# Executive Summaries
# 2018

California Pistachio Research Board
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# 2018 Manager's Report

Bob Klein

The 2018 crop year was filled with contra-indications where preliminary observations suggested one outcome that was entirely different than what ended up happening. When the CPRB met in early February 2018 to consider and award funding for research proposals, we had to consider the 2018 crop so we could have some assurance that assessment income would be adequate to continue ongoing projects. The 2018 crop situation seemed dire and the mood was grim; while we were hoping for an on-year crop, there had been little quality chill. Yield analyses on past crops by Craig Kallsen suggested that for every hour over 65°F between November 15 and February 15, yield declined by 13 pounds per acre. There had been over 200 hours above 65°F from November 15, 2017 to our February meeting and past research had suggested that chill accumulated after February 15 was not effective. We were wondering if 2015 was going to repeat itself. A week later, the weather turned cold and wet for the next 2 months and we instead had a record crop of over 990 million pounds with the lowest insect damage in the last decade or more. Overwintering populations of navel orangeworm (NOW) were high in 2018 and there was record heat that could drive NOW populations higher, but we ended the year with perhaps the lowest NOW damage since the early 2000s. There were excellent reasons to believe 2018 was going to be a small crop with high insect damage but we instead had a record crop of over 990 million pounds with very low insect damage. There is a lot more we need to learn about pistachios.

The CPRB funded 30 research projects in 2018 and the Executive Summaries of those projects are in this booklet. The CPRB has a broad research and education program that covers a range of projects from rootstock and variety development through nutrition, water relations, pest and disease management, and harvest. Multipage full reports will be posted on the website as the researchers finalize their data and submit the reports.

Navel orangeworm (NOW) is arguably the most serious problem facing the pistachio industry. NOW control relies on overwintering sanitation, population monitoring and timely pesticide applications, mating disruption, and prompt/early harvest. When all these efforts are accounted for and we tally up the yield losses and the problems resulting from NOW-related aflatoxin contamination, NOW costs the pistachio industry alone over $100 million annually. Consequently, the CPRB is interested in additional control tools. The largest research and development project, the NOW Sterile Insect Technique (SIT) was begun in 2015. NOW SIT is the massive inundation of an established NOW population with sterile NOW so the reproduction rate is depressed and the pest controlled. This technique has been used for other moth pests of other commodities, most notably on the pink bollworm of cotton in the San Joaquin Valley and then nationally. However, like the stock market, past successes are no guarantee of future success and it will likely require several more years of research to determine the feasibility and integrate it into the control strategies.

The project is a cooperative effort of the CPRB, USDA-APHIS, CDFA, USDA-ARS and UC Cooperative Extension. Funding is from the CPRB, USDA-APHIS, and bridge money from US Cotton. In 2018, the SIT project addressed several technical issues critical for the success of the project. These included mass rearing (up to 1 million per day), labeling with a red dye to insure identification in the field, irradiation, shipping from Phoenix to Shafter, aerial release over test plots in Lost Hills, trapping of released moths, and evaluation of mating behavior.

When the project was begun, it was not clear if NOW could be mass reared. The project has demonstrated that NOW can be mass-reared with the potential of 8 million moths per day with current technology and space. Like pink bollworm, NOW larvae will take up a red dye in their artificial diet and adult moths can be identified in the field after release. Improvements are being made in the labeling, but it currently is adequate although not optimal. The moths were successfully irradiated but shipping was difficult due to the basal metabolism causing deleterious increases in temperature during shipping. This was successfully resolved and over 95% of the moths survive shipping and 7 days in bio-chambers. Females do become mated in laboratory quality checks and in the field but have a low mating rate the first night in the field, possibly due to the stresses of shipment. Male behavior is uncertain and, while mobile, do not appear to be highly sexually active. The males will mate in laboratory quality checks but have not been observed to mate in the field. The sterile NOW appear to fly well and have been trapped over a mile from the release site but the trap numbers have been disappointingly low. While over 180 million moths were released from early April until mid-November on the 2000 acre test plot, a decrease in damage could not be measured due, at least in part, to the exceptionally low NOW damage observed across the industry in 2018. Research plans are being formulated for the 2019 crop year to resolve the problems encountered in 2018. A full written report for 2018 will be published on the CPRB website when all the data is analyzed, probably in February or March.

The CPRB has 16 members and alternates plus a Technical Advisory Committee to identify emerging problems and review proposed research. Being human, some things may slip past us and if you have concerns or suggestions, please let us know so we can respond. Best wishes for an excellent 2019 crop!

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# Improving the Online Pistachio Educational Program to Train Pistachio Production for New Growers and Handlers (2017-2018)

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## Introduction

The CDFA SCBGP (2015-2018) and the California Pistachio Research Board funded the project Online Nuts and Tree Fruits Education Program to Train California Specialty Crops Growers. The objective is to train California's beginning and transitioning farmers in improved orchard management practices and sustainable fruit production, with topics such as principles of tree biology, commodity production, food safety, technology tools, environmental conservation, fruit maturity and harvesting techniques. The project consists in developing interactive courses for each crop, including a series of online videos as well as quizzes, engagement activities and supplemental information. Courses on a modules fashion in English and Spanish for Pistachio, Stone Fruits, Walnut and Almond and postharvest handling went public on August 2017.

## Results and Discussion

Pistachio online program in English and Spanish was launched in October 2017. Since this time, the courses have been offered to ~240 Californian growers among people who either took or were interested in our "hands on" classroom extension course "Principles of Fruit and Nut Tree Growth, Cropping and Management" in the past and offered to a few extra test groups in our network. The students' progress and knowledge gain in the FNRIC-pistachio online program is evaluated by comparing results of the registration-entry questionnaire and of the tests at the end of each module.

In order to measure expected outcomes and collect feedback from students, we will gather baseline data using an entrance demographic survey and a finale evaluation survey. In this last year, based on our 2017-18 user review surveys, updating photos and technical materials (new ANR Pistachio Production Manual) were added to improve these modules quality. All shorter videos of five minutes each approximately, with more photographs were created according to the feedback received from a group of experts during the last reporting period. Supplemental information (reading material or any other useful tool), engagement activities (quiz or specific exercise) and entrance and exit tests were also prepared for each module and section in each of these courses. All this material and tools were uploaded to our Canvas platform, along with the entrance/demographic survey previously prepared and the scripts provided in pdf format for each video.

## Conclusion

The inexpensive and time-flexible FNRIC online fruit and nut production program will provide beginner farmers and others with the knowledge and tools to become sustainable and productive growers. Experts (UC Davis Faculty, UCCE Specialists and Farm advisors) in each field have been reviewed scripts, while pictures were collected and/or taken for video production.

# Comprehensive ecological and economical modeling of pesticide spray applications in pistachio orchards

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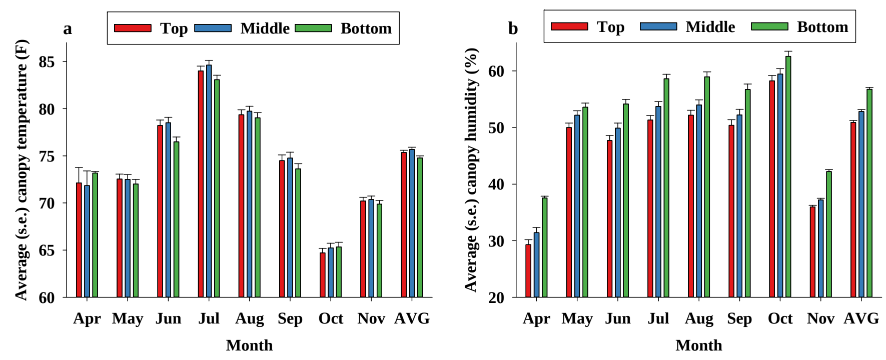
## Introduction

Insecticide spray applications in pistachio orchards represent both significant labor commitment and overall costs. In large pistachio orchards, it may take several weeks to complete insecticide spray applications. Growers primarily use existing degree-day models to predict NOW emergence, however these models do not assist in predicting when to apply pesticides. Equally important, these models do not take into account variation in temperature and humidity within each portion of pistachio trees that may affect both the NOW distribution and the obtained spray coverage. Low and inconsistent spray coverages increase the risk of spray failure, which is one of the major short-term challenges associated with effective navel orange worms (NOW) (*Amyelois transitella*) management. Long-term low and inconsistent spray coverages are also likely to increase the risk of NOW populations developing insecticide resistance.

## Results and Discussion

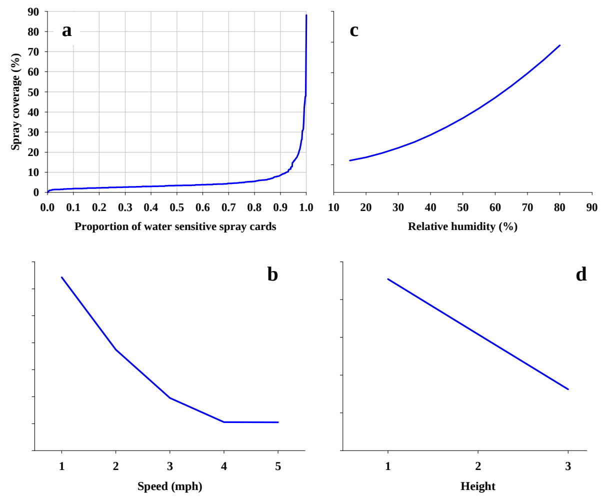
This project was initiated in 2018, and a PhD student, Miles Dakin, started in September 2018 and is conducting his research as part of this project. The project is composed of the following components: 1) Develop a damage model component describing the seasonal phenology of NOW population dynamics and of pistachio flower and fruit development. 2) Develop an insecticide spray application model component describing the influence of operations spray settings and weather conditions on insecticide spray applications. 3) Develop an economic model component describing the costs of insecticide spray applications, yield value, and economic losses incurred by navel orange worm. 4) Integration of the three model components and accompanying field validation. 5) Convert the comprehensive ecological and economic model into a user-friendly decision support tool and, through presentations at relevant meetings and conferences, disseminate it to groups of stakeholders. Important progress has been made within the first two project components, see below.

**Fig. 1.** Vertical profiles of temperature and relative humidity in pistachio canopies




**Develop a damage model component describing the seasonal phenology of NOW population dynamics and of pistachio flower and fruit development**. As an initial approach to development of models to describe seasonal phenology of NOW population dynamics and of pistachio flower and fruit development, we deployed temperature and relative humidity data loggers at three vertical positions (top, middle, and bottom third of the canopy) within a representative pistachio canopy in a commercial orchard in Hanford. The data loggers recorded temperature and relative humidity in hourly intervals from April 27$^{th}$ to November 4$^{th}$, 2018. Fig. 1 shows the monthly averages of temperature and relative humidity, and it is seen that: 1) the middle portion of the pistachio canopy is slightly warmer than the top and bottom portions (Fig. 1a), and 2) there was a strong and negative relationship between height and relative humidity (Fig. 1b). More weather data will be acquired in year 2 of this project. The vertical difference in canopy temperatures may seem subtle, but we are in the process of linking these temperature and relative humidity data to existing population models of NOW (Sanderson et al., 1989; Soderstrom et al., 1986). Thus, we will be able to predict NOW population dynamics within pistachio canopies.

**Fig. 2.** Multi-regression of spray coverage data




**2) Develop an insecticide spray application model component describing the influence of operations spray settings and weather conditions on insecticide spray applications**. Five times during the 2018 growing season, water sensitive spray cards were placed inside pistachio canopies at three vertical positions and in four cardinal directions (12 water sensitive spray cards per tree) during actual insecticide spray applications. With three replications and data collected based on five tractor speeds (1, 2, 3, 4 and 5 mph), data from a total of 900 water sensitive spray cards have been acquired. However at this point, only 528 water sensitive spray cards have been processed (spray coverage quantified). Temperature and relative humidity data were collected during insecticide spray applications. More data will be acquired in year 2 of this project, but we are already identifying very important trends in the spray coverage data (Fig. 2a): 1) 90% of the water sensitive spray cards had <10% spray coverage, and 2) only 5 spray cards (<1%) had spray coverage exceeding 40%. Thus, we have obtained strong indication of spray applications frequently leading to low and inconsistent coverage. In addition, we performed a multi-regression analysis, in which obtained was the response variable and temperature, relative humidity, height (top = 3, middle = 2, and bottom = 1), and tractor speed (in mph) were used as explanatory variables. More data is needed before definitive conclusions can be drawn, but Figs 2b-d show the identified and highly significant trends: 1) tractor speed negatively affects spray coverage (Fig. 2b), 2) spray coverage is positively associated with relative humidity (Fig. 2c), and 3) spray coverage is negatively associated with height (vertical position) (Fig. 2d). Based on this preliminary analysis, temperature alone did not significantly affect spray coverage.

## Conclusion

In year 1 of this project, we have generated comprehensive field data sets of both vertical distribution of weather conditions in pistachio canopies, and we have identified published models, so that we – as part of project component 1 - can develop reliable NOW population models. The same data can also be used to model the phenology of flower and fruit developments. The results presented in this report were based on 528 of the 900 spray coverage data points collected so far. We have collected data from a night spray application and know that the spray coverages were considerably higher. We are almost certain that a main recommendation from this project will be to spray at night (low temperature and high relative humidity).

## Literature Cited

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Soderstrom EL, Mackey BE & Brandl DG (1986) Interactive effects of low-oxygen atmospheres, relative humidity, and temperature on mortality of two stored-product moths (Lepidoptera: Pyralidae). Journal of Economic Entomology 79: 1303-1306. doi:10.1093/jee/79.5.1303.

# Spray Drift Mitigation Using Opposing Air-Blast Sprayers

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## Introduction

Pesticide drift is the airborne movement of pesticides away from the intended target. Off-site movement of pesticides from orchard air-blast sprayers is of particular concern when an orchard is adjacent to an environmentally sensitive site or adjacent to a crop in which the pesticide is not registered. Many studies have been conducted and recommendations have been made by private industry, academic and government agencies in an attempt to mitigate the off-site movement of pesticides. However, these studies have shown less coverage as compared to the grower standard. For grower acceptance of spray drift mitigation techniques, there must be excellent pesticide coverage of the outside rows as well as greatly reduced off-site drift.

This study investigated off-site spray deposition (drift) as well as coverage, contact bioassay using navel orangeworm (NOW) and insecticide deposition from an engine driven air-blast sprayer using two novel spray techniques. These techniques use two synchronized sprayers driving and spraying parallel to each other. In one technique (Double Spray), one engine driven air-blast sprayer drives between the first and second row and sprays both rows one and two. A second air-blast sprayer drives parallel with the inside sprayer along the outside of the orchard and sprays the first row with the inside spray solution and air on/baffles open and the outside spray solution off and air on. In the other technique (Air-In), an engine driven air-blast sprayer drives between the first and second row and sprays both rows one and two. A second engine driven air-blast sprayer drives parallel with the inside sprayer along the outside of the orchard and sprays the first row with the inside spray solution off and air on and the outside solution and air off /baffles closed. Then an engine driven air-blast sprayer returns along the outside of the orchard and sprays the first row with the spray solution and air on and the spray solution and air off/baffles closed towards the outside of the orchard. These two techniques were compared to a grower standard and an untreated check.

## Results

A study was conducted on a mature pistachio orchard near Three Rocks, CA. Insecticidal drift, coverage, bioassay of navel orangeworm and insecticide deposition of two novel spray techniques were compared to the grower standard spray technique and an untreated check. Drift was dramatically reduced in the Air-In spray technique compared to the grower standard while the Double Spray technique significantly reduced drift as compared to the grower standard. No drift was observed with the untreated check. Drift as indicated by the coverage of water sensitive cards at 25 ft. from the drip line was about 20% in the grower standard while only 10% in the Air-In application technique. Coverage of water sensitive cards was near zero at 100 ft. from the drip line with the Air-In application technique and 150 ft. with the grower standard. Drift with the Double Spray technique was between the Air-In and grower standard.

Foliage coverage high in the tree canopy was greater in the grower standard compared to the Air-In technique while coverage of the Double Spray technique was between the grower standard and Air-In technique. Foliage coverage low in the tree canopy was greater in the Air-In compared to grower standard, which was greater than the Double Spray technique. Regardless of spray technique, coverage was greater low in the tree canopy compared to high in the tree canopy. NOW mortality high in the tree canopy was greater in the Air-In technique compared to grower standard or the Double Spray technique, which was the differ. NOW mortality low in the tree canopy was greater in the Air-In technique compared to the Double Spray technique, which in turn, was greater than the grower standard. Regardless of spray technique, NOW mortality was greater low in the tree canopy compared to high in the tree canopy. The amount of methoxyfenozide (Intrepid 2F) high in the tree canopy was greater in the Double Spray technique compared to the grower standard, which in turn, was greater than the Air-In technique. The amount of methoxyfenozide low in the tree canopy was greater in the grower standard compared to the Double Spray or Air-In techniques, which did not differ. Regardless of spray technique, the amount of methoxyfenozide was greater low in the tree canopy compared to high in the tree canopy.

**Conclusion and Practical Applications**

Drift into adjacent areas can be significantly reduced by the Air-In technique compared to the grower standard. The insecticide coverage, NOW mortality and the amount of methoxyfenozide with the Air-In technique was as good as the grower standard. Drift into adjacent areas can also be significantly reduced by the Double Spray technique compared to the grower standard. The Double Spray technique was not significantly different from the Air-In technique; however drift was numerically higher than the Air-In technique. The insecticide coverage, NOW mortality and the amount of methoxyfenozide with the Double Spray technique was as good as the Air-In technique or grower standard. Thus, simple equipment adjustment and an alternative spray technique can significantly reduce the amount of drift while maintaining similar insecticidal control. Further improvements in drift mitigation are likely with spray equipment modification.

# Comparing the feeding damage of the invasive brown marmorated stink bug to native large bugs

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## Introduction

The brown marmorated stink bug (BMSB), *Halyomorpha halys*, is an invasive pest from Asia that is now found in California, including the Central Valley. In North America, BMSB was first found in Pennsylvania in 1996 and has since spread to much of the US fruit and vegetable regions (see www.stopbmsb.org/). In mid-Atlantic states, BMSB caused severe damage to tree and small fruit crops, in part because of large overwintering populations in that region that can migrate into crop systems. In other US regions, such as Oregon and California, BMSB populations are either more moderate or may still be increasing; but their impact on agricultural crops has yet to be fully understood.

Stink bugs are not new to California pistachios and these large bugs represent one of the more important pest groups that orchard managers must monitor and, when needed, treat. Most stink bugs found in California pistachios are native to North America; in fact, a redshouldered stink bug (*Thyanta pallidovirens*, the green stink bug (*Chinavia hilaris*), and the Uhler and Say stink bugs (*Chlorochroa uhleri* and *C. sayi*) were all identified prior to 1890. The invasive BMSB may be different than these native stink bugs, perhaps causing more damage because of its feeding behaviors, or perhaps becoming less of a pest because of lower population densities resulting from the Central Valley's hot, dry summers. Here we sought to determine if BMSB can survive on pistachios in the San Joaquin Valley, and if BMSB will cause the same amount of damage as our native stink bugs and leaffooted bugs.

## Results and Discussion

BMSB survival and pistachio nut damage were studied at the Kearney Agricultural Research and Extension Center (Parlier, California). For all trials, terminal branch ends with pistachio clusters were caged in April to enclose 1-3 clusters. BMSB, a leaffooted bug (*Leptoglossus zonatus*), and the green stink bug were placed inside cages and removed after each trial period.

Survival of BMSB or leaffooted bug was tested by placing eggs or first instars into cages on 28 June and 2, 10 and 21 August to determine if these pests could survive on pistachio only. In the June trial, 46.7±11.4% of leaffooted bug eggs developed to adults, whereas only 18.5±4.2% of BMSB eggs survived and developed to the adult stage. Similarly, in the early August trial, 48.1±1.9% of leaffooted bug eggs survived to the adult stage (no BMSB eggs were available to test). Later August trials used first instar nymphs and found lower survival; only13.3±8.8% of leaffooted bugs developed to adults and no BMSB survived. Almost all mortality occurred as eggs or first instar nymphs. We conclude that there was greater survival and development of leaffooted bug eggs and nymphs than BMSB eggs and nymphs and that the hot dry summer periods may limit the survival of BMSB on pistachios.

BMSB, green stink bug, and leaffooted bug adults, and BMSB nymphs (typically 3$^{rd}$ instars) were caged with pistachio clusters for 5-day feeding periods from May through August. Results found a single BMSB adult caused >15 new epicarp lesions per cage in early and mid-May inoculation periods and considerably less damage thereafter (Figure 1). BMSB nymphs, during the same period caused less damage (similar to earlier work with native stink bug adults and nymphs, see discussion below). In comparison, green stink bug feeding resulted in about 8 new epicarp lesions per cage and leaffooted bug adults caused slightly more damage; all trials are compared with a no insect control (Figure 1). Based on epicarp lesions, BMSB appeared to cause more damage than the native bugs.

There were more 'new-feeding' wounds than epicarp lesions, but as this damage is difficult to accurately assess we focused on harvest data. After the 5-day feeing period, there was also considerable nut drop. At harvest time, across all inoculation dates, the percentage of clean nuts was highest in the control (71.1%), followed by BMSB nymphs (65.1%), green stink bug adults (65.0%), BMSB adults (53.7%) and leaffooted bug adults (48.9%). Most cluster damage resulted from aborted nuts (13.9%) rather than kernel damage (3.1%) or damage by other means (0.3%). There was nearly two-fold greater rachis damage in the insect treatments (7.6%) and this often led to damaged nuts. Generally, nut damage decreased during the successive inoculation dates, but on the first inoculation date (2 May) there were fewer clean nuts, most likely from nut drop that occurred more than a week after insect inoculation.

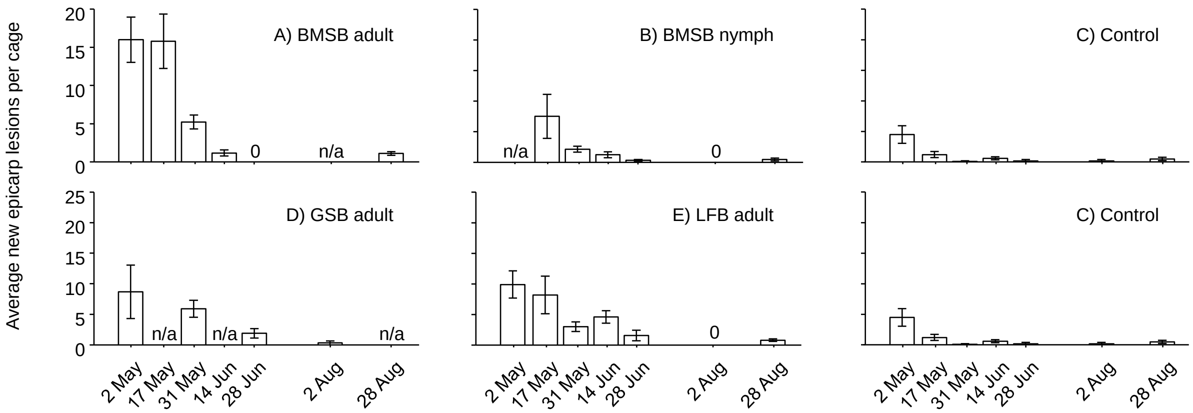


Figure 1. Insect were caged for 5-day feeding periods, from May to August and the amount of epicarp lesions were used as an indication of damage from (A) brown marmorated stink bug (BMSB) adults, (B) BMSB nymphs, (C) no insect control, (D) green stink bug adults and (E) leaffooted bug adults (n/a = no insects tested).

Results are similar to earlier studies that showed two factors are key to determine hemipteran damage levels. First, how large is the insect? Basically, the larger the bug, the more damage it causes because it has longer and stronger mouthparts (e.g., adults are more damaging than nymphs). Second, at what time of the season does the insect feed? Stink bug feeding early in the season can result in fruit drop and epicarp lesion (damage to the outer shell). Often, crop loss from bug damage in April and early May is compensated by natural fruit drop. Around mid-May, cluster size is set, and stink bug feeding will cause epicarp lesions on fruit that remain in the cluster and stain the outer shell and kernel necrosis at harvest time, lowering market value. BMSB is larger than green stink bug, but smaller than leaffooted bug.

## Conclusion

BMSB had a difficult time developing from an egg or first instar to an adult on pistachios during June and August; the native leaffooted bug had better survival. This suggest that pistachios are an unlikely season-long host and the BMSB will likely be more important migrating into the pistachio orchard early in the season or moving between other food sources into the pistachio canopy later in the season.

BMSB adults caused more feeding damage immediately after a 5-day feeding period, as measured by epicarp lesions, than either green stink bug or leaffooted bug. Damage was greater with adults than nymphs and decreased as the season progressed. The trial appears to have started too late (2 May 2018) to capture the number of dropped nuts, although percentage clean nuts was lowest in the 2 May trial, indicated dropped nuts more than a week after the feeding event. This preliminary work suggests that, overall, BMSB adult damage appeared to be most comparable with leaffooted bug adult damage, and slightly greater than the green stink bug adults.

# The use of trap crops to monitor and suppress large bug damage

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## Introduction

Pistachios are attacked by a variety of insect pests, including "small" and "large" bugs that can be a damaging group requiring annual treatments. Small bugs include several species of Miridae and Rhopalidae, most importantly *Calocoris norvegicus*, *Phytocoris relativus* and lygus (*Lygus hesperus*). The small bugs may be abundant early in the season and can cause significant epicarp lesion (damage to the outer shell) and fruit drop but cease to cause damage after the shell begins to harden. The large bugs are composed of stink bug species such as a redshouldered stink bug (*Thyanta pallidovirens*), Uhler's and Say's stink bugs (*Chlorochroa uhleri* and *C. sayi*), the flat green stink bug (*Chinavia hilare*), and leaffooted bugs (*Leptoglossus zonatus* and *L. clypealis*). The large bugs can cause the same damage as their smaller relatives during the first half of the season. However, during the latter half of the season (from shell-hardening until harvest) the large bugs continue to puncture the nut-meat through the shell, causing epicarp lesion, kernel necrosis (damage to the nut meat) and stigmatomycosis (a mold that infests the nut).

Here, we began a study of novel, irrigated strips of annual ground covers to increase the abundance of large bug natural enemies, improve large bug monitoring, and hold large bugs away from the pistachio canopy (e.g., a trap crop). This work was initiated by an organic grower-collaborator seeking to reduce weeds by using a novel middle-row, buried irrigation system. We sought to determine if strips of season-long ground vegetation provide a monitoring tool or increase bio-controls (both can reduce pesticide use) thereby providing multiple benefits to pistachio pest management. The pests' migratory behavior, low economic injury threshold, poor monitoring systems, and inefficient biological controls dictate the primary goal of an IPM program should be to prevent the arrival of hemipteran pests in the pistachio canopy. Our 2018 objectives were to (1) follow the seasonal population levels of small and large bugs on the seeded ground cover strips and the pistachio tree itself and (2) determine the levels of pistachio damage throughout the season and at harvest.

The field trial took place in a 230-acre pistachio block that was subdivided into 5 replicate blocks with 2 plots each (10 plots total, 23 acres/plot). Within each block one of the plots was randomly assigned to the "trap crop" and the other to the "control" treatment. Each plot was 19 rows wide with 180 trees/row. In March, the center 7 row-middles of the trap crop plots were seeded to a mix of alfalfa (*Medicago sativa*), vetch (*Vicia sativa*) and mustard (*Brassica* sp.). Ground covers were present in the control plots, but they consisted of resident weedy vegetation. All plots were sampled 2x/month between May and October. Sweep nets were used to sample insects on the ground covers and beat sampling was used to sample insects in the tree canopy. Visual estimates of crop damage were conducted 1x/month, and at harvest subplots were commercially harvested and graded according to industry standards.


## Results and Discussion

**1. Seasonal populations of small bugs, large bugs, and beneficial insects.** Populations of small and large bugs on the trap crop were greater than on the resident weedy vegetation in control plots, but this did not necessarily lead to lower densities in the tree canopy of plots with the trap crop (Fig. 1). In fact, small bugs were at times more abundant in the tree canopy in the trap crop plots compared to the tree canopy in control plots. Beneficial insects were in greater abundance in the trap crop plots. Predatory insects were more abundant on the trap crop itself than on weedy vegetation while parasitoid populations were increased in the tree canopy in trap crop plots (Fig. 2).

**2. Crop damage.** There was no difference in crop damage or insect infestation across nuts collected from the plots with and without the seeded trap crops.

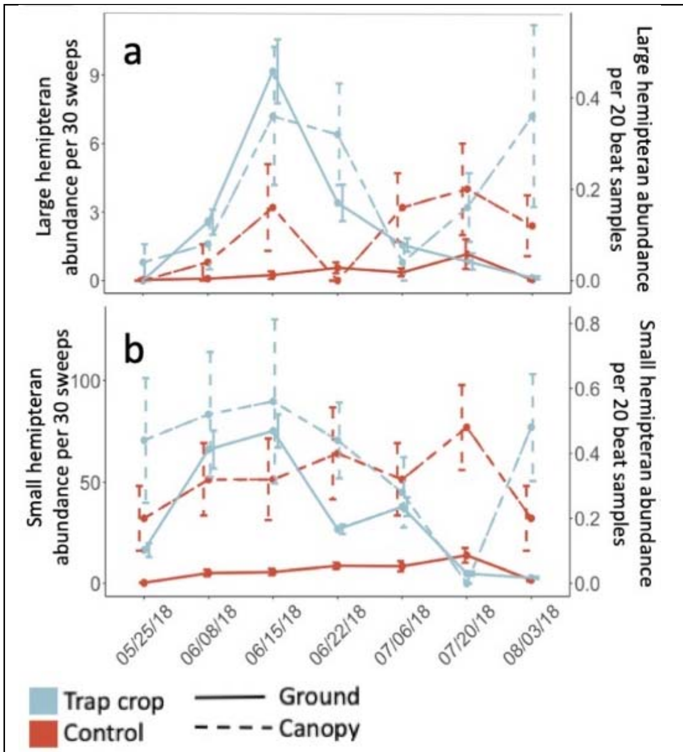


*Figure 1. Densities of large (1A) and small (1B) hemipterans in the ground covers and tree canopy.*

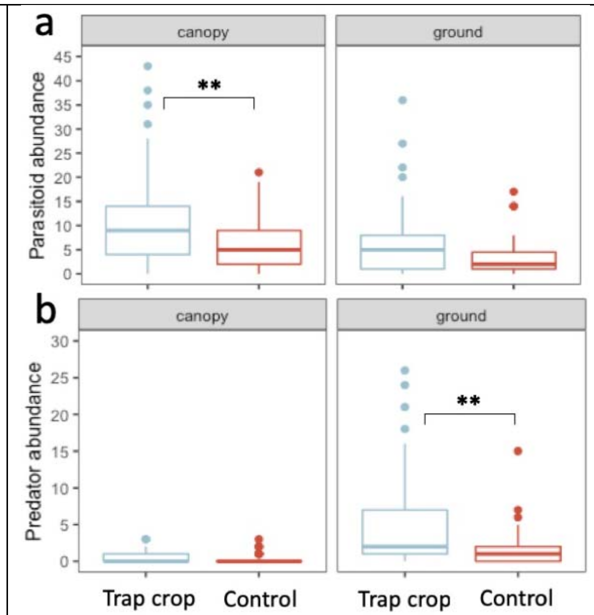


*Figure 2. Beneficial insect densities were higher in the trap crop plots, with more predators on the trap crops themselves and parasitoids in the tree canopy.*

__**Conclusions and Practical Implications**__

Trap crops can influence small and large plant bug populations, but this did not necessarily lead to reduced densities in the tree canopy itself. This study will be repeated in Year 2, with improvements to trap crop species used, including weedy species, that are being screened as candidate trap crops for future studies. It may be that alternate trap crop species could be selected based on key pest preferences (i.e. a more attractive trap crop). The development of a novel, center-line drip irrigation strategy has presented a unique opportunity to evaluate the use of summer trap crops to manipulate plant bug populations and possibly serve as an early indicator of orchard colonization by these pests.

# Another look at pheromonal and related attractants for leaffooted bugs infesting California nut crops

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## Introduction

Leaffooted bugs (LFB, primarily *Leptoglossus zonatus* and *L. clypealis*) are some of the most damaging insect pests in California's pistachio and almond crops. LFB overwinter primarily as adults, then move to feeding sites and start to oviposit in spring. There are typically 3 generations per year, although a partial or complete 4$^{th}$ generation is possible in some regions. Damage is unpredictable because bug populations immigrate into nut crops from surrounding crops or native vegetation. In the congeneric species *L. australis*, there is evidence that males move into a crop first and begin producing an aggregation pheromone that accelerates the buildup of bugs in the crop (Yasuda and Tsurumachi 1994). Because of these rapid buildups, and because bug damage may only become apparent sometime after the bugs have left, continuous monitoring is a key factor in timing treatments. Current monitoring methods rely primarily on beat tray or sweep net sampling. Trapping systems based on attractants would be of great value for monitoring. Thus, our overall goal is to reexamine the pheromone-mediated behavior of leaffooted bugs, with the aim of identifying any insect-produced compounds that could be exploited as attractants for traps, or in control methods such as attract-and-kill, for IPM programs.

LFB could use at least four different types of pheromones. First, both sexes produce alarm pheromones and defensive secretions which are not species specific, and so are unlikely to be involved in sexual interactions. Second, male LFB produce compounds such as benzyl alcohol which are not attractants (Wang and Millar 2000) but instead, appear to be aphrodisiacs, rendering females receptive to mating. Third, there is some evidence that male, summer-form LFB produce aggregation pheromones that attract both sexes into orchards. Finally, LFB form overwintering aggregations in sheltered spots, and it is likely that pheromonal signals assist in the formation and maintenance of these overwintering aggregations. These signals are likely to be different from the signals used to attract bugs into a crop for feeding and mating. The possible source and structures of the latter two types of pheromones are not yet known.

**A primary project objective is to identify and verify the function of the pheromones involved in the formation of the summer and overwintering aggregations, with the goal of developing practical applications for these pheromones for LFB management**. **An equally important, parallel objective is to optimize traps and trapping protocols for LFB.**

## Results and Discussion

**1. Identification of potential attractant pheromones from male *L. zonatus*.** We identified nine compounds which are consistently produced only by sexually mature, summerform males, and so are likely pheromone candidates. These include benzyl alcohol (**1**), nonanal (**2**), decanal (**3**), β-caryophyllene (**5**), *E*-β-farnesene (**7**), *cis*- and *trans*-α-bergamotene (**4** and **6**), sesquiphellandrene (**9**), and an unknown (**8**). Compounds **1**, **2**, **3**, **5**, and **7** are commercially available, and we have synthesized compounds **4**, **6**, and **9**. We have not yet fully identified compound **8**. In electroantennogram bioassays, the antennae of females responded strongly, in descending order, to compounds **8**, **3**, **4**, and **1**, even though **8** is present in lower quantities than most of the other compounds. Thus, a large part of our efforts for the past year have been focused on trying to identify compound **8**. In June, we combined 54 separate extracts collected from summerform males over several months, and purified out a few micrograms of **8**, sufficient to provide some basic nuclear magnetic resonance (NMR) spectra. From these spectra, we narrowed down the structural possibilities to six most likely structures, but we were not able to determine the exact structure.

Thus, we are proceeding on two fronts. First, we have begun the synthesis of the structure that we consider most likely. Second, we are collecting and stockpiling more extracts from summerform males, to make a second attempt at isolating enough of compound **8** to get the full gamut of NMR spectra that we need to narrow the structural possibilities down to one structure, i.e., to fully identify it.

**2. Work with possible attractant pheromones of *L. clypealis*.** We were not able to establish a colony of our second target species, *L. clypealis*, until November of 2017, when we collected specimens from an overwintering aggregation near Palm Springs to begin a lab colony. We were able to shift them back to summerform using long daylength, and made a few collections from males before the colony died out. Those extracts were enough to show that *L. clypealis* produce many of the same compounds as *L. zonatus* males, including unknown compound 8. We are currently stymied with this species until we can collect enough to start a robust lab colony.

**3. Field evaluation of candidate pheromone blends.** We conducted three trials in almond (4/24 – 5/17), two trials in pistachio (7/10-7/30, 8/29-9/5) and one trial in pomegranate (8/29 – 9/5), using partial reconstructions of the male-produced compounds. In each trial, replicate pairs of fluon-coated hanging panel traps with and without pheromone lures were set up at the edge of the orchard. Paired traps were 10 m apart and each replicate pair of traps was 18 – 100 m apart, depending on the trial. Traps were checked weekly. In all trials the partial reconstructions of the blend did not appear to attract LFB adults.

**4. Field trials to evaluate LFB attraction to aggregations.** In a first field trials in pistachio, we evaluated attraction to artificial LFB aggregations consisting of 6 adults (all male, all female, or 3 of each), placed inside a 5-gallon paint strainer bag (Trimaco), with and without a mummy pistachio cluster, and hung from the center of a fluon-coated cross vane panel trap. Negative controls included empty paint strainer bags with and without mummy clusters. Treatments were replicated 5 times, with replicates spaced 18 m apart and treatments within replicates spaced 10 m apart. Traps were deployed August 8 and acounted on August 15. In the 2nd trial, larger aggregations were used (20 total adults, instead of 6) and aggregations were provided either green beans or pomegranate as food. Baited traps were set up on August 22 and counted on August 28. In both trials the artificial LFB aggregations did not attract wild LFB adults, although the presence of a mummy pistachio cluster seemed to result in fairly consistent catch of LFB adults.

**5. Field trials to improve the use of hanging panel traps.** In 2017 we determined that a black cross vane panel trap coated in fluon could effectively trap LFB in orchards. In 2018 we evaluated the effect of trap color, comparing black, white, red, yellow, green and blue traps. Traps were set up along the edge of a heavily infested pomegranate hedgerow. There were 5 replicates in total, spaced 20 m apart, and within each replicate the different colored traps were spaced 3 m apart. Traps were set up on August 28 and checked weekly until November 20. Results indicated that yellow and blue traps are more attractive than the black trap, with yellow being most attractive.

## Conclusions and Practical Applications

1. The identification and synthesis of all the compounds produced by summerform male LFB is continuing, with the goal of having complete blends to test in 2019.
2. Testing of the effects of cuticular hydrocarbons from summer and winterforms is on hold until we can get vigorous, healthy colonies of each form in the lab simultaneously.
3. There was no attraction to partial reconstructions of the male-produced compounds, nor to the artificial aggregations, although mummy pistachio clusters may be attractive.
4. We continue to improve and refine the use of the hanging panel trap. Yellow and blue traps were more effective than black traps.
5. Work to follow LFB from pomegranates to their overwintering aggregations is ongoing. We are also in the process of dissecting all females collected in 2018 to determine reproductive status and egg loads.

# Investigating pheromones for the Leaffooted bug, *Leptoglossus zonatus*: developing an alternative control to insecticides

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## Introduction

The leaffooted bug species, *Leptoglossus zonatus*, is a large seed-feeding insect. In California, this insect has been collected from Butte County to Kern County in the Central Valley, and can be damaging to pistachios and other crops (Michailides 1989, Joyce et al 2014, 2017). Although there are at least two species of leaffooted bugs which occur in pistachios, *Leptoglossus clypealis* and *Leptoglossus zonatus*, *L. zonatus* is substantially larger than *L. clypealis*. A field-cage study of feeding damage found that *L. zonatus* was more damaging than *L. clypealis* (Joyce et al. 2015). There is a need to develop traps or monitoring devices to detect the presence of this insect in orchards, before bug feeding is observed and before it causes economic damage. *L. zonatus* are difficult to monitor as they are elusive. Effective insecticides to control these insects are being further restricted; additional monitoring and management tools are needed. The research in this study will contribute to understanding the biology and behavior of *L. zonatus* as well as to the ability to trap and monitor this insect.

Potential attractants for leaffooted bugs include pheromones, host plant volatiles or induced plant volatiles associated with bug feeding (Aldrich et al. 1979, Yasuda 1998, Wang and Miller 2000). Our work last year found that mating pairs of *L. zonatus* were attractive to other adult *L. zonatus*, and relatively more attractive to adult males than to adult females. The goal of this project was to further investigate the attraction of *L. zonatus* to volatiles and the environmental conditions that influence formation and dispersal from aggregations. The ability to attract, detect and quantify adult *Leptoglossus* spp. in the field before damage is observed in pistachios or in almonds could improve timing of controls and could also reduce the use of preventative insecticide applications.

## Results and Discussion

We continued to raise large colonies of the insect *Leptoglossus zonatus* in the lab during the year, so that insects were available for lab bioassays. Insects were produced to conduct the studies described below, which were 1) to examine attraction to volatile odor sources in the wind tunnel and 2) to investigate dispersal from aggregations. Each week we prepared a group of newly emerged adult females and newly emerged adult males, so that unmated adult insects were available for standardized behavior trials. The age of sexual maturity of adult *L. zonatus* was previously investigated, and subsequently 4-week old unmated adults were used for remaining lab behavior bioassays.

Last year, we found that 5 mating pairs of *L. zonatus* were the most attractive odor source for males. This year, we further explored whether increasing the number of mating pairs in a choice trial would result in a higher frequency of adult insects choosing this odor source. Four additional experiments were conducted. Insects were only used for one behavioral trial to avoid pseudo-replication. Dual choice trials were run to compare the following 1) the attraction of males to 15 mating pairs vs. a control, 2) the attraction of females to 15 mating pairs vs. a control, 3) the attraction of males to 20 mating pairs vs. a control and 4) the attraction of females to 20 mating pairs vs. a control. Data recorded included the number of insects taking flight, number of landings on each odor choice, the time spent on each odor source, and the time from the start of the experiment until a test insect landed on an odor. At least 25 replicates were run for each of these experiments. Insects were observed for at least 15 minutes in experiments.

Preliminary trials using 15 mating pairs as the attractant source found a result similar to when 5 mating pairs; about twice the number of males were attracted to the odor source compared to a control. The number of mating pairs was increased again, and 20 mating pairs were used in the following experiments.

The third experiment was testing the attraction of males in a dual choice trial, to 20 mating pairs of *L. zonatus* or a control. Approximately twice the number of males landed on the branch with 20 mating pairs of *L. zonatus*, rather than on the control; there was a significant difference between the two choices. In the fourth experiment, adult females were similarly offered a dual choice, of either 20 mating pairs or a control branch. In this experiment, females landed on the branch with mating pairs more frequently than landing on the control, but the difference in the choice test was not statistically significant. Overall, the wind tunnel trials found that males appeared to have a stronger attraction than the females to the volatiles associated with mating pairs. The time spent by a male on the two odor sources was compared, and there was no significant difference in time on the two choices. However, females spent significantly more time on the control than on the branch with the mating pairs. Above experiments suggest that the mating pairs are more likely to attract males than females.

Aggregations of leaffooted bugs could be influenced by environmental conditions such as changing light and temperature in the spring and the fall. Last spring 2017, we investigated the effect of light and temperature on the formation of aggregations in the lab. Changing light conditions (when temperature was held constant) did not cause formation of aggregations; temperature may a critical factor which causes insects to join or disperse from aggregations. This coming spring, aggregations will be placed outdoors in field cages and will be monitored daily. We will determine the number of insects leaving an aggregation each day and relate it to temperature.

## **Conclusion**

Results suggest that the most attractive odor source in this project to date is associated with mating pairs of *L. zonatus.* These experiments used unmated *L. zonatus* which were 4-weeks old for behavior bioassays. Volatiles should be collected from this odor source and similarly tested for the ability to attract adults. Traps using adult insect mating pairs as lures still need to be tested in the field.

# Monitoring and overcoming pesticide resistance in navel orangeworm (*Amyelois transitella*)

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## Introduction

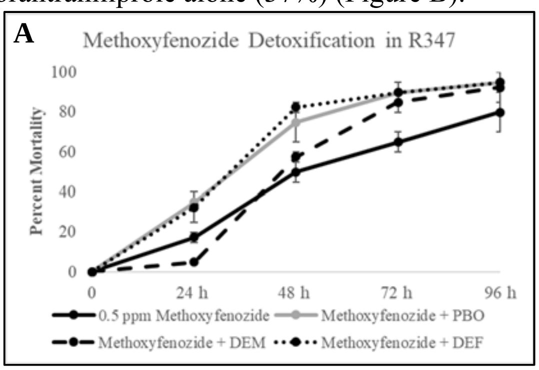
Ever since navel orangeworm (NOW) pyrethroid resistance was first reported in Kern County almond orchards in 2013, we have investigated mechanism(s) associated with resistance evolution using populations collected from the same orchards in Kern County. Although we originally reported contributions by detoxification enzymes such as the cytochrome P450 monooxygenases and carboxylesterases toward pyrethroid resistance in NOW, recent experiments have suggested that modifications of the insect cuticle may also facilitate resistance through reduced penetration of insecticides. A combination of these resistance mechanisms could cause devastating losses for growers because of the potential for cross-resistance to insecticides from other classes beyond the pyrethroids. Our work this year has focused on two insecticides frequently applied as alternatives to pyrethroids – methoxyfenozide (Intrepid) and chlorantraniliprole (Altacor). In these experiments we compared a pyrethroid-resistant (R347) population collected from Kern County with a susceptible population from Madera County almond orchards (ALM) where pyrethroid resistance has not been reported. Both populations were collected in summer 2016 and have been maintained in the Berenbaum Lab at the University of Illinois. Using our established feeding assay protocols with first instar larvae, we assessed the toxicity of methoxyfenozide at different concentrations to both populations. When insects acquire resistance to insecticides as a result of enhanced metabolism, this frequently occurs as a result of enzymes produced from detoxification superfamilies such as cytochrome P450 monooxygenases (P450s), glutathione-S-transferases (GSTs), and carboxylesterases (COEs). We continued feeding assays with first instar larvae using methoxyfenozide in combination with synthetic inhibitors that prevent P450, GST, and COE detoxification of methoxyfenozide; a non-toxic inhibitor dose targeting each of these three detoxification pathways separately in NOW that increases methoxyfenozide mortality would suggest involvement of the enzymes blocked by the inhibitor in methoxyfenozide detoxification. Previous research by from the Berenbaum Laboratory had determined that methoxyfenozide was not detoxified by P450s because synergism was not observed using the P450 inhibitor; however, these experiments were conducted using a laboratory population acclimated to controlled conditions and more susceptible to insecticides than field populations. We chose to examine the R347 population collected recently from the field to reassess the involvement of P450s, GSTs, and COEs in the metabolism of methoxyfenozide.
In a separate series of experiments, we investigated the potential for chlorantraniliprole to be synergized by kaolin clay. The mechanism of action for kaolin is unknown, although it probably disrupts insect cuticle integrity as a sorptive dust. If kaolin is abrasive to the NOW cuticle, then it may allow for an insecticide to penetrate and kill larvae/adults when sprayed in combination with an insecticide. Our preliminary assessment of the potential for kaolin to synergize chlorantraniliprole was carried out by infestation experiments with almond kernels. We obtained some promising results from these experiments and intend to assess kaolin-insecticide interactions on egg toxicity, contact toxicity, and infestation trials with pistachios.

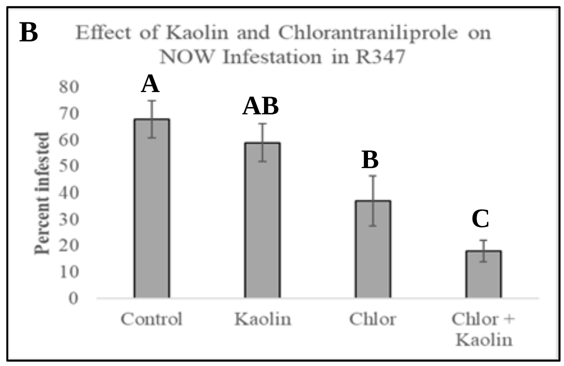
## Results and Discussion

Methoxyfenozide concentrations tested in feeding assays included 0.1 ppm, 0.2 ppm, 0.5 ppm, and 1 ppm. There was no difference in mortality between populations at a high concentration ($\geq$ 0.5 ppm); however, at 0.2 ppm, the resistant population experienced lower mortality (5%) than the susceptible strain (28%) ($N = 40$, $\chi^2 = 7.44$, $df = 1$, $P < 0.01$). These results suggest there may be cross-resistance potential at low concentrations of methoxyfenozide. We were unable to identify median-lethal concentrations with

($LC_{50}$) confidence intervals because the mortality data were not well-distributed based on methoxyfenozide concentrations examined. In our feeding assay protocols, insecticides are stirred into an artificial diet and larvae receive exposure through ingestion as well as surface contact with the diet. The reduced mortality observed at 0.2 ppm in the resistant R347 population may have resulted from cuticular modifications that reduce methoxyfenozide penetratation. We plan on adding replicates and additional concentrations because we did not observe any differences between strains beyond 0.2 ppm. Further investigation of methoxyfenozide toxicity between resistant and susceptible populations will allow us to generate $LC_{50}$ values as a more reliable measure to differentiate these populations. In assays with methoxyfenozide and the PBO, DEM, and DEF inhibitors, we selected a 0.5 ppm dose of methoxyfenozide because we expected approximately 50% mortality after 48 h and this allowed us to test the mortality effects of each inhibitor in combination with methoxyfenozide. The P450 inhibitor and carboxylesterase inhibitor DEF significantly increased larval mortality after 72 h ($\chi^2 = 7.17$, df = 1, $P = 0.0074$), an indication that methoxyfenozide may be detoxified by P450 and/or carboxylesterases (Figure A). The DEF inhibitor also significantly increased methoxyfenozide mortality after 48 h ($\chi^2 = 9.45$, df = 1, $P = 0.002$)

Almond infestation experiments were conducted using a 1% field concentration of chlorantraniliprole and the max label rate for kaolin. A total of 200 neonates were placed on 100 nuts each of which was treated with kaolin, chlorantraniliprole, and chlorantraniliprole + kaolin. The addition of kaolin significantly reduced infestation ($P < 0.05$, one-way ANOVA + Fisher's LSD) by 2-fold (18%) relative to chlorantraniliprole alone (37%) (Figure B).




Figures. (A) Determination of methoxyfenozide detoxifcation pathways using the P450 inhibitor PBO, GST inhibitor DEM, and COE inhibitor DEF with a 0.5 ppm dose of methoxyfenozide (N = 40 per treatment). (B) Infestation of almond kernels (N = 100 kernels, 200 larvae per treatment) treated with control solution (33% acetone), kaolin (30mg/mL), chlorantraniliprole (0.01mg/mL), and chlorantraniliprole + kaolin. Error bars represent the standard error.

## Conclusion

Our finding that PBO increases the toxicity of methoxyfenozide has implications for resistance management of the navel orangeworm. Focusing on mode of action alone without considering the metabolic pathway of detoxification may lead to increased selection pressure and facilitate resistance development. Because pyrethroids are detoxified by P450s and COEs in NOW, growers may need to consider alternative management strategies with methoxyfenozide applications as insecticides detoxified by the same mechanism present a higher potential loss of efficacy via cross-resistance, especially in resistant genotypes with enhanced P450 or COE activity. Although the mechanism of action for kaolin is unknown, our results demonstrate potential for kaolin to synergize chlorantraniliprole in field applications and warrant further investigation with other insecticide classes used in NOW management. Because Altacor sprays provide extended control for NOW, the addition of kaolin to sprays may enhance knockdown of early populations during first and second flights. Moreover, the potential benefits of kaolin with insecticides may extend beyond NOW and provide an option for additional insect pests of pistachio orchards. However, we need to examine egg toxicity and larval contact toxicity through bioassays and test for synergism with formulated products such as Altacor, Intrepid, Intrepid-Edge, Bifenture, and LambdaCy before suggesting use of kaolin in field sprays.

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

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## Introduction

Mass rearing and irradiation of navel orangeworm (NOW, *Amyelois transitella*) provides a unique opportunity to explore the use of sterile insect technique (SIT) as part of an area-wide integrated pest management (IPM) program for pistachio and other tree crops affected by NOW. This endeavor also may provide large quantities of NOW that can safely be released into commercial fields in large-scale mark-release-recapture experiments to improve monitoring techniques and mating disruption. In its current form, moths for this NOW-SIT project will be produced and irradiated at a USDA-APHIS facility near Phoenix, AZ, and then shipped to California for release. In order to fully capture the opportunities offered by a NOW-SIT program, it is necessary to first verify the viability and performance of irradiated/shipped moths relative to wild moths. As such, this project will evaluate the effects of both irradiation as well as the shipping process on NOW flight, dispersal and mating competitiveness under both laboratory and field conditions.

## Results and Discussion

**1. Recovery of irradiated NOW – Kearney Ag. Center.** Field trials were conducted in a 2-acre block of pistachios with no sprays or mating disruption. Four wing-traps (Trece Pherocon IC) with pheromone lures (Suterra Biolure) were setup on 3/19. Traps were placed 50 feet apart in a square pattern in the center of the block. Trap liners were replaced weekly (3/19-8/7) and then daily (8/7-10/15). Lures were replaced every 4 weeks. Additionally, nine mating tables were hung at about 1.5 m towards the center of the block in a 3x3 grid. A single virgin female NOW with a clipped wing was placed into each mating table at the end of the day. Tables were checked the following morning at sunrise to record copulation status and, later, moths were dissected to determine mating success. Unirradiated females used in these mating table studies came from a colony maintained by Burks that was initiated from material collected near Mendota, CA. This "Mendota" strain subsequently served as a control population in comparative studies with the irradiated "Phoenix" strain (see below). Weekly release of approximately 6,000 irradiated "Phoenix" moths began on 6/10. The Phoenix moths are marked internally with a red dye and recapture is determined by dissecting moths to look for presence of the dye. Recovery of marked NOW in the wing-traps was extremely low (0.24%, 11/4,455 NOW). At the same time, no marked males were ever recovered in copulation with the sentinel Mendota females from the mating tables. On 9/7 flight-traps with a combination pheromone and phenyl-proprionate (PPO) lure were placed into the block, but this too did not lead to any greater recovery of Phoenix NOW.

**2. Recovery of irradiated NOW – Murray.** This field trial was conducted in a 640-acre section of land comprised of 480 ac. pistachio (NW, NE, SE quadrants) and 160 ac. almond (SW quadrant). The orchards were under conventional management without mating disruption. A grid of 64 wing-traps with pheromone lures (Trece L2) were setup in an 8x8 grid evenly spaced over the entire 640 acres (1 trap every 10 acres). In early July, aerial release of irradiated moths was initiated over the 160 acres of pistachio in the NE quadrant. 750,000 moths were released 1x/week over the target area. Similar to what was observed at the Kearney Ag. Center, recovery of marked moths was low (0.35%, 54/15,493 NOW). Notably, some moths were recovered up to 0.5 miles outside of the release block.

**3. Recovery of irradiated NOW – Lost Hills.** As part of the broader NOW-SIT program there was a large-scale pilot study in which 750,000 irradiated moths were released 5-6x/week over approximately

1,900 acres of mostly pistachios (and a few almond blocks) near Lost Hills between 4/1 – 10/15. All blocks were under mating disruption. Within this area, subplots were established in July in an almond and pistachio block with and without sterile moth releases (4 blocks total). These subplots were monitored with a grid of 16 flight traps baited with a combined pheromone and phenyl-proprionate (PPO) lures capable of attracting NOW even in the presence of mating disruption. Recovery of marked/irradiated moths in these subplots was relatively low (0.8 – 5.5%) and follows findings from the Kearney Ag. Center and Murray site.

**4. Comparison of Mendota and Phoenix NOW in mating tables – Kearney Ag. Center.** On 7/17, an additional 33 mating tables were added to the 2-acre pistachio block in order to compare the performance of Mendota and Phoenix females. Individual moths from the Phoenix and Mendota strains were exposed for 1, 2, or 3 nights following the release of irradiated moths. Each experimental treatment as replicated 7 times across the block. This comparison was then repeated over multiple weeks/releases. Results indicate that a lower proportion of the Phoenix moths successfully mate on the first night of exposure, but then appear to perform equivalent with the Mendota strain on the second and third nights. In another iteration of this mating tables experiment, female Mendota and Phoenix moths were paired together with either their own strain or the comparison strain and then again exposed for 1, 2 and 3 nights. This experiment was also repeated over multiple weeks. Results indicate that pairing together moths of the same or different strains does not appear to influence mating success. Finally, from 8/14 – 8/17 hourly observations of the Phoenix and Mendota female moths in the mating tables were made over a 72-hour period. Results indicate that the irradiated Phoenix moths mate at approximately the same time as the Mendota strain. These data again show that a lower proportion of the Phoenix moths successfully mate on the first night, but then make up for this on the second and third night. Alternately, most of the Mendota moths were mated after the first night and so very few of them were recorded in copula on the second and third nights.

## Conclusion and Practical Applications

Comparison of the aerial release data and those at Kearney also provide useful information. The similarly poor recapture from the aerial releases at Murray and the hand releases at Kearney indicate that the problems documented for the aerial release were not caused principally by the stressors accompanying the aerial release process. Irradiated mass-produced females at Kearney called almost as successful as a local strain after acclimating follow shipping stress. Other experiments (data not shown) indicated that both male and female sterile navel orangeworm bred well under laboratory conditions. These observations indicate that, at the present state of knowledge for navel orangeworm and SIR, the objective of parallel field and laboratory assays should be to determine which laboratory assays best predict field competitiveness for this insect. Conversely, laboratory assays should not be used at this point as a pre-screening measure to guide field studies.

# Population dynamics and epidemiology of navel orangeworm damage to pistachios: Effect of adjuvants on application efficacy

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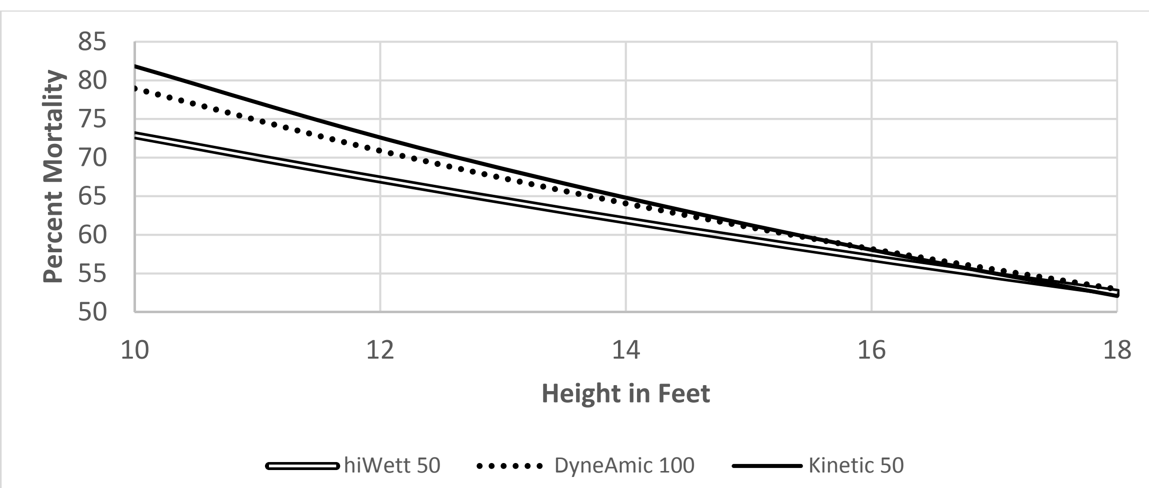
## Introduction

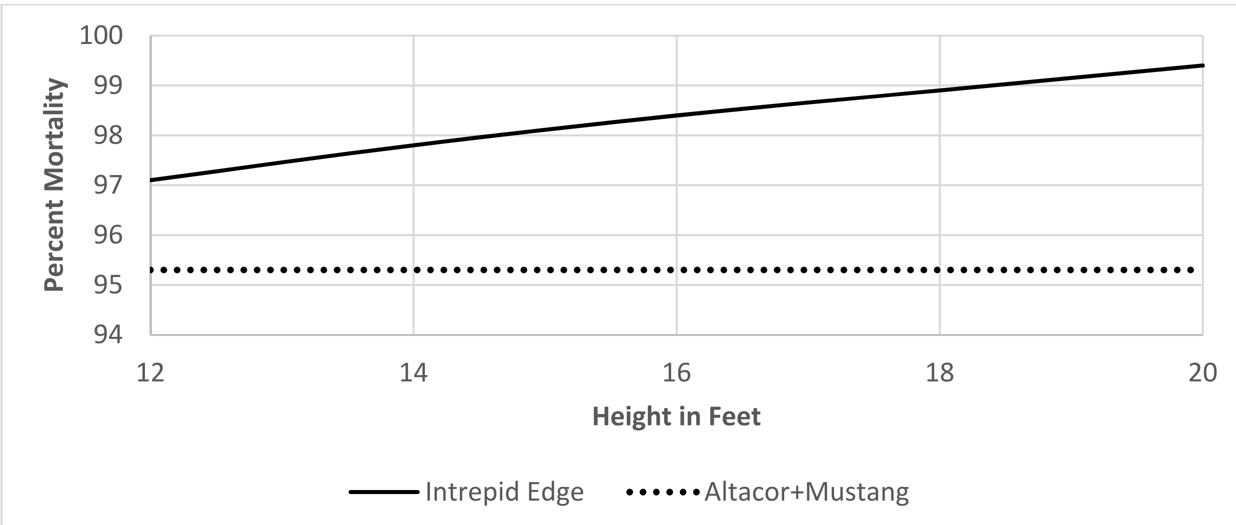
The purpose of this research is to improve control of navel orangeworm by a combination of increased application efficacy and insecticide choice. Any product named is for specific information purposes and does not constitute an endorsement by the researchers. The experiments reported are a follow-up to the study conducted last year (Summer 2017) evaluating the ability of two organosilicone adjuvants (Hi-Wett and Kinetic) to reduce the amount of water needed for insecticide application. The initial study contrasted ground applications made by Air-O-Fan power take off (PTO) air- blast sprayers at 50 gallons per acre (gpa) with a fixed wing aircraft application at 10 gpa using Cohere as the adjuvant. What was noteworthy was that both organosilicone adjuvants provided greater coverage in the upper canopy than the lower canopy, which is atypical. In the study conducted this summer, using the same organosilicone adjuvants, we contrasted ground applications made with an Air-O-Fan PTO air-blast sprayer at 50 gpa with a ground application made at 100 gpa using a methylated seed oil (MSO) adjuvant, Dyne-Amic. The pyrethroid insecticide Lambda-Cy (lambda cyhalothrin) was used at 5.12 oz/ac. Later in the summer, we evaluated two ground applications using Kinetic (4 oz/100 gal) with the insecticides Intrepid Edge (methoxyfenozide+spinosyn) at 19 oz/ac, and the combination of Altacor (chlorantraniliprole) at 4.5 oz/ac+Mustang (zeta cypermethrin) at 4.3 oz/ac, separated by two weeks. Insecticide coverage in all assays is evaluated by contact toxicity bioassay. Filter papers are placed in the canopy using PVC poles, with the papers spaced from 10-20 feet. The filter paper is then collected one day after commercial application, placed in petri dishes containing navel orangeworm diet, and then challenged by placing egg masses in the center of the paper. Newly hatched larvae crawl over the filter paper to reach the diet and mortality is scored 18 days later. Control plates using unexposed filter paper are also used. The following graphs report the mortality from the filter paper placed at two-foot intervals on PVC pipe.

## Results and Discussion

This first graph reports the results from the trial using lambda cyhalothrin. When the data were pooled for all heights there is no statistically significant difference among the treatments. There is an approximate loss of 60% mortality between 10 and 18 feet in height. The maximum mortality of 73-83% at the height of 10 feet is disappointing because mortality using this insecticide has been higher in previous studies



We could not determine why there was a loss of activity in Lambda Cy and note that because we use a susceptible line of navel orangeworm in our bioassays insecticide resistance is not an issue. This second graph reports the results from the two trials using Intrepid Edge and Altacor+Mustang applied at 100 gpa with Kinetic as adjuvant. For Intrepid Edge mortality was highest at 20 feet (98.4%) and fell to 96.8% at 12 feet, but this coverage is almost uniform and the efficacy assessed by bioassay was excellent. For Altacor+Mustang coverage was uniform at the heights tested and efficacy was also excellent. For all intents and purposes both applications were close to perfect and this degree of coverage at heights greater than 12 feet with PTO is unprecedented in our experience. The only new item in these assays is our choice of an organosilicone adjuvant.




## Conclusion

Although it is difficult to compare applications made on different days because of changes in wind, humidity, and operator, several factors stand out. First, both applications made using the organosilicone adjuvant at 100 gpa with the PTO sprayers provided far greater coverage in the upper canopy than the application using the methylated seed oil adjuvant at the same rate. Second, based on overall mortality, these later applications were far more effective than the earlier application that used Lambda Cy. In fact, mortality of 50% is essentially a failure in our bioassay system, so the ground applications using Lambda Cy at both 50 and 100 gpa essentially failed above 14 feet. In contrast, the later applications provided control at all heights. We do not think this involves insecticide resistance because a susceptible line of navel orangeworm is used in the bioassays. Third, using organosilicone adjuvants, we successfully reduced our water use from 100 gpa to 50 gpa, as shown in the first graph. However, since all three applications were unsatisfactory, that portion of the study needs to be repeated in order to determine if we can in fact achieve a satisfactory ground application using 50 gpa. The more interesting conclusion from this study is that by incorporating an organosilicone adjuvant with a volume of 100 gpa, coverage in the upper canopy was dramatically improved. This study needs to be repeated to confirm this unexpected but highly encouraging finding.

# Factors Affecting the Efficacy of AF36, Improvement of the Biocontrol Agent, and Monitoring Commercial Applications

**Authors:** **Themis J. Michailides, Ryan Puckett, Juan Moral, Teresa Garcia-Lopez, Yong Luo, Dan Felts, Lorene Doster, John Lake, and Ramon Jaime,** University of California-Davis, Kearney Agricultural Research and Extension Center.

## Introduction

Aflatoxins occasionally contaminate pistachio and other nuts in California, but they constitute a high economical treat to pistachio industry due to strict regulations of aflatoxin contamination in food and feeds. Aflatoxins are toxic metabolites produced by fungi in the *Aspergillus* section *Flavi* group, including *A. flavus* and *A. parasiticus.* Even though contamination is sparse, the risk of product being rejected by the market makes aflatoxin an issue that need to be addressed. Currently the only reliable method of control of aflatoxin is the use of atoxigenic (unable to produce toxins) strains of *A. flavus* to displace or exclude the aflatoxin-producing fungi and *A. flavus* AF36 is the only atoxigenic strain registered for use in pistachios in California. The *A. flavus* AF36 strain is naturally widespread in California, occurring in all of the major pistachio-growing areas (Doster et al., 2014), but at very low rates to significantly reduce the aflatoxin contamination potential of the natural population of *Aspergillus*. However, when the biocontrol *Aspergillus flavus* AF36 is applied on the pistachio orchards, the rate of the atoxigenic strain AF36 is increased and is able to compete and displace the toxin producing strains and consequently reducing the aflatoxin producing potential of the population.

Experimental applications of the atoxigenic biocontrol agent *A. flavus* AF36 to reduce aflatoxin contamination in pistachios in California started in 2002. The application of the AF36 product was successful in substantially increasing the proportion of the atoxigenic strain AF36 within the population of *A. flavus*/*A. parasiticus* fungi (Doster et al., 2014). In addition, the nuts from the orchards treated with the AF36 product did not have a higher incidence of kernel decay by *A. flavus* compared to nuts from untreated areas, suggesting that applying AF36 will not affect the quality of the pistachio. Nut samples from the orchards treated with the AF36 product were also less likely to be contaminated with aflatoxin than those from untreated orchards. To better protect crops from becoming contaminated with aflatoxins, more research is required to optimize the application of the biocontrol and change the population structure of the aflatoxin producing fungi towards a population dominated by atoxigenic strains. Information about optimum conditions for sporulation and dispersal of the biocontrol product can help facilitate the displacement of toxigenic strains and ultimately can reduce aflatoxin in pistachios. Additionally, introducing new atoxigenic isolates will facilitate the change of population structure in an area-wide long-term bases.

## Results and Discussion

Commercial applications of the biocontrol *Aspergillus flavus* AF36 Prevail® under orchard floor conditions are subjected to predation by several organisms, including ants, roly polies and birds, especially when the orchards conditions are not conducive for the fungus to sporulate. Some predators will partially eat the product and leave the remaining on the soil. We collected partially eaten product and after 4 days of incubation at the optimal conditions they were able to sporulate normally. This indicates that if conducive conditions for the biocontrol fungus return, the remaining pieces of the product on the soil will still sporulate. However, more research under field conditions is necessary to assess the impact of predation under suboptimal conditions.

Studies in cotton indicate that the earlier applications of the biocontrol are best, even though sporulation is very low. This might be due to a funder effect occurring when the biocontrol infects the earlier available points of infection and then serves as a source of inoculum for secondary infections, before toxin producing isolates inhabiting the orchard's soil are able to reach the canopy. This allows the biocontrol to be established as the dominant population on the crop. Sporulation studies under orchard conditions in the Central Valley of California indicate that the biocontrol *A. flavus* AF36 Prevail product sporulate faster on applications made on the month of July. Sporulation studies under controlled conditions simulating orchard conditions in the Central Valley during May, June and July indicate that sporulation of *A. flavus* AF36 Prevail is delayed under low night temperatures, especially under low soil humidity. In this experiment, we also evaluated sporulation of Afla-Guard (another atoxigenic product looking registration for use in pistachio in California), which had a good sporulation even under low temperatures.

The overall goal of the use of atoxigenic strains to reduce aflatoxin contamination in crops is to change the population structure of the aflatoxin-producing *Aspergillus* fungi from toxin-producers to atoxigenic isolates in the soils of all crops subjected to aflatoxin contamination, including pistachio and almond. To monitor the success of this change we need to quantify the proportion of the applied *A. flavus* AF36 in both the crop and the soil after harvest. Currently this procedure is done by Vegetative Compatibility Group (VCG) Analysis, which is time and resources consuming. Using DNA techniques, including Real Time PCR, can greatly reduce both the time and the costs of the analyses. We developed a specific (SNP) qPCR assays to quantify frequencies of the atoxigenic strain biocontrol *A. flavus* AF36 compared to the orchard-native population of *A. flavus* and *A. parasiticus*. This SPN-qPCR is being validated for use in epidemiological studies in orchards treated with the biocontrol. The reduction of time and costs by using this SNP-qPCR will allow us to expand epidemiological studies aimed to implement an area-wide and long-term management to reduce risks of aflatoxin contamination in pistachio.

Additionally, we continued analyzing pistachio library samples for aflatoxins and evaluate the effect of commercial application of AF36 Prevail® on both the percentage of the applied AF36 on the soil and aflatoxin content in the nuts. Also, we started the analysis of the samples for Ochratoxin content. Ochratoxin is another mycotoxin of concern to the pistachio industry, caused mainly by different species of *Aspergillus*. Results indicate a positive effect of the commercial applications on both the percent of AF36 (displacement) and aflatoxin content. Last year's results indicate a reduction of aflatoxin content on the treated fields (8.6 ppb) compared to the untreated control (18.9 ppb) and an increase on the percentage of the AF36 in the treated fields (70.5%) compared to the untreated controls (27.1 %). Results on ochratoxin indicate that contamination is much lower in the Eastside Valley, north of Bakersfield, CA. Also, there was no correlation between contamination with aflatoxins and ochratoxins.

## Conclusion

The overall goal of the aflatoxin research in nut crops in California is to reduce the risks of aflatoxin contamination and product rejection by the market. Considering that the fungi causing aflatoxin contamination are able to move long distances the best strategy to reduce the risks of contamination is the implementation of an area-wide and long-term aflatoxin management program intended to change the overall population structure of the aflatoxin producing *Aspergillus* from toxin-producing strains to atoxigenic strains. Information presented here indicate that an area-wide program is possible to implement but more research is necessary to optimize applications of the biocontrol products. Future research will focus on new formulations (i.e. Afla-Guard) and application strategies to deliver the biocontrol to the orchard environment in a timely matter. Also, we are looking for other atoxigenic strains that can be developed (even in mixtures of 2 to 3 strains), which may have advantages in different environmental conditions.

# Efficacy trials of new dormancy-breaking treatments in pistachio

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## Introduction

In our first year of research on dormancy-breaking agents (DBAs), we turned our attention to potential late-winter treatments for pistachio. We used a combination of field trials and growth chamber experiments to identify candidates that could advance and compress the bloom window without adverse yield effects. Our main objective this year was to identify promising candidate DBAs for more extensive trials in the winter of 2018-2019.

## Results & Discussion

### *Field study*

We sprayed 'Kerman' trees with ethephon, $GA_3$, and AVG on March 6, 2018 (64 CP at this site). Effects on yield, bloom, and bud respiration of these new treatments were compared to standard treatments with 6% IAP 440 oil and with water (control). Oil advanced budbreak by 2-3 days but expanded the bloom window relative to water.

Ethephon is the most promising new treatment. A spray at rest completion of 500 ppm ethephon advanced budbreak by 2-3 days and increased 1st-shake fresh weight per tree by 20 pounds over water-sprayed control (7 pounds over oil). Although that yield difference was large, due to the low number of trees examined the difference is still not statistically significant.

$GA_3$ cannot be recommended at this time. Treatment with 0.2% $GA_3$ advanced budbreak by 5-6 days, compressed the bloom window by 2-3 days, and regularized bloom variation among north-, south-, east-, and west-facing branches, but yielded less than oil. Clusters initially developed excellently, but fruit were small. We suspect overdosage and loss of pollenizer synchrony (only females were sprayed) contributed to yield loss in this $GA_3$ trial. Lower or earlier doses and multiple applications could be tried next.

125 ppm AVG slightly delayed budbreak, and its yields were very similar to water control. AVG may interfere with the accumulation of heat during the delayed-dormant period.

Bud respiration rates ~3 weeks before full bloom (March 25; 19 days after treatment) predicted the observed blooming order: (earliest) GA > ethephon ~ oil > water ~ AVG (latest). Bud respiration increases to a maximum before bloom begins, then temporarily decreases to a minimum just before flowers open, before resuming increase as fruits set.

### *Growth chamber study*

Shoots were cut and the cuttings were treated from March 8 through 11. Ethephon (500 and 1000 ppm), $GA_3$ (0.2% and 2%), AVG (125 and 250 ppm), and IAP 440 oil (4% and 6%), as well as water were applied by spray. Dormex (2.5% and 5%) was applied by painting the first-year wood. The cuttings were placed in a growth chamber set to impose strongly forcing conditions (a daily cycle of 12 hours light at 25 C and 12 hours dark at 15 C.)

The smaller doses had the same effects as the large ones for all treatments except AVG. AVG 250 ppm advanced budbreak, but AVG 125 ppm retarded budbreak. The observed order of budbreak was (soonest) AVG 250 ppm = $GA_3$ > water control = ethephon > oil > Dormex = AVG 125 ppm (latest/failed to push). The growth chamber study was partially compromised by the lack of a separate growth chamber for the ethephon treatment; ethephon fumes (i.e. ethylene) seem to have a slightly advancing effect.

With most treatments, only one or two buds would break on each cutting, with the others aborting by growing a short, brown peduncle and abscising. On $GA_3$-treated cuttings, either all the buds would break, or all the buds would fail to achieve green tip without aborting. Application of $GA_3$ may interfere with the trees' own concentration of resources to support the optimal number of inflorescences, resulting in worse yield and yield components as seen in the field study.

Bud respiration was monitored after treatment. Dormex and oil treatments immediately induced large, temporary increases in bud respiration; respiration decreased thereafter with time. Ethephon induced a slight increase in bud respiration that was maintained until ~11 days after treatment; respiration decreased thereafter. All other treatments showed a peak in respiration at ~11 days after treatment. DBA effects on bloom were uncorrelated with their immediate effects on respiration. Growth chamber advancement results only agree with field advancement results for the treatments that did not induce increased respiration.

Interestingly, painting Dormex onto 1-year-old wood on cuttings may promote lateral bud break at the node between 1-year-old and 2-year-old wood. If this effect is reliable and can be reproduced on young trees, it might be useful in their training.

## Conclusions

Ethephon is the most promising new treatment, giving bloom advancement similar to oil but with improved bloom compactness and no apparent carbohydrate depletion. This year yields with this treatment were larger than with oil.

$GA_3$ shows promising physiological activity, but its use needs to be refined to avoid adverse yield effects.

Although AVG will likely not be useful as a dormancy-breaking treatment for California pistachios, knowledge of its effects will be important for future mode-of-action studies.

Oil application immediately increases bud respiration.

Energy depletion due to induced increases in respiration compromises growth chamber studies on cuttings and likely adversely affects yield in the field.

DBA-induced increases of respiration were uncorrelated with effects on bloom and should generally be considered a side effect unless part of a known mode of action.

# Monitoring the physiological status of pistachio trees by gene activity measurements to optimize the timing and improve our understanding of rest-breaking treatments

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## Problem

The effect of rest-breaking treatments in pistachio trees (*Pistacia vera*) strongly depends on the moment of application. Differences in growing conditions, specific cultivation methods, and temperature variations between winters affect the optimal moment of application, making it difficult for growers to forecast the seasonal development and guarantee a successful rest-breaking treatment.
Gene activity measurements provide a powerful tool to monitor the physiological status of plants. Changes in gene activity are often among the first event to occur whenever a plant is adapting to changing environmental conditions or progressing to the next developmental phase. NSure, a biotech company based in The Netherlands, has successfully developed several test kits for agriculture based on gene activity measurements. One of these tests is the BreakNSure for kiwifruit that is currently available in New Zealand. This test enables growers to monitor the physiological status of their kiwifruit vines, making it possible to apply Dormex at the optimal moment in each individual orchard. This leads to increased and synchronised bud break and flowering and, subsequently, improved economic returns.
This research project is aimed at developing a BreakNSure test for the pistachio industry in California. This test can be used to determine the optimal moment of application of rest-breaking agents. First, the effect of several differently timed rest-breaking treatments on pistachio bud break and flowering will be determined in a field trial. Secondly, from samples collected in parallel during the field trial, relevant pistachio genes will be identified. A practical and user-friendly test will be subsequently developed based on these indicator genes.

## Objective

The goal of this project is to develop a rapid molecular diagnostic test to determine the rest status and flowering readiness of Pistachio so that bud-break enhancing treatments can be optimized. The development of a rapid, easy to use procedure to determine the rest status and flowering readiness of Pistachio will have immediate benefits for the Pistachio industry by allowing for more effective use of bud-break enhancing sprays. The development of a molecular diagnostic test of rest-status and the identification of molecular processes underlying rest-break, also has potential benefits for freeze management and breeding of low-chill varieties.

## Approach

### *Year 1: Identification of candidate indicator genes*

During the first year of the project, field trials will be executed. These trials are focused around monitoring the activity of dormancy related genes and applying bud-break enhancing agents to Pistachio trees at different moments. Kerman trees will be selected in 3 different orchards located in Kings and Kern county spanning a diversity of known chilling accumulation and with well-placed local weather stations. Treatments with selected bud-break enhancing compounds (PHT 470 Supreme Spray Oil; though other materials may be tried on an experimental basis) should include a very early treatment (2 weeks before the anticipated optimal moment of application according to experience, typically mid-January) followed by one weekly treatment for the next 4 weeks. Mineral oils will be applied as a foliar spray, using standard industry protocols and under suitable weather conditions. The effect of each treatment on final bud break and flowering will be carefully evaluated by monitoring commencement, full and last bloom on each cultivar, in each orchard/block. Temperature, sunlight hours, and humidity conditions at each location will be carefully monitored, either from an appropriate nearby weather station and by installation of our own climate installations. Models for Chill Unit and Chill Portions calculation developed by Luedeling, Brown and Pope will be used (see summary at http://ucanr.edu/sites/PistachioShortCourse/files/274455.pdf).

For bud sampling and molecular determinations, at weekly periods commencing from 9 weeks prior to historic average bloom dates in each orchard, samples consisting of 25 pooled flowering buds will be collected from throughout the orchard/block (5 buds per tree, 5 different trees). Buds will be immediately frozen using liquid nitrogen (-196 °C) or dry ice (-79 °C) and stored at -80 °C. A subset of these samples will be used for genome-wide gene expression analysis (RNA-seq) to identify genes whose changes in activity correlate with the changing physiological status of the buds and, hence, with optimal or sub-optimal application moments. The remaining samples will be kept for (technical) validation of a limited set (30) of carefully selected individual genes.

The exact process for gene extraction and selection is a proprietary process, however it is based upon finding correlations between changes in gene activity and the effects of the differently timed rest breaking treatments. In addition, NSure will be able to include extensive molecular knowledge on dormancy release from other species, including kiwifruit, sweet cherry, and table grape. The RNA-seq data will form the basis of a draft Pistachio transcriptome that can be subsequently exploited to identify candidate indicator genes from Pistachio that significantly change their activity during the rest-breaking season.

Besides determining genes that predict responsiveness to bud-enhancing applications, year 1 will also be used to establish the inherent variability in tree response, which is essential to determine the number of tests that will be required in a given orchard. For this, variation between different samples collected within the same orchard/block will be compared to the variation between different orchards/blocks.

Also, in the first year, a start will be made developing the so-called NSure sampling card protocol. Samples for commercial testing are collected using a practical and user-friendly protocol (and not using liquid nitrogen or dry ice). This protocol can be executed readily in the field by non-specialists.

***Year 2: Validation of indicator genes***

During the 2nd year of the project, an identical field trial will be executed. Again, the trial will include applying bud-break enhancing agents to Pistachio trees at different moments. Similarly, the effect of each treatment on bud break and flowering will be carefully evaluated. Time of first/last and peak flowering of all trees in the experimental block along with cumulative chilling units will be recorded.

In year 2, bud samples will be collected on similar timepoints, only from untreated trees, using the sampling card protocol established in year 1. These sampling cards will subsequently be used to validate 20 candidate indicator genes from year 1 by Q-PCR. The best genes, together with the sampling protocol and obtained information about maximum sampling area, are the basis of the prototype test.

***Year 3: Implementation of the test***

The 3rd year of the project is aimed at collecting large-scale data from a 3rd season, and from a more commercial setting. Block trials will be executed in 30 orchards/blocks. Block trials are trials where one sampling area is split into 2 test plots that are treated and tested at 2 different moments, preferably 1 week or more apart. Bud break and flowering of both parts are monitored and related to the test results.

***Potential Benefits to the Industry***

- Development of a rapid cost-effective tool for grower decision making.
- Improved understanding of the molecular aspects of rest breaking in pistachio.
- Improved utilization and efficacy of rest breaking treatments.
- Technology to better test and validate available and potential rest breaking products for use in California.

# Assessing Bud Differentiation of Kerman and Golden Hills Pistachios

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## Introduction

In July of 2017 we observed an interesting phenomenon: bud differentiation appeared to vary between cultivars. Flower buds on a current year Randy shoot matured earlier than buds on a Peters shoot. Analyses of the non-structural sugar and starch contents of both wood (xylem) and bark (phloem) in an adjacent twig section demonstrated both were significantly higher in the Peters shoot than in the Randy shoot. It suggested that during the rapid early summer differentiation the Randy buds consumed more of the available nonstructural carbohydrates from the shoot. This result also triggered our interest in investigating the bud differentiation of female trees' flower buds as the bloom and harvest timing also varies between the cultivars. We investigated the early maturing cultivar Golden Hills and later maturing cultivar Kerman. Simultaneously, in another experiment we demonstrated a close correlation among female flower bud drop, crop load and non-structural carbohydrate levels of the shoot bearing these flowers. These results led us to investigate bud differentiation as a function of carbohydrates status.

In application, the ability to distinguish high quality vegetative buds would greatly enhance both young tree training and mature tree pruning. Based on our previous demonstrations of bud drop on female bearing and non-bearing shoots, we hypothesized that both the vegetative and pistillate bud differentiation are affected by the carbohydrate status of shoot on which the buds are borne as well as differing between cultivars.

Our 2018 research objectives were: 1) explore the differentiation characteristics of both apical vegetative and lateral pistillate buds on the current year shoot in both Kerman and Golden Hills; 2) determine the correlation between bud differentiation and non-structural carbohydrates of current year shoots of bearing and non-bearing Kerman trees; the objective is to provide better decision tools for pruning

## Methods

Current year's shoot growth of three shoot types were collected three times, from June through October. The three shoot types were: non-bearing Golden Hills, and bearing and non-bearing Kerman shoots. Each bud from top to bottom on the twigs was cut longitudinally and the structure were observed and measured by Scanning Electron Microscope. The length of buds was measured based on SEM pictures as showed in Figure 1. A 5 cm shoot section proximal to the individual buds was analyzed for non-structural carbohydrates, the sugar and starch present in the xylem and phloem.

## Results and Discussion

Generally, on current year shoots there was substantial growth on both apical vegetative and floral buds from the end of June to August, thereafter differentiation slowed in September and October in both Kerman and Golden Hills. On the first sampling date, June 22$^{nd}$ the buds were obviously smaller than buds on August 25$^{th}$ and October 27$^{th}$. This indicates the primordium initiation of pistachio buds was almost totally arrested in September.

In October, the apical vegetative buds of on the non-bearing Golden Hills current year's shoot growth were approximately the same size as the floral buds located in the middle and bottom of the same shoot. On the current year's growth of non-bearing Kerman, the apical vegetative buds were on average 20% smaller than the flower buds; on a Kerman tree with crop on the one-year-old wood the apical vegetative buds were approximately 50% smaller (Fig 1.) in length than the middle and bottom flower buds on the same shoot.

The apical vegetative buds of Golden Hills developed more quickly than the buds of Kerman which is consistent with the early bud-break and earlier nut maturity of Golden Hills. The apical vegetative buds from Kerman trees without crop on the one-year old wood were larger than the buds of trees with crop. These results suggest that the crop on one-year-old wood deprived the apical vegetative bud of carbohydrates needed to support optimal bud development, resulting in weaker new shoot growth the following spring.

## Conclusion and Practical Applications

Due to the unexpected closure of the UC Davis campus from smoke we are delayed in assessing the carbohydrate levels of shoots proximal to the individual buds and will update the information in the final report. The results analyzed thus far demonstrate bud differentiation of both vegetative and flowering buds was influenced by tree bearing status, and fewer bud locations on the twigs; these differences were measurable as the bud entered dormancy in late September.

Until all the data is fully analyzed we hesitate to offer practical applications. However, some preliminary conclusions can be made. 1) The apical vegetative buds of non-bearing shoots are generally better developed; therefore, theoretically dormant pruning should avoid tipping these shoots as they will bear crop the following year. 2) The results suggest any production practice that promotes leaf area and function to increase current year's shoot growth will increase the shoot carbohydrate levels in July and August which benefits the bud differentiation of the apical vegetative bud as well as well as the current year's nut growth.

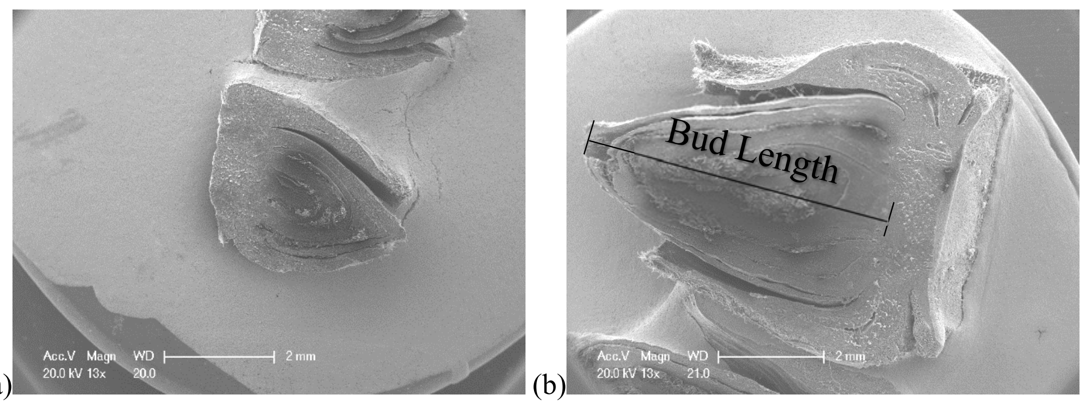
(a) (b)



Fig 1. Vegetative buds on the current year's shoot growth of Kerman on (a) bearing shoot, and (b) non-bearing shoot. Bud (a) is 2.4mm longer than bud (b).

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

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## Introduction

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

Currently, evaluating the physiological status of trees for guiding orchard management decisions are limited to analyses of tree water status, leaf nutrient levels, and visual observations. Until recently, these methods were adequate and produced dramatic improvements in pistachio yields. However, as the climate becomes more erratic and the abiotic stresses more severe, these proven approaches may become less effective. Research efforts described here focus on the development of new approaches to measure trees' physiological status that complement the current aforementioned methods. Specifically, we aim to understand tree non-structural carbohydrate (NSC – sugars and starch) management in the context of dormancy, chilling requirements and yield performance. We have taken a state-wide citizen science approach to rapidly and thoroughly study carbohydrate seasonal dynamics specific to pistachios. Our goal is to determine best carbohydrate management in relation to climate, tree age, and geographic distribution. Specifically, we want to study the dormant carbohydrate status of orchards which may be useful for management or predicting yield..

## Results and Discussion

We have developed a high throughput facility for the analyses of sugars and starch (total nonstructural carbohydrates (NSC)) in pistachio branch samples. Using a citizen science approach to gather large amounts of data we collaborate with tens of farmers across the Central Valley to collect a temporal series of samples from over a hundred orchards. Near real-time results of NSC content are presented via Carbohydrate Observatory website:
http://www.plantsciences.ucdavis.edu/plantsciences_faculty/zwieniecki/CR/cr.html.
Our website is freely accessible and allows participating growers to analyze and compare NSC content in specific orchards over values observed in the entire Central Valley, CA. We are currently completing the second full year of data collection (October 2016 till September 2018). An initial analysis of the seasonal pattern of NSC in pistachio twigs shows an interesting feature that might be linked to alternate bearing. In the winter preceding an OFF year (2017) NSC content in twigs was half of that observed in the following winter that preceded an ON year (2018; Figure 1). This observation has to be tested for individual farms and over multiple years (work underway). If proven, NSC analysis at the peak of the pre-dormancy accumulation could be used to estimate specific orchard yield potential in next year and provide means for the optimization of winter management.

Additionally, we have observed large and persistent differences in NSC content between individual farms that are related to geographical location (climate), tree age, rootstocks, salinity, and potentially management practices (irrigation and fertilization). It remains an open question if this variation is related to yield of specific orchards. If so, there is a high potential to find major parameters that determine NSC content and yield potential of the orchard. Interestingly, parameters might be different for each orchard and yield potential might be associated with varying NSC content, i.e. same NSC content in different orchards might result in different yields.

Our analyses also allowed for the development of a novel chill/heat model for bloom time prediction that uses soluble sugar and starch content dynamic changes in winter as a proxy for dormancy progression.

This model is based on a mechanistic understanding of sugar metabolic pathways in response to temperature. It also explains multiple aspects of observed variation in bloom time caused by unusual winter thermal conditions including low chill, occurrence of frost, or unusual high temperatures.

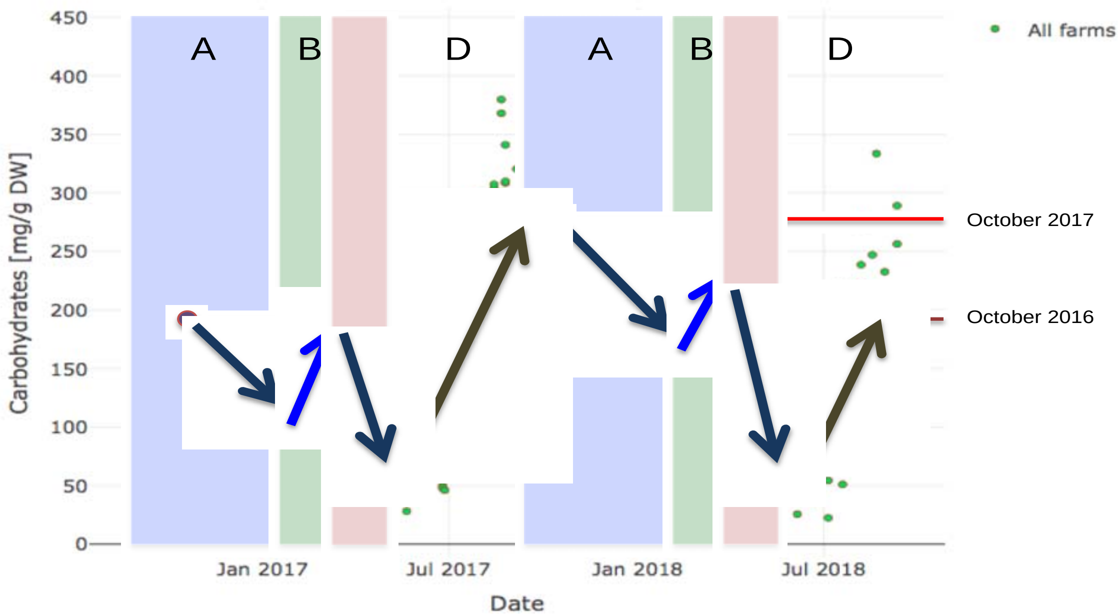


Fig. 1. NSC content in twigs of Pistachio trees over two consecutive years 2016 (October) till 2018 (September). Each season can be characterized by four specific periods of NSC trends: A) the period of dormancy characterized by the starting point of maximum accumulation of reserves prior to leaf senesence and loss of reserves during winter. B) the period prior to budbreak that is characterized by a sudden increase of NSC content, most likley an influx from reserves towards distall locations (trunk or roots). C) a short period of bloom and an intial vigerous spring growth is reflected as a singificant loss of NSC reserves. D) a period of accumulation of reserves for the next dormancy period. Two years of observations reveal that the conent of NSC in the fall preceding an OFF year was almost 50% lower than maximum content observed in a year preceding an ON year.

## Conclusion

- Seasonal observations of NSC content strongly suggests that year-to-year variation in November levels of NSC might be used as a predictor of next year's yielding potential as high NSC levels during November (2017) was associated with an ON year (2018) and low levels of NSC in November (2016) preceded an OFF year (2017).
- NSC variation allowed for the development of a dynamic model for predicting bloom time. This model is based on the analysis of enzymatic activity of the transformation of sugars to starches and starch to sugar in response to diurnal variation in air temperature and allows for near real-time observations of winter chill progression and prediction of timing and uniformity of bloom.

# Characterization of root plasticity in pistachio rootstocks for better nutrient uptake and stress response

**Authors: Georgia Drakakaki[1],** Associate Professor, **Oliver Betz**[1], Student, **Shahab Madahhosseini**[1], visiting Professor; Collaborators: **Thomas Wilkop**[2], **Judy Jernstedt**[1], **Deborah Golino**[3], **Louise Ferguson**[1] ([1]Department of Plant Sciences, University of California Davis, [2]Light Microscopy Core, University of Kentucky Lexington, [3] Foundation Plant Services, University of California Davis)

## Introduction

Pistachio currently is one of most rapidly expanding crops in California and a major contributor to its economy. With the current climate change already affecting the production areas in California, there is a great need for robust plants that can sustain adverse environmental conditions. Under the current combination of environmental changes and increased soil salinization in California, the time lag in responding to such changes using conventional methods is long, thus a combination of alternative approaches is required. Pistachio plants with environmental adaptability afford the possibility to use marginal land and improve yield on current acreage. Plant roots are the first contact to nutrients and microorganisms and are important for robust and sustainable growth. So far most of the rootstock characterization is based on aerial part (leaves and shoots) while little is known about the uptake and translocation of nutrients and salts throughout the pistachio root system under environmental stresses. Thus, it remains challenging to select for superior rootstocks without taking into account their root system.

Understanding the mechanism of root adaptation to adverse environmental conditions at the tissue, cellular and molecular level is invaluable in assessing mechanisms of salinity, drought tolerance as well as many other stress responses in pistachio rootstocks. The phenotypic variability observed in the aerial parts of pistachio rootstocks is associated with a diverse response of pistachio plants to biotic and abiotic stresses; however, currently there is no systematic characterization of their root plasticity.

Our project aims at the establishment of universal cellular and molecular methodologies to assess pistachio root adaptation under environmental stress including drought and salinity. Characterization of cellular structures in pistachio roots that are altered under stress response will allow the development of cellular and molecular markers for selection of best sustainable genetic material. Root endodermis and exodermis represent important apoplastic barriers for radial transport of water and ions to the vascular system of the plant. The endodermis, the innermost layer surrounds the vascular tissue, forms barriers controlling root water and ion transport. The tight control of the endodermal barrier formation is modulated in response to external stress thereby fine-tuning nutrient acquisition and endodermal integrity. To achieve this, endodermal cells undergo two specialized differentiations states consisting of deposition of two impermeable polymers in the cell wall: lignin, forming the Casparian strips, and suberin lamellae. The Casparian strips correspond to localized lignin depositions at the junction between adjacent endodermal cells that form a ring, sealing the apoplastic space. This endodermal layer is differentiated by suberin build up, a hydrophobic polymer forming a secondary cell wall deposition at the inner surface of primary cell walls, covering the entire surface of endodermal cells.

The exodermis is a common, although not always present, apoplastic barrier of the outer cortex of angiosperm roots. Exodermal differentiation is clearly subject to dynamic impacts from the environment, enhanced by stress. As in the endodermis, the Casparian band of the exodermis is a barrier to the apoplastic movement of ions, providing a filtration of ions from the soil solution.

Understanding the developmental plasticity of roots in response to various stresses including salinity can provide a convenient way of identifying desired plant “characteristics” to be selected for in rootstocks in order to achieve optimal plant performance and composition. Insights gained will be assisting in the development of better agricultural practices under saline and other biotic and abiotic stress conditions.

## Results

*Salinity treatments showed increased sodium accumulation in the endodermal and exodermal barriers.* In order to characterize root plasticity in pistachio rootstocks and improve nutrient uptake and stress responses, we have established methodologies for in vivo imaging of Na+, Cl- and K+ ions involved in salinity stress, using a variety of preparation techniques and microscope modalities. Salinity treatment resulted in increased sodium staining in endodermis and exodermis of the hybrid *P. atlantica* x *P. integerrima* (UCB-1) and the parent *P. atlantica* while this increase was not pronounced in *P. integerrima.* Interestingly, our ongoing research has also demonstrated the saline tolerant *P. atlantica* and the hybrid UCB1 have the ability to exclude sodium ions from being transported to the leaves. Taken together, the enhanced sodium accumulation in the apoplastic barriers such as endodermis and exodermis pointed us to their important role in creating a selective barrier that controls ion transport.

*Suberin deposition is increased under salt stress.*

We based our follow up experiments on the knowledge that endodermal barriers are modified in response to a multitude of abiotic stresses to shield plants from environmental stress. Thus, we further developed methodologies and examined suberin deposition under control and stress response conditions in pistachio root sections. Under non-salt treatment (control conditions), our data showed strong suberin deposition in the endodermis and the exodermis of UCB-1 and *P Atlantica* compared to *P. integerrima.* More interestingly, salt treatment induced suberization of the endodermis and exodermis in UCB-1 and *P. atlantica*. However *P. integerrima* did not follow the same induction pattern compared to *P. integerrima* and UCB 1. Instead, *P. integerrima* showed a minimal increased in suberin deposition.

Taken together, the data suggest that suberization is induced under salt stress and it is significantly higher in UCB-1 and *P. Atlantica* compared to *P. integerrima*. Our results are supported by previous studies in which abiotic stress is known to affect the width of endodermal Casparian strips and to induce suberization in many plant species.

*Lignin deposition in apoplastic barriers under salt stress*

To further understand root plasticity under salinity stress we developed methodologies of detecting lignin accumulation via histochemical staining and confocal imaging in the root apoplastic barriers. Our preliminary data show enhanced lignin staining in salt treated pistachio roots. Quantitative analysis is currently in place to compare this effect between the three genotypes. We will continue with follow up studies in gene expression analysis of pistachio root segments starting from the root tip under salt treatments and controls to dissect the cellular pathways that control apoplastic barrier differentiation and halotolerance. We expect to identify enhanced expression in genes involved in biosynthesis and deposition of suberin and lignin and ion specific transporters under salinity stress.

## Conclusion and Practical Applications

The results suggest that both ion exclusion and sequestration are mechanism contributing to salinity tolerance. More importantly, structural differences observed in root tissues within the different genotypes can be related to halo-tolerance. The increased suberin deposition in UCB-1 and *P. Atlantica* correlated with salt ion exclusion, suggests that these barriers contribute to halotolerance in pistachio rootstocks. With our established methodologies, we will conclude the evaluation of different rootstock genotypes and contribute to the understanding of mechanisms contributing to salinity tolerance. Differential accumulation of polymers such as lignin and suberin in the endodermis and exodermis helps roots cope with both biotic and abiotic stresses. Thus, understanding the genetic and cellular mechanisms and the of endodermis and exodermis development in pistachio rootstocks and correlating them with overall plant phenotypic responses can provide an effective way for the identification of the most suitable genotypes with anatomical plasticity in adult roots. Genotypes, with these phenotypic characteristics, will be examined for altered gene expression of specific transporters and biosynthetic genes, allowing the identification of molecular markers for salinity tolerance.

Application of these methods in genotype characterization efforts affords a unique opportunity to assess rootstocks and elite cultivars in an efficient way. This could help us to better understand how altered apoplastic barrier differentiation in roots affect/regulate water and solute transport and will aid in the improvement of future breeding programs to select for salt and generally stress tolerant pistachio.

# Pistachio Improvement Program

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

## Introduction

The goal of this ongoing project is to re-initiate the public pistachio breeding program at UC Davis. This multi-faceted, long-term project is expected to create resources - germplasm, data, and expertise - that foster research collaboration between pistachio researchers engaged in different areas of study including entomology, pathology, physiology, and orchard management, as well as breeding and genetics. Accomplishments during this first year of the project are listed below, with major program goals listed in order of descending priority.

## Results and Discussion

*1. Establishment of germplasm blocks*. We obtained 503 clonal rootstocks (250 UCB-1 and 253 Platinum). UCB-1 trees were purchased from Sierra Gold, and Platinum rootstocks were obtained from an anonymous donor. 270 trees (135 each of UCB-1 and Platinum) were planted at Davis (38.541160 N, -121.791021 W) on April 18, 2018 and 233 trees (115 UCB-1 and 118 Platinum) were planted at Winters (38.500684 N, -121.975523 W) on May 3rd, 2018. Spacing at Davis was 20' x 12' and at Winters 16' x 12'. Both plantings are on microsprinkler irrigation. The Winters site was fallowed for several years before planting, but the Davis site was planted immediately following removal of a walnut block. No mortality has been observed at Winters, but 3 trees have died at Davis from unknown causes. In both plantings, UCB-1 and Platinum trees were alternated within rows in sets of 9 (Figure 1). The budding season began on July 20th, following our participation in a budding workshop held near Harris Ranch, and ended on September 20th. Budwood was obtained from Craig Kallsen, Dan Parfitt, and Foundation Plant Services (FPS). Three blocks were defined within each planting, and within each block we first budded our "high-replication" entries: 2 trees for each of 32 females and a single tree for each of 8 males (Table 1), for a total of 12 trees of each female entry and 6 trees of each male entry. Male entries display a very wide range of flowering times, and are evenly-spaced across blocks in an attempt to provide pollen to all female trees continuously throughout the flowering season. Female entries represent released varieties, elite breeding material, and varieties of historical interest. Budwood for released varieties was obtained from FPS when supplies permitted (Golden Hills, Lost Hills, Randy) and from Craig Kallsen otherwise (Gumdrop, Famoso, Tejon). Altogether these "high-replication" male and female entries are budded to just over 50% (264/503) of the available rootstocks. While we have left many rootstocks ungrafted this season in anticipation that more elite varieties may soon become available, we also began including additional varieties on one tree each at Winters and Davis (2 trees total). Overall our budding success rate was only 68% (224/330), but increased dramatically over the season as we became more proficient, and was >90% when budwood was readily available for multiple budding attempts and/or multiple buds per tree. These germplasm blocks are aimed primarily at scion breeding with *P. vera*, but we have reserved trees at the west end of the Winters block for grafting members of other *Pistacia* species that may be useful for rootstock breeding.

*2. Characterization of molecular and phenotypic diversity in Pistacia*. We collected leaf tissue and extracted DNA from every *Pistacia* tree at the National Clonal Germplasm Repository in Winters, and also noted whether each tree was a male or female, as some of the previously recorded data is incorrect. As of November 2018, we have constructed reduced-representation Illumina libraries for approximately half of those trees, with the remainder to be completed in winter 2018-2019. In October 2018, we collected samples of 50-100 nuts from 100 different trees at Winters, and measured nut size and weight, % splits, hull and shell color, insect damage, and gumminess. The main objectives of this data collection were to identify promising parents for breeding work and to construct a preliminary marker-trait association model, though this analysis has not been completed yet.

3. *Breeding crosses*. We crossed pollen from 2 males to 5 females in a factorial design, then harvested and phenotyped the resulting nuts. In winter 2018-2019 we plan to stratify these nuts, screen germinated seedlings using a marker for sex, and plant all female individuals and 1-3 male individuals from each family.
4. *Tissue culture*. We obtained microshoot cultures of clonal UCB1 rootstock from a commercial source and continued culturing of these in our lab to gain experience with pistachio tissue culture, and for future use in developing improved multiplication and rooting procedures. We also extracted and cultured zygotic embryos from immature *P. vera* seed from Golden Hills and Lost Hills to begin developing methods for introducing seedling material to culture and for initiating pistachio somatic embryo cultures.
5. *Synergistic activities*. We obtained a CDFA grant to characterize salinity tolerance in diverse *Pistacia* and *Juglans* species. We also set up a greenhouse salinity screen in which we exposed ~750 UCB-1 seedlings (kindly donated by FPS) to 100-200 mM NaCl over 4 months, phenotyped growth rate and leaf symptoms, extracted DNA, and constructed Illumina libraries for each individual. These data will be used for marker-trait association analysis in winter 2018-2019.

## Conclusions

Inclusion of additional diverse germplasm is desirable to make full use of the germplasm blocks established this year, and to increase the number of entries for greater power in marker-trait association studies.

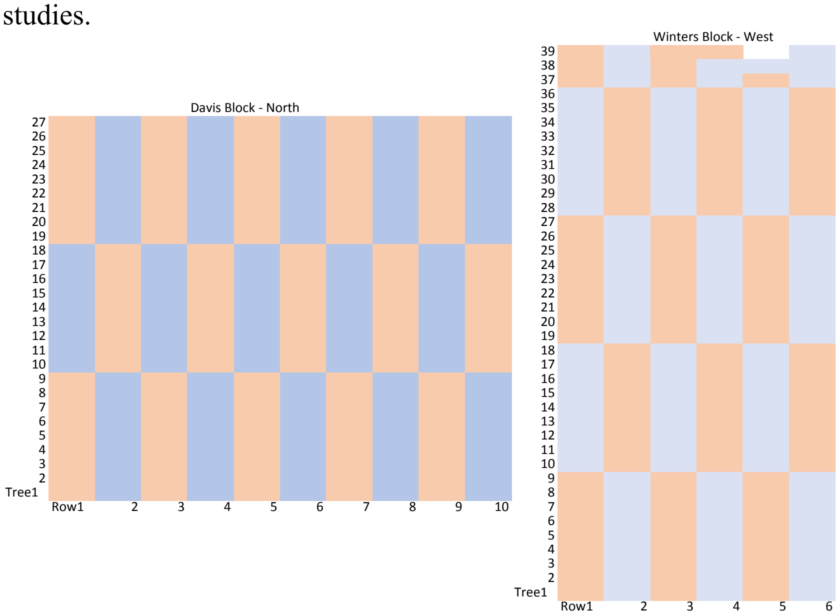


Figure 1. Layout of Davis and Winters plantings. Orange cells represent UCB-1 rootstocks and blue cells represent Platinum rootstocks.

| Entry | Name | Entry | Name | Entry | Name |
|---|---|---|---|---|---|
| F01 | Gumdrop | F10 | N-51 | M01 | Zarand |
| F02 | C2-35 | F11 | S-18 | M02 | Tejon |
| F03 | B15-69 | F12 | Aria | M03 | B2-27 |
| F04 | S-34 | F13 | Joley | M04 | Famoso |
| F05 | Golden_Hills | F14 | Kalehghouchi | M05 | Randy |
| F06 | Lost_Hills | F15 | Aegina | M06 | S-45 |
| F07 | Kerman | F16 | Trabonella | M07 | S-48 |
| F08 | K25-78 | F17 | Bronte | M08 | Peters |
| F09 | S-51 | F18 | Damghan | | |

Table 1. List of replicated entries in field plantings. Entries F01-F18 are females on 6 trees per planting (12 total) and entries M01-M08 are males on 3 trees per planting (6 total).

# Evaluation of Pistachio Rootstock-Breeding Selections, 2018-19

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

## Introduction

The U.C. breeding program began with the original crosses made in 1989 by Dr. Dan Parfitt and Joseph Maranto. Since 2009, the breeding program has included development of experimental rootstocks (please see a separate report on the U.C. Scion-Breeding Program). As new male and female pistachio cultivars are released to the industry, the focus of the program shifts from these to evaluation of novel U.C. breeding crosses and other potential cultivars of interest to the industry. New trials are created, often with the indispensable and long-term donation of land, labor, equipment and time by interested, gracious and generous private, cooperating growers.

As part of the breeding program, potential seedling rootstocks originating from breeding crosses made in 2009 and 2011 and later, have either been planted in rootstock-selection trials (three of these) or in randomized and replicated evaluation trials in comparison with UCB-1 seedling and other rootstocks (five of these). These eight trials are all located in Kern County or at the Westside Research and Extension Center in Fresno County, with one east of the Sierra Mountains near Rosamond. Most of these trials are in orchards with high sodium, chloride and boron salts. All of these trials are now budded to Kerman, Gumdrop, Golden Hills or Lost Hills, the earliest in the fall of 2011, the latest in 2018. Additional rootstock trials are planned. The objectives of the rootstock evaluation will be to identify breeding lines or individual rootstocks that produce higher early yields, have a reduced pruning requirement with a closer tree spacing, may confer greater cold and salt tolerance, comparable Verticillium wilt and Phytophthora root and crown rot resistance to that possessed by existing commercial rootstocks, and which will form a smoother, more uniform graft union with new cultivars such as Golden Hills, Lost Hills, Gumdrop and Kalehghouchi, than do existing rootstocks. The first harvests of two of these rootstock trials occurred in 2017.

## Results and Discussion

The breadth of the breeding program does not lend itself to brief summarization. However, some research areas will be discussed based on the degree of interest and rate of developing information from our trials.

The rootstocks from the U.C. breeding program are novel in that the parentage is different from UCB1 or pure *P. integerrima* rootstocks. The two rootstock trials harvested in 2017, were harvested again in 2018. One of those harvested was a seedling rootstock-selection trial planted in a colder area of the SJV in 2013 and which was reclaimed from high salinity. Each of the rootstocks in this trial originated from a seed and each is grafted to the Lost Hills scion cultivar. While each rootstock is genetically distinct, they are somewhat similar based on their parentage. The selected individual trees harvested in 2017 were harvested again this year (with some additions). Based on harvestable green field weight (nut quality results were not available as yet at the time of this writing) the yield from these experimental rootstocks was significantly larger, as it was in 2017, than that of the UCB1 seedling control trees. These experimental rootstocks, generally, produce a more compact tree, which has produced higher yields for its age than UCB1, but with sufficient new shoot growth to support optimism for its ability to maintain yields in the future. This rootstock, at this stage, suggests that it may be amenable to planting at a closer tree spacing without future crowding than current commercial rootstocks and with a reduced pruning requirement. Its small size may be easier to shake efficiently with less energy. One of these seedling trials with similar rootstock crosses is located near Rosamond, east of the Sierra Mountains, and the rootstocks appear to have very good cold tolerance. These rootstocks do not sucker to any extent. Due to the nature of the genetic crosses, the best of the individual rootstocks from these trials will have to be selected and cloned to produce a viable commercial candidate for further testing.

In another trial, planted in the fall of 2011, two other novel UC experimental seedling rootstocks are being compared to UCB1 seedlings. The parentages of these experimental rootstocks are different from that of the rootstocks discussed above. The scion is Golden Hills. Both the soil and irrigation water are high in boron (up to 5 ppm or more in both cases). In 2017, average edible yields were not significantly different among the rootstocks at this the first commercial harvest, but in 2018, yield (at this point, based on green-weight material harvested in the field only) of the experimental rootstocks were significantly better than those of the UCB1 seedlings. The canopy of Golden Hills on UCB1 rootstock demonstrated large areas of leaf-tissue necrosis with leaf drop, with few or no leaf symptoms on scions grafted to the novel rootstocks. In 2018, replicated leaf tissue analysis showed that leaves on UCB1 rootstock had average dry tissue levels of 1997 ppm, compared to 583 and 593 for the two novel rootstocks. This result was similar to that obtained in 2017 testing. Additional evaluations are being made in replicated trials, using seedlings or cloned trees in large pots, to further document observations made in the field trials.

## Conclusions

These rootstock trials are in the initial stages and what the differences in performance among our experimental rootstocks and seedling UCB1 rootstocks that we have noted the first two bearing years might mean are not clear. However, currently, the commercial rootstocks available to the industry are limited. Exploring new interspecific genetic combinations for rootstocks appears to be a useful exercise, both in general knowledge, and novel nut production possibilities inherent in diversity.

# Evaluation of Pistachio Scion-Breeding Selections, 2018-19

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

## Introduction

The U.C. breeding program began with the original crosses made in 1989 by Dr. Dan Parfitt and Joseph Maranto. The program continues with breeding and evaluation of novel scions, but also, as of 2009, experimental rootstocks (please see separate report for the rootstock summary). As new male and female pistachio cultivars are released to the industry, the focus of the program shifts from these to evaluation of novel U.C. breeding crosses and other potential cultivars of interest to the industry. New trials are created, often with the indispensable and long-term donation of land, labor, equipment and time by interested, gracious and generous private, cooperating growers.

Currently, we are evaluating nine advanced male and/or female scion-selection trials. These trials were planted from 2007 to 2016. Five of these trials have the objective of identifying male cultivars, several from the precocious seedling trial planted in 2008, that demonstrated robust flower development and close bloom synchrony with Kerman, Golden Hills or Gumdrop in years with insufficient winter chill/excessive winter heat. Two of these five trials are located near Inyokern, east of the Sierra Mountains in the high desert. The other four scion selection trials compare or will compare, novel advanced female scion selections with existing commercial cultivars such as Gumdrop, Golden Hills, Lost Hills or Kerman for yield, nut quality characteristics, and bloom and harvest timing. These trials are located in areas with varying amounts of winter chill. We continue to evaluate the cultivar Gumdrop in the oldest trial containing this U.C. cultivar.

In addition, the breeding program has two active seedling scion-selection trials. Our seedling-selection trials, primarily, are composed of trees planted from seeds, and thus, are on their own roots. The seeds originate from crosses made as part of the activities of the U.C. Breeding Program, with the objective of producing advanced selections that target specific traits that may have value to the pistachio industry. Some of the seedlings in this trial are the result of backcrosses to other *Pistacia* species. One of these selection trials was planted in 2012, from which, advanced selections have been made beginning in 2017. The major focus for selecting trees from this trial were, and will be precocious, potentially commercial cultivars with novel traits that should increase resistance/avoidance to navel orangeworm damage and greater tolerance to low-chill/high-winter-heat climatic conditions. Obviously, other traits with possible applications to the industry are, also, of interest. These selections are, or will be, included in additional advanced, replicated scientific trials, now in preparation for the 2019 growing season. An additional scion seedling-selection trial, including grafted experimental trees or commercial cultivars and interesting selections from other trials, was initiated in 2017, in the Coachella Valley. The purpose of this trial is to identify seedling trees with germplasm with a reduced winter rest period (i.e. low chilling) requirement. Some of the selections in this trial were made from the seedling selection trial planted in 2012 and the precious seedling selection trial planted in 2008.

## Results and Discussion

The breadth of the breeding program does not lend itself to brief summarization. However, some research areas will be discussed based on the degree of interest and rate of developing information from our trials.

The pistachio cultivar called Gumdrop is the most recently released female variety from the U.C. breeding program. We continued to harvest Gumdrop, in comparison to the performance of Kerman and Golden Hills, in a trial established near Buttonwillow in the SJV in 2007. Gumdrops's most noteworthy characteristics is its early harvest (approximately 10 days earlier than Golden Hills and 20 days earlier than Kerman), while maintaining acceptable commercial nut quality characteristics. The earlier harvest of Gumdrop should reduce navel orangeworm infestation as pressure from this insect increases later in the harvest season. The earlier flowering data, and some observations made during leaf-out and bloom, suggest that this cultivar may be more tolerant of 'insufficient winter chill/high winter heat' conditions. As research continues data and observations suggest the following regarding producing pistachios with Gumdrop:

1. At this time, Gumdrop, should only be planted by those who want an earlier harvest and who will have access to a processing plant that will be open early enough to accept the harvested nuts.
2. Later in the season (July) some nuts produce a drop of gum on the hull (hence its name). A Gumdrop' harvest is 'stickier' than a 'Kerman' harvest.
3. Gumdrop nuts to not hold well on the tree and air temperatures can be hot when 'Gumdrop' is ready for harvest. A timely 'double shake' harvest is suggested. In the San Joaquin Valley, depending on season and location, the first harvest will occur in early August with a second shake a week to ten days later. 'Gumdrop' is an alternate bearer, maybe even more so than Kerman and Golden Hills.
4. Gumdrop will perform best on well-drained soils where water 'ponding' does not occur.
5. Gumdrop has shown more growth variability on UCB1 seedling rootstock than Golden Hills. Growth among Gumdrop trees has been much more uniform on Platinum® clonal rootstock in an observation trial planted in 2014.

A number of existing cultivars and grower selections are being compared in a trial planted on UCB1 clones in 2010 in an orchard located in the citrus belt near the junction of State Highways 155 and 65, which generally, receives less winter chilling than many lower-elevation areas in the SJV. This trial has been harvested in 2016, 2017 and 2018. Nut quality information was not available to calculate yield, as of this writing in 2018, but will be available in the Full Report. Based on the green-weight yields, however, significant differences exist among cultivars, and the highest yielding cultivars, to date, are not necessarily those that have been the best performers in other trials of the past. The results continue to demonstrate that cultivars with Red Aleppo heritage are slow to produce economic yields in this area.

Some of the other advanced scion-selection trials have not yet come into bearing or are just beginning to bear. One of these trials include trees that were selected for a later harvest date than Kerman and for precocity.

Chill hour and chill portion accumulations are very low in the Coachella Valley where we planted an advanced selection trial in 2017. Initial data collected in the spring of 2018, in this trial, strongly suggest that the winter rest period in this area is not adequate for 'normal' March or April bud 'push' or uniform development of the leaf canopy for many of the existing cultivars used in the California pistachio industry. The canopy development and early push of vegetative buds on some of the novel selections from the breeding program, however, foster some optimism that development of cultivars with a greatly reduced chill requirement is possible. Additional genetic material was planted in 2018, as will more material in 2019. The data is extremely preliminary and results should be more meaningful the trees age, and we learn how to farm the deep granitic sand of the U.C. Riverside Coachella Ag Station more effectively. Many of the selections in the trial are clones from trees located in trials in the SJV that are available for comparison of vegetative and reproductive growth characteristics.

# Evaluating new training systems for pistachio

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## Introduction

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

Three pruning trials were initiated as part of this project. The first was initiated in a 'Golden Hills' on 'PG1' seedling rootstock orchard on double line drip irrigation in Kings County. The rootstocks were planted in early winter of 2016 and budded in July of that year. Treatments were imposed in the spring of 2017. The second trial, also in Kings County, and is similarly in a 'Golden Hills' block on 'PG1' seedling rootstock. The rootstocks were planted in the fall of 2016 and budded in the summer of 2017. Treatments were imposed in the spring of 2018. This site was flood irrigated three times in 2018, once in January, once on June 14th and once on August 1st. In 2018, a third site was established in an orchard in Yolo County near Woodland. The orchard is nursery budded 'Golden Hills' on seedling 'UCB1' rootstock and was planted in mid-February 2018. Irrigation was supplied with double line drip with in-line emitters. Dataloggers with Watermark and temperature sensors were installed in one replication of each treatment at all three pruning sites. Pruning treatments were: 1) The industry standard for training young orchards, as described in the Pistachio Production Manual, including in–season tipping (Beede and Ferguson, 2016), 2) a modified central leader training system, and 3) an unpruned control. Selected data trees met a minimum height requirement of 50 inches. The conventional trees in the two Kings County trials had metal stakes rather than the traditional wooden stakes while the Yolo County trial had metal stakes for the unpruned and modified central leader treatments but traditional wood stakes for the conventional training treatment.

To study the impacts of irrigation on the following year shoot formation, a fourth trial was set up on the Davis campus with four irrigation treatments including 50%, 100%, 150% and 200% ET treatments. These trees were nursery grafted 'Kerman' on 'UCB1' seedling rootstocks and were planted on May 1, 2018. Irrigation was via microsprinkler. Measurements taken were similarly to those described for the earlier three trials.

## Results and Discussion

Trial #1 Kings County- Midday stem water potential was measured approximately every month in 2017 and every two weeks in 2018. There were no significant treatment differences in midday stem water potential on any date in 2017 or 2018 but the conventionally pruned treatment tended to be the most stressed on most dates. By the fall of 2018, although there were no differences in scion diameter among treatments, the unpruned were significantly taller and had a significantly larger rootstock diameter compared to modified central leader or conventionally trained trees. Unpruned trees had significantly more female flower clusters in the second leaf compared to either the modified central leader or conventionally trained trees, suggesting the potential for earlier productivity in this treatment.

Trial #2 Kings County- This orchard was only flood irrigated on 3 dates in 2018 yet trees were generally not stressed. Trees tended to run about 2 bars below the fully watered (almond) baseline numbers which was very similar to the range in Kings County Pruning Trial #1. Once again, the conventionally pruned trees tended to be the most stressed on most dates, but the differences were not statistically different. There was a trend for unpruned trees to have the largest scion diameter, but the differences were again not significant. The unpruned trees did have significantly larger rootstock diameters compared to the modified central leader tree and both had larger rootstock diameters than the conventionally trained trees. In May of 2018, unpruned, modified central leader, and conventionally trained trees had 46, 20, and 11 shoots opening, respectively.

Trial #3 Yolo County- This trial utilized nursery grafted trees. There were more problems with leaning trees than at either of the other trials described above. This has been previously observed by others and likely these trees are more flexible due to having been raised in crowded conditions in the nursery. 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. Trees trained to metal stakes were much stronger and were largely strong enough to have stakes removed by the end of the first year. Trunk circumference and height had not been measured at this site by the due date for this summary but will be reported later.

On-campus irrigation trial- Trees grew well in this trial as well. Despite water applications levels of about 12, 30, 90 and 120 inches in 2018, there were only minor differences in tree size. The highest water treatment trees exhibited many leaf damage symptoms by late summer and defoliated about a week earlier than the lowest water treatment. The 100%ET treatment had significantly larger rootstock and scion diameter compared to any of the other treatments in December 2018. Since a major goal of this trial is to look at the impacts of current year irrigation practices (including over-irrigating) on shoot formation in the following year, the most interesting data should come out of this trial in 2019.

## Preliminary Conclusions

Although these trials are in their infancy, the results to date look encouraging. Trees in all treatments grew well with the unpruned treatment having significantly larger rootstock diameters compared to the conventional and modified central leader trees in both Kings County trials. Conventionally pruned trees tended to be more stressed on most dates. Although some unpruned trees had tops that were bending over (since they were often taller than the stakes), they appear to be straightening themselves out by resprouting branches that balance the lean by the second leaf similar to results we have seen in walnut. Data collection will continue in all four of these trials in 2018.

# Integrated Conventional and Genomic Approaches to Pistachio Rootstock Development

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## Introduction

University of California Berkley 1 (UCB 1 – *Pistachio atlantica* x *P. Integerrima*) interspecific hybrid seedling rootstock has been successfully used in pistachio production in California since its release in 1960. However, recently some of the clonal seedling selections have performed poorly exhibiting severe deformities at the graft union causing stunting and bushy appearance often resulting in girdling causing death of trees in orchards. Other currently used rootstocks including *Pistacia atlantica* seedlings, PG-1 (*P. integerrima* seedling), and the interspecific hybrids PG-2 (Platinum - *P. integerrima x P. atlantica;* seedling and clonal). With the narrow genetic base that these rootstocks represent, the California pistachio industry remains vulnerable to biotic and abiotic stresses affecting production. While causes for poor performance of some UCB 1 clonal rootstocks are not clear, it is important to explore the possibility of developing improved rootstocks with superior field performance and durable resistance to soil borne diseases and to address the current genetic vulnerabilities. The species in the section *Eu Terebinthus* in the genus *Pistacia; P. khinjuk, P. integerrima, P. palestina, P. terebinthus*, and *P. vera* offer a great opportunity to develop improved rootstocks with durable resistance to soil borne diseases, tolerance to drought and salinity. Wild pistachio species are excellent sources of genes for resistance to soil borne diseases and adaptation to wide range of growing environments. The wild pistachio genepools at the National Clonal Germplasm Repository, USDA-ARS, offer a wide range of germplasm to recombine many important horticultural traits and disease-pest resistance in rootstocks. The current project focuses on the following: (1) Production of genetically diverse pistachio interspecific hybrids involving potential donors of resistance to soil borne pathogens and pests, (2) Evaluate rootstocks for tolerance to Verticillium wilt, Phytophthora root and crown rot, and Nematodes to produce high quality disease data; (3) Develop and use genomic tools to characterize pistachio wild gene pools and rootstock breeding populations to decipher genetic basis for disease resistance; and (4) Develop and implement effective marker assisted selection strategies for rapid development of improved rootstocks.

## Results and Discussion

*Interspecific hybrids, embryo culture, and micropropagation.*

During the first year of the project we attempted 20 different cross combinations among the species within the section *Eu Terebinthus* and successfully produced 17 hybrids with good fruit set (Table 1). Of particular interest is the usage of UCB-1 as pollen parent to create three-way crosses. Efforts were made to rescue/culture hybrid embryos *in vitro* and successfully produced ~80 plants of 12 different hybrids. About 150 mature seeds of different hybrids are stored ready to be put into culture. Successfully embryo rescued plants are currently in shoot proliferation, and cultures from 2017 crosses are in rooting. Micropropagation of Pistachio hybrids through embryo culture is time consuming and labor-intensive. Pistachio species grow and multiply slowly in tissue culture (TC) and often require standardization for different species and hybrid combinations. *In vitro* rooting of TC plants can be difficult and may require microcuttings to be rooted *ex vitro* under controlled conditions before transferring them to greenhouse with variable levels of success.

*Disease resistance evaluation of hybrids.*

Four interspecific hybrids produced in collaboration with the Sierra Gold Nurseries during spring, 2017 are being screened for resistance to Verticillium wilt caused by *Verticillium dahliae*, Phytophthora crown and root rots caused by *Phytophthora neiederhauserii*, and root-knot, root lesion, and ring nematodes. For

Verticillium wilt screening, inoculations were made on both sides of small (4-10 mm dia) trees after wounding the bark with dissecting needle. One drop of inoculum containing > 500,000 spores/ml was placed on the wounds with a 23-gauge hypodermic needle. Fans were positioned to blow on the plants to increase transpiration and conidial uptake. Four trees of each genotype were used as control group. Evaluation of resistance/tolerance to *Phytophthora and Macrophomina crown and root rots* experiments are ongoing and assessment of the different rootstock resistance will be assessed in March 2019. Four rootstocks (*P. vera x P. atlantica*) were planted along with a number of UCB 1 clones in a sandy loam field and inoculated with *Pratylenchus vulnus* and *Meloidogyne* sp. for resistance evaluation in 2019. All experiments were laid out in a standard statistical design to compute the analysis of variance comparing different experimental rootstocks.

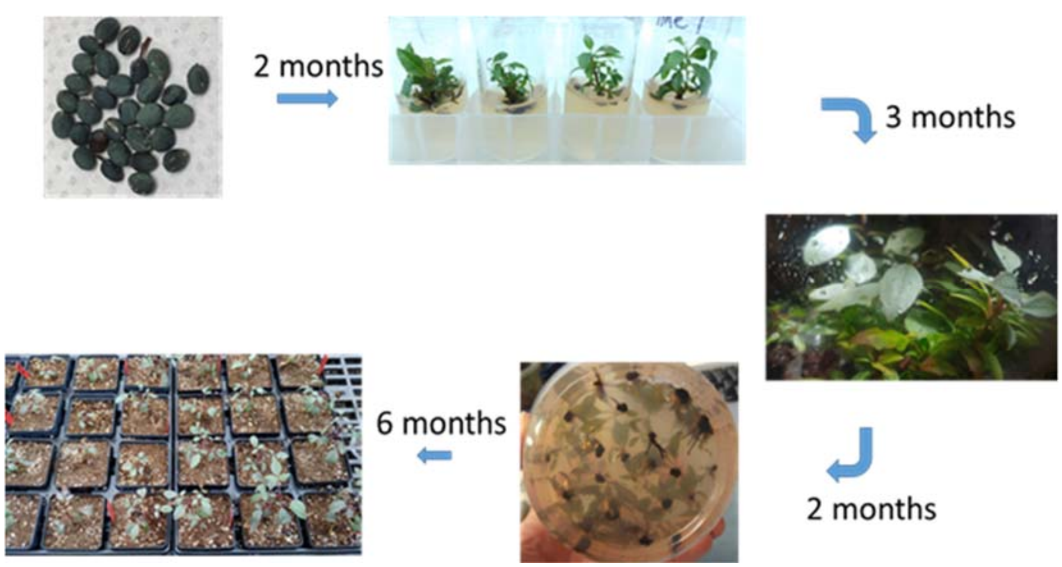
## Conclusion

We would like to emphasize here that micropropagation of interspecific hybrids is the major challenge and the key to the success of this project. We need to produce large enough numbers of plants of each of the hybrids that we produced in 2018 and we are currently in various stages of production. We will continue to make progress as we proceed further in this project.

Table 1. Interspecific hybrids produced in spring, 2018.

| Seed Parent | Pollen Parent | Seed Parent | Pollen Parent |
|---|---|---|---|
| *P. integerrima* | Hybrids 51C | *P. vera* | *P. afghanistania* |
| *P. palestina* | *P. atlantica* | *P. vera* | *P. integerrima* |
| *P. palestina* | *P. vera* | *P. vera* | *P. lentiscus* |
| *P. palestina* | *P. khinjuk* | *P. vera* | *P. atlantica* |
| *P. palestina* | *P. integrrima* | *P. vera* | UCB-1 |
| *P. palestina* | *P. palestina* | *P. vera* | *P. palestina* |
| *P. palestina* | *P. atlantica* | *P. vera* | *P. terebinthus* |
| *P. palestina* | *P. lentiscus* | *P. khinjuk* | *P. integerrima* |
| *P. palestina* | UCB-1 | *P. khinjuk* | UCB-1 |

Figure 1. Embryo culture and production of micropropagated plants of pistachio hybrids



# Development of new, reliable, vigorous, clonal rootstocks 2018 Update

**Authors**: **John Preece,** Research Leader, USDA, National Clonal Germplasm Repository, Davis; **Deborah Golino**, Director and **Franklin Lewis**, Technician, Foundation Plant Services, UC Davis; and **Florent Trouillas,** Assistant Cooperative Extension Specialist, Department of Plant Pathology, KARE, UC Davis

## Introduction

There is a need for new superior UCB-1 clonal rootstocks that are reliable and give rise to vigorous and high yielding orchards. Because recent "off-types" have occurred in clonal UCB-1, collectively referred to as Pistachio Bushy Top Syndrome (PBTS), a system is necessary where new, vigorous clones can be continuously released to replace older ones. This should be on a schedule that will eliminate or greatly reduce the chance of new, "off-types" showing up in orchards.

Once seedling pistachios become established in the greenhouse or field, they become infected with bacteria that live in the xylem (endophytes). The role of these endophytes in the growth and health of the plant is unknown; however, these bacteria will grow out of pistachio shoots and contaminate tissue cultures used for micropropagation of clonal rootstocks. Therefore, it is difficult to use material from proven trees in the field.

## Results and Discussion

We have simplified the procedure for UCB-1 seed germination in vitro in a sterile environment. During the previous year, we scarified the seeds in concentrated sulfuric acid (18 Molar = 36 Normal) for 7 hours, followed by three 15-minute sterile water rinses. The acid was an excellent sterilant, so no bleach or other treatment was necessary. The drawbacks are working with a caustic agent and the long time necessary to sufficiently weaken the shell to allow cutting in half with a scalpel.

We eliminated the necessity of the 7-hour sulfuric acid treatment by cutting the seeds in half using pruning shears prior to surface sterilization. The same technique for disinfestation also works for UCB-1 seeds cracked in a vise; however, vise-cracking is a slow process. Using the pruning shears is quick. After cutting or vise cracking the seeds are disinfested with Sodium Dichlorocyanuric acid (NaDCC) in a 3-hour agitated soak and is not rinsed off. Non-cold stratified seeds germinate at about 90%.

Our seed germination experiments have resulted in 120 clonal seedling lines with 4 culture tubes being maintained of each. Leaf samples of each line have been collected and DNA was extracted at the UCDavis Genomics Center. The genetic markers developed to predict growth in the Genomics and Phenomics associated study will be used to make selections for field testing. The markers should be able to predict which trees will remain small, which medium, and which will be the most vigorous. Representative from all groups will be rooted for field comparisons. This will also help confirm the robustness of the molecular markers.

Data are being collected on differences in the in vitro growth among these clonal UCB-1 seedlings. There are differences in height growth, branching, and overall vigor in the culture environment. These data can be correlated to subsequent field data to determine any relationships between the growth in the two different environments.

We were successful using shoot apical meristems to establish cultures from three field-grown trees more than one year ago and they have thus far been free from any sign of contamination caused by bacterial endophytes. We have tested the resulting shoots on various microbiological media and they appear to be clean of endophytes and other microbial contaminants. Currently There are approximately 300 microshoots among these three clones. So far, rooting success has been limited. Rooting is possible, the percent success is currently low.

## Conclusion

Non-stratified, dormant UCB-1 seeds are relatively easy to cut either following a 7-hour soak in concentrated sulfuric acid or with pruning shears on non-scarified seeds. The highly effective disinfestant NaDCC does no noticeable damage to the seed or subsequent seedling.

There are observable differences in growth and multiplication among clonal UCB-1 seedlings in an in vitro environment. These will be correlated with field growth.

It appears possible to establish field-grown UCB-1 apical meristems in vitro that have few or no endophytes. The advantage is that desirable trees can be established in vitro and their clonal progeny should also grow vigorously.

# Clonal UCB-1 Pistachio Rootstock Micropropagation: Is pistachio Bushy top syndrome a variant that occurred in tissue culture? 2018 Summary

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## Introduction

Clonal UCB-1 pistachio rootstock has been preferred by growers over seedling restocks to produce more uniform, vigorous, and higher yielding orchards. Recently, problems appeared in orchards grafted to clonal UCB-1 rootstock. Stunting, bark overgrowths at the nodes, abnormal growth and cracking at some graft unions became known as Pistachio Bushy Top Syndrome (PBTS). These symptoms may be caused by *Rhodococcus faciens,* a bacterial plant pathogen, or they may be the result of a bud sport or somaclonal variant that formed in vitro on a clonal line that had been micropropagated for years. The focus of this proposal is to obtain subclonal shoot culture lines from commercial labs of the clonal UCB-1 where some of the resulting plants have exhibited PBTS symptoms. This study will be to regenerate shoots to be rooted and tested for freedom from *Rhodococcus*. Those free from this bacterium will be planted in a replicated study in the field to determine if the symptoms can be traced to one or more individual shoot subclonal lines.

## Results and Discussion

Shoot cultures of the UCB-1 clone that has been used historically were obtained from 3 California nurseries. DNA tests conducted by FPS show that all three labs have been culturing the same UCB-1 clone with profile values identical to "Duarte Old Clone 1." These shoot cultures have been multiplied and there are observable differences in branching of the shoots depending upon the laboratory of origin. This has persisted for more than one year under our culture conditions.

From these cultures, subclonal single shoot descent lines were subcultured and multiplied and observed for potential variations among different lines. Selections were made by us and by one of the nurseries based on potential phenotypic differences observed in vitro or ex vitro. Our selections were based on in vitro growth differences, including growth rate, branching, leaf expansion, and general appearance. Eight subclonal lines have been selected, 5 by us and 3 by a commercial lab. Two shoots from one of our selected lines have been fascinated (flat, fused stems), which is an obvious new variant. These fasciated shoots are also being multiplied for further observations.

Shoot multiplication and acclimatization to the greenhouse environment have not been difficult. However, this UCB-1 clone is not easy to root and research is focused on improving rooting of the microshoots. We are seeing differences in rootability depending on the laboratory of origin, with shoots from one laboratory easiest to root. This may be related to differences in original culture conditions in the commercial labs, including ingredients in the medium, such as cytokinin that is used to promote shoot growth and multiplication. It will be a benefit of this study and to commercial labs if their rooting rates can be improved. Currently rooted plantlets are acclimatized to the greenhouse environment from selections from two of the three laboratories. Growth rate and multiplication is slow from one lab and that has delayed rooting.

## Conclusion

General shoot multiplication is not difficult with the UCB-1 clone. There are interesting differences in growth and response of the clone depending upon laboratory of origin.

Rooting and acclimatizing protocols continue to be revised to understand rooting and thereby improve success. Plantlets continue to be produced to provide planting stock for the field planting and growth comparisons portion of this study.

We will continue to screen selections for *Rhodococcus* and new variations that appear during micropropagation. Cultures of the selections have been delivered to KARE for testing to determine if they contain *Rhodococcus*; none was detected. All plants will be screened for *Rhodococcus* before planting in the field.

# Identification of Superior UCB-1 Rootstocks Using DNA Markers Phase 2: 2018 Report

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## Introduction

The majority of pistachio rootstocks are currently produced from seed and are genetically variable due to segregation in the gametes of each highly heterozygous parent. This results in differences in morphology among individual rootstocks and in performance in the field. The UCB-1 hybrid is the main pistachio rootstock used in the US and is produced from controlled crosses between specific clones of *Pistacia atlantica* (female) and *P. integerrima* (male). The male was selected by Dr. Lee Ashworth (UC Berkeley) in the 1980s for its resistance to Verticillium wilt, a serious soil-borne pathogen of pistachio. For over 20 years, variation has been observed in orchards planted on seedling UCB-1 rootstock. Reduced vigor and stunting of some trees are of particular concern. The cause of this variation is unclear. Stunting is a significant economic problem and results in decreased nut yield and/or quality.

We are characterizing the genome of UCB-1, its parents and collecting detailed phenotypic data in order to dissect the causes leading to differences in vigor in orchards that are planted with UCB-1 seedling rootstocks. We are separating out the effects of environment and genetics on vigor and providing molecular markers to allow the early identification of inferior seedlings that need rogueing. We are currently completing the second year of Phase 2 of a multi-year project that started in 2013.

## Results and Discussion

In early 2013, 961 UCB-1 seedlings were planted in an experimental orchard at UC Davis followed by another 263 in 2014. In a collaboration between FPS and the USDA-ARS National Clonal Germplasm Repository at Davis, measurements of phenotypic variability among these $F_1$ individual trees began immediately. Phenotypic measurements have been made annually since January 2014, including measurements of variation in growth, branching, and active growth period. Subsequently, we sequenced the DNA of these trees using several approaches including GBS and skim-seq. This year we collected another round of multi-trait phenotyping data in this orchard (such as trunk caliper, tree height, and canopy diameter) and were also able to record flowering time for the small number of trees that reached sexual maturity. This dataset continues to demonstrate that tree height and trunk caliper in early years is a poor predictor of tree size in later years. However, both trunk caliper and tree height from last year were predictive of both in this year’s data. From March 2018, we began regular drone flights over the orchard to capture multispectral imagery in collaboration with Sean Hogan (UCANR) and Alireza Pourreza (UC Davis). These data will be used to generate 3D computer models of the orchard to accelerate and enhance our collection of phenotyping data.

Since 2013, we have also sequenced and collected trunk caliper measurements from over 1,600 UCB-1 trees from commercial orchards in the Central Valley. These rootstocks range in age from 7 to 14 years, are grafted with either *P. vera* cvs. *Kerman*, or *Golden Hills*, and span orchards from near Merced to Bakersfield. Data from 600 of these were collected this year; in addition, we sequenced and phenotyped 197 PG I rootstocks grafted to *P. vera* cv. *Kerman*. The latter will help us establish if the results seen in UCB-1 are applicable to other rootstocks. Interestingly, trees in all UCB-1 orchards sampled have exhibited a bi-modal size distribution. This likely reflects the ‘stunting’ phenomenon observed by growers. Phenotypic data also indicated a strong correlation between rootstock and scion caliper, in both

UCB-1 and PG I orchards. This summer we also captured multispectral drone imagery for four of these commercial orchards.

We now have chromosome-scale genome assemblies for three key *Pistacia* species: *P. atlantica*, *P. integerrima,* and *P. vera* (in collaboration with Salih Kafkas, Cukurova University, Turkey). The *P. vera* sequenced in collaboration with Salih Kafkas is the *'Siirt'* cultivar, which is not grown in the US; therefore, this year we generated new sequencing data from both cvs. *'Kerman'* and *'Golden Hills'* to identify DNA sequences unique to these two US scion cultivars. Using our *P. atlantica* and *P. integerrima* assemblies and UCB-1 sequencing in five orchards (one experimental, four commercial), we have identified DNA polymorphisms segregating in UCB-1 rootstock. Using a new association testing workflow, we have validated two chromosomal locations that control growth of UCB-1 seedlings in commercial orchards. We have also identified four loci observed to associate with trunk caliper in our un-grafted experimental trees at FPS in Davis (only two of which are observed in commercial orchards). In order to analyze such a large dataset, we developed a novel high throughput approach that allows rapid association testing in low coverage sequence data, with many traits and thousands of individuals in a cost-effective manner.

**Conclusion**

This project aims to provide the foundational resources needed for next-generation rootstock development. Together with our collaborators we are developing genetic and phenotypic tools to enable next-generation pistachio genetics. These tools and resources will be made available to the wider pistachio research community with a view to accelerating the development of superior rootstocks.

Over five years we have collected a large amount of phenotypic and genetic data from both experimental and commercial orchards and have now collected data for more than 2,000 trees. This year we continued our multi-year multi-trait phenotyping of the experimental orchard in Davis, as well as sampled over six hundred new trees from older Central Valley orchards. We have begun collaborations with Sean Hogan (UCANR) and Alireza Pourreza (UC Davis) to conduct multispectral aerial imaging surveys of the orchards we have sampled. This will accelerate and enhance our phenotyping efforts. We have completed chromosome-scale genome assemblies for three key *Pistacia* species: *P. atlantica, P. integerrima*, and *P. vera* (*cv. 'Siirt'* in collaboration with Salih Kafkas, Cukurova University, Turkey) and have developed a novel high-throughput, low-cost, genotyping and trait association approach that allows us to handle data from thousands of trees and dozens of traits simultaneously. We are using these data to separate out the effects of environment and genetics on stunting, as well as better understand the growth characteristics of UCB-1.

It is common practice for nurseries to rogue as many as 10 to 15% of their UCB-1 seedlings based on early growth parameters, such as tree height and other visual clues. This selection is being made on seedlings that are only a few weeks to months old and before planting in commercial orchards. Our data show that such traits in very young trees are poor predictors of size and vigor in older trees and therefore seedling selection in this way is unlikely to be effective. Furthermore, we have a observed a bimodal size distribution of trees in all commercial orchards sampled. This likely reflects the stunting phenomenon as observed by growers. We have identified molecular markers for two chromosomal regions that determine trunk caliper and explain this size distribution. These markers will enable the selection of young UCB-1 seedlings that will result in rootstocks with predictable sizes and the culling of inferior genotypes prior to planting in orchards. It will also allow the selection of vigorous genotypes for clonal propagation.

# Examination of Seedlings from Open-Pollinated Female *Pistacia atlantica* Parent Trees of UCB-1 Seed: Paternity Testing, Phenotypic Characterization and Development of Improved DNA Markers

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## Introduction

The UCB-1 seedling rootstock has been a staple of the California pistachio industry for decades. It is produced by crossing a single female selection of *Pistacia atlantica* (UCB-Female) with a single male selection of *Pistacia integerrima* (UCB-Male). For seed production, UCB-Female trees are isolated from exogenous pollen and manually pollinated with pollen collected from UCB-Male trees.

A row of UCB-Female trees planted at FPS in 2013, too young for UCB-1 seed production, are not yet protected from outside pollen and are not manually pollenated. These trees offered an experiment of opportunity to study pollen contamination pressure at a site of UCB-1 production by examining a seedling population from the open-pollinated UCB-Females. We examined flower and fruit development on the open-pollenated *P. atlantica* female trees and the phenotype of the resulting seedlings. Using DNA markers, we determined the identity and location of the male parent of each seedling.

## Results

In mid-March 2018, when the unprotected female *P. atlantica* trees were blooming, we surveyed the *Pistacia* spp. males within a ~ one-mile radius. We identified feral males along the roadside (west of the female trees), along the creek bed and levee road (south of the female trees) and trees in the FPS and Pomology Department field collections (north of the female trees). The closest feral male tree was on the levee road, ~300 feet (90 meters) from the unprotected UCB-Females, and the most distant feral male was nearly one mile (1,444 meters) north of the unprotected UCB-Females. We also found shedding males in the Pomology Department field collection, 0.8 miles (1,269 meters), north and east of the unprotected UCB-Females. Each male tree was tagged and photographed. In late April, leaf samples were collected from each tagged male for DNA analysis.

Clear, unambiguous DNA profiles were generated for 22 feral male trees and 31 male trees in the Pomology Department field collection. DNA testing of the feral males indicates they are predominantly *P. chinensis,* as expected. There are indications that some may be *P. chinensis* x *P vera* hybrids. The DNA profiles of the trees in the Pomology Department field collections are consistent with the record. As expected of different species, the profiles of the Pomology collection show much more variation than the feral males, which as a group are rather similar to one another. DNA profiles for the *Pistacia atlantica* UCB-Female and the *Pistacia integerrima* UCB-Male were also included in the analysis. These profiles are easily distinguished from one another; they are both very different than the feral males as a group.

We monitored flower and fruit development on the unprotected UCB-Female trees. In October 2017, seed was collected from four unprotected UCB-Female trees. Fifty-nine seeds germinated out of the initial 132 seeds collected, a germination rate of ~45%, much lower than the 90% or more typical pure of UCB-1 seed. All seedlings were planted in individual pots and labeled for identification.

DNA was extracted from leaf samples of each open-pollenated seedling. The marker type we use, simple sequence repeats (SSRs), shows inheritance from the parent generation into the progeny. Removing the portion of the DNA profile inherited from the known mother allows us to determine the male parent of each seedling, if the male parent was sampled. Of the 59 seedlings, 32 were standard UCB-1 siblings. This result is consistent with the fact that the unprotected UCB-Female trees are adjacent to our seed production UCB-Females and our UCB-Male pollen source trees. As such, they are exposed to UCB-Male pollen directly from the male trees and pollen venting from the enclosures protecting the seed-production females. This consistent result validates our method of identifying male parents.

We identified the male parent of 18 out of the 27 non-UCB-1 open-pollenated seedlings (UCB-Female x X). Ten were fathered by a single feral male, Levee Rd. #1. This tree, ~300 feet (90 meters) south of the unprotected UCB-Females, was the closest feral male to the unprotected UCB-Females. The next-most successful feral male, Pedrick #17, fathered three UCB-Female x X seedlings. It is 0.87 mile (1,400 meters) north of the UCB-Females: a long distance, but not the most distant successful male. Among other trees growing to the north, Pedrick #9 fathered two UCB-Female x X from 0.66 mile (1,070 meters) away. Two adjacent feral males at 0.56 miles (890 meters) away, fathered one seedling each. Hutchison #1, the most distant male parent, at 0.91 miles (1,460 meters) north and east of the UCB-Females, fathered one seedling. In the same direction, but closer at 0.8 miles (1,269 meters), males from the Pomology Department collection were not successful pollinators. It appears that pollen distribution from these trees was limited by the dense canopy and buildings close to the collection block. We were unable to determine the male parent of nine seedlings. It is possible that one or more males were overlooked. However, given that Pedrick #17 and Hutchison #1 could successfully pollinate flowers from almost a mile away, it is also possible that the father(s) of these nine seedlings is more than a mile away.

At 11 months in containers, there are clear phenotypic differences between the UCB-Female x X seedlings. Leaves of the UCB-Female x X seedlings show curling and the leaflets are ovate to elliptic, while UCB-1 siblings universally show no curling and have lanceolate to falcate leaflets.
Vertical bark striations are observed on ~50% of UCB-Female x X seedlings and absent on UCB-1 siblings. In general, UCB-1 seedlings are more robust. Plant height averaged a rather uniform 56 cm and trunk diameter averaged 5.52 mm compared to a more variable average height of 44 cm and average diameter of 3.07 mm for the UCB-Female x X seedlings.

## Conclusion and Practical Applications

The unprotected *Pistacia atlantica* UCB-Female trees examined in this study are adjacent to our seed-production UCB-Females and our UCB-Male pollen source trees. Though UCB-Male pollen typically shed two to three weeks prior to female flower receptivity, the unprotected UCB-Females were exposed, while receptive, to large amounts of UCB-Male pollen venting from the enclosure protecting the seed-production females. Similar conditions would be expected in a UCB-1 seed production setting where the UCB-Females are isolated from exogenous pollen by distance to male trees but not isolated with a physical barrier. Despite the abundance of UCB-Male pollen, we found that 46% of the seedlings we tested were fathered by feral males. The finding is surprising given the small number of seedlings tested (59). Obviously, an "isolation-by-distance system" would not include a wild male within a hundred meters of the production females, but we clearly demonstrated successful pollination by two trees nearly a mile from the UCB-Females with one fathering two seedlings. Though both of the distant fathers were primarily north of the UCB-Females, one was also to the east (30°). The final report will contain prevailing wind data. These will be augmented by data from a second year of study.

The final report will also contain detailed photographs documenting the phenotypic differences between UCB-1 and the UCB-Female x X seedling populations. These differences could be very useful in efforts to rogue non-UCB-1 seedlings in a nursery setting.

To date, the project has generated 27 open-pollenated UCB-Female x X; we will produce more in the second year of the study. These are potentially an interesting genetic resource. Anyone interested in further study of these UCB-Female x X plants should contact the authors.

# Understanding the impacts of soil-water salinity on water uptake and consumptive use of mature pistachio orchards grown in the San Joaquin Valley with micro-irrigation

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## Introduction

In the last 15 years, pistachio acreage in the San Joaquin Valley has rapidly expanded and now includes land affected by salinity that was previously used to grow field crops, such as cotton. In addition to being more profitable than field crops, pistachios express both drought resilience and higher salt tolerance than most other tree crops grown in the valley.

In the recent past, some research studies investigated the tolerance of different pistachio rootstocks to various salinity levels and assessed their performance under field conditions. However, no information is currently available on how salinity affects the actual water use of micro-irrigated pistachio orchards. This information is becoming increasingly important to help growers better manage irrigation and leaching practices in salt-affected areas, as in the future the San Joaquin Valley will face fresh water limitations due to recurring droughts and environmental regulations.

In 2015-2017 our team conducted a 3-year study to develop updated information on ET and Kc of mature well-watered pistachio orchards grown in the San Joaquin Valley with micro-irrigation on non-saline and increasingly saline soils.

With the present project, we continued the field data collection of biophysical parameters over the course of the crop season 2018 in the same four commercial pistachio orchards as those of the 2015-2017 study, and instrumented an additional mature pistachio orchard grown on non-saline soil for systematic field data collection along the 2018 season. At all five study orchards, in addition to biophysical parameters (actual ET, soil evaporation, applied water, soil moisture, light interception, midday stem water potential, canopy reflectance in different spectral bands, nut yield and quality), our team also gathered complementary crop-related information, i.e. canopy temperature and trunk size variations, that could enable a more detailed understanding of the effects of soil-water salinity on tree performance. Finally, we planned to monitor the soil electrical conductivity (ECe) and the soil oxygen concentration in continuous, as hypoxia and asphyxia may strongly affect tree performance under saline conditions in the long term. Through the extended field data collection, our aim was to gain better understanding of salinity-induced plant stress with regard to water use in salt affected orchards, and obtain more reliable and accurate information on how soil-water salinity can affect pistachio tree performance relative to non-saline orchards. We also wanted to minimize the influence of year-specific and orchard-specific growing conditions and variations on ET and Kc to increase the reliability of water use information for non-saline conditions. This information can directly benefit pistachio growers, farm managers and irrigation consultants operating in the valley.

## Results and Discussion

Our research team completed the collection of field data for the proposed biophysical parameters during the crop season 2018 in the five pistachio study orchards, which are all located in Kings County. Three of these orchards are non-saline, two of which are located in Hanford area and the other in the area of Coalinga, whereas the two salt-affected orchards are located in Lemoore area.

Unfortunately, we could not find on the market adequate and reliable sensors to monitor soil EC and soil oxygen diffusion rate in the study orchards. However, during the last part of the crop season, we instrumented one non-saline and one salt affected orchard with sensors of soil oxygen (Figaro SK25F) from a Japanese manufacturer, for testing and evaluation. Soil oxygen sensors have been rarely used for

data collection under field conditions, but the test data we collected seem promising for systematic monitoring of $O_2$ in the soil during the crop season 2019.
Our team is currently processing, analyzing and interpreting the various biophysical and crop-related datasets collected in all study orchards in 2018. As such, we are not yet able to report final results, articulate proper discussion, and outline the practical implications of our study. However, a few key aspects stemmed from the preliminary data analysis and interpretation, as indicated below.

Key aspects:
#) The non-saline orchard in Coalinga has 15-20% higher actual ET and Kc than the non-saline orchard in Hanford, although the two orchards have a very similar light interception by the tree canopy (fPAR ~ 76%), i.e. very similar canopy size and density. This significant difference in ET and Kc could be due to windier conditions in Coalinga area than in Hanford area, as well as to greater incoming radiation resulting from lower air turbidity in Coalinga than Hanford. However, site-specific condition such as different soil type and different orchard floor management, probably contribute to increasing water use in the Coalinga orchard. Using a 4-channel net radiometer, we observed consistently higher values of outgoing radiation at the Coalinga orchard, which could be possibly due to the bright cover cropping reflecting and scattering part of the incoming solar radiation in multiple directions. Some of the reflected and scattered radiation could possibly be re-captured by the trees' canopy, and thus trigger higher water use in the orchard through transpiration.

#) Our preliminary data analysis indicates that pistachio water use is impacted by the combined effects of salinity and sodicity. The size of trees grown under medium-to-high salinity/sodicity was 10% to 40% smaller than those in non-saline conditions. This was indicated by lower light interception (fPAR) values. Moving from non-saline to increasing saline/sodic conditions, the actual ET decreased linearly with fPAR, since ET is mainly driven by the amount of light intercepted by the tree canopy, which in turn depends on canopy size and density.

#) The field data from our study show low correlation between soil EC and orchard ET, suggesting that soil osmotic potential may not be the main stressor for pistachio trees exposed to long-term salinity conditions. While salinity may contribute to osmotic stress and perhaps to specific ion stress, sodic conditions are likely imposing a secondary stress on the trees.

#) We found a relatively high correlation between ET, sodium adsorption ratio (SAR) and adjusted SAR (SARadj). High values of SAR and SARadj correspond to higher concentration and activity of sodium ions ($Na^+$) on the soil exchange complex relative to calcium ($Ca^{2+}$) and magnesium ($Mg^{2+}$), that may adversely affect soil physical properties and water infiltration. Tree growth and performance under saline-sodic conditions may be affected by poor soil aeration and hypoxia due to poor oxygen diffusion in the soil. Our results suggest that these secondary effects due to sodicity may predominantly impact tree growth, and thus light interception and actual evapotranspiration.

We reserve to confirm these key aspects in the final project report, once the analysis and interpretation of field datasets and information will be finalized.

## Conclusions

The preliminary analysis and interpretation of field datasets from our study shows that the actual water use of pistachio significantly decreased as soil-water salinity and sodicity increased. Salinity and sodic conditions adversely affect tree growth in the long term, thus reducing radiation interception and ET. Our preliminary analysis also indicates that secondary effects of sodicity (soil degradation leading to poor aeration and reduction of plant growth) possibly have stronger impact on pistachio ET than salt concentration alone in the long term.
Overall, we think that more in-depth and longer research studies are necessary to understand the soil-plant-water relations of tree crops grown on marginal soils with micro-irrigation, as well as the tree response to different soil-water conditions during the crop season.

# Management of Alternaria Late Blight of Pistachio (Fungicide Trial 2018)

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## Introduction

Alternaria late blight (ALB) mainly caused by *Alternaria alternata,* continues to be a major disease in California pistachios. Favored by high relative humidity and dew, the disease is worse in late summer (August and September) when these conditions have longer duration. On foliage, ALB causes severe premature defoliation of trees and it can affect the orchard productivity. On fruits, this disease can cause black shell staining, thus reducing the product quality and in some cases causing mold of the kernels. Despite cultural practices such as tree hedging to increase orchard ventilation and irrigation management to help reduce the incidence of ALB, the use of fungicides is the most effective method for controlling this disease. The objective was to determine the fungicide efficacy against ALB.

## Methods

In an experimental Kerman pistachio orchard established in 1990 at Kearney Agricultural Research and Extension Center in Parlier CA, we tested spray programs in addition to evaluating single fungicide compounds. Treatments consisted of three sprays approximately four weeks apart (30 May, 2 July – critical time for spray-, and 2 August). All the fungicides were applied at label rates recommended by the manufacturer (Table 1). Each treatment consisted of five single-tree replications. Sprays were applied with a handgun sprayer at 400 gallons per acre. The orchard was irrigated using fanjet micro-sprinklers. Symptoms of disease developed only very late in the season, and for this reason evaluation was performed on 11 October. Fungicide efficacy was rated using the whole tree evaluation method or efficacy score, where 1 = the least control, 5 = the best control, and 2, 3, and 4, are intermediate levels of increasing ALB control. Two people rated the trees and their scores were averaged to arrive at a final value. Mean comparison was made using LSD test with $\alpha = 0.05$.

## Results and Discussion

As previously mentioned, ALB disease symptoms developed late in the season forcing us to perform the evaluation on 11 October. In this year's fungicide trial, the sole application of the experimental product WXF17002 (T19) resulted in the highest score for ALB control (3.9), but it was not statistically different from the other experimental products such as UC-1 (T7), V-10424 (T15) and WXF17001 (T17) with score of 3.5, or UC-2 (T8), A20259E (T12) and the commercial product Quash (T14) with scores of 3.4, 3.4, and 3.2, respectively (Table 1). All other fungicides tested presented statistical similarities and their scores ranged from 2.4 to 3.1. The Control (T24) where no fungicides were applied showed the lowest score (1.6) differing from all other treatment made during the season of 2018.

## Conclusion

Though the disease was late in appearing and not as severe as in some past years, there is evidence that spray treatments significantly reduced visible disease symptoms in the orchard. The weather data plots for temperature (Fig. 1A) and relative humidity (Fig. 1B) retrieved from CIMIS Station 39 (cimis.water.ca.gov) confirmed that the 2018 weather conditions were not very conducive for ALB disease in Fresno County where the trial was performed.

**Acknowledgements** We thank the KARE field staff for taking care of the cultural practices of our orchards, and various fungicide companies for providing unrestricted gifts to support our research.

**Table 1.** Efficacy of fungicides against Alternaria Late Blight in an experimental pistachio orchard in Fresno County – 2018.

| Treatment | Fungicide(s)[1] | Active Ingredient (FRAC#) | Rate | 1st Spray (5/30/2018) | 2nd Spray (7/2/2018) | 3rd Spray (8/2/2018) | ALB Score[2] |
|---|---|---|---|---|---|---|---|
| T1 | Fontelis (Fon) | Penthiopyrad (7) | 20 fl oz | Fon | Fon | Fon | 3.1 b-e |
| T2 | Fontelis / Quadris Top (QT) | Penthiopyrad (7) / Azoxystrobin (11) & Difenoconazole (3) | 20 fl oz /14 fl oz | Fon | QT | Fon | 2.7 c-e |
| T3 | Fontelis / Quash (Qs) | Penthiopyrad (7) / Metconazole (3) | 20 fl oz + 4 oz | Fon | Qs | Fon | 2.5 de |
| T4 | Luna Sensation (LS) | Fluopyram (7) & Tryfloxistrobin (11) | 5 fl oz | LS | LS | LS | 2.7 c-e |
| T5 | Luna Sensation + Baythroid (Bay) | Fluopyram (7) & Tryfloxistrobin (11) + Insecticide | 5 fl oz + 2.8 fl oz | LS + Bay | LS + Bay | LS + Bay | 2.4 e |
| T6 | Luna Experience (LE) + Baythroid | fluopyram (7) & Tebuconazole (3) + Insecticide | 6 fl oz + 2.8 fl oz | LE + Bay | LE + Bay | LE + Bay | 3.0 b-e |
| T7 | UC-1 | Not Disclosed | 5 fl oz | UC-1 | UC-1 | UC-1 | 3.5 ab |
| T8 | UC-2 | Not Disclosed | 7 fl oz | UC-2 | UC-2 | UC-2 | 3.4 a-c |
| T9 | Pristine (Pri)/MBI-10612 | Boscalid (7) & Pyraclostrobin (11) / Not Disclosed | 14.5 oz/1 quart | Pri | 10612 | Pri | 2.9 b-e |
| T10 | Pristine/Stargus (St) | Boscalid (7) & Pyraclostrobin (11) / *Bacillus amyloliquefaciens* (44) | 14.5 oz/2 quart | Pri | St | Pri | 2.4 e |
| T11 | Pristine | Boscalid (7) & Pyraclostrobin (11) | 14.5 oz | Pri | Pri | Pri | 2.6 de |
| T12 | A20259E | Not Disclosed | 13.7 fl oz | A20259E | A20259E | A20259E | 3.4 a-c |
| T13 | A20560C | Not Disclosed | 9.1 fl oz | A20560C | A20560C | A20560C | 3.0 b-e |
| T14 | Quash | Metconazole (3) | 4 oz | Qs | Qs | Qs | 3.2 a-d |
| T15 | V-10424 | Not Disclosed | 4 fl oz | V-10423 | V-10424 | V-10424 | 3.5 ab |
| T16 | Actinovate (Ac) | *Streptomyces lydicus* (25) | 8 oz | Ac | Ac | Ac | 2.6 de |
| T17 | WXF-17001 | Not Disclosed | 0.25% w/w | WXF-17001 | WXF-17001 | WXF-17001 | 3.5 ab |
| T18 | WXF-17001 | Not Disclosed | 0.35% w/w | WXF-17001 | WXF-17001 | WXF-17001 | 3.0 b-e |
| T19 | WXF-17002 | Not Disclosed | 0.25% w/w | WXF-17002 | WXF-17002 | WXF-17002 | 3.9 a |
| T20 | WXF-17002 | Not Disclosed | 0.5% w/w | WXF-17002 | WXF-17002 | WXF-17002 | 3.0 b-e |
| T21 | Rhyme (Rhy) | Flutriafol (3) | 7 fl oz | Rhy | Rhy | Rhy | 3.1 b-e |
| T22 | Rhyme + Koverall (Kov) | Flutriafol (3) + Mancozeb (M3) | 7 fl oz/2.4 lbs | Rhy + Kov | Rhy + Kov | Rhy + Kov | 2.6 de |
| T23 | Control | NA | Untreated | NA | NA | NA | 1.6 f |

[1] Dyne-Amic at 0.0625% vol/vol was added, except T17-20. Fungicides separated with "/" were alternated and "+" were mixed. [2] Efficacy score is an average of five replicate trees, evaluated in Oct 11. Number followed by different letters are different according to LSD test at α = 0.05.

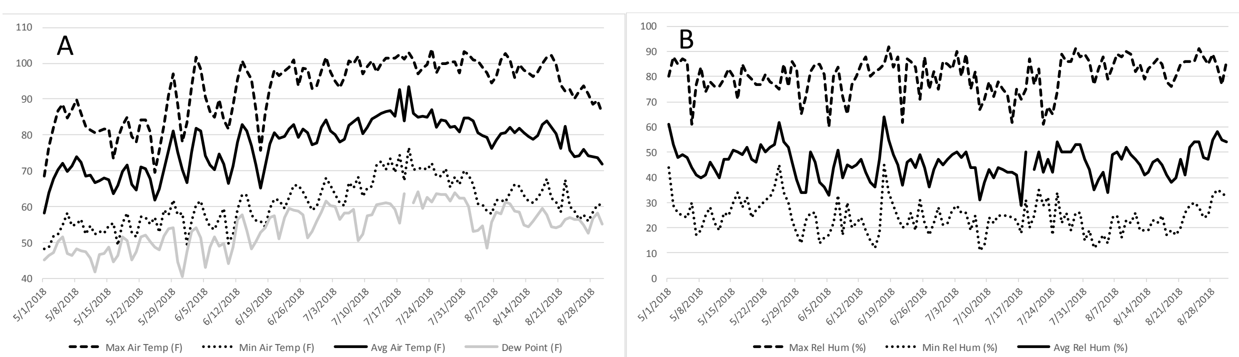


**Figure 1.** Weather conditions from May 1st until August 31st 2018 (retrieved from CIMIS Station 29 (Parlier CA), (A) maximum (dark dash), average (solid dark), minimum (doted dark) and dew (solid gray) temperature in Fahrenheit (°F), (B) Relative humidity (maximum, average and minimum) in percentage.

# Management of Botryosphaeria panicle and shoot blight of pistachio (Fungicide Trial 2018)

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## Introduction

In the last several years, the extreme drought conditions have limited the severity potential of the Botryosphaeria panicle and shoot blight in the central and southern valleys, where pistachios are grown. However, in the Sacramento Valley this disease is still a problem, and frequent fungicide sprays are needed to control this disease. Also, the disease could cause significant losses if conditions were conducive in an orchard and the grower failed to follow a fungicide program. We are continuing the testing of new fungicides formulations against this disease as requested by various chemical companies. In contrast to the Alternaria late blight of pistachio, there are no concerns about fungicide resistance in the Botryosphaeriaceae pathogens. The objective of the 2018 spray trial was to determine the fungicide efficacy against Botryosphaeria blight of pistachio.

## Material and Methods

Butte County Botryosphaeria fungicide spray trial. Four rows of a commercial pistachio orchard in Butte County were used for the fungicide efficacy study. The orchard was irrigated using drip emitters. Treatments consisted of five single-tree (cv. Kerman) replications using a randomized complete block design. The fungicide treatments, rate of each fungicide, and spray timing are listed in Table 1. All sprays were applied with a handgun sprayer using 400 gallons of water per acre. All treatments consisted of four applications performed from 19 April to 19 July, with a late dormant spray for one material on 27 March. Disease was recorded on 4 September by counting the blighted panicles among 100 fruits panicles per replicated tree.

## Results and Discussion

The most effective treatment was obtained by spraying the mixture of Fontelis (active ingredient penthiopyrad – FRAC#7) and Tebucon 45DF (a.i. tebuconazole – FRAC#3), listed as T3 in Table 1, resulting on 0.6% of blighted panicles. There were eight other treatments with less than 2% blighted clusters. They were: Pristine (T17) with 1.1%; Luna Sensation (T8), Luna Experience + Baythroid (T10) and Fontelis + Abound (T2) with 1.2%; Approach (T6) with 1.3%; Fontelis (T1) and Viathon (T20) with 1.4%. The experimental fungicide product UC-2 (T12) showed 1.2%. The rest 19 treatments were not showed to be significantly different in efficacy (Table 1). All treatments provided better control than the untreated control (12.3%).

## Conclusion

The 2018 fungicide trial revealed the good efficacy of many fungicide products currently registered for pistachio usage. Great disease control was obtained with products formulated with carboxamides, now called succinate dehydrogenase fungicides (FRAC#7).

**Table 2.** 2018 Efficacy of fungicides against Botryosphaeria panicle blight in a commercial pistachio orchard in Butte County.

| Treatment | Fungicide(s)[1] | Rate | Dormant Spray (27-March) | Full Bloom (19-April) | 17-May | 14-Jun | 19-Jul | Disease incidence on tree (%)[2] |
|---|---|---|---|---|---|---|---|---|
| T1 | Fontelis (Fon) | 20 fl oz | - | Fon | Fon | Fon | Fon | 1.4 a-d[2] |
| T2 | Fontelis + Abound (Ab) | 20 fl oz + 12.0 fl oz | - | Fon + Ab | Fon + Ab | Fon + Ab | Fon + Ab | 1.2 ab |
| T3 | Fontelis + Tebucon 45 (Teb) | 20 fl oz + 8 oz | - | Fon + Teb | Fon + Teb | Fon + Teb | Fon + Teb | 0.6 a |
| T4 | Fontelis / Quadris Top (QT) | 20 fl oz + 14 fl oz | - | Fon | QT | Fon | QT | 2.1 a-e |
| T5 | Fontelis / Quash (Qs) | 20 fl oz + 4 oz | - | Fon | Qs | Fon | Qs | 2.2 a-e |
| T6 | Aproach (Ap) | 12 fl oz | - | Ap | Ap | Ap | Ap | 1.3 ac |
| T7 | Indar (In) | 6 fl oz | - | In | In | In | In | 3.0 b-e |
| T8 | Luna Sensation (LS) | 5 fl oz | - | LS | LS | LS | LS | 1.2 ab |
| T9 | Luna Sensation + Baythroid (Bay) | 5 fl oz + 2.8 oz | - | LS | LS | LS + Bay | LS + Bay | 3.0 b-e |
| T10 | Luna Experience (LE) + Baythroid | 6 fl oz + 2.8 fl oz | - | LE | LE | LE + Bay | LE + Bay | 1.2 ab |
| T11 | UC-1 | 5 fl oz | - | UC-1 | UC-1 | UC-1 | UC-1 | 2.9 b-e |
| T12 | UC-2 | 7 fl oz | - | UC-2 | UC-2 | UC-2 | UC-2 | 1.2 ab |
| T13 | Ph-D | 6.2 oz | - | PH-D | PH-D | PH-D | PH-D | 3.0 b-e |
| T14 | Ph-D + Tebucon 45 | 6.2 oz + 4 oz | - | PH-D + Teb | PH-D + Teb | PH-D + Teb | PH-D + Teb | 2.7 a-e |
| T15 | Pristine (Pri) / MBI-10612 | 14.5 oz/ 1 quart | - | Pri | 10612 | Pri | 10612 | 2.4 a-e |
| T16 | Pristine / Stargus (St) | 14.5 oz/ 2 quart | - | Pri | St | Pri | St | 2.1 a-e |
| T17 | Pristine | 14.5 oz | - | Pri | Pri | Pri | Pri | 1.1 ab |
| T18 | Pyraziflumid (Py) + Silwet (Sil) 3 oz/100gal | 3.1 fl oz | - | Py + Sil | Py + Sil | Py + Sil | Py + Sil | 2.2 a-e |
| T19 | Pyraziflumid + Silwet 3 oz/100gal | 4.7 fl oz | - | Py + Sil | Py + Sil | Py + Sil | Py + Sil | 2.4 a-e |
| T20 | Viathon (Vi) | 1 quart | - | Vi | Vi | Vi | Vi | 1.4 a-d |
| T21 | Viathon | 2 quarts | - | Vi | Vi | Vi | Vi | 2.3 a-e |
| T22 | A20259E | 13.7 fl oz | - | A20259E | A20259E | A20259E | A20259E | 2.0 a-e |
| T23 | A20560C | 9.1 fl oz | - | A20560C | A20560C | A20560C | A20560C | 2.3 a-e |
| T24 | Quash | 4 oz | - | Qs | Qs | Qs | Qs | 3.1 b-e |
| T25 | V-10424 | 4 fl oz | - | V-10423 | V-10424 | V-10424 | V-10424 | 4.1 c-e |
| T26 | Actinovate (Ac) | 8 oz | - | Ac | Ac | Ac | Ac | 5.2 e |
| T27 | WXF-17001 | 0.25% w/w | - | WXF-17001 | WXF-17001 | WXF-17001 | WXF-17001 | 4.2 de |
| T28 | WXF-17001 | 0.35% w/w | - | WXF-17001 | WXF-17001 | WXF-17001 | WXF-17001 | 3.2 b-e |
| T29 | WXF-17001 | 0.35% w/w | 0.5% w/w | WXF-17001 | WXF-17001 | WXF-17001 | WXF-17001 | 4.3 de |
| T30 | Control | Untreated | - | - | - | - | - | 12.3 f |

[1]Non-ionic surfactant (NIS) was added at 0.0625% (vol./vol.) to all treatments, except 27 to 30. [2] Blighted fruit panicles/100 panicles for each of 5 replicated trees were recorded on 24-September. Number followed by different letter are significantly different according to the LSD test at α=0.05.

# Biology, Epidemiology, and Management of Anthracnose Blight and Stigmatomycosis of Pistachio in California and Phoma Blight in Arizona (Second year)

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## Introduction

The pistachio anthracnose in California is caused by two *Colletotrichum* species, *C. fioriniae* found in Glenn County, and *C. karstii* sporadically found in Tulare County. From 2016 to 2018, severe pistachio losses were caused by the *C. fioriniae* in commercial pistachio orchards in California where the cultivar Red Aleppo was grown. This pathogen can infect different plant parts including leaves, twigs and fruits. On fruits, lesions start with several small black spots that coalesce and become black and sunken lesions leading to severe shriveling of kernel and hulls. To determine the best timing of fungicide treatment to control the anthracnose disease, it is necessary to know the period in which pistachio trees present the highest susceptibility to the pathogen infection. For that, spore suspensions of *C. fioriniae* (11K11) and *C. karstii* (3G24) were prepared at $5\times10^4$ spores/ml into a 500 ml volume, used to inoculate 30 different pistachio clusters for five months starting in mid-April. Inoculation dates were April 18$^{th}$, May 21$^{st}$, June 21$^{st}$, July 18$^{th}$ and August 20$^{th}$. In total, 300 clusters were harvested on September 10$^{th}$ and each fruit was visually inspected for the presence of anthracnose lesions. Frequencies were recorded and analyzed statistically by means comparison, using the Fischer's LSD test with the statistical software R-studio.

## Results and Discussion

*C. fioriniae inoculations.* The inoculations made in April presented the highest ($P$=0.023) frequency of blighted fruits (69.1%) when compared with inoculations from May (50.5%) and August (54%). However, April inoculations were statistically similar to those obtained in June (61.4%) and July (60.6%) (Figure 1, dotted bars). According to our field observations, *C. fioriniae* inoculated in April caused a severe cluster collapse at the fruit onset (Figure 2). In 2017, this event was not observed because inoculations started in June through August. Higher numerical disease incidence was observed in June and July (when not considering the April infections), and this result was like the results obtained in 2017 when inoculating Kerman cultivar (See last year CPRB report).

*C. karstii inoculations.* The blighted fruit frequency obtained from inoculations made in July (65.9%) was higher ($P$ = 0.023) than inoculations made in April (48.5%) but was not distinguished from infections obtained from May (54.9%), June (53.1%) and August (55.6%) (Figure 1, line bars). This result is in accordance with last year's results, where *C. karstii* caused more fruit lesions in July (See 2017 CPRB report).

## Conclusion

Infections of *Colletotrichum* species can occur from April through August, and the absence of significant difference among the studied months make it difficult to determine an accurate timing for fungicide application. However, if we consider the results from 2017, we can narrow the main period for infection from June to August, and it is possible anthracnose control may overlap the period for controlling the Alternaria late blight (from early June to the first week of August). It may be that climatic conditions in our experimental station in Parlier (Fresno County) are not as conducive to the anthracnose disease as the conditions from the northern counties where *Colletotrichum* currently occurs. The cluster collapse observed in consequence of mid-April inoculations (caused by *C. fioriniae*) is an important observation and should be an alert for growers in northern San Joaquin Valley. A similar study should be repeated in 2019, and we strongly suggest a physiological characterization for the optimal infection conditions of *C. fioriniae* and *C. karstii.*

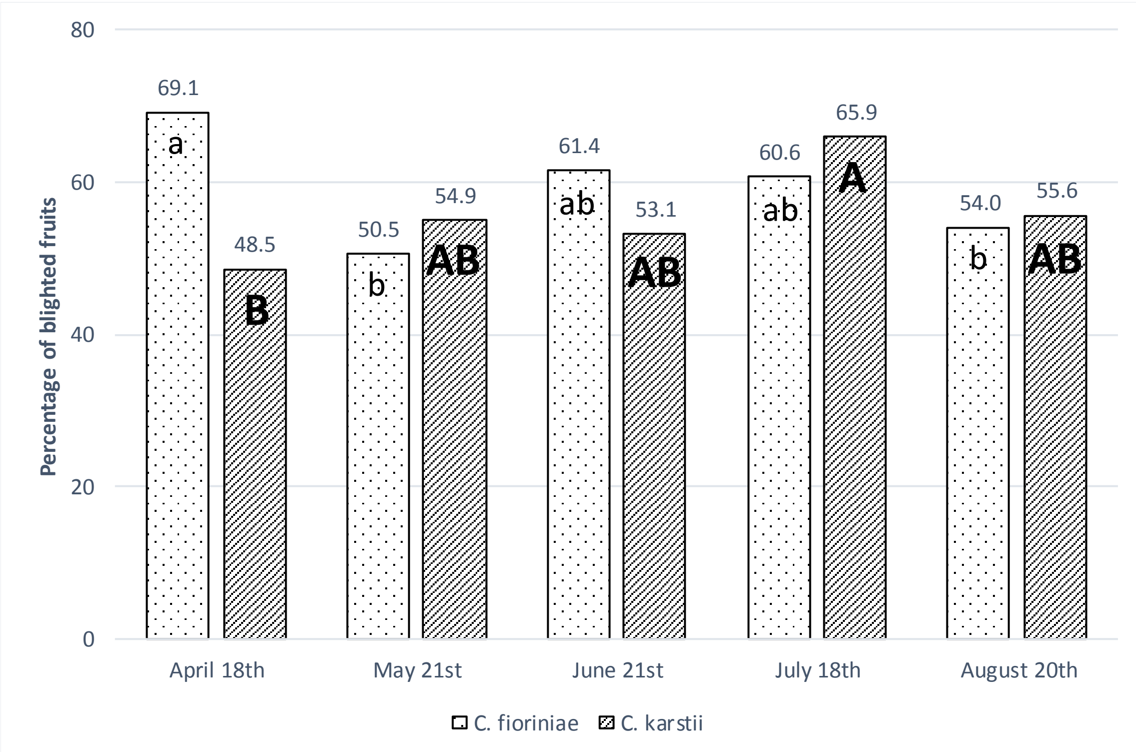


Figure 2. Period of pistachio (cultivar Red Aleppo) susceptibility for *C. fioriniae* and *C. karstii* infections, tested from April through August 2018. Bars with similar letters are not different at α=0.05 using the Fisher's LSD test. Low capital letters compare the *C. fioriniae* inoculations while bold capital letters compare the *C. kasrtii* inoculations. Values correspond to the frequency (%) of blighted fruits inspected from a total of 30 pistachio clusters.


Figure 3. Image of Red Aleppo yuoung pistachio clusters photographed on April 21st 2018 after fruit set (A). Image of Red Aleppo pistachio cluster showing total colapse due to the infection of *C. fioriniae*, photographed on May 18th 2018 (B). Notice in picture (B) the regular size of fruits not infected by the *C. fioriniae*.

# Early Detection of Pistachio Botryosphaeria Panicle Blight Disease Using High-throughput Plant Phenotyping

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## Introduction

Botryosphaeria Panicle Blight (caused by *Botryosphaeria dothidea*), a major disease of pistachios in California, first became a serious problem in Sacramento Valley in late 1980s. However, a couple of decades later, the disease caused a significant yield reduction in pistachio orchards of the San Joaquim Valley as well. Botryosphaeria is known as the major threat to the California pistachios. Unfortunately, Botryosphaeria disease has a latent period and the earliest symptoms appear in late April to May, if the temperature is warm enough. Dead or partially infected buds can show symptoms such as dead areas as early as mid-summer. However, a significant portion of potentially infected buds remain non-symptomatic. A portion of these contaminated buds will likely develop infected shoots in spring.

Current early detection practice suggests collecting up to 100 buds from random locations throughout the grove and processing them using BUDMON, a bud monitoring technique. The BUDMON technique is highly sensitive and capable of diagnosing the samples with little or no visual symptoms. However, it requires sample collection/preparation, and laboratory effort/cost, and it takes at least one week to have the diagnosis results. Additionally, the number of samples collected from a grove is not even comparable to the number of buds in one orchard. Although, the BUDMON technique is a highly accurate and reliable diagnostic method, the test is expensive, and it requires laboratory effort. Therefore, number of samples to be collected for BUDMON test is limited by the available budget for disease monitoring; while, an optimum Botryosphaeria management requires a maximized monitoring in which high special and temporal resolution data must be available.

A portable hyperspectral measurement tool, called KOBIN Proximity (2$^{nd}$ prototype), with high spectral resolution was developed in this project that is able to conduct a real-time and in-field spectral measurement of pistachio buds, conduct spectral analysis, and compute the probability of the Botryosphaeria infection. KOBIN proximity has a fiber optic and probe head that is customized for pistachio bud spectral measurement. It consists of two separate spectrometers and covers the range of wavelengths from 180 nm to 1650 nm. KOBIN Proximity also includes a light source, a micro-computer and a touch screen display that illustrates the measurement and analysis results. Several datasets of spectral reflectance of pistachio buds were created in 2017, and 2018. The 2017 datasets were created by the first prototype of KOBIN Proximity which covered the range of 180-1030 nm divided into 2048 wavelengths. These datasets included pistachio bud samples with *Botryosphaeria* infection, healthy buds, and a few samples with Phomopsis infection. In 2018, the second prototype of KOBIN Proximity was used for spectral measurement that covered an extended range of 180-1650 nm divided into 2176 different wavelengths. The bud samples in the 2018 datasets were infected with *Botryosphaeria dothidea* (Bd), *Botrytis cinerea* (Bc), *Alternaria alternata* (Aa), *Aspergillus niger* (An), *Aspergillus flavus* (Af), and *Fusarium* (Fus). No healthy bud samples were found in the 2018 dataset. The infection statuses of all pistachio buds were assessed by BUDMON test.

Spectral data were preprocessed before conducting the supervised machine learning. Preprocessing was conducted in several steps including (1) range selection to remove the noisy signals; (2) normalization to avoid the impact of variable light on the spectral data; (3) outlier removal to avoid the negative effect of outliers on the machine learning models; (4) oversampling to avoid the negative impact of class imbalance; and (5) wavelength selection to determine to most important wavelengths. Four supervised learning models were trained and their accuracies for disease detection were obtained.

## Results and Discussion

The range of 412 nm to 904 nm were selected for the 2017 datasets after removing the noisy part of the spectrum. Also, for the 2018 datasets, two ranges of 444-691 nm and 936.72-1659.5 nm were selected as not noisy. The Standard Normal Variate (SNV) method was used to normalize the spectral data. Afterwards, the outliers were spotted and removed from the dataset. To solve the problem of class imbalance, we use an oversampling technique called Synthetic Minority Over-Sampling Technique (SMOTE). An important part of using SMOTE is that to develop truly robust models, the testing dataset must have only original samples and not mixed with the synthetic oversampled data points, as they can cause minority class bias in accuracy and can give misleading results. Since all datasets contained very few data points (samples) with large number of features, it was imperative to use feature selection methods, namely Boruta and Successive Projections Algorithm. Only top features determined by the feature selection algorithm was used in the classification process.

Four supervised learning models were used in this research including Multi-layer Perceptron, Decision Tree Classifier, K-Nearest neighbors, and Random Forest Classifier. Each dataset was split into training and test sets and 10-fold cross validation was used to avoid the bias effect. The confusion matrix for each split was added to get the final confusion matrix and prediction accuracies. The best classification accuracy (healthy vs Botryosphaeria**)** obtained for the 2017 data was 61% using the Random Forest Classifier. For the 2018 dataset, the best classification accuracy of 79.2% was achieved using the Decision Tree Classifier and the spectral data in the range of 936.72-1659.5 nm.

## Conclusion

We created a portable diagnosis tool that is customized for non-destructive spectral measurement of pistachio buds. Additionally, we developed a general preprocessing and machine learning pipeline to classify different fungal infection and distinguish them from healthy pistachio buds using hyperspectral sensing.

A few challenges were encountered during the data analysis: first, the datasets were highly imbalanced. This makes learning very difficult as models tend to often predict the majority class. Second, the number of samples in each dataset were not enough, so that the results depended on the samples conditions in each dataset and cannot be generalized. Finally, the 2018 datasets did not include any healthy sample, so that the classification was only among different fungal infections.

In conclusion, we stablished the methodologies for spectral data collection and data analysis. In the spring 2019, we will create a library of pistachio buds' spectral data with large number of samples (about 4000 samples). We will confirm the fungal infection status of all pistachio buds using BUDMON assay. Finally, we will generate a generalized classification model and implement it in the KOBIN Proximity for the real-time on-site diagnosis.

# Evaluating pistachio rootstock tolerance to soil-borne diseases

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## Introduction

Since the Verticillium wilt crisis in the 1970s, UCBI rootstocks (clonal or seeded) have been the most widely utilized pistachio rootstocks in California. As the pistachio industry continues to expand, more UCBI trees are being planted in marginal grounds including soils with high salinity and poor water infiltration. This has resulted in greater stress to pistachio trees, rendering them more prone to diseases caused by soil-borne pathogens. Recently, our laboratory identified Phytophthora root and crown rots as emerging new threats to pistachio, particularly in areas where soil conditions or cultural practices favor water saturation. Three Phytophthora species including *Phytophthora niederhauserii, P. cinnamomi* and *P.* taxon walnut were identified from diseased pistachio trees in California. The fungus *Macrophomina phaseolina* also was detected as an emerging soil borne pathogen causing crown rot in newly planted UCBI pistachio rootstocks. *Fusarium* species including *F. oxysporum, F. proliferatum, F. falciformis* and *F. solani* were found occasionally in association with crown rot or stem cankers of UCBI rootstocks. The present research aimed to (i) confirm the pathogenicity of the various fungi to pistachio rootstocks; (ii) develop methodologies for rapid screening of pistachio rootstock resistance to soil-borne pathogens; (iii) determine the relative resistance of commercial pistachio rootstocks to the new soil borne pathogens in order to provide sustainable management strategies against soil borne diseases. All experiments were conducted at the Kearney Agricultural Research and Extension Center in Parlier, CA.

## Results and Discussion

In order to demonstrate the ability of the various soil borne fungi to cause root rot, a final pathogenicity assay was conducted on October 24, 2018. Inoculation tests were carried out using 2-year-old UCBI clonal rootstocks with size corresponding to that of trees used for field planting. After 37 days of incubation period following soil/root inoculation, results revealed that all three *Phytophthora* species as well as *Macrophomina phaseolina* were pathogenic to clonal UCBI rootstocks causing root rot, with *P.* taxon walnut being the most virulent species. The ability of *Fusarium* species to cause root rot was not shown in our experiments.

In this study, two inoculation methods were tested and compared for efficient screening of pistachio rootstock resistance to the soil-borne pathogens *Phytophthora* spp., *M. phaseolina* and *Fusarium* spp. Assays included soil/root inoculations with spores as well as direct inoculation of rootstock stems with mycelium plugs. Stem inoculation of 2 to 3-year-old pistachio rootstocks using mycelium plugs produced vascular discolorations that can be easily quantified and thus compared among various treatments. This method appeared very efficient to assess pistachio rootstock susceptibility to crown rot pathogens and appeared adapted for routine resistance screening of rootstocks. Soil/root inoculation using a spore or microsclerotia suspension resulted in rapid wilting of inoculated plants suggesting that this method also can be adopted routinely to screen pistachio rootstocks for disease resistance.

This research revealed significant differences of susceptibility (expressed as the lesion length produced in stems inoculated with mycelium plugs) among UCBI, PGI and Platinum rootstocks to the various soil-borne pathogens. Platinum rootstock was most resistant to all pathogens (2.89 cm average lesion length), followed by PGI rootstock (4.07 cm average lesion length) (Fig. 1). UCBI appeared most susceptible rootstock with lesion length averaging 8.03 cm across all pathogens (Fig. 1). Isolate recovery rates from the inoculated rootstock ranged between 60 and 100% for all isolates used in the experiment.

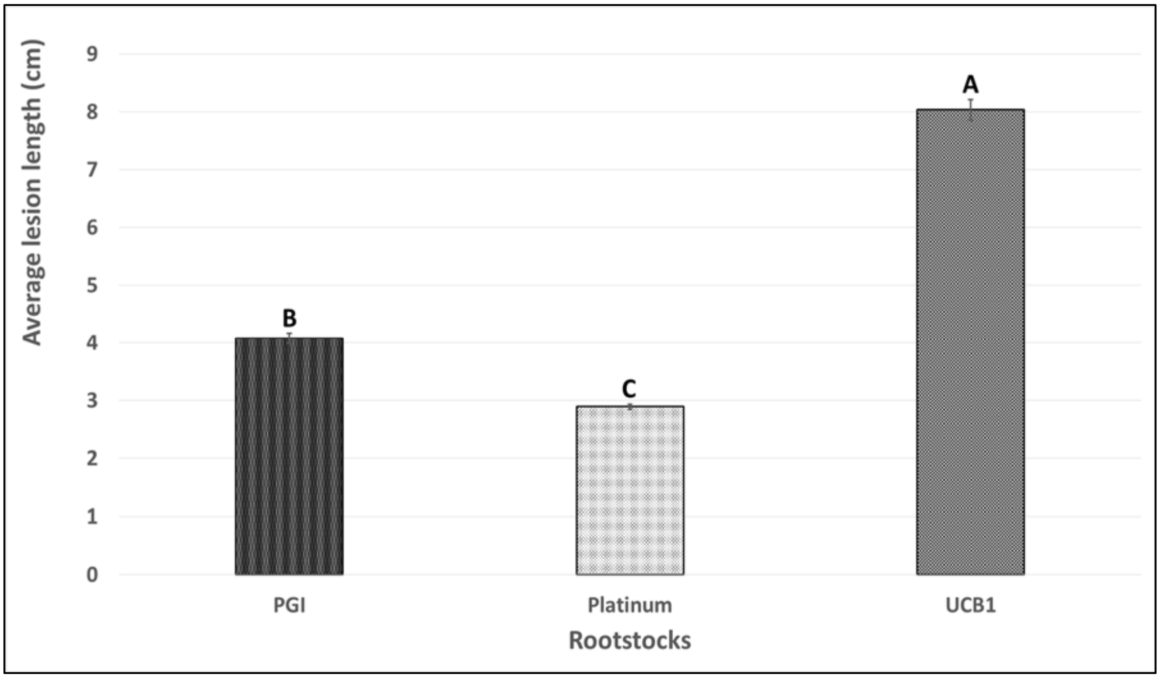


**Fig. 1.** Lesion length (mm) produced in stems of UCBI, PGI and Platinum rootstocks inoculated with mycelial plugs colonized with *Phytophthora, Macrophomina* and *Fusarium* fungal isolates. Lesion sizes were averaged across all fungal isolates for each rootstock. Bars topped with different letters indicate treatment means that are significantly different ($P < 0.05$). Data showed higher resistance of Platinum rootstock to soil-borne pathogens whereas UCBI appeared as the most susceptible rootstock. PGI shows intermediate level of disease tolerance.

## Conclusion

- This study confirmed the ability of *Phytophthora* spp. and *M. phaseolina* to cause root and crown rots of pistachio. *Fusarium* spp. did not affect root health but produced crown rot and stem cankers in pistachio rootstocks.

- Outcomes from this research include the development of methodologies that can be used for rapid screening of pistachio rootstock resistance to soil-borne pathogens. These protocols could be used in further pistachio rootstock improvement programs.

- This study reports significant differences in the resistance of UCBI, PGI and Platinum (a PGII decent) to soil-borne pathogens *Phytophthora, Macrophomina* and *Fusarium* species. Platinum was the most resistant rootstock across all various soil-borne pathogens. On the other hand, UCBI appeared as the most susceptible rootstock whereas PGI showed intermediate level of disease tolerance. Additional experiments were established last fall to validate these findings.

- Although, this result needs to be confirmed with additional experiments, Platinum may be the preferred rootstock choice for pistachio trees planted in areas at risks for Phytophthora, Macrophomina and Fusarium soil-borne diseases.

- Research continues to provide the California pistachio industry with knowledge of the resistance of commercial rootstocks to soil-borne pathogens and to identify most resistant rootstocks as a management strategy to control soil-borne diseases.

# Characterizing pistachio rootstocks for host status to plant-parasitic nematodes

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## Introduction

In California, Pistachio (*Pistacia* spp.) is often cultivated by the use of the female cultivar 'Kerman' and the pollinating male 'Peters'. At initiation of the pistachio industry, these scions were grafted onto rootstocks of *Pistacia atlantica* and *P. terebinthus.* While apparently resistant to *Meloidogyne* spp. and *Pratylenchus vulnus* these rootstocks were highly susceptible to Verticillum wilt that occurred widely in California (Michailides and Teviotdale, 2014; Crane and Maranto 1988; McKenry and Kretsch, 1984). A controlled cross of *P. atlantica* x *P. integerrima* resulted in the 'UCB1' clonal rootstock that was developed to combat increasing challenges with Verticillium. Different genotypes of this controlled cross are used as UCB1 rootstocks.

Overall, nematode problems in pistachio have been considered minimal may be because in a California survey, only low population densities of plant-parasitic nematodes were found (McKenry and Kretsch, 1984). Susceptibility to *Meloidogyne* spp. (root-knot nematode, RKN) is generally reported as low (Westerdahl, 2015). *Xiphinema index* was found to infect *Pistacia vera* and *P. mutica* (Weiner and Raski, 1966).

Culver and coworkers (1989) chose *P. atlantica* as a resistant standard when screening woody perennials. In preliminary screens of UCB1 clones, large differences between defined clones of this cross have been identified (McKenry, unpublished). In recent work, interaction of *Pratylenchus vulnus* with *Mesocriconema xenoplax* (ring nematode) on pistachio illustrated the susceptible host status of one clone of UCB1 (Westphal et al., 2016). Previous crops were probably planted to nematode-free soils but todays plantings often follow cotton crops or vineyards, both of which frequently leave noticeable populations of plant-parasitic nematodes behind. Similarly, nut crops are often infected with root lesion nematode, *Pratylenchus vulnus* (RLN).

It is the aim of this project to determine the relative host suitability to *Pratylenchus vulnus*, *Meloidogyne incognita,* and *Mesocriconema xenoplax* of currently available pistachio rootstocks, including multiple clones of UCB1 that are marketed by various nurseries. In addition to the UCB1 genotypes, there were *P. atlantica*, Pioneer Gold I, and in 2018 three crosses provided by Dr. Mallikarjuna Kuma Aradhya (USDA-ARS Davis). Controls were *Prunus* rootstocks with known susceptibility to the respective nematodes or *Juglans* rootstocks. Two sets of rootstocks were planted into sandy loam field plots (first planting 2017, second planting 2018) for inoculations with *Pratylenchus vulnus* and *Meloidogyne* sp. Two sets were planted to sandy soil in contained plots for inoculation experiments with *Mesocriconema xenoplax* (first planting 2017, second planting 2018).

## Results and Discussion

<u>The field screen for susceptibility to RLN and RKN planted in 2017</u> included nine clonal UCB1, one seed derived UCB1, two *Pistacia atlantica*, one *P. terebinthus*, one *P. integerrima* and one hybrid of *P. atlantica* and *P. integerrima* (15 genotypes total). In 2017, plants had been planted in pairs of two per genotype in four replicate plots. After the second year of growth, all plants were measured for height and diameter. For each genotype, one of the plant pair plants was removed, and root systems were rated for nematode-induced galling before fine roots for nematode extraction were excised. When examining the Pistacia species, in general taller plants were also thicker but variability of the relative increase of the two

parameter was larger with taller plants. Especially clonal UCB1_4 was taller than eleven other *Pistacia* spp., the other plants, including the seed-derived UCB1, were similar like UCB1_4. UCB1_5 had the largest trunk diameter but was not the tallest plant suggesting a somewhat more stout growth habit. *P. atlantica* and *P. terebinthus* that had been raised from seed before field planting were much smaller at planting and did not reach the level of growth of the genotypes that had been planted as liners. Root-knot nematode numbers in the Pistacia rootstocks were lower than in the known susceptible Prunus and Juglans species. Numbers on UCB1 ranged from 0.1 to 10.5 infective second stage juveniles (J2) per gram of root whereas Prunus reached from 70.8 to 92.4 J2 per gram of root, and Juglans had 115.4 J2 in these calculations. UCB1_4 had significantly fewer RKN J2 per gram of root than UCB1_8. *Pistacia terebinthus* had 29.3 J2 per gram of root thus elevated compared to UCB1. In root lesion nematode, there were only trends within UCB1 but numbers varied from 2.6 to 40.0 vermiform per gram of root compared to 44.3 to 75.3 in the susceptible Prunus, and 27.4 in the Juglans. So overall, infection and reproduction rates of RLN and RKN were somewhat lower on the UCB1 clones than on the known susceptible Prunus and Juglans rootstocks.

<u>One screen for susceptibility to *Mesocriconema xenplax* was planted in single-plant plots in sand tanks in 2017.</u> In 2018, soil cores were taken from the root zone of the test plants, and nematodes extracted with a sugar flotation-centrifugation method that aims at nematode stages in the soil matrix. Despite using the most efficient extraction method for *M. xenoplax*, ring nematode numbers were low in all testers and the susceptible standards. There was variability among the different lines, but no significant differences were detected. A longer incubation will be necessary for obtaining a comprehensive assessment.

<u>Plantings in 2018 in root-knot and root lesion nematode infested field plots and in sand plots for ring nematode evaluations.</u> Replicate experiments were planted and inoculated in 2018. These included three genotypes of the breeding program of Dr. Mallikurjana Aradhya (USDA-ARS). These plantings will be evaluated in 2019. In addition, experiments are ongoing that expose rootstock genotypes to different population densities of the root lesion nematode.

## Conclusion

Overall, nematode numbers in the second-year evaluation were lower in pistachio roots than in known susceptible crops. There were differences among the UCB1 clones, but these were only significant for root-knot nematodes. The typical slow development of pistachio will require further monitoring of nematode population development.

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# Phenotype characterization of PBTS affected trees entering maturity: investigation of tree size, yield potential, and variability of 8th leaf PBTS trees.

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## Introduction,

Pistachio bushy top syndrome (PBTS) is a problem that has affected orchards planted between 2011 and 2016 in California, Arizona, and New Mexico. Because of the unprecedented nature of PBTS, no research-based data are available to predict the long-term productivity of PBTS-affected trees in orchards. In fact, yield potential is only one factor growers consider when assessing the economic viability of maintaining PBTS trees or affected orchards. Other factors include i) the common failure of PBTS trees to bud, and, as a consequence, lack the potential to set a commercial crop, and ii) and the potential for PBTS trees to break at the cracked graft union upon shaking at harvest. Even if PBTS trees exhibited adequate yield potential at maturity, the net returns could be eroded if symptomatic trees require hand harvest. Due to the unknown productivity and management costs associated with PBTS trees, most growers and land managers opted to either remove entire orchards or individual trees, depending on the level of PBTS incidence in affected orchards. Symptoms of PBTS have largely been observed in clonally propagated 'UCB-1' rootstock and include stunting, shortened internode length, swollen nodes, gall formation, cracked graft union, and failure to bud. PBTS-affected trees develop abnormal tree morphology, but the syndrome does not manifest as necrosis or mortality. As a consequence, the decision to rouge trees or orchards is not based on tree mortality, as is typical of other diseases of perennial tree crops, but rather on concern for the long-term economic productivity of the orchard.

To assess the risk of not removing PBTS-affected trees from orchards, we gathered phenotypic data (including yield) on PBTS trees over two consecutive years, in an orchard entering its 7th and 8th leaf. The test site was a commercial block 'Golden Hills' on clonal 'UCB-1' rootstock, planted in 2011; consequently, it is one of the first blocks in which the irregular plant morphologies associated with PBTS were observed. A comparative analysis of phenotype (ie. suckering, trunk and scaffold diameters, bark texture, and yield) between symptomatic and asymptomatic trees was completed in 2017 and 2018.

## Results and Discussion

Trees symptomatic of PBTS had significantly smaller mean trunk diameter and mean total scaffold diameter than asymptomatic trees during both years ($P \leq 0.0001$). In 2018, symptomatic trees exhibited 26% lower trunk diameter and 38% lower total scaffold diameter than asymptomatic trees. The variance ($s^2$) of both trunk circumference and total scaffold diameter were greater for symptomatic trees then asymptomatic trees ($P \leq 0.0001$). These data indicate that trees symptomatic of PBTS are both smaller and more variable in size then asymptomatic trees.

Symptomatic trees had statistically more suckers ($P \leq 0.0001$) per tree and more suckers per unit tree circumference ($P \leq 0.0001$) then asymptomatic trees in 2017. Additionally, the PBTS symptomatic trees had more variability in suckering then asymptomatic trees ($P \leq 0.0001$) in 2017. Sucker data was not collected during 2018 because work crews had removed suckers in the research plot. These results support early reports from growers regarding the propensity for suckering on symptomatic trees in PBTS-affected orchards.

Symptomatic trees exhibited a 73% and 55% reduction in yield in 2017 and 2018, respectively ($P \leq 0.0001$). The variance in yield, however, was statistically similar between symptomatic and asymptomatic trees over both years. In 2017, nut quality differed between symptomatic and asymptomatic trees. Quality subsamples exhibited 1.8% and 3.5% blanks from asymptomatic and symptomatic trees, respectively ($P \leq 0.01$), and blanking was more variable in nuts from symptomatic trees than asymptomatic trees ($P \leq 0.0001$). Percent edible yield was 7% higher from samples from asymptomatic trees than symptomatic trees, though not statistically different ($P \leq 0.05$). Nut quality data from 2018 is not yet available.

## Conclusion

The results of the phenotyping study indicate that trees symptomatic of PBTS are both smaller and more variable than asymptomatic trees. Trees exhibiting the syndrome produced at least 50% lower yield than asymptomatic trees, and nut quality was adversely affected by the syndrome. No trees were broken during harvest; however, all trees within the experimental plot were harvested by hand with mallets. Growers assessing loss due to PBTS-affected trees need to consider the incidence of the syndrome in a block (ie. percent affected trees), as well as the anticipated yield reduction and influence of nut quality on price.

The variability in phenotype characteristics exhibited in the population of symptomatic trees suggests that the PBTS population in this block did not result from the propagation of a single off-type clonal genotype. In contrast, the phenotypic variability is indicative of a disease or disease complex, where multiple factors may influence disease development (i.e., host, pathogen, environment, and time).