving residue on the ground during the summer months with the aim to provide mulching function. However, to our knowledge, no research work evaluated how cover crops affect the actual evapotranspiration in commercial nut production orchards, and specifically in newly-planted orchards. Alongside, several irrigation agencies (irrigation districts and GSAs) recently adopted satellite remote sensing (SRS) methods to estimate crop evapotranspiration for SGMA-related assessments and for adaptive strategies of surface/groundwater conjunctive use, but without solid ground-truthing and validation. Relying on SRS-based methods without appropriate validation and ground-truthing may lead to uncertain, unrealistic, and highly inaccurate ET estimates for pistachio, and especially for young orchards, where both the large soil background captured by satellite imagery (due to the small trees), the green vegetation of cover-cropped orchard floor, and the subsequent residues, represent highly confounding factors.

In this multi-faceted water context, growers, farming corporations, and water agencies need reliable and accurate information on ET and Kc of pistachio during the tree development stages up to maturity to improve allocation, delivery, and management of limited and highly-variable water supplies, and devise irrigation scheduling decisions for maintaining profitable pistachio production.

Results and Discussion

In April 2023, one young pistachio study orchard (4th leaf) located in Yolo County was instrumented with two energy-flux measurement stations, each consisting of a suite of sensors for collecting continuous micro-meteorological measurements to determine ET and Kc with the residual of the energy balance method (REB) using a combination of eddy covariance and surface renewal equipment. Specifically, two energy-flux measurement stations were installed at this study orchard, one in a 37-ac block where young trees are grown with winter cover cropped floor, and one in a 37-ac block where the young trees are grown on clean-cultivated ground, respectively (Figure 1).

Another young pistachio study orchard located in Kern County was selected for this project, and it was already instrumented with an energy-flux measurement station since 2022 by the contractor Land IQ, which made the micro-meteorological measurements collected over the crop season 2023 available to the UC Davis research team.

Figure 1 – Energy Flux measurement stations installed in the young pistachio study orchard in Yolo County

Following the original plan of activities, the UC Davis research team has collected continuous micro-meteorological measurements in the two young micro-irrigated pistachio study orchards (Yolo and Kern counties) indicated above along the period from May through November 2023.
The field datasets from the two pistachio study orchards are currently being subjected to a quality control procedure to remove data gaps and unrealistic values of the surface energy balance parameters (Net Radiation, Rn; Sensible Heat Flux, H; and Ground Heat Flux, G). Once the data quality control is finalized, the quality-ensured field datasets will be analyzed and interpreted to determine the values of actual evapotranspiration (ETa) and crop coefficient (Kca) at various time steps (daily, weekly, bi-weekly, monthly and seasonal) for the crop season 2023.

At the same time, the project team has been collecting daily values of actual ET estimated with seven different SRS-based models encompassed within the OpenET Web Platform for the two selected pistachio study orchards for the entire crop season 2023, and is aggregating the ET estimates to the same time-steps considered for the ground-based measurements (daily, weekly, bi-weekly, monthly and seasonal).
The data collection of daily ET estimates from SRS-based models entailed the determination of the footprint area of the ground-based energy-flux measurement stations located in the two pistachio study orchards using state-of-the-art methods, as well as the consideration of an adequate number of pixels from satellite imagery to match the ET station's footprint areas for setting the comparisons between ground-measured ET values and SRS-based ET estimates on the same spatial extent.

The UC project team is also determining the number of days characterized by satellite overpass versus the non-satellite overpass days during the crop season 2023, and also the number of clear-sky days (cloud-free days) out of the total number of satellite overpass days. These parameters will be necessary in later analyses for comparative evaluations based on statistical parameters.

Once these preliminary determinations will be finalized, the SRS-based ET estimates will be compared against the ground-measured ET values determined with the residual of energy balance from micro-meteorological measurements to quantify discrepancies, uncertainties, and possible sources of errors. Such comparisons will enable to develop recommendations for further validation, correction measures, and potential accuracy enhancements of SRS-based ET estimates as major outcomes from this project.

It is worth it pointing out that the UC Davis research team accrued some delays in the execution of this project over the course of 2023 due to a temporary reduction in capacity (loss of a project collaborator who was hired by a State Agency in February 2023), which did not allow progressing as originally planned. In consideration of that, the Project PI will request a No-cost Time Extension to the Pistachio Research Board in order to conduct and finalize all the planned activities.

Pistachio Improvement Program

Authors: **Pat J. Brown**, Assoc. Professor; **Ivan Bermudez**, Grad. Student; **Chuck Leslie**, Specialist; **Franklin Lewis,** Asst. Specialist; **Laia Menendez Diaz**, Jr. Specialist; **Kristina McCreery**, Asst. Specialist; and **Zach Uebelhor**, Jr. 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 genotyping and phenotyping technologies are used cost-effectively to cull inferior individuals earlier and more accurately, enabling field costs to be concentrated on replicated trials of superior individuals.

Results and Discussion

Germplasm blocks in Davis and Wolfskill: Most grafted trees in the Davis and Wolfskill germplasm blocks have matured enough to begin flowering yearly. Nut yield in these blocks is low, especially at Davis, due to bird and squirrel pressure. Mesh bags constructed from pet-resistant screening material were used successfully for the first time this year to exclude pests from controlled crosses. Hedging of the USDA-NCGR Pistacia blocks A-D and the departmental pistachio germplasm block (9 rows of pistachios left over from the Parfitt/Kallsen breeding program) was performed in February 2023. Because own-rooted *P. vera* trees in the USDA-NCGR C and D blocks ae succumbing to an unknown soil-borne pathogen, we continued budding surviving trees onto UCB-1 rootstocks.

Seedling blocks at Wolfskill: 2,389 *P. vera* seedlings are being maintained in three blocks planted in 2020 (n=469), 2021 (n=630), and 2022 (n=1,290). These seedlings represent 86 families with a mean size of 28 individuals. Fifty-four of these are full-sib families resulting from controlled crosses, and 32 of these are half-sib families resulting from open pollination of a female. The five female parents represented most often in the seedling individuals are Golden Hills (n=341), Gumdrop (n=310), Lost Hills (n=203), B15-69 (n=158; female parent of Gumdrop), and C2-35 (n=72; female parent of Golden Hills and Lost Hills). The five male parents represented most often in the seedling individuals are Tejon (n=169), Zarand (n=151), B22-20 (n=102), B15-58 (n=88), and Famoso (n=61). Because of uncertainty over farm management, the 2023 seedlings from 2022 crosses were maintained in the shade house this year rather than being planted. 2023 crosses followed a diallel design with 8 female parents and 5 male parents. Crosses from both 2022 and 2023 will be planted into the 2024 seedling block. Fourteen individuals in the 2020 seedling block flowered in 2023; seven of these were males and seven were females, and all fourteen matched the sex predicted by the sex marker. Three quarters of this block was planted to predicted females, suggesting that sex markers will be successful in enriching for females and that males are more precocious than females.

UCB-1 subclone experiment: Ten UCB-1 subclones from three commercial nurseries are being maintained on campus in a small (2-row) field experiment with four 2-tree reps of each subclone in a randomized complete block design with UCB-1 seedlings interspersed between blocks. Two of the ten clones show distinct “bushy” and “cracky” phenotypes, and all 10 appear to be virtually identical across a set of ~50K GBS markers. We note that it is now possible to ascertain genome-wide methylation directly from sequence data, so the same dataset could now be used both to generate a high-quality UCB-1 genome assembly and to investigate a possible epigenetic basis of the “bushy” and “cracky” phenotypes.

Westside precocious seedling trial: This block, inherited from Craig Kallsen in summer 2023, consists of ~600 mature seedlings of P. vera on close spacing. We harvested nut samples from 272 female trees in fall 2023 and measured nut and kernel weight and kernel yield (kernel wt/nut wt). Most (79%) of these females are progeny of the S-51 female. We are collecting kernel oil (%) data using NMR and will pass these samples on to the Diepenbrock lab for determination of oil, protein, and carbohydrate using NIR.

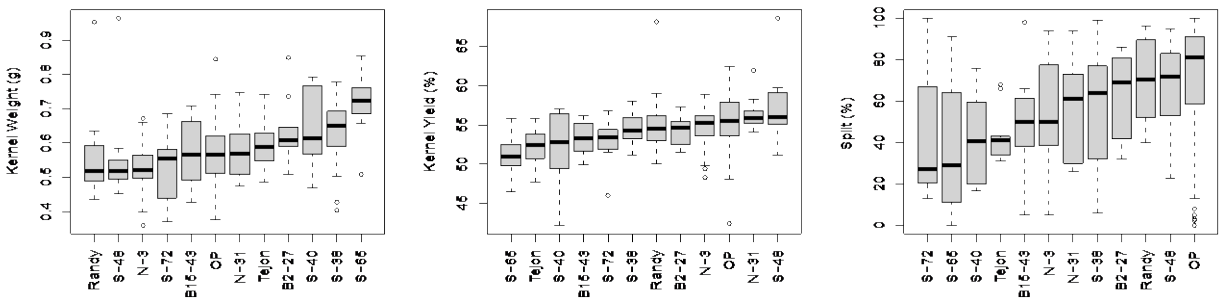


Figure 1. Effect of male parent on kernel weight, kernel yield, and split percentage of S-51 progeny at Westside (n=214). Pedigree data were provided by Craig Kallsen, open-pollinated (OP) progeny (n=93) are shown as a separate category, and the remaining 11 male parents are represented by 6-23 progeny.

In vitro germplasm maintenance and propagation: We continue to maintain microshoot cultures of commercial clones of UCB-1 and Platinum, UCB-1 seedling selections, and additional diverse material. This year we successfully established a microshoot culture of the *P. atlantica* parent of UCB-1. The culture of the *P. integerrima* parent become contaminated and we will re-introduce it in 2023. We are improving our production of UCB-1 seedling selections for future rootstock trials.

Development of embryogenic cultures: Open-pollinated immature kernels of Aegina were collected in early July and are being maintained as three stable and repetitively embryogenic cultures. Embryogenesis was confirmed with both shoot and root development. We are currently multiplying embryo cultures for future testing and distribution to collaborators. Next season we propose to attempt to establish similar embryogenic cultures of UCB-1 seedlings and open pollinated progeny of Golden Hills, to continue testing selection media, and to attempt pistachio transformation and editing experiments.

Development of a chill requirement assay: In the winter of 2022-2023 we scaled up our chill requirement assay to 1600 trees sampled five times for a total of 8000 sticks harvested. We also moved the assay from the growth chamber to the greenhouse in order to accommodate this larger experiment. Results will be presented in more detail at the research meeting in January.

Genetics of internal kernel defect (IKD): In 2023 we received Golden Hills samples for genotyping from Drs. Phoebe Gordon and Giulia Marino. Kernels from Dr. Gordon were germinated *in vitro* to generate seedling leaf tissue for DNA sampling, but the success rate was low due to high rates of contamination. Kernels from Dr. Marino are being sampled directly into plates for DNA extraction. Data will be combined with IKD datasets from 2021 and 2022 to assess whether there is a genetic component to IKD.

Erik Wilkins provided open-pollinated and budwood of "Arena", a volunteer tree on the east side of I-5 with high yield and split percentage in 2023 despite having no irrigation, fertilizer, or neighboring pistachios within at least a mile. Arena has been grafted onto two UCB-1 rootstocks, open-pollinated seed are being germinated for genetic analysis. Predicted females will be saved for planting into available spaces in the 2024 seedling block.

<u>Conclusions:</u> Phenotype and genotype data from mature *P. vera* seedlings can now be used to develop prediction models for choosing both parents and progeny based on genotype.

Collaborative Pistachio Rootstock Breeding

Authors: **Pat J. Brown,** Assoc. Professor, and **Franklin Lewis**, Asst. Specialist, Plant Sciences, UC Davis; **Victor Gabri**, Graduate Student, CSU Fresno and **Themis J. Michailides**, Plant Pathologist, Plant Pathology, KARE; **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 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 2023 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 continued performing crosses in both directions between *P. integerrima* and *P. atlantica* ("UCB-1 type" and "PG-II type" crosses), to further explore this heterotic combination, and 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 also collected open-pollinated seed from *P. integerrima* and *P. atlantica* accessions to serve as negative and positive controls in Verticillium screens. The USDA collection was hedged again in February 2023. Seed yields in the collection were higher in 2023 than in 2022.

Introduction, maintenance, and distribution of micro-shoot cultures of clonal rootstocks: We continue to maintain microshoot cultures of UCB-1 seedling selections, including very early and late leafing selections, to test whether phenology drives vigor and increased stem diameter; and precocious selections from a commercial orchard, to test whether rootstock genetic variation affects precocity in the scion.

Salinity screening: In 2022 we received >1,000 UCB-1 seedlings from Foundation Plant Services, genotyped them using GBS, and identified 237 seedlings with recombination events in one of two genomic intervals containing a locus previously shown to affect salinity tolerance. In summer 2023, recombinant individuals were subjected to increasing salinity (from 0-200 mM NaCl) over a period of 8 weeks in the greenhouse. At the end of the experiment, eight different tissues were sampled: upper, middle, and lower leaves; stem, and bark. Upper leaves emerged during the experiment itself; middle leaves typically showed the most severe symptoms (discoloration and/or spotting); lower leaves were those below a pruning cut made during the winter of 2022-2023. We are currently measuring chloride content in dried samples from this experiment, which we hope will provide insight into the genes and mechanisms underlying these two salinity tolerance loci.

Phytophthora Resistance Evaluation: The relative susceptibility of UCB-1 seedlings and *Pistacia integerrima* x *P. khinjuk* seedlings to Phytophthora crown and root rot was evaluated. Seventy-six potted UCB-1 seedlings were root inoculated on July 25th, 2023 using 100 CC of vermiculite-oat-10%CV8 medium infested with *Phytophthora niederhauserii* (KARE2555) and incubated for 4 months. Plants were flooded for 48 hrs. every two weeks for 1-month. After 1-month, plants were placed in plastic bins so that there was always in 1-2 inches of water near the lower roots, and flooded once a month for the next 3 months. After 4-months the plants were assessed based on survivability and root fresh weight. The same protocol for inoculation was used for the *P. integerrima* x *P. khinjuk* seedlings with inoculation on July 25th. For the UCB-1 seedlings 2 plants out of the inoculated 76 plants died. Variable root weight was seen among the several UCB-1 seedlings with the lowest root weight being 22 grams and the highest being 76 grams, suggesting differences in seedling susceptibility to Phytophthora (Figure 1). As for the *P. integerrima* x *P. khinjuk* seedlings, 3 out of the 14 inoculated plants died, suggesting high susceptibility of these individuals to Phytophthora. Variable root weight was seen among these seedlings with the lowest root weight being 23 grams and the highest being 68 grams (Figure 2).

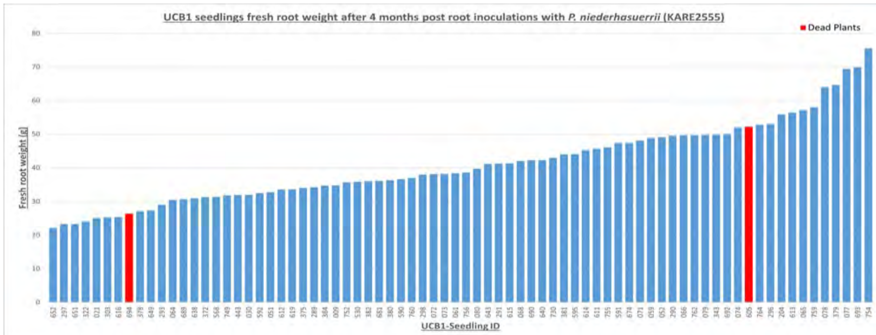


Figure 1. Root weight of inoculated UCB-1 seedlings with *P. niederhasuerrii* after 4 months.

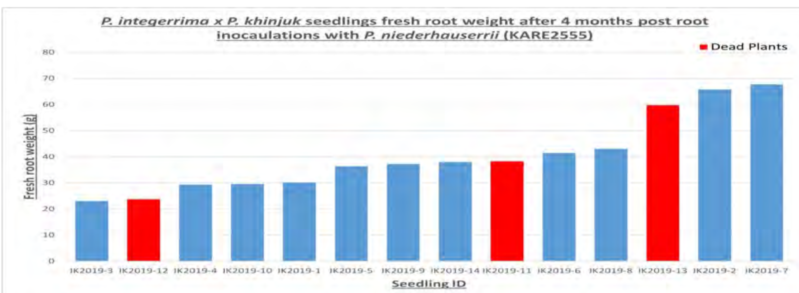


Figure 2. Root weight of inoculated *P. integerrima* x *P. khinjuk* seedlings with *P. niederhasuerrii* after 4 months.

Verticillium Resistance Evaluation: No new accession were provided or inoculated in 2023. However, the seedlings provided to us in 2022 were inoculated with a spore suspension of 1x 10^7 conidia intermixed with 5,000 microsclerotia of *Verticillium dahliae* per ml of suspension. Inoculations were done on 5 July 2023, using the traditional inoculation procedure of seedlings. Briefly, the roots of the seedlings were cut with scissors to create wounds and dipped in the conidial+microsclerotial suspension for about 30-45 minutes. Then, the inoculated plants with the jiffy were planted into large pots and watered regularly. The young seedlings were cut and examined for stem streaking in cross section. Table 1 shows the incidence of infection. These inoculations need to be repeated to confirm results.

Table 1. Seedling infection (%) with conidial + microsclerotial suspension of *Verticillium dahliae*.

Seedling code	Genetic background	Seedlings infected (%)
E-21-1 OP	Open-pollinated seed from UCB-1 tree	0%
B-9-5 OP	Open-pollinated seed from *P. lentiscus*	100%
D-8-2 OP	Open-pollinated seed from *P. integerrima*	0%
A-2-6 OP	Open-pollinated seed from *P. terebinthus*	80% (light streaking)
P20-101	*P. integerrima* X *P. atlantica*	10% (light streaking)
UCB1 seedlings (TJM)	Commercial UCB-1	0%

Conclusions: A root inoculation assay for *Phytophthora* and a conidial+microsclerotial suspension inoculation for *Verticillium* both appear promising. *Pistacia X saportae* hybrids (*P. lentiscus* X *P. terebinthus*) have been previously reported to be resistant to *Verticillium*; however, individual sources of both these species appear highly susceptible in our data.

Clonal propagation of U.C. Experimental Rootstock Selections to develop field evaluation trials with Existing Commercial Rootstocks

Authors: Giulia Marino, Plant Sciences Dept UCD; Franklin Lewis Plant Sciences Dept UCD, **Craig Kallsen**, UCANR; **Pat J. Brown -** Plant Sciences Dept UCD, Charles Leslie- Plant Sciences Dept UCD, **Dr. Dan Parfitt**, Plant Sciences Dept UCD

Introduction

The genetic diversity among the US pistachio scions and rootstocks is extremely limited with the entire California pistachio industry being dependent on only two genetically similar hybrid rootstocks. This leaves the industry vulnerable to unexpected environmental and biotic challenges in the future.

Hence, the overarching objective of this long-term project is to make available new and diverse rootstocks options for the pistachio industry. The project builds on the previous work made by Craig Kallsen and Dan Parfitt. They developed a series of novel clonal experimental rootstocks, called Endeavor, with different parentage than UCB-1. These seedling hybrid rootstocks have demonstrated early yield, lower vigor and pruning requirement, smoother graft-union than UCB1, cold and salt tolerance. In addition, they showed a very high boron tolerance that makes them a valuable option for some of our high boron locations in the San Joaquin Valley. This information is very promising but has been developed so far only in limited trials, with few replicates and seedling individuals. These novel rootstocks should be evaluated rigorously with clonally propagated trees planted in replicated and randomized field trials. The goal for this first stage of the project is to micropropagate these rootstocks to obtain enough clonal individuals to be planted in field trials for commercial evaluation in the future.

Results and Discussion

Three genotypes of the Endeavor 1 class rootstock (#1, #3 and #4) were delivered as in vitro material from Sierra Gold in December of 2022. This delivery comprised one container of the #1, one container of the #3, and two containers of the #4. Information regarding previous work performed with these clones was provided the same week. Each clone required different amounts of work to return to a usable state in the lab after delivery. Materials appeared clean upon arrival but contamination may have been picked up during transport and became apparent days to weeks later. The #3 was unable to be recovered, the #1 was successfully disinfested, and the #4 only required minor media additives to recover.

Budded or grafted material arrived from Sierra Gold in February 2023, with plant survival assessments in April. At the end of May the plants that were confirmed to not have bud take were discarded, and successfully budded plants had UCB-1 growths removed to allow only the Endeavor buds to continue growth. We currently have at least one replicate of all six genotypes established in the greenhouse at UC Davis. Trees in the greenhouse were allowed to grow throughout the 2023 growing season to increase the number of nodes available for future in vitro introductions without affecting tree health.

In May, field material was delivered to UC Davis from West Side, Kearney, and Kern County for introduction of new plant material to tissue culture. Five genotypes (Endeavor1 #1 and #4, and Endeavor 2 #10, #15, and #18) were delivered and nodal segments of each were surfaced disinfested with a solution of 3000 ppm NaDCC for up to 3 hours before introduction to culture. Due to the long travel time of this shoot material, meristem excisions were not attempted. In July a second attempt to introduce Endeavor2 #10 was necessary and sticks were forced in an environmental chamber to later clean and introduce to culture. Endeavor1 #3 was successfully introduced to culture from material located in Davis. Currently, all three Endeavor 1 class genotypes and all three Endeavor 2 class genotypes are in culture and clean with at least one shoot.

Endeavor1 #4 arrived with the largest number of shoots and continues to be the best performing culture. This has allowed us to experiment with etiolated shoots for multiplication and compare their growth and

rooting performance to standard cultures grown under light conditions. The few rooting successes observed thus far have not survived greenhouse acclimation, more work will be necessary in this area.
Endeavor1 #3 and #4 and Endeavor2 #18 are currently in sufficient numbers to experiment with media salt compositions. Continued media improvements are necessary to improve the propagation efficiency of these genotypes that have different parentage.

Figure 1 – newly introduced material (left); best performing rootstock Endeavor 1 # 4 (right) demonstrating that multiplication rates can be improved with shoot orientation changes

Endeavor1 #1 and #4 and Endeavor2 #10 and #15 are not multiplying well and shoot numbers for each genotype are extremely low. Endeavor2 #15 was unresponsive to etiolation and may need to be reintroduced from greenhouse material in the spring. Endeavor1 #1 and Endeavor2 #10 appear to have been slow to stabilize in culture, but seem to be responding well to the new media composition despite the low shoot count. If they continue to multiply slowly, they will also need reintroduction in the spring.

Conclusion
Overall, more time will be needed to have enough material to be planted and grafted in well-bordered, replicated and randomized field trials within commercial orchards for comparison with existing commercial rootstocks. The need to re-introduce in vitro most of the material from mother plants slowed down progress. Considering the higher performance of the Endeavor1 #3 and #4, we are now targeting a spring 2025 planting with these two genotypes, assuming that we develop more efficient rooting protocols. Giving the high genetic variability of these rootstocks with respect to the more widely micropropagated UCB1, adjustment at the growing media and rooting protocols are needed per each single genotype. In the long term, this project will provide more diverse rootstock options for growers, that will make the industry less susceptible to unforeseen stresses including new pests and pathogens, declining winter chill and limited water supplies.

Pistachio Pan-Genome for Accelerated Breeding

Authors: Grey Monroe, Assistant Professor, Dept. of Plant Sciences, UC Davis; **Chaehee Lee,** Postdoctoral Scholar, Dept. of Plant Sciences, UC Davis; **Matthew Davis,** Ph.D. Candidate, Dept. of Plant Sciences, UC Davis

Introduction

The current project marks a significant advancement in pistachio genomic resource development for accelerated cultivar improvement and other foundational pistachio research. This initiative is particularly crucial for addressing the challenges posed by climate change, such as the increasing warmth of winters that negatively affect pistachio yield. We aim to use advanced genomic technologies to generate Pistachio reference genome of 'Kerman' and pan-genome using genetically diverse cultivars to help rapidly develop pistachio varieties that are resilient to these environmental shifts.
A key objective of the project is to establish a high-quality reference genome for the 'Kerman' variety, which is the primary female pistachio tree cultivated in California. Additionally, the project seeks to construct a comprehensive pan-genome of pistachio. This effort extends beyond just analyzing chromosomal genomic data; it involves an in-depth examination of the genetic diversity within pistachio germplasms and key traits vital for pistachio cultivation in California, including the winter chill requirements. A thorough genetic understanding of these traits is crucial for breeding pistachio varieties that can adapt to and thrive in changing climate conditions.

This project is set to generate and integrate these new pan-genomic resources into existing breeding strategies. We are exploring functional genomics encompassing the full spectrum of genes and structural variations found in diverse pistachio genotypes and ultimately contributing to developing new pistachio cultivars that not only withstand but also remain productive under the warmer conditions projected for California.

Results and Discussion

Since the chromosome-scale 'Kerman' genome was generated with chromatin contact information in the previous year (Figure 1), we continued to improve the quality of the 'Kerman' genome. The manual correction was made based on telomere location and pseudo-duplication information on the arm regions of each chromosome. We put extra effort into improving the 'Kerman' genome to a better version and putting all of the findings together with a manuscript being submitted (accompanied by public release of genomic tools) in collaboration with Dr. Barbara Blanco-Ulate lab. We found that one arm of 11 chromosomes contained the highly repetitive 180 bp sequences reaching up to ~9 Mbp, which is very atypical and makes the fully complete 'Kerman' genome extremely challenging, likely limited previous genomic efforts in pistachio. Even genome-wide chromatin interaction information from Omni-C data could not fully resolve these regions likely due to the limitation of read length. Therefore, highly repetitive regions remained to be improved with more genomic data, such as ultra-long reads from Oxford Nanopore, to encompass repetitive regions with longer reads, which provides true biological evidence. Since these repetitive regions do not include any protein-coding genes, the quality of gene annotations for all six pistachio genomes has been still maintained at exceptionally high quality, enabling future functional discoveries for breeding. The updated version of the 'Kerman' genome also improved five additional genomes and macrosynteny analysis shows that six genomes are collinear with substantial structure variation. Since the development of pan-genome construction tools is in its infancy, the construction of sequence-based pan-genome is under way. Nonetheless, a gene-based pan-genome of six pistachio genomes was constructed using the most complete pistachio gene models and presence and absence variation (PAV) in gene content was characterized . We examined the gene PAV and functions of those genes among six genotypes with focus on low-chill cultivars 'Mateur' and 'T-41'. We have been comparing gene PAV and structure variation in 'DORMANCY-ASSOCIATED MADS-BOX (DAM)' and 'FLOWERING LOCUS C' genes, which are known to be associated with chill requirements (CR) in orchard crops.

1. **The comprehensive genomic data for 'Kerman' was complete is currently being published.**
   - The 'Kerman' genome has been signficantly improved
   - The manuscript titled '**In a nutshell: pistachio genome and kernel development**' is submitted to bioRxiv and is in the process of submission to Nature Plants.
   - Comprehensive pistachio genomic data will be publicly available at pistachiomics.sf.ucdavis.edu upon publication.
2. **The pan-genome of diverse pistachio genotypes**
   - Assemblies of 5 additional genotypes are anchored into 15 chromosomes
   - Core (present in all six genomes), dispensable (present in two to five genomes), and private (present in one genome) gene clusters were identified.
   - Genes associated with chill requirements are identified and being analyzed.
3. **Functional annotations of pistachio pan-genome**
   - All six pistachio gene annotations have been functionally annotated.
   - Functional annotation was matched with gene PAV in each genotype.
4. **GWAS panel with agricultural traits**
   - DNA extraction of 153 diverse pistachio accessions with different species in the genus *Pistacia* was completed.
   - Whole genome sequencing of these accessions was submitted for sequencing at 20X depth of coverage.
   - Phenology data (flowering and leafing dates) of these accessions have been analyzed.
5. **The online hub for pistachio genomics resources is being finalized.**
   - Pistachiomics.sf.ucdavis.edu webpage in UC Davis Site Farm.
   - As we progress the submission of 'Kerman' genome manuscript to Nature Plants in January, the web portal will be actively updated with details about the project.

Conclusion

In conclusion, we are publishing an improved version of high-quality reference genome for the 'Kerman' cultivar, the main female tree in California, with a focus on pistachio fruit development as a collaboration with Dr. Barbara Blanco-Ulate lab. This development, coupled with the construction of a comprehensive pistachio pan-genome, has laid a solid foundation for future breeding efforts. The more advanced pan-genome construction tools will be tested to improve pistachio pan-genome. The vast genomic data for a diverse GWAS panel was generated and in-depth analysis on discovering alleles useful for pistachio improvement has been in progress by associating genomic variation with agronomic traits, such as flowering time, using our pistachio pan-genome. Our ongoing analysis of gene presence and absence variation and structure variation associated with important traits underscores the project's commitment to addressing breeding challenges in the changing environmental conditions.

Dissecting the genotypic and environmental basis of kernel compositional traits in pistachio

Authors: Christine Diepenbrock, Assistant Professor, Department of Plant Sciences, UC Davis; **Grey Monroe**, Assistant Professor, Department of Plant Sciences, UC Davis; **Patrick J. Brown**, Associate Professor, Department of Plant Sciences, UC Davis; **Elton Kane**, Graduate Student, Plant Biology Graduate Group, UC Davis.

Introduction

In this project, we are developing calibrations (i.e., prediction models) on a benchtop near-infrared spectroscopy (NIRS) platform for high-throughput phenotyping of compositional traits (protein, fat, starch, total mineral (ash), and moisture %) in pistachio kernels. We are collecting spectral reflectance data on ground kernels in the 400 to 2500 nm range (with 0.5 nm steps) and cross-analyzing those spectra with wet-chemistry reference values on a subset of samples to train calibrations that can accurately predict these traits in the full sample sets of interest. Performance metrics for NIRS calibrations include Global H (the spectral distance of a given sample from the mean sample in the database) and Neighborhood H (the spectral distance of a given sample from the next closest sample in the database), alongside metrics such as standard error of cross-validation and standard error of prediction. We are also simultaneously using the developed calibrations to assess the effects of Genotype, Environment, and Management on kernel compositional traits in California production settings.

Results and Discussion

A total of 74 samples were analyzed from 2022 harvests. These samples included both commercial cultivars and breeding germplasm. More than twice that number of samples was collected in 2023 harvests for analysis this fall/winter, with continued thanks to project collaborators.

Pistachio samples were ground and scanned, and trait values were predicted using a pre-existing 'ground peanut' calibration (which was the best-performing pre-existing calibration available from the NIRS instrument manufacturer, in a pilot study conducted on pistachio samples from a collaborator before the project began). Global and Neighbourhood H values were generally quite high, except on samples of the Kerman genotype from one growing location (suggesting that the ground peanut calibration was more suited for those samples). Further adaptation of the existing ground peanut calibration and/or the development of a custom set of calibrations for pistachio will be needed before the predicted trait values are used in decision-making. The calibration adaptation/development process relies on wet-chemistry reference values; 35 of the 78 samples from 2022 harvests were submitted to the UC Davis Analytical Lab this fall for wet-chemistry reference analyses, and those values are expected to be available by early winter 2024. We will also select samples from the 2023 harvests for wet chemistry this winter/spring based on their spectra and predicted trait values, as it is important that the samples sent for wet chemistry span the relevant range of values for each given trait.

With the caveat mentioned above—that calibrations optimized for pistachio are still being developed—preliminary results based on the samples harvested in 2022 are as follows. The following results will need to be re-examined once wet-chemistry data are returned, once the optimized calibration has been applied, and once having data from the 2023 harvests in addition to the 2022 harvests. Fat and protein % were each found to vary by a few percentage points (on the original trait scale) across these samples. (Protein and fat, alongside moisture, are three of the traits of focus in this project that are available in the pre-existing ground peanut calibration, albeit with suboptimal performance metrics. Additional traits are being brought into the pistachio calibrations by conducting additional types of wet-chemistry reference analyses.) A non-negligible negative correlation was observed between fat and protein % in two of the three environments tested. The effect of sample source (where each sample source specifically represented one or multiple unique growing locations) was significant for protein and moisture. The effect of cultivar was significant for protein.

A subset of the 74 samples that represented three common cultivars was also analyzed (n = 27 samples). In that subset, the effect of cultivar was significant for fat %. The main effects of both cultivar and sample source were significant for protein %, but their interaction effect was not significant. Lastly, the effect of lab source was significant for moisture %.

Principal component analysis was conducted on the trait data (in each of the 74 and 27 samples) to examine major axes of variation in the data sets, and will be informative for understanding the relatedness of these samples in terms of both their predicted trait values and their raw spectra. Post hoc tests (in this case, Tukey's Honestly Significant Difference; HSD) were conducted to compare the means of each cultivar, lab source, or combination thereof for a given trait if the respective term (cultivar, lab source, or their interaction) was significant. If an interaction effect was significant, we only conducted Tukey's HSD for that interaction, and did not conduct Tukey's HSD for either of the main effects (so as to not interpret main effects in the presence of a significant interaction). This analysis will be informative for understanding which cultivars, lab sources, or combinations thereof are exhibiting significantly different values than others.

Major activities for this winter/spring include building and deploying the calibrations once the wet-chemistry reference data have been provided by the UC Davis Analytical Labs. Newly developed or adapted calibrations can be re-run on the spectra of previously scanned samples to re-predict trait values in those same samples, such that re-scans will not be needed. Subsampling (from master sample containers) and analysis of 2023-harvested samples is also underway so that samples from that year can also be scanned and (a subset) sent for wet chemistry, and with data available sooner this year than last. We also plan to roast dry samples provided from a commercial source to emulate a commercial roasting process and examine kernel compositional traits before and after roasting.

Conclusion

Thus far, NIRS appears to be a promising technique that has distinguished cultivar and lab source (representing one or more unique growing locations). Key advantages of NIRS are 1) that collection of sample spectra and provision of predicted trait values is nearly instantaneous once having a suited calibration, and 2) that multiple traits can be predicted simultaneously from those spectra (rather than needing to run a separate analysis/protocol or instrument/platform for each trait). The grinding of kernels for this analysis (and freeze-drying if needed, so that the samples form a homogenous powder upon grinding) is the slowest step involved but can be conducted with reasonable throughput now that method refinement for these steps and for the transitioning of samples into the NIRS workflow has been conducted.

Comprehensive preliminary reports were prepared both within and across sample sources and were shared with the collaborators who provided samples in 2022; the same will be conducted for those who provided samples in 2023. We will look forward to discussing these results in more detail with the California Pistachio Research Board and research and agricultural practitioner communities once the calibrations optimized for pistachio have been developed (such that absolute trait values can be discussed in detail; it is important not to do so until the calibrations' performance metrics are in a favorable range following the adaptation/development process). We are also preparing a manuscript to report on the pistachio calibrations that are in development and the effects of Genotype, Environment, and Management on kernel compositional traits.

We would be glad to receive additional samples from other interested parties from their 2023 harvests if of interest to the readers of this report and/or to others in the research and agricultural practitioner communities.

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

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

Introduction

Since our last executive summary in November 2023, we have conducted significant work identifying metabolites and altered physiological pathways associated with pistachio cultivars treated with Rest Breaking Agents (RBAs) - horticultural oils. Farmers have long used these agents to induce bud breaking for pistachio plants that do not receive adequate chill portions during winter. Despite their widespread use, little is known about the mode of action of these oils or how they affect the metabolism of plants.

To address these questions, we utilized a metabolomics approach at Fresno State. Specifically, we used proton nuclear magnetic resonance (^{1}H NMR) spectroscopy with a recently acquired 600 MHz JEOL NMR spectrometer that the National Science Foundation funded. Our research team has worked to analyze the samples and generate data that can provide insights into how these RBAs work and how they affect the metabolism of pistachio plants.

The research conducted so far has mainly involved the analysis of samples from previous temporal studies carried out by Brar et al. These studies assessed the bud and bark pistachio tissues treated at different spray periods and locations. The executive summary submitted in November last year focused on comparing the Cantua sites for 2019 and 2021. This summary is an update on the work done since the previous summary. It will concentrate on the samples collected at the Madera site in 2019 and 2021, specifically on time-dependent and budswell samples.

Over the past year, 103 samples were analyzed - 64 from the Madera temporal study and 39 from the Madera budswell study. The temporal study involved four spray treatments (S1, S3, S4, and F5) in mid-January, early February, mid-February, and late February, respectively. Ten days after treatment, Bud and Bark Tissue were collected and kept at -80 °C until metabolomics analysis. Similarly, the Madera budswell study collected bud and bark tissue from trees of all spray treatments at the end of March, and like the temporal study, samples were kept at -80 °C until metabolomics analysis.

All the samples were prepared following protocols established previously. Starting from roughly 1000 mg tissues, metabolites were extracted. A 700 uL extracted metabolites with 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 used. The ^{1}H NMR experiments were conducted on a 600 MHz JEOL NMR Spectrometer at 30 °C. Raw NMR data was processed using MestReNova software, which included phase correction baseline correction. The binned spectral data was further processed using metaboanalyst.ca, which involved log10 transformation, Pareto Scaling, and running the partial least squares-discriminant analysis (PLS-DA).

Results

PLS-DA analysis was performed on metabolite data to compare control tissues with their respective spray treatments for each spray interval. There was a slight overlap between the control and treated bud and bark tissues. Still, most controls had distinct clusters, indicating different metabolic profiles than the spray treatments. The temporal studies may help determine how horticultural oils alter the metabolome of pistachio tissues. All the spray treatments except the last one had already been treated for over a month when the budswell samples were collected.

The project has currently gathered and curated 384 independent samples from two different sites, Cantua and Madera, in two additional years, 2019 and 2021. With the increased data, it has become possible to investigate the effect of two treatments at a given site. A multivariate analysis was conducted to compare the control samples of bark and bud. This analysis led to the identification of nine metabolites, including Creatine, Aspartate, Sucrose, Asparagine, Succinate, Fumarate, Leucine, Adenosine, and Uridine, that show a significant change in at least one of the comparisons.

The figure highlights the significance of how selective metabolites exhibit characteristic changes due to treatment. As shown in the figure, there is a variation in the levels of Asparagine metabolite. Moreover, the relative changes in the Asparagine levels tend to increase in 2021 compared to 2019. However, oil treatment seems to have less modulation as compared to Dormex. These results suggest that it is worth extending the study specifically to understand the role of Dormex as an effective RBA rather than oil.

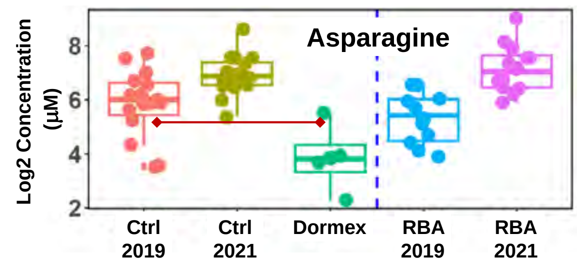


Figure: Variation in the levels of Asparagine due to RBA treatment

<u>Conclusions:</u>

Our work has yielded significant insights thus far. We have observed differences in the metabolic profiles of pistachio tissues subjected to various oil treatments during the dormant period. However, our findings suggest that the impact of Dormex on these profiles is more significant than that of oil treatment. Our results indicate that horticultural oil applications at different chill accumulation milestones can alter the metabolome of pistachio tissues. Furthermore, our samples taken at the budswell stage did not show any significant differences in their metabolic profiles, which suggests that the levels of pistachio tree metabolites remain similar as they approach bloom. Therefore, we anticipate that completing samples collected in Colusa during 2019 and 2021 (budswell and time-dependent) will enable us better to understand the interlinkages between geographical locations and RBA treatments. The sample processing for Colusa is still ongoing and is expected to be completed by 2024.

Our findings have been informative and will be of value to farmers who use RBAs to grow pistachios. By identifying the specific metabolites and altered physiological pathways associated with these agents, we can help farmers optimize their use, leading to better crop yields and more sustainable practices.

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

Authors
Gurreet Brar, Associate Professor, Department of Plant Science, Jordan College of Agricultural Sciences and Technology, California State University Fresno; **Gurbendir Kang**, Graduate Student, Department of Plant Science, Jordan College of Agricultural Sciences and Technology, California State University Fresno; **Dave Goorahoo,** Professor, Department of Plant Science, Jordan College of Agricultural Sciences and Technology, California State University Fresno; **Florence Cassel Sharma,** Associate Professor, Department of Plant Science, Jordan College of Agricultural Sciences and Technology, California State University Fresno; **Faranak Hadavi**, Lecturer, Department of Plant Science, Jordan College of Agricultural Sciences and Technology, California State University Fresno.

Introduction
Dormancy induction in temperate zone trees is a critical adaptation to winter conditions, marked by complex hormonal signaling and metabolic adjustments. Recent challenges in California's pistachio orchards, attributed to insufficient chill accumulation, have prompted growers to explore strategies like rest-breaking agents. However, limited research exists on dormancy induction in pistachios. In addressing this gap, this study was conducted at the Fresno State pistachio field with mature female 'Kerman' trees and male 'Peters' trees on UCB-1 rootstock. Late October applications of ABA (500 mg/L), $ZnSO_4$ (40 lb./acre), and ABA + $ZnSO_4$ in two different regimes of irrigation were implemented in a randomized complete block design (RCBD). Scheduled visits to the site were carried out to determine the bloom window, ranging from the 50% bud swell stage to the 80% full bloom stage.
Yield and yield components, abscisic acid (ABA), and structural and non-structural carbohydrate content in buds and barks of pistachio shoots were periodically analyzed, alongside observations on tree defoliation. Data were analyzed using SAS (ver. 9.4) and Duncan's multiple range test (P<0.05) was used to separate the means.

Results
Zinc application resulted in a significant increase in tree yield compared to other treatments and non-sprayed control trees (Table 1). Additionally, the application of Zinc led to an advancement of the bud swell stage by two days compared to the control, while also extending the female trees' bloom window by two days (Table 2). Analysis of ABA content in both November and February samples revealed no significant differences among the treatments Nevertheless, a statistically significant increase in ABA content was observed in February samples compared to those from November (data not shown). The 2022-23 fall and winter were characterized by cooler and wetter conditions compared to a normal Fresno dormant season, so minimal difference was anticipated between the irrigated and non-irrigated plots. However, notably lower blank percentages were observed in the irrigated plots (Table 1). This trial will be repeated in 2023-2024 and the effect of the chemical and irrigation treatments will be investigated on bloom and ABA contents as well as carbohydrates and yield components, and leaf defoliation after applications.

Table 1: Effect of ABA, Zinc, and ABA+Zinc 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.

Chemical Treatment	Yield/tree (lb.)	Total edible (%)	Closed Shell (%)	Blank (%)
Zinc	39.8 a	86.0 a	33.4 a	8.8 a
ABA	28.9 b	86.8 a	33.7 a	7.4 a
ABA+Zinc	31.1 b	75.5 a	35.3 a	10.8 a

Control	34.5 ab	84.0 a	39.3 a	11.7 a
Irrigation Treatment	**Yield/tree (lb.)**	**Total edible (%)**	**Closed Shell (%)**	**Blank (%)**
Irrigation	33.1 a	83.3 a	33.4 a	7.9 b
Withhold	34.1 a	82.8 a	33.7 a	11.5 a

Table 2. The effect of ABA, Zinc, and ABA+Zinc sprays on bud swell and bloom advancement of 'Kerman' female pistachio trees

Chemical Treatment	Bud Swell (@50%)	Full Bloom (@80%)	Bud Swell Advancement over Control (day)	Bud Break to Full Bloom (day)
Zinc	April 3	April 18	+2	15
ABA	April 5	April 18	0	13
ABA+Zinc	April 3	April 18	+2	15
Control	April 5	April 19	0	14
Irrigation Treatment	**Bud Swell (@50%)**	**Full Bloom (@80%)**	**Bud Swell Advancement over Control (day)**	**Bud Break to Full Bloom (day)**
Irrigation	April 5	April 19	-	14
Withhold	April 4	April 18	-	14

Table 3. The effect of ABA, Zinc, and ABA+Zinc sprays on bud swell and bloom advancement of 'Peters' male pistachio trees

Chemical Treatment	Bud Swell (@50%)	Full Bloom (@80%)	Bud Swell Advancement over Control (day)	Bud Break to Full Bloom (day)
Zinc	April 1	April 17	0	17
ABA	April 3	April 17	-2	15
ABA+Zinc	March 31	April 16	+1	17
Control	April 1	April 17	0	17
Irrigation Treatment	**Bud Swell (@50%)**	**Full Bloom (@80%)**	**Bud Swell Advancement over Control (day)**	**Bud Break to Full Bloom (day)**
Irrigation	April 1	April 17	-	17
Withhold	April 1	April 17	-	17

Effect of bloom time and growing season temperatures on pistachio hull integrity and nut quality (Year 2)

Authors: Barbara Blanco-Ulate, Associate Professor, Plant Sciences Dept., UCD; **Giulia Marino**, CE Specialist, Plant Sciences Dept., UCD**; Paula Guzmán-Delgado,** Project Scientist, Plant Sciences Dept., UCD; **Collaborators: Robert Beede,** CE Specialist (Emeritus) Kings County, UC ANR; **Joseph Coelho**, Maricopa Orchards; **Ian Humrick**, Maricopa Orchards; **John Gebhardt**, Gebhardt Farm Management; **Muhammad Ismail Siddiqui**, Delano-2665; **Selina Wang**, CE Specialist, Food Science and Technology Dept., UCD; **Louise Ferguson**, CE Specialist, Plant Sciences Dept, UCD; **Georgia Drakakaki**, Professor, Plant Sciences Dept., UCD.

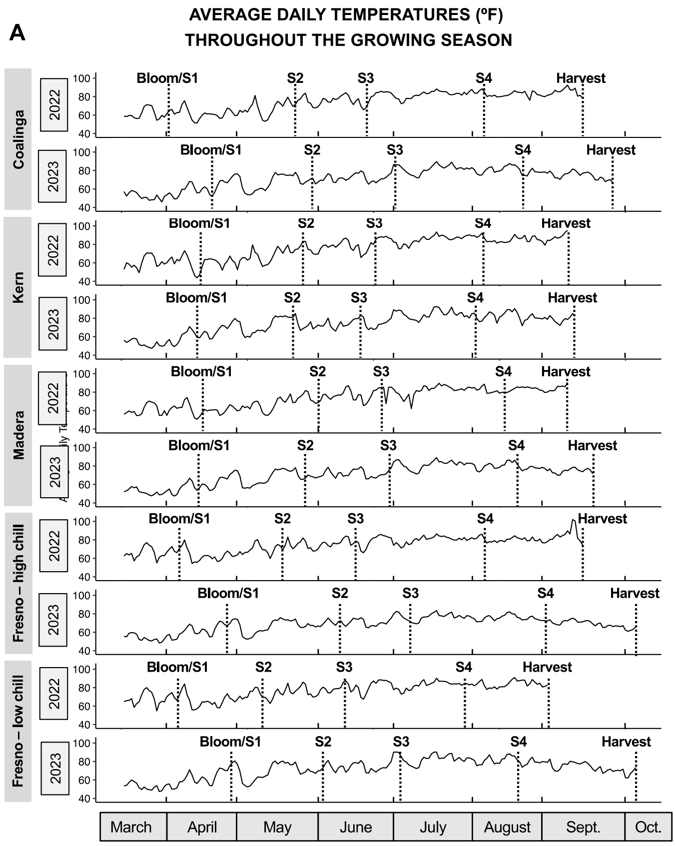
Introduction

Last year, 2022, was an odd year in terms of low chill and high early spring temperatures that continued through the summer. We observed that bloom time and the interaction with growing season temperatures strongly affected nut quality. In 2023, we focused on comparing 2022 and 2023 temperature data in multiple locations to assess if different temperature patterns have the same effects on nut development and quality. We selected commercial orchards ('Kerman') in four locations across the San Joaquin Valley: Coalinga, Kern, Madera, and Fresno. We chose two orchards in Fresno because of their differences in chill accumulation (low and average chill) and seasonal temperatures (one area was hotter). The Coalinga orchard has low chill, while the Madera orchard has high chill. Finally, one Kern orchard was studied because it presents nut quality issues similar to those related to low chill. We also studied a 'Golden Hills' orchard in Fresno during 2022 and 2023 to compare the responses to temperatures across both varieties. Temperature sensors and cameras were installed at each location to track bloom time in spring and accumulated heat during spring and summer. We sampled eight trees from each orchard with three clusters (16-30 nuts/cluster) per tree. We collected nuts at four sampling points per location: (i) initial nut growth (~120 growing degree days, GDD), (ii) complete nut filling (~1,800 growing degree days (GDD)), (iii) start of hull softening and shell split (~2,100 GDD), and (iv) harvest time (~2,400 GDD). Sampling times were determined based on the accumulated heat at each location. A preliminary trial using light diffusing treatments (kaolin sprays) was done in a 'Kerman' orchard in Fresno to test if reducing nut temperatures could offset the adverse effects of high temperatures in spring and summer, and subsequently improve nut quality. We included three treatments: non-spraying control (C), spraying only nuts (SN), and spraying nuts and leaves (SNL). The SNL treatment was intended to mimic a commercial spray while the SN was selected to isolate the effect of kaolin on nut temperature from that on leaf photosynthesis.

Results and Discussion

Given the space limitations in this report, we are presenting only data on the seasonal temperatures of the 'Kerman' orchards and some key nut quality traits at harvest. We measured more nut traits across the two seasons for all orchards, including a 'Golden Hills' one. Additional data will be made available in the final report. Some highlights of our work are:

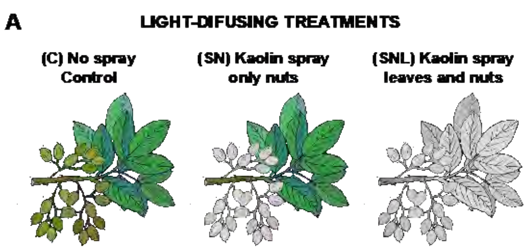
- In 2023, the winter and spring temperatures were lower compared to 2022, which delayed bloom in some locations. High temperatures in spring can reduce the percentage of shell split from 80% to 40% and increase the percentage of blanks from 20% to 50% across sites and years.
- Spring temperatures did not appear to have consistent effect on kernel dry weight, and no relation between kernel weight and shell split was found.
- Hull firmness was very site specific, and in some cases the mild temperatures of 2023 allowed for a reduction of hull firmness.
- Preliminary data suggest that reducing nut temperature by applying sun reflecting product on the nuts improved kernel dry weight, probably by mitigating the impact of high summer temperatures.



B NUT QUALITY PARAMETERS AT HARVEST

Location	Year	% Blank	Kernel weight (DW g)	% Split	Hull firmness (g of F)
Coalinga	2022	46.09 *ab*	0.55 *ab*	42.44 *c*	1,231 *b*
Coalinga	2023	29.44 *cd*	0.56 *a*	74.19 *a*	854 *cd*
Kern	2022	55.26 *a*	0.43 *d*	53.71 *bc*	1,680 *a*
Kern	2023	53.21 *a*	0.44 *cd*	79.88 *a*	1,167 *b*
Madera	2022	40.36 *bc*	0.36 *e*	52.61 *bc*	937 *c*
Madera	2023	53.56 *a*	0.52 *ab*	40.99 *c*	843 *cd*
Fresno – high chill	2022	20.55 *d*	0.52 *ab*	89.61 *a*	757 *cd*
Fresno – high chill	2023	22.96 *d*	0.51 *abc*	66.92 *ab*	649 *d*
Fresno – low chill	2022	44.55 *ab*	0.53 *ab*	40.14 *c*	696 *d*
Fresno – low chill	2023	33.00 *bcd*	0.48 *bcd*	40.71 *c*	637 *d*

Fig. 1. 2022 and 2023 temperatures and nut quality of five 'Kerman' orchards in the San Joaquin Valley. **Panel A** compares the average daily temperatures across locations and years, the dotted vertical lines denote the start of the four nut stages: S1- hull and shell growth, S2 - transition, S3 - kernel growth and shell hardening, and S4 - kernel maturation and hull ripening, plus the time of harvest. **Panel B** shows key nut quality parameters measured at harvest. The letters next to the numbers represent significant differences (*P*< 0.05, n= 24-48) between all locations and years for a given parameter.



B NUT QUALITY PARAMETERS AT HARVEST

	% Blank	Kernel weight (DW g)	% Split	Pull Test (N Force)
C	18.81	0.51	62.17	1.13
SN	↓ 16.32	↑ 0.62	↓ 57.38	↓ 0.87
SNL	18.76	0.54	↓ 55.95	1.17

Fig. 2. Preliminary trial using light diffusing treatments to improve nut quality at harvest. **Panel A** illustrates the kaolin spray treatments. **Panel B** displays the differences in quality parameters among treatments, arrows show the trend (increase or decrease) when compared to the Control (C). The trends were statistically significant (*P*< 0.05, n= 12) only for kernel weight.

Conclusion Thanks to the CPRB funding we started creating the first large dataset of pistachio nut quality traits across multiple seasons and locations. The data clearly shows that temperature has a primary impact on nut quality that can strongly reduce growers' income by increasing blanks, reducing split, and delaying ripening. We urgently need to develop a better understanding of these physiological processes and science-based management strategies to improve quality under variable and extreme temperatures.

Effects of winter cover crop on pistachio canopy temperature, bloom, and yield

Authors
Gurreet Brar, Associate Professor, Department of Plant Science, Jordan College of Agricultural Sciences and Technology, California State University Fresno; **Susana Lopez,** Graduate Student, Department of Plant Science, Jordan College of Agricultural Sciences and Technology, California State University Fresno; **Ranjit Riar,** Assistant Professor of Agronomy and Crop Nutrition, Department of Plant Science. Jordan College of Agricultural Sciences and Technology, California State University Fresno; **Masood Khezri,** Research Director, MARI Agricultural Research Institute, Fresno, CA. **Faranak Hadavi**, Research Scientist, Department of Plant Science, Jordan College of Agricultural Sciences and Technology, California State University Fresno.

Introduction

The pistachio industry in California is grappling with challenges associated with warm winters and insufficient chill accumulation, affecting bud break and yield. The literature highlights the significance of winter cover crops in lowering soil temperature and improving soil health. Despite this, the impact of winter cover crops on the advancement and synchronization of male and female pistachio trees has remained unknown. This study explores the implementation of winter cover crops in a commercial pistachio orchard in Madera, CA, where 8-year-old female and male trees ('Kerman' and 'Peters,' respectively, all on 'UCB1' rootstocks) were investigated. The research focuses on temperature variations, chill accumulation, and their effects on bud break, bloom progression, yield components, and carbohydrate content (not presented here). Conducted as a randomized complete block design (RCBD) with two treatments including Control (no cover crop) and Cover Crop, the planted cover crop was a mix of triticale, peas, vetch, and radish, covering a total of 3.5 acres. Each treatment comprised three blocks, with an average of 165 trees per block. Regular site visits were conducted to determine the bloom window, ranging from the 50% bud swell stage to the 80% full bloom stage.

Results

In the cover crop plots, bud temperature and ambient temperatures were consistently, on average, 0.5°C lower than in control plots (data not shown). It is worth mentioning that in the fall and winter seasons of 2022-23 in Central Valley, the weather exhibited cooler and wetter conditions compared to a typical dormant season. Notably, advanced male trees experienced a 2-day earlier bud swell, as indicated in Table 2. Furthermore, it's interesting to note that the male tree 'Peters' initiated the bud swell stage approximately 5 days earlier than the female tree 'Kerman'. However, the full bloom date for both male and female trees coincided. This implies that, despite male trees starting bud swell sooner than female trees, they remained in the bud swell stage longer than the female trees. This suggests that, in a high-chill year, we observed appropriate bloom synchrony between male and female trees in both cover-cropped and control plots. An additional point of significance is the impact of the cold early spring, which led to an extended bloom window. Specifically, the duration from bud swell to full bloom was around 19 days for the male 'Peters' and 14 days for the female 'Kerman' (Tables 1, 2) These timelines significantly exceeded the normal bloom window time in the Central Valley, underscoring the unique conditions experienced during this particular season. The yield of pistachio trees in cover-cropped plots with 25.2 lb./tree was higher compared to the control treatment with 23.6 lb./tree, although the difference was not statistically significant (Table 3).

Cover cropping did not have any significant effect on yield components like total edible, closed shell, and blank percentages (Table 3). These results suggest potential temperature moderation effects of cover cropping and a marginal impact on overall yield, yet further investigation is warranted to comprehend its comprehensive influence on yield components within varying climatic contexts. This trial will be repeated in the fall and winter of 2023-24 in the same field to explore the effects of cover crops in a typical fall and winter year, characterized by marginal chill conditions. This continuation of the study will further investigate the impact of cover crops on chill portion accumulation, the bloom synchrony of male and female trees, and yield.

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

Treatment	Bud Swell (@50%)	Full Bloom (@80%)	Bud Swell Advancement over Control (day)	Bud Break to Full Bloom (day)
Control (No Cover Crop)	April 10	April 24	0	14
Cover Crop	April 10	April 24	0	14

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

Treatment	Bud Swell (@50%)	Full Bloom (@80%)	Bud Swell Advancement over Control (day)	Bud Break to Full Bloom (day)
Control (No Cover Crop)	April 5	April 24	0	19
Cover Crop	April 3	April 24	2	20

Table 3. The effect of cover crop 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 DW/Tree (lb.)	Dry to Green (%)	Total Edible (%)	Closed shell (%)	Blank (%)
Control (No Cover Crop)	23.6 ± 1.7 a	42.1± 1.0 a	85.4 ± 0.7a	5.1 ± 1.1 a	7.2 ±1.0 a
Cover Crop	25.2 ± 1.3 a	42.8± 0.3 a	86.7 ±1.4 a	6.2 ± 1.9 a	7.2 ± 0.7 a

Investigating Chemical Composition and Morphogenesis of Pistachio Internal Kernel Discoloration

Authors: Georgia Drakakaki[1], professor; **Minmin Wang**[1], staff; **Shuxiao Zhang**[1], **Hannah McCurry**[1], graduate student; **Rolando Lopez**[1], undergraduate student.
Collaborators: Phoebe Gordon[2], Judy Jernstedt[1], Themis J. Michailides[2], Giulia Marino[1], Larry Lerno[3], Thomas Wilkop[4]
[1]Department of Plant Sciences, UC Davis; [2]UC Cooperative Extension, Madera, CA; [3]Food Safety and Measurement Facility, UC Davis; [4]MCB Light Microscopy Imaging Facility, UC Davis

Introduction

A recently observed phenotype related to the quality of pistachio fruit is the pistachio internal kernel discoloration (IKD), appearing as a brown concentric stripe pattern in longitudinally opened nuts. This partial staining extends to only 3 cell layers deep in cross sections. The cultivar of the largest expanding acreage, "Golden Hills", shows a rate of IKD that can reach 10-20% in some orchards. California pistachio growers are seeking evaluation of the chemical composition and morphogenesis of IKD, whether IKD can have any potential effect on human nutrition, and how to manage IKD incidence. Cotyledons, embryonic storage organs of plants, are the edible kernel of tree nuts such as almond, walnut, and pistachio. Yet, there is limited knowledge on the embryo development of tree crops, including the morphogenesis of the cotyledon. It is unknown if IKD development in pistachios is tied to kernel development. However, studying pistachio embryo development in relation to IKD will not only benefit the pistachio industry but also other tree crop industries by identifying developmental mechanisms responsible for kernel quality. We monitored the relationship between IKD occurrence with multiple biotic and abiotic factors in this study. We also evaluated the aflatoxin and mycotoxin levels in IKD samples, in order to provide insights into health concerns associated with IKD. We established the pistachio kernel developmental profile and determined the timing of the associated polyphenol deposition shown as IKD.

Results and Discussion

IKD rate monitoring and its relationship with biotic and abiotic factors

In collaboration with Dr. Phoebe Gordon, we extensively monitored a Yolo County orchard (Orchard A) which was reported for IKD incidents in the last two consecutive years. Trees located near the diagonal line of the orchard were selected and branches containing over 3 clusters were caged, to exclude the factor of large bug herbivory as a possible cause of IKD. **(1)** At 35 DPA of fruit development, leaf samples of Orchard A were sent to Michailides Lab and analyzed for the pathological source of leaf black spots. Neither *Botryosphaeria spp.* or *Alternaria spp.* infections were found in this orchard. **(2)** We tested the correlation between heat stress and IKD occurrence rate by installing aluminum cones and temperature sensors on fruit clusters at 77 DPA of fruit development in Orchard A. Accumulated heat units were significantly increased in coned clusters compared to clusters in the shade, with significant hull color changes. However, no increase in IKD occurrence rate was found in coned clusters with higher accumulated heat units compared to the rest of the tree, suggesting that heat stress during Stage II and III of fruit development does not induce a higher IKD occurrence rate. **(3)** The relationship between plant water stress level and IKD occurrence rate was also monitored. Stem water potential of Orchard A was at 12-15 bar throughout the growing season at a biweekly sampling interval, suggesting that Orchard A trees did not experience drought stress. Notably, we observed an IKD rate of over 18% in caged nuts on 133 DPA, a few days before the commercial harvest of this orchard. The water content of IKD nuts in caged samples was not different from that of non-symptomatic nuts in Orchard A, suggesting that the water content level of individual nuts is also not correlated with IKD. In collaboration with the Marino Lab, we measured the IKD rate of uncaged samples in another Yolo County orchard, and found that regulated deficit irrigation did not induce higher IKD occurrence in the treatment group compared to the control. These results do not show a significant relationship between stem water potential and IKD. The relationship between IKD occurrence and high irrigation remains to be investigated.

Chemical analysis of IKD regions to address the nature of staining.
We identified polyphenols, which are natural antioxidants, as the major color contributor to the IKD brown stripes. Quantification of polyphenol and precursors of phenylpropanoid pathways on a kernel developmental basis is in progress. We prepared the LC-qTOF quantification method for aflatoxin and ochratoxin A at the Food Safety and Measurement Facility of UC Davis. The limit of detection (LOD) is below 10 ppb in our method, in accordance with the EU standards (10 ppb) and FDA recommendations (20 ppb) for monitoring the aflatoxin and ochratoxin contamination. We did not detect levels of aflatoxins or ochratoxin A above EU standards in any nut samples, including non-symptomatic and IKD nuts from Orchard A or a Merced County orchard we sampled. These results suggest that IKD is unlikely to be caused by fungal infection.

***Pistacia vera* kernel developmental profile and cellular analysis of IKD samples**
We monitored the kernel development at a biweekly interval throughout the season. We have established a pistachio kernel development profile as an anatomical guide and identified key development events such as endosperm expansion, embryo development, and deposition of lipid and starch in the kernel. We have discovered that kernel maturation appears to proceed along the dorsal-ventral axis as opposed to the apical-basal axis as we previously expected, and that the attachment point of the funiculus to the kernel occurs at the dorsal end of the fruit (**Figure 1**).

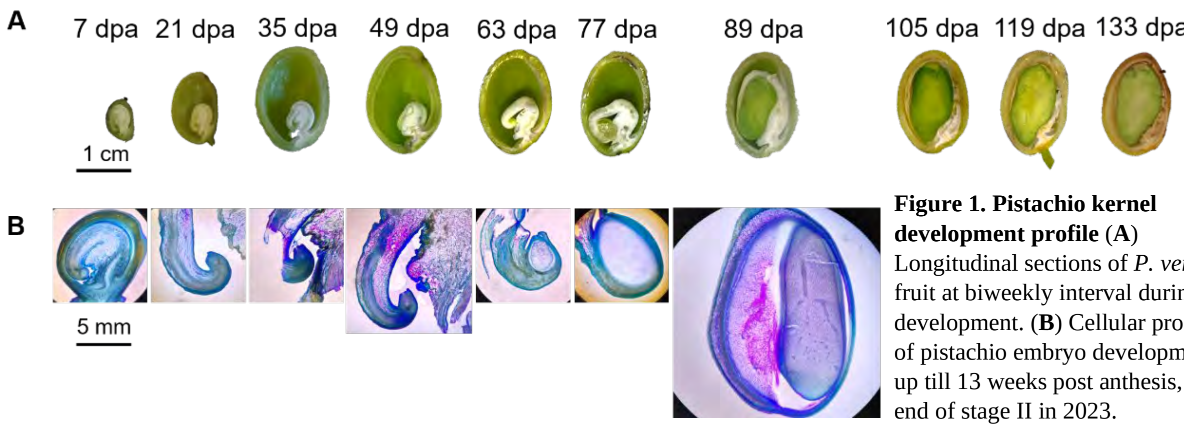


Figure 1. Pistachio kernel development profile (**A**) Longitudinal sections of *P. vera* fruit at biweekly interval during development. (**B**) Cellular profile of pistachio embryo development up till 13 weeks post anthesis, end of stage II in 2023.

IKD incidence was not found until the cotyledon completely filled the endocarp, which is consistent with last year's observations. The rate of IKD occurrence in stage III increased as the developmental stages progressed (from 5.3% to 18.3% on 105 and 133 DPA, respectively, in caged fruit clusters). We observed polyphenol accumulation as punctae at the initiation of IKD in cotyledon cells, suggesting that the stripe pattern is likely to develop from the expansion of the initial polyphenol spot deposition.

Conclusion
Monitoring abiotic factors in three California orchards suggests that IKD is not correlated with heat stress or drought stress. The relationship between IKD occurrence and high irrigation remains to be investigated. No compounds of general public health concern as aflatoxins and ochratoxin A were detected in IKD samples. We identified the incidence of IKD development at the beginning of stage III. We have established the kernel developmental profile for pistachio, which will serve as a guide for kernel quality monitoring and future pathological analyses for the pistachio industry.

Determining severity of Internal Kernel Discoloration Incidence in Pistachio Cultivars, year 2

Authors: Phoebe Gordon, Orchard Systems Advisor, Madera County, UCANR; **Georgia Drakakaki**, Professor of Plant Sciences, Department of Plant Sciences, UC Davis; **Minmin Wang**, Associate Project Scientist, Department of Plant Sciences, UC Davis; **Susan (Shuxiao) Zhang**, Ph.D. Candidate, Department of Plant Sciences, UC Davis; **Doug Amaral**, Orchard Systems Advisor, Kings County, UCANR; **Elizabeth Fichtner**, Pomology Advisor, Tulare County, UCANR

Introduction

Internal kernel discoloration (IKD) is a kernel disorder that presents as concentric rings of darkened tissue, resembling growth rings. There is no indication that this affects pistachio kernel quality, however there is a desire within the industry to understand the cause of this discoloration. Survey work done in 2022 showed that it appears more in Golden Hills than Kerman, and that it does occur in Lost Hills and the genetic mother of Golden and Lost Hills. There is a need to better understand this discoloration to determine the cause and whether its severity can be managed.

Results and Discussion

This year, we sampled 22 locations and a total of 44 blocks. Most locations contained at least two cultivars. A small number contained all three or only one, and two of the locations are cultivar trials. This is an increase in sampling sites from last year, when we sampled 19 locations and 39 blocks. In order to improve the likelihood that we detect IKD within an orchard, we increased the number of trees we sampled in each block from 5 to 15, but reduced the number of kernels sampled per tree from 20 to 10. Kernels were sampled and mounted on a tray and then recorded via a photograph. All classifications of IKD or no IKD were done based on these photographs (Figure 1).

Table 1: Preliminary sampling counts for the three cultivars of interest, and incidence of "concentric ring/classic" type IKD among each cultivar. Only samples collected around cultivar harvest are included.

Cultivar	Number of orchards sampled[1]	Number of kernels collected	"Classic IKD[2]"	Orchards with IKD
Golden Hills	19	2415	11%	100%
Kerman	17	1725	4%	76%
Lost Hills	6	574	5%	83%

[1]Two of the locations we sampled were variety trials, in this chart each variety is classified as a separate "orchard"

[2]Numbers based on a preliminary analysis. This may change in the full annual report.

IKD appears to be more prevalent in 2023 (Table 1) than in 2022, when Golden Hills, Kerman, and Lost Hills had 4.5, 0.5, and 1.1% IKD, respectively. Additionally, the number of orchards where at least one kernel with "classic IKD" was collected increased significantly from 2022 (Table 1), when only 75, 23, and 25% of Golden Hills, Kerman, and Lost Hills orchards had IKD.

While it is known that insect damage does not cause IKD, there were many instances last year where it was unclear whether some discoloration was due to insect damage or IKD. We called this "black spot IKD." To significantly reduce the chance of plant bug damage, we bagged clusters with paint straining bags between fruit set and the end of May. We were not able to visit all sites before the end of hull expansion, however we bagged clusters at 83% of the locations we sampled from. We recovered kernels that had "black spot IKD" from bagged clusters, suggesting at least some of the discoloration we saw last year was not bug damage, however at this point we do not know if it is IKD. Unfortunately, processing more than 10,000 fruit required a rapid way to process fruit and record data before the kernels began to deteriorate, and a close examination of each kernel during this time was not possible. We will be re-examining these kernels more closely and reporting results in the 2023 annual report.

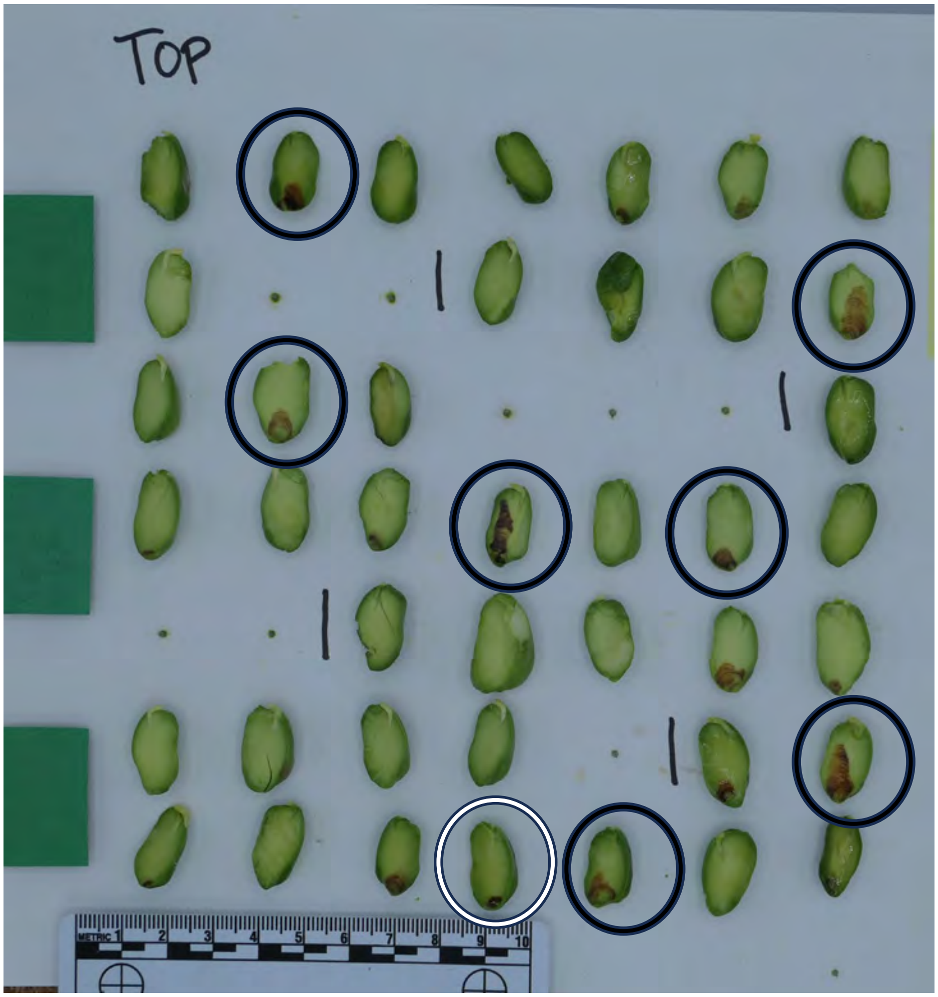


Figure 1: Golden Hills mounted on a tray. Both "Classic IKD" (black circles) and "black spot type IKD" (white circle) are visible

<u>Conclusion</u>

We have confirmed again that Golden Hills gets more IKD than Kerman and Lost Hills. As in 2022, there was a wide variation in IKD severity between orchards. Work done in 2023 suggests that applied water may influence IKD severity, but this needs to be confirmed in 2024.

<u>Acknowledgements</u>

Thank you to Richard Saldate, Kelsey Galvan, and Rito Medina for your work on this project. Thanks to all of the growers, farm managers, and consultants who helped identify locations. A big thank you to Joe Coelho, Ian Humrick, and Maricopa orchards for hosting research trials, and thanks to Gulia Marino, Louise Ferguson, Blake Sanden, and Mukesh Mehata for allowing us to sample from their research trials.

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

The pistachio (*Pistacia vera* L.) is a deciduous, woody perennial crop that is rapidly expanding its production, currently ranking among the top commodities in California. A critical stage in the growth and development cycle of pistachios takes place when their buds enter dormancy after the trees drop their leaves in the fall. Upon exposure to a specific number of low-temperature hours ("winter chill"), the buds are released from dormancy ("bud break") in the spring—a key process that significantly impacts pistachio yields. Bud dormancy involves two sequential phases: endodormancy, which is regulated by developmental factors within the bud, and ecodormancy, which is controlled by environmental factors. Meeting the winter chill requirement facilitates the release of buds from endodormancy and their transition to ecodormancy.

To gain insights into the physiological and biochemical mechanisms driving bud endodormancy release in pistachio cv. Kerman, we conducted a transcriptome analysis on flower buds collected from trees grown in three orchard locations, which were subjected to varying durations of winter chill (Yu et al., 2023). Our study revealed the upregulation of *β-1,3-glucanase* and *β-amylase* genes for breaking down callose and starch, respectively, during endodormancy release. This result indicates their roles in symplasmic trafficking and energy provision in the endodormancy release process. Additionally, as winter chill accumulated, there was a concurrent decrease in the expression of *nine-cis-epoxycarotenoid dioxygenase* (*NCED*), which suppresses abscisic acid (ABA) biosynthesis and relieves endodormancy inhibition. Our metabolite analysis supported these findings in gene expression analysis by showing elevated carotenoid precursor levels and reduced ABA content in buds undergoing endodormancy release (Yu et al., 2023).

Our temporal transcriptome and biochemical analyses collectively established that the degradation of structural and non-structural carbohydrates, along with attenuated ABA biosynthesis, are key processes driving endodormancy release in pistachio buds. Interestingly, changes in the gene expression patterns of *β-1,3-glucanase*, *β-amylase*, and *NCED* were consistently observed in buds collected from different orchard locations (Yu et al., 2023). These results suggest that they can potentially be used as gene expression markers for the bud endodormancy release process and for the application of bud-break enhancing chemicals. In the current research, our objectives are to conduct field trials with and without the application of horticultural oil along with bud sample collection. We also aim to verify the effectiveness of the identified gene expression markers through molecular and biochemical analyses using the collected bud samples.

Results and Discussion

To ensure that the previously observed gene expression patterns are primarily determined by genetic factors rather than environmental conditions, we expanded our investigation to include buds from three additional orchard locations, each subjected to varying durations of winter chill during the 2022-2023 growing season. We carefully monitored the temperature and other environmental conditions at each orchard location with our own climate installation and used the Chill Portions model for the calculation of chilling accumulation. It should be noted that, due to a severe winter storm in early 2023, bud collection at CP45 was not feasible as the orchards were not accessible. Despite this setback, we successfully collected bud samples at CP50, CP55, CP60, and CP65. All collected buds were sent to our lab in Davis on ice or dry ice for analysis. We conducted real-time qPCR analysis on the candidate gene expression markers and also performed metabolite analysis. The gene expression and metabolite accumulation patterns were consistent with our previous report, affirming the reliability of these markers for indicating the endodormancy release process. During the 2022-2023 growing season, we also conducted a trial to evaluate the effects of applying horticultural oil to pistachio cv. Kerman trees at these three orchard locations. An intriguing observation from this trial was the increase in nut abscission and the ease of nut harvest in trees sprayed with horticultural oil, which adds a compelling dimension to our findings.

Conclusion

Our expanded investigation in multiple orchard locations during the 2022-2023 growing season revealed consistent gene expression and metabolite accumulation patterns, which validates the use of these gene expression markers in reflecting the endodormancy release process in pistachio buds. Additionally, the intriguing observation of increased nut abscission in pistachio trees treated with horticultural oil suggests its potential application in nut harvest practices.

Yu, S., Amaral, D., Brown, P.H., Ferguson, L., and Tian, L. (2023) Temporal transcriptome and metabolite analyses provide insights into the biochemical and physiological processes underlying endodormancy release in pistachio (*Pistacia vera* L.) flower buds. Frontiers in Plant Science 14, 1240442.

Phenotype selection for tolerance to low fall/winter chilling and hot-bloom climatic conditions in the Coachella Valley of California

Authors: **Craig E. Kallsen,** UCCE Farm Advisor emeritus, Kern County and **Dan E. Parfitt** Pomologist-AES emeritus, UC Davis

Introduction

The San Joaquin Valley (SJV) of California is, arguably, the premier pistachio growing area of the world based on total acreage, per acre yields and crop quality. Nevertheless, in this area, inadequate and borderline winter chilling has been a problem some years, and remains a concern to the industry. Increasingly, warm temperatures during the winter chill period and hot temperatures during bloom have been implicated in negatively impacting bloom and eventual nut yield and these effects require further research. Current future climate predictions are for increasingly warmer air temperature in the SJV. To better quantify the effects of warmer temperatures on production, we decided to evaluate existing California cultivars (*Pistacia vera*) and interspecific crosses between *P. vera* and *P. integerrima* for growth, foliation and fruiting under low winter-chill conditions. The rational for making the interspecific crosses were based on observations that *P. integerrima* appears to have a lower chill requirement than *P. vera*. *Pistacia integerrima* has a short dormancy period in the SJV and exits dormancy in the spring well before *P. vera*. Chill calculations from the CIMIS station there suggested that the Coachella Valley Agricultural Research Station in Thermal, California would be an appropriate site for this trial. The included cultivars, grafted onto UCB-1 rootstocks, included the female cultivars Kerman, Golden Hills, Lost Hills, Gumdrop, Aria, Kalehghouchi, Aegina and commonly used male cultivars. Additionally, advanced selections from existing Kallsen/Parfitt breeding trials, which appeared to have a lower chill requirement, were grafted onto UCB-1 rootstocks and included in the trial. The cultivars were planted in 2017, with additional selections grafted in 2018 and 2019. The majority of the interspecific hybrids were planted in 2019 and as they were planted from seed, are on their own roots. As of 2023 there were approximately 140 P. vera trees in the trial and 250 interspecific hybrids. The trees were evaluated annually for earliness of foliation and for fruiting, if present. The objective of this trial was not to move pistachio production to the low desert, but to determine how pistachio might perform in an increasingly low chill environment in the SJV.

Results and Discussion

As of fall 2023 (7th leaf), only two cultivars had flowered. The males Tejon and Zarand produced one or two inflorescences in 2022 and 2023. It is unlikely the flowers were fertile. None of the other selected and grafted *P. vera* in the trial have flowered. In comparison, in a seedling selection trial at the Westside Research and Extension Center, two seedling trees flowered at 4th leaf and a significant percentage at 5th leaf. Although planted later in the Coachella, the interspecific hybrids have not yet produced any flowers either. A comparison between air temperature variables in Thermal versus a closely-monitored low-chill test trial in the San Joaquin Valley called Jasmine suggests why. The variables "Fall/winter chill hours < 45 °F" and "Fall/winter chill portions" in other pistachio and tree fruit research have been correlated with higher nut yields, while higher values for "Fall/winter heat hours" and "Bloom heat hours > 85 °F" were correlated with reduced yield. Compared to the Jasmine trial site in the SJV, the CV site during dormancy had only 57% of the hours < 45 °F, 43% of the chill portions, 368% of the hours greater than 65 °F, and, during bloom, 2609% of hours greater than 85 °F. The differences in the magnitude for any one of these variables compared to what occurs in the SJV may be sufficient to prevent pistachio trees from flowering in the Coachella Valley (CV). However, having all of the model variable values strongly indicating reduced yields, in combination with the absence of flowering observed in our trial, likely makes *P. vera* unsuitable for pistachio production in the current climate of the CV.

So, what are the chances that the SJV may end up with similar values for the important temperature-related variables shown for the CV as described above? Generally, from 2017 – 2023, the CV, on any given day, was approximately 10 °F warmer than the SJV regardless of month. As noted above, to date, our flowering results strongly suggest that this average air temperature difference prevents pistachio nut production in the CV. Judging by the difference in the four variables above between the CV and the Jasmine test site, the effect that a 10 degree increase in air temperature has on important chill and heat accumulations is huge. Historically, in the SJV, small perturbations in air temperature at critical crop developmental periods can negatively affect the degree of fall/winter chilling and air temperature during bloom and decrease yields at harvest. Thus, it is unlikely that a full climate-change induced 10 °F average increase in air temperature change in the SJV would be necessary to make pistachio production unprofitable. Yields from the disastrous SJV 2014-2015 crop year are a case in point. A smaller average increase in air temperature in the SJV may be sufficient to prevent normal flower bud development and flowering most years. The following quote is from a recent report from the California Energy Commission (Fernandez-Bou et al., 2018: Link> San Joaquin Valley Region Report for California's Fourth Climate Change Assessment), "*With respect to historical data (1961-1990), the San Joaquin Valley annual average maximum temperatures increased by about 1 °F (0.5 °C) (Abatzoglou et al., 2009; WRCC, 2021), and they are projected to increase 4 °F to 5 °F (2.2 °C to 2.7 °C) by midcentury, and 5 °F to 8 °F (2.7 °C to 4.4 °C) by the end of the century depending on the actions we take to reduce heat trapping gas emissions (Figure 6) (Cal-Adapt, 2019).*" So, if the predictions regarding air temperature increases in this report and other reports (Leudeling et all, 2009: Link> https://doi.org/10.1371/journal.pone.0006166) are accurate, profitable pistachio production may be untenable in the SJV even before midcentury. Note that the increased temperature predictions in these reports are only based on current climate models, which are associated with significant uncertainty.

Additionally, this project included evaluating the performance of *P. vera x P. integerrima* hybrids growing on their own roots. Having trees growing on their own roots may be advantageous as evaluation of the *P. vera* cultivars has been complicated by the UCB-1 rootstocks they are grafted to. The UCB-1 rootstocks appear to have a lower chill requirement, and their trunk suckers vigorously outgrew their P. vera scions. Past research has shown that *P. vera x P. integerrima* hybrids grown from seed, under conditions in the SJV, take longer from planting to the time of first flowering than do *P. vera* grown from seed. Since most of the hybrids were not planted until 2019 they may not have had sufficient time from planting to produce flowers due to their apparently longer juvenility stage. To date, none of these hybrids have flowered, but appear to leaf out more uniformly, in general, than do *P. vera* on rootstocks in this trial. Further evaluation of the hybrids is planned.

Tentative Conclusions

1. Current California pistachio cultivars, even those that may require less winter chilling than others in the SJV, cannot be grown profitably in the CV, since, apparently, they do not produce viable flowers (or even flower buds).
2. The amount of winter chilling may not be the only air temperature variable limitation associated with adequate bloom of pistachio, or the most limiting. Air temperatures pre-bloom and during bloom appear to be more significant than previously considered. The negative effects of freezing temperature during bloom is well known, however, the negative effects of temperatures over 85°F during bloom requires further investigation.
3. Based on existing temperature and chilling models, warmer air temperatures during the fall, winter and spring appear to be the reason for poor or non-existent flowering in the CV.
4. Current predicted changes in climate for the SJV, if accurate, will result in air temperatures in the SJV more similar to those of the CV, resulting in greater expectations of temperature-related crop failures as early as mid-21st century.

Evaluating fungicide efficacy and resistance levels in the management of Alternaria late blight in California pistachio orchards

Authors: **Boris X. Camiletti**, Postdoctoral Scholar, Department of Plant Pathology, UC Davis and UC Kearney Agricultural Research and Extension Center (KARE); **Victor M. Gabri,** Staff Research Associate, UC Davis and KARE; **Thiago A. Carraro**, Visiting Student, UC Davis and KARE; **Roman Pandey,** Visiting Scholar Researcher, UC Davis and KARE; **Apostolos Papagelis,** International Scholar Researcher**,** UC Davis and KARE; **Themis J. Michailides**, Principal Investigator, Department of Plant Pathology, UC Davis & KARE.

Introduction

The primary measure to reduce Alternaria late blight (ALB) in pistachios involves applying multiple sprays per season. The prevalence of fungicide-resistant isolates in *Alternaria* populations is a major factor contributing to the unsuccessful control of ALB. The frequency of mutations associated with resistance to SDHI (H134R) and QoI (G143A) fungicides can serve as an indicator of resistance levels in orchards. The goals of this study were to evaluate the efficacy of various commercial fungicides and to assess resistance levels in commercial orchards throughout California. Field trials were conducted from 2013 to 2023 in a Kerman experimental plot located at the Agricultural Research and Extension Center in Parlier, CA. Treatments comprised three applications per season, approximately four weeks apart (early June, July – a critical time for spraying – and early- to mid-August). All fungicides were applied at the maximum rate specified on the label. Each treatment consisted of five single-tree replications, with non-sprayed trees used as controls. Sprays were applied using a handgun sprayer at a rate of 400 gallons per acre. Disease evaluation occurred close to harvest, typically in early September. Severity was assessed using a 0-to-5 rating scale, where 0 represents no disease, 5 indicates several branches with lesions on leaves, and 1, 2, 3, and 4 represent intermediate levels of disease. During the 2021, 2022, and 2023 seasons, leaf samples were collected from 33 orchards/locations. In each orchard, 60 symptomatic leaflets were randomly collected. DNA extraction was performed using commercial kits. The qPCR assays developed by Camiletti et al. (2023) and Luo et al. (2007) were applied to determine the frequency of the mutations G143A and H134R. Statistical analysis was conducted using R Studio software, with a cumulative link mixed model fitted to severity data using the *clmm* function in the *ordinal* package. Treatment marginal means were estimated using the *emmeans* function from the *emmeans* package, and post hoc comparisons were conducted with Tukey's test (P=0.05).

Results and Discussion

As expected, the non-treated control exhibited the highest severity scores. With the exception of Rhyme, all fungicide treatments reduced the severity of ALB (Fig. 1). The fungicide treatment using Mibelya resulted in the lowest severity scores. According to the statistical analysis, three fungicide products (Cevya, Miravis Prime, and Luna Experience) demonstrated a performance similar to the aforementioned treatment. The remaining fungicides showed intermediate efficacy in controlling the disease. Mutations conferring resistance to QoI and SDHI fungicides are widespread in California (Fig. 2). The highest frequencies of resistance were found in orchards that are usually treated with fungicides. Conversely, the lowest frequencies of resistance were observed in orchards located in areas where conducive conditions are not usually present, and therefore, orchards are not treated against ALB.

Conclusion

Fungicide products Mibelya and Cevya, formulated with the active ingredient mefentrifluconazole (FRAC# 3), provide high disease control and could be used to combat fungicide resistance. SDHI (FRAC# 7) and QoI (FRAC# 11) fungicides should be used in alternation or combination with other chemical classes to minimize the impact of resistance.

References

Camiletti et al. 2023. Plant Disease 107: 1433-1441.
Luo et al. 2007. Pestic. Biochem. Physiol. 88:328–336.

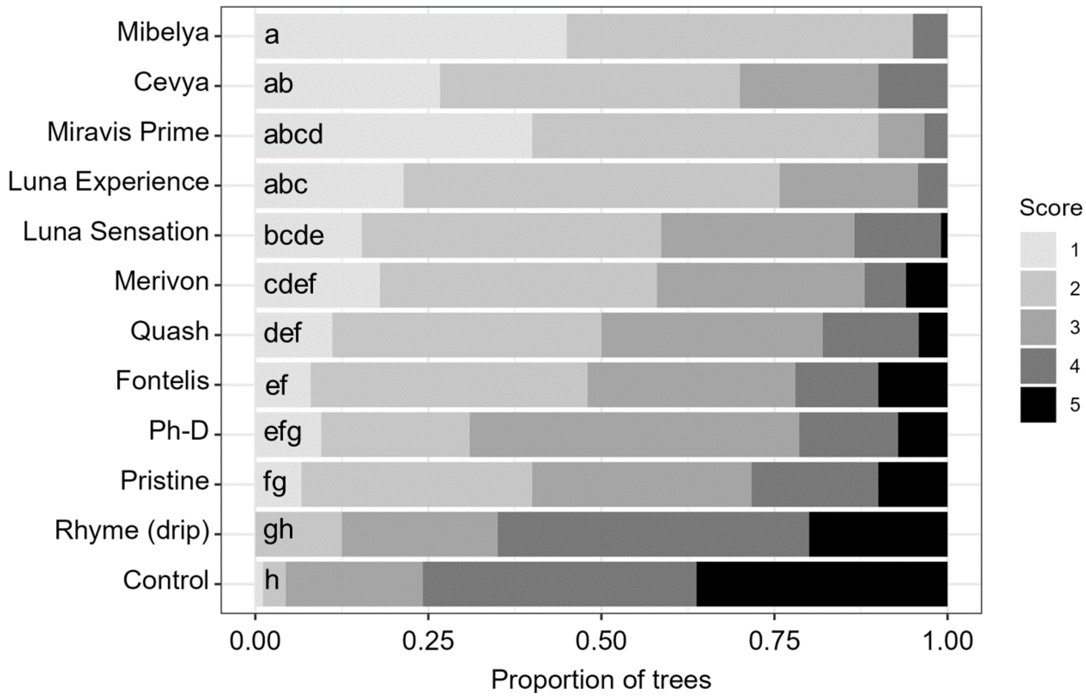


Fig. 1. Effect of fungicides on ALB severity in an experimental pistachio orchard in Parlier, CA. A higher score indicates higher disease severity. Different letter indicates significant differences (Tukey's test; *P*=0.05).

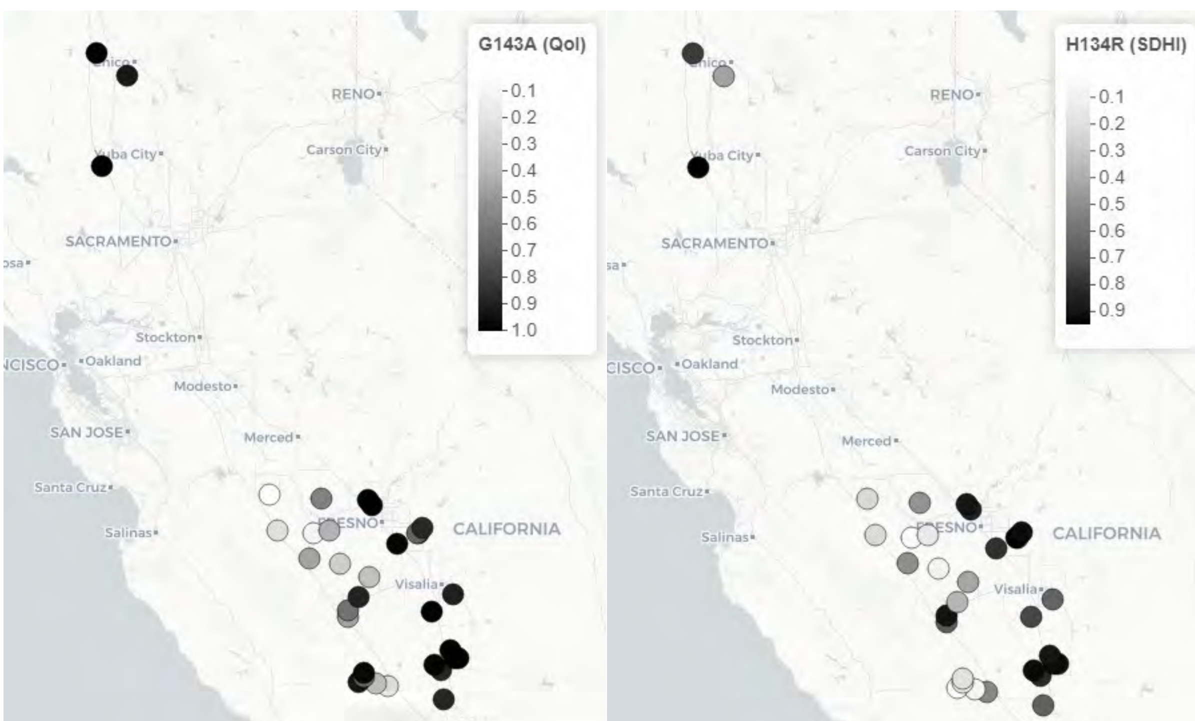


Fig. 2. Frequency of G143A and H134R mutations (indicating resistance levels) in California pistachio orchards. Values range from 0 to 1, with 1 representing the highest resistance levels.

Evaluating the Efficacy of Phosphites, Mefenoxam, and New Oomycota fungicides for Managing Phytophthora Crown and Root rot of Pistachio

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

Introduction

Phytophthora root and crown rot of pistachio is widespread in California and has been an increasing threat to pistachio trees in recent years. Main pathogens associated with this disease include *Phytophthora niederhauserii*, *P*. taxon walnut and *P. mediterranea*. Registered fungicides used for control of Phytophthora root and crown rot in pistachio have included potassium phosphite or other phosphonate compounds, and mefenoxam. New fungicides belonging to different FRAC groups with different modes of action specifically targeting oomycetes have recently become available. These fungicides include oxathiapiprolin, fluopicolide, ethaboxam, and mandipropamid. Our goal is to assess the efficacy of these new fungicides as compared to mefenoxam and potassium phosphite. The efficacy of each fungicide to manage Phytophthora crown and root rot was first assessed in green house experiments using 6-month-old rootstocks. Each plant was root-inoculated with *P. niederhauserii* (KARE2555) and 1 week later it was treated with either potassium phosphite, mefenoxam, oxathiapiprolin, mandipropamid, ethaboxam, fluopicolide, or water. After incubation in the green house for 20 weeks, the percent of root rot in each treatment was assessed. Systemic activity was also investigated for all fungicides, except mandipropamid, using 2-year-old UCB1 clonal plants planted in the field and 4-year-old potted UCB1 clonal plants kept under natural conditions. The plants were treated with each fungicide via drench application. One week later a mycelial plug (6mm) of *P. niederhauserii* (KARE2555) was wound-inoculated about 27 inches and 6 inches above the soil line for field plants and potted plants, respectively. Three total drench applications were made at 1-month intervals starting in May. Canker length was measured 1 month after each treatment. An additional experiment to determine best application timings and method of applications of potassium phosphite was carried out under field conditions using Golden Hill trees grafted onto UCB1. Six different application treatments were compared as follow: 3 foliar, 3 drip, 2 foliar, 2 drip, 1 drip +1 foliar, and water control. Treatments were applied at monthly intervals with the first application on June 1st, just one week after pathogen inoculation. *P. niederhauserii* (KARE2555) was wound-inoculated using a 6mm mycelial plug placed onto a 2 to 3-year-old branch for each replicate per treatment. Canker length was assessed at monthly intervals following the initial fungicide application.

Results and Discussion

Green house fungicide efficacy experiments. After 20 weeks post fungicide treatment, the percent of root rot incidence was assessed in the inoculated 6-month-old trees. Root rot incidence was calculated based on the percent of roots infected from isolating 20 root pieces in culture medium. Incidence of root rot was 27.5%, 10%, 2.5%, 1.25%, 0%, 2.5%, and 1.24 % percent for control, potassium phosphite, oxathiapiprolin, mandipropamid, ethaboxam, fluopicolide, and mefenoxam, respectively (Figure 1A). All treatments showed a reduction of root rot incidence relative to the water control. Overall, the efficacy of the new fungicides tested was equivalent to or superior to that of potassium phosphite or mefenoxam with all products significantly reducing root rot.

Systemic activity experiments. Systemic activity was measured as the reduction of Phytophthora canker length in plants treated with the various fungicides relative to plants treated with the water control. Measurements were recorded 1 month after the last fungicide application was made. In potted plants, canker length was greatest in fluopicolide and lowest in mefenoxam treated plants. The two fungicides with statistically smaller cankers than the control plants were potassium phosphite and mefenoxam, indicating these fungicides are systemic (Figure 1B). For field plants, canker length was also greatest in fluopicolide and lowest in mefenoxam treated plants. Plants treated with mefenoxam and potassium phosphite had cankers that were significantly smaller than the control. In both potted and field conditions, mefenoxam and potassium phosphite showed the most systemic activity, reducing canker length relative to the untreated control by 58% to 83%.

Phosphite Experiments. Measurements were taken 1 month after the last fungicide application was made. Application of potassium phosphite via foliar or chemigation showed a reduction in canker length, relative to the non-treated water control. Foliar applications were significantly more effective at reducing canker length caused by *P. niederhauserii*, compared to chemigation treatments (Figure 1D). Two foliar applications were as effective at reducing canker length as three foliar applications. Similarly, two chemigation applications were as effective at reducing canker length as three chemigation applications.

Conclusion

Green house experiments indicated that oxathiapiprolin, fluopicolide, ethaboxam, and mandipropamid as well as mefenoxam and potassium phosphite applied as soil drench greatly reduced Phytophthora root rot incidence. Systemic activity following soil applications was greatest for mefenoxam and potassium phosphite in both potted plants and field-planted experiments. Oxathiapiprolin was not translocated in trunks at 6 or 27 inches above the soil line, but more work is being conducted to detect partial translocation of this product near the root crown. Both chemigation and foliar applications of potassium phosphite were effective at reducing canker length caused by *P. niederhauserii*. However, foliar application of potassium phosphite was more effective at reducing canker length than chemigation, regardless of the number of applications (2 vs 3).

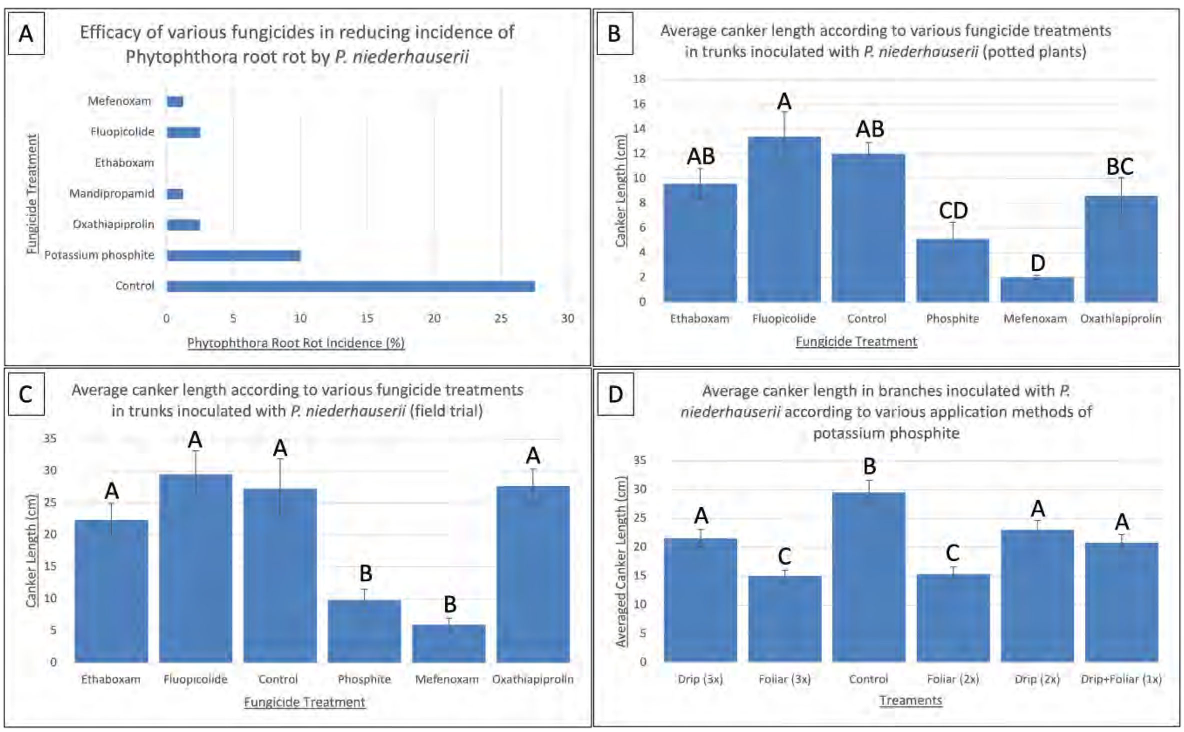


Figure 1. Summary of 2023 fungicide experiments. (A.) Efficacy of Oomycota fungicides in reducing Phytophthora root rot in green house trials. (B.) Systemic activity in potted plants as measured by canker length in trunks of UCB1 rootstocks. (C.) Systemic activity in field plants as measured by canker length in trunks. (D.) Comparison of multiple foliar vs drip application of potassium phosphite in reducing cankers caused by *P. niederhauserii* (KARE2555).

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

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

Introduction

Typically, 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 breadth of currently used rootstocks is somewhat limited. The California pistachio industry started with using *Pistacia atlantica* and *P. terebinthus* as rootstocks. These rootstocks seemed resistant to *Meloidogyne* spp. and *Pratylenchus vulnus* but were highly susceptible to Verticillium wilt that occurred widely in California (Michailides and Teviotdale, 2014; Crane and Maranto 1988; McKenry and Kretsch, 1984). Alleviation of the Verticillium problem came with different accessions of a controlled cross of *P. atlantica* × *P. integerrima* named 'UCB1' clonal rootstock. The name UCB1 identifies the cross but different siblings of this cross were taken into rootstock production. They all mitigate increasing challenges with Verticillium wilt.

The understanding of the role of plant-parasitic nematodes on pistachio in California needs further understanding. 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 previous preliminary screens of UCB1 clones, large differences between defined clones of this cross had been identified (McKenry, unpublished). In more recent greenhouse experiments, interaction of *Pratylenchus vulnus* with *Mesocriconema xenoplax* (ring nematode) on pistachio illustrated the susceptible host status of one clone of UCB1 (Westphal et al., 2016). With the expansion of pistachio, these orchards frequently follow a crop of cotton, grapes or walnut, 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 was determined. Evaluations of genotypes generated by Dr. Mallikarjuna Kuma Aradhya (USDA-ARS Davis) had been completed earlier. Now, the development of novel rootstocks to broaden the genetic base of pistachio rootstock by Dr. P.J. Brown is safeguarded for possible susceptibility to *P. vulnus*, *M. incognita* and *Mesocriconema xenoplax.*

Results and Discussion

<u>Field screening for *Pratylenchus vulnus* and *Meloidogyne incognita* host status</u> After several years of observing low population densities in pistachio roots compared to other nut crop hosts, numbers of *P. vulnus* increased in the 2017 planting when sampled in January 2022, similarly numbers increased in the 2018 planting of these UCB1 genotypes at the end of 2022. At these sampling times after five years of planting and inoculation, *P. vulnus* 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). Numbers of *M. incognita* remained low in UCB1 rootstock genotypes. Thus this surge in *P. vulnus* population densities after five years of cultivation was reproducible. In younger screening

plantings, the nematode numbers appeared lower than in the other nut crop rootstock comparatives. Summarizing these data sets, we hypothesized that *P. vulnus* reproduces on California UCB1 rootstocks but that it takes several years to reach high reproductive capacity on these hosts.

In the screening of novel rootstock breeding families from Dr. P.J. Brown's program, variability within each family in plant vigor noticeable. Population densities of *P. vulnus* were low two years after planting and inoculating but easier detectable in some families than others. Population densities of *M. incognita* in the roots of these trees were low, close to the detection level. These diversities of growth and nematode reproductive potential suggested the possibility to select different vigor types with limited nematode susceptibility.

<u>Microplot screen for susceptibility to ring nematode</u> 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. Ring nematode population densities appeared to decline to almost non-detectable under most rootstock cultivars from year to year for undetermined reasons.

<u>Microplot experiments to determine root lesion nematode damage potential</u> In two microplot experiments (experiment 1: planted in 2018, experiment 2: planted in 2021), the quantitative growth responses 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 were determined. Each of the trials was half planted to sandy loam soil and half to sand. The tentatively assigned resistant rootstock cultivar was more vigorous but response to nematode infection was similar among rootstock cultivars. When summarizing the data of both experiments, a biological threshold level was determined above which damage to pistachio rootstocks was measured. The population density determined was equivalent to those frequently encountered when an old walnut orchard is removed and the field repurposed for pistachio cultivation. Using the information of this principal damage potential would be best corroborated under field conditions. Care should be exercised when following a crop with high residual population densities of *P. vulnus* with pistachio.

<u>Microplot experiments with dagger nematodes</u> 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. The long life cycle of *Xiphinema index* makes it difficult to discern some trends on the soil infestation to plant growth responses.

<u>Conclusion</u>

A distinct role of plant-parasitic nematodes in the vigor and performance of pistachio rootstocks was defined for *P. vulnus.* Population densities comparable to those left behind after a walnut or almond orchard can damage pistachio rootstocks in experimental context. In screens for nematode susceptibility, the build-up of root lesion nematode on pistachio roots to levels comparable to susceptible Prunus or Juglans took five years. Further information is necessary to corroborate the damage potential of *P. vulnus* under commercial conditions. The principal susceptibility and the principal sensitivity to infection by *P. vulnus* call for caution when pistachio follows walnut. It seems prudent to take soil samples to learn about infestation levels if new pistachios are to be planted. Depending on detected population densities, mitigation actions should be considered.