Executive Summaries 2019

California Pistachio Research Board
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2019 Manager's Report

Bob Klein

The California Pistachio Research Program was created as a state marketing order by a referendum of the pistachio growers in 2007 as a vehicle for pistachio production research and grower education. It is administered by the California Pistachio Research Board (CPRB) which is made up of nine members and four alternates, all of whom are pistachio growers. The CPRB also has a public member who is not affiliated with any pistachio producer/handler.

The research and education projects approved by the CPRB are funded by mandatory assessments based on production. This report contains summaries of the funded research projects for the past fiscal year (2018-2019). Many of the research projects in this report are multiyear projects. Past reports, including full reports containing more information, are available on the CPRB website (https://calpistachioresearch.org). The scope of the research projects reflects the concerns of the pistachio industry as determined by the CPRB as limited by the availability and interests of potential researchers. The CPRB engages researchers within the University of California system (campuses and Cooperative Extension), the California State University system, USDA Agricultural Research Service, as well as other universities outside California. Many production issues must be addressed in the California production area and consequently, most researchers are based in California.

A significant amount of the field research is conducted by Cooperative Extension Farm Advisors under UC Division of Agriculture and Natural Resources (ANR). The years have not been kind to the ANR budget, both due to the amount of state funding and to the priorities of UC administrators, including UC President Janet Napolitano (she has resigned and, in my opinion, any replacement cannot be worse for agriculture than she was). Over the years, California agriculture has lost Farm Advisors due to retirement and few have been replaced. We are thus woefully short of Farm Advisors throughout the Central Valley. The CPRB is working with other commodity boards to develop programs to improve the effectiveness of the Farm Advisors who remain. You should expect to hear more about this in the future.

Navel Orangeworm (NOW) is the principal insect pest of pistachios. Due to its direct impact on production and its indirect impact in export markets, much of the CPRB research emphasis has been on NOW and its control. After two years (2016 and 2017) of historically severe problems, the industry has been blessed by two historically low damage years (2018 and 2019). Nevertheless, the NOW problem will almost surely return. Over the past few years, the CPRB has been investigating the potential of using sterile insect technology (SIT) as a component of an areawide NOW control program in cooperation with USDA-APHIS, Wonderful Farming, California Department of Food and Agriculture, ANR, and USDA-ARS. We have demonstrated that NOW can be mass-reared, irradiated to sterility, shipped from Phoenix to Shafter without excessive mortality, and aerially released over a large treatment area without excessive mortality. However, recapture following release has been minimal and reduced NOW damage in pistachios has not been documented, in part due to the very low damage levels of 2018 and 2019. There has now been a federal appropriation of $6 million that will help cover some of the costs of this program. This should allow additional research on improving the vigor of NOW moths following shipment, release mechanisms for sterile moths, and other research on NOW population dynamics critical to the success of the program. While the CPRB will continue to fund some aspects of this program, our expenses should decrease significantly.

Two years of low NOW damage have supported observations suggested by the 2010 crop, that NOW damage in pistachios is significantly affected by crop susceptibility. Unlike almonds where Nonpariels become susceptible every year with hull split, pistachios only become susceptible as the hull degrades. In 2018 and 2019, there was very little hull degradation. Without hull degradation and the resultant susceptibility to NOW infestation, almost any NOW control program yielded the same result – little or no insect damage. Very little is known about hull composition and how it varies during the season, what physiological changes occur with maturity, what predicates hull slip, what volatiles are released that attract NOW egg laying,… and with that ignorance, how to predict susceptibility and how to control and manipulate the hull. As you read through the executive summaries, you will notice several projects that have started to address our fundamental lack of knowledge about hulls. While this hull project will likely take many years (we have to rely on the crop to create years of high and low susceptibility), there are many potential spinoffs that could reduce NOW control costs.

California pistachio production continues to expand and encounter new conditions. In addition, we must deal with climatic variability and invasive pests. We are particularly concerned with Brown Marmorated Stink Bug (BMSB) and Spotted Lantern Fly (SLF). BMSB continues to expand its range in California and we are monitoring its effects, particularly around urban areas that it seems to prefer. SLF has not yet appeared in California but its effects on the East Coast have been severe.
If there are particular concerns that you may feel are not being addressed adequately, please let the CPRB know. Our Members, Alternates, and Advisors cover operations across the Central Valley, but localized issues can sometimes escape our attention.

Thank you for your support of CPRB programs and best wishes for a productive 2020 season.

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Improving and Verifying Quality of Mass-Reared Navel Orangeworm for Sterile Insect Technique

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

Introduction

Mass rearing and irradiation of navel orangeworm (*Amyelois transitella*) (NOW) 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 almond, 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 are 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 evaluates the effects of moth strain, irradiation, shipping/handling process and field release mechanism on NOW flight, dispersal and mating competitiveness under both laboratory and field conditions.

In 2018, data from aerial and ground releases of irradiated/sterile NOW in the absence of mating disruption provided little if any evidence that released males were able to fly to and/or locate pheromone sources. In contrast, irradiated/sterile NOW females were generally reproductively competitive (i.e. they effectively called and mated with wild males). Objectives at the start of the 2019 season were to parse the relative importance of insect strain, radiation dose, shipping/handling effects (cold and darkness) inherent in the production/transport procedures used to date, as well as field release mechanism. We also refined the mark-release-recapture assays used to test these effects in the field.

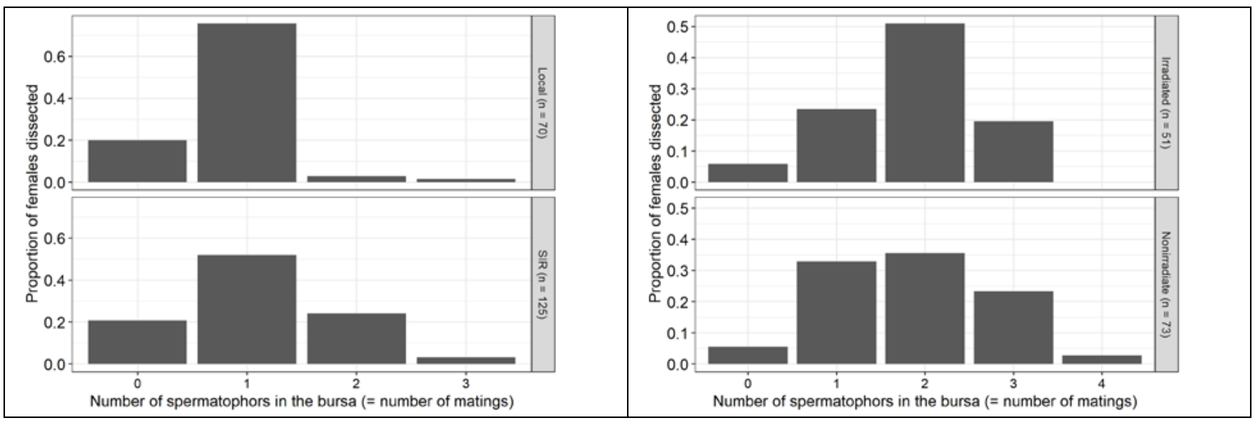
Results and Discussion

Field mark-release-recapture experiments were carried out weekly in almond and pistachio plots at the UC Kearney and Westside Research and Extension Centers between March – October 2019. Recovery of male NOW shipped from Phoenix was initially very low, even when releasing non-irradiated males (recovery <1%). Changes to the release mechanism led to significant increases in recovery of males, including irradiated males (recovery ~2-6%). Past mark-release-recapture studies with NOW (in 2006 and 2017) yielded recovery rates of 10-20% of males and 1-2% of females released. In 2019, we similarly made multiple releases of non-irradiated, locally produced NOW (including a strain isolated in winter 2019) in the Kearney and Westside experimental plots and found that, under these conditions, recovery was widely variable (1-10%). Recovery of these locally-produced moths was also highly variable in a pomegranate orchard, with up to 20% recovery during flowering but <5% afterwards. There was a significant difference in recovery between moths never shipped and moths shipped locally (i.e. shipped from ourselves in Parlier back to ourselves in Parlier), albeit under conditions of low recovery (table below).

Sex	**Not Shipped**	**Shipped**	***P***
Male	15	3	0.075
Female	14	0	0.006

Male and female moths captured following three releases (August 20, September 9, and September 17) that were marked with either green fluorescent dye (not shipped), or pink dye (shipped). Totals released were 1400 not shipped and 1400 shipped.

Laboratory studies provided corroborating data. An extensive flight mill study (n = 369 total moths flown over 24 separate nights) found no difference in flight time or distance flown between locally produced moths from a control strain of NOW collected from Mendota in 2010 and the Phoenix SIT strain when both were reared locally in Parlier. In contrast, all moths produced and shipped from the Phoenix facility flew significantly shorter times and distances than the locally produced moths, and there was no significant difference in flight performance between irradiated and non-irradiated moths produced/shipped from Phoenix to Parlier. Under these forced conditions, the NOW examined were capable of mean cumulative flight distances of around 9-10 km per night for locally produced moths, compared to around 5-6 km per night for moths produced and shipped from Phoenix.
Likewise, a wind-tunnel study found that locally-produced males from the strain of moth reared at the Phoenix mass-rearing facility (n = 208) effectively responded to a female pheromone source, albeit slightly less vigorously in some aspects than moths from the Mendota strain collected in 2010 (n = 143).
Laboratory assays of mating in close quarters found that, over the first three days of adult life, irradiated NOW females shipped from Phoenix mated more often than their counterparts from the local Mendota strain ($P < 0.001$, below left). However, non-irradiated NOW shipped from Phoenix mated more often than their irradiated counterparts ($P < 0.001$, below right). These observations suggest that greater multiple mating occurs in Phoenix strain NOW compared to other strains (or presumably wild females), and that this effect is due to the strain and not to irradiation.



In summary, field and laboratory data to date suggest that, compared to effects of moth strain or radiation dose, the shipping/handling methods have a stronger negative influence on the field performance of the sterile/irradiated NOW males compared to the effects of strain or irradiation. Additionally, the way in which moths are released under field settings appears to also have some effect on their performance. While research and pilot studies on sterile insect technique have been conducted for over a dozen Lepidopteran species, full production programs have been implemented only for three species—the codling moth (Canada), pink bollworm (USA), and the false codling moth (South Africa). The false codling moth proved more sensitive to chilling and cold storage than the other two species, and engineering adjustments based on physiological studies improved the performance of sterile moths in that species.

Conclusion

- The effect of shipping and handling effects is a more important limiting factor for male performance compared to irradiation or strain effects.
- Chill injury is likely the most important aspect of shipping and handling effects.
- Future research should compare laboratory and field assays of recovery from chill for 24 to 48 hours at temperatures between 40 and 60°F.
- Continued refinement of release mechanisms and recapture protocols is also needed.

Identification of pistachio associated volatiles for the control of navel orangeworm

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Introduction

The navel orangeworm (NOW), *Amyelois transitella*, is a major pest of pistachios and utilizes a strategy of overwintering in pistachio fruits that have remained on trees or on the orchard floor after harvest (mummies) and then re-emerging in the spring to start a new generation. Various approaches have been used to monitor NOW populations in pistachio orchards during the growing season, such as ovipositional traps with almond meal as the attractant, traps with a kairomone attractant based on almond orchard host plant, traps with NOW pheromone, and pistachio mummies (Peterson bait). However, such traps were either too labor intensive, did not work well throughout the growing season or did not work well in mating disruption conditions. Previous attempts to use pistachio plant and nut volatiles did not result in usable lures. Given the strong association of NOW with pistachio mummies, we hypothesized that mummy volatiles could serve as NOW lures. Volatiles from pistachio mummies were collected on a solid-phase microextraction (SPME) fiber, identified by gas chromatography/mass spectrometry (GC/MS), and were then tested individually or in combination for male and female NOW olfactory response using an electroantennogram (EAG) apparatus. Lures were then prepared based on mummy volatiles with high EAG response, especially for females, and tested in pistachio and almond orchards throughout the 2019 growing season.

Results and Discussion

Pistachio mummies with varying degrees of overwintering degradation were collected from 4 different commercial and experimental orchards; additional samples included Peterson baits and the rachises from pistachio mummies. Volatile emissions from these mummy tissues were identified and compared with the volatiles generated by these same mummies incubated in an atmosphere of increased humidity that increased the water activity of the tissues and allowed for the production of microbial volatiles. The volatile profiles of these samples were compared with the volatiles of developing pistachio fruits and 120 compounds could be identified as unique to the mummy tissues. Monoterpenes, primarily limonene, make up the bulk of volatiles from developing pistachio fruits. As pistachio fruits undergo environmental degradation during overwintering, the level of limonene and other monoterpenes decreases and new volatiles are produced consisting primarily of oxidized monoterpenes, microbially-transformed monoterpenes, and saturated and unsaturated aliphatic alcohols, aldehydes, and ketones from fatty acid degradation. These unique pistachio mummy volatiles were tested for EAG response, and female NOW generally had high sensitivity to the oxidized and transformed monoterpenes while male NOW had high sensitivity to the fatty acid degradation volatiles. A total of 41volatiles with high EAG response were grouped in varying combinations of 3 to 10 volatiles per lure and tested for field attractivity to NOW.

Field tests of candidate lures were carried out in San Joaquin Valley almond and pistachio orchards between April – October 2019. For each round of trials, a replicated complete block design was used with 7 replicates. Replicate were at least 16 m apart and, within replicates, treatments were spaced at least 9 m apart (see Table 1 for more details). All traps were hung on the same side of the tree at a given site, either the east or west side of the tree. Each treatment consisted of a wing-trap (Trece Pherocon 1C) with sticky liner baited with a specific lure or combination of lures. There were multiple control treatments, which consisted of (1) a trap with pheromone lure (Suterra Biolure), (2) a trap with a Peterson bait bag (Peterson Trap Co.) and (3) a trap with no lure at all. Every round of trials included 6 candidate volatile lures that were each tested alone and in combination with a pheromone lure (6 candidates with and without pheromone = 12 volatile treatments + 3 controls = 15 treatments/trial). Sticky liners were replaced weekly and candidate volatiles were evaluated for a 1-3 week period, depending on the trial. Pheromone lures and Peterson bait bags were never in the field longer than 3 weeks and were replaced with each new trial.

Table 1. Summary of Field Trials in 2019

<table>
<tr><th>County</th><th>Crop</th><th>Round</th><th>Dates</th><th>Weeks</th><th>Replicate Spacing</th><th>Treatment Spacing</th></tr>
<tr><td rowspan="8">Tulare</td><td rowspan="8">Pistachio</td><td>1</td><td>May 1 – May 22</td><td>3</td><td rowspan="6">18 m</td><td rowspan="6">10 m</td></tr>
<tr><td>2</td><td>May 22 – June 11</td><td>3</td></tr>
<tr><td>3</td><td>June 11 – July 2</td><td>3</td></tr>
<tr><td>4</td><td>July 2 – July 16</td><td>2</td></tr>
<tr><td>5</td><td>July 26 – Aug. 9</td><td>2</td></tr>
<tr><td>6</td><td>Aug. 16 – Aug. 22</td><td>1</td></tr>
<tr><td>7</td><td>Aug. 23 – Sept. 5</td><td>2</td><td rowspan="2">36 m</td><td rowspan="2">20 m</td></tr>
<tr><td>8</td><td>Sept. 13 – Sept. 27</td><td>3</td></tr>
<tr><td rowspan="2">Fresno</td><td rowspan="8">Almond</td><td>1</td><td>April 30 – May 21</td><td>3</td><td rowspan="6">16 m</td><td rowspan="6">9 m</td></tr>
<tr><td>2</td><td>May 21 – May 31</td><td>1</td></tr>
<tr><td rowspan="6">Madera</td><td>3</td><td>June 12 – July 3</td><td>3</td></tr>
<tr><td>4</td><td>July 3 – July 18</td><td>2</td></tr>
<tr><td>5</td><td>July 26 – Aug. 9</td><td>2</td></tr>
<tr><td>6</td><td>Aug. 16 – Aug. 23</td><td>1</td></tr>
<tr><td>7</td><td>Aug. 23 – Sept. 6</td><td>2</td><td rowspan="2">32 m</td><td rowspan="2">18 m</td></tr>
<tr><td>8</td><td>Sept. 13 – Sept. 27</td><td>2</td></tr>
</table>

Lure blends based on the pistachio mummy volatiles did not prove to be attractive to male or female NOW in pistachio or almond orchards throughout the growing season. However, one 3-component lure blend used in conjunction with the pheromone lure was tested multiple times throughout the growing season and did increase trap captures of male NOW by 30%. Since multi-component blends prepared from mummy volatiles were not proving effective at attractants under field conditions, more complex blends were prepared from pistachio mummies, mummy rachises, and Peterson baits. Hydrodistillates that included the entire volatile profiles of these tissues were prepared under conditions of low and high water activity. In addition, lure blends based on mummy volatiles were prepared in pistachio-based carriers, including pistachio oil extracted from kernels and solvent extracts of Peterson baits. These efforts to increase the diversity and complexity of volatiles in the mummy-based lures did not result in NOW field captures when placed alone or with pheromone lures.

Conclusions

Dozens of volatile compounds unique to pistachio mummies have high EAG response for both male and female NOW; however, these volatiles did not prove to be effective in attracting NOW to field traps in the 2019 growing season. Efforts to increase the volatile complexity of the lures did not improve attractivity. Additionally, lures consisting of the entire volatile profile of pistachio mummies, mummy rachises, or Peterson baits did not result in NOW trap captures in either pistachio or almond orchards. Although additional field testing of the pistachio mummy volatiles is warranted, one can conclude from these results that female NOW attraction to pistachio mummies and Peterson baits is not driven solely by the volatile emissions of these tissues. The mummy-based lure blend that increased the trap capture of NOW males in conjunction with a pheromone lure will continue to be tested and optimized as this lure may prove attractive in a mating disruption environment. This blend also produced the highest female EAG response out of all the individual volatile compounds and blends that were tested and therefore provides a foundation for continued work on a lure that will attract female NOW.

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

Authors: **Judith M. Stahl**, Postdoctoral Scholar, Department of Environmental Science, Policy and Management, UC Berkeley; **Davide Scaccini**, Graduate Student, Department of Agronomy Food Natural Resources Animals and Environment, University of Padova, Italy; **Kent M. Daane**, Cooperative Extension Specialist; Department of Environmental Science, Policy and Management, UC Berkeley

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 Central 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 to enclose 1-3 clusters. BMSB, and, for the damage study, a leaffooted bug (*Leptoglossus zonatus*; LFB), and the green stink bug (GSB) were placed inside cages and removed after each trial period.

Survival of BMSB was tested by placing first instar nymphs into cages to determine if this pest could survive on pistachio only. This was repeated three times synchronous with the BMSB natural phenology in California: in May, in July, and in September. In the May trial, no BMSB eggs developed to adults. In July, 4.4 ± 2.9% survived to the adult stage, and in September, most likely due to a low sample size, again no adults developed. Mortality was highest in the first (July setup, 70.6 ± 9.0%) and second (May setup, 49.2 ± 8.2%) instars. Survival on other crops, such as peach and almond, and a combination treatment with peach, almond, pistachio, and grapes, was overall higher than on pistachio, but nymphal mortality was always >80%. Higher survival under laboratory conditions indicates climatic limitations of BMSB nymphs on pistachios in the Central Valley.

BMSB, GSB, and LFB adults were placed with pistachio clusters that had been caged since April for 5-day feeding periods from end of April through July. The inoculation periods were shifted forward based on the 2018 data to better represent natural feeding after bud break and focus on early to mid-season feeding that seems to have more impact on nut damage than late-season feeding after shell hardening. Epicarp lesions as an indication of insect damage were as high as 10 ± 2 (BMSB) to 16 ± 4 (LFB and GSB) (mean ± SE) per cage at the end of April – significantly more than in the no insect control cages (Figure 1). In successive inoculation periods epicarp lesions considerably decreased (Figure 1). This corroborates the results from 2018, even though this year there were no significant differences between insect species, BMSB caused the same amount of epicarp lesions as LFB and GSB. After the first 5-day feeding period, there was considerable nut drop without indication that natural nut drop was increased by insect feeding.

With 20% of the harvested clusters processed, the percentage of clean nuts across all inoculation dates seems highest in the no insect control (53.2%), followed by BMSB (50.6%), GSB (46.0%) and LFB (44.6%). The proportion of harvested clean nuts in the controls seem stable over time, while it seems to decrease in the insect treatments after the first inoculation period, most likely due to the compensation nut drop after the first inoculation. Like in 2018, most cluster damage resulted from aborted nuts (22.4%) rather than kernel necrosis (11.9%).

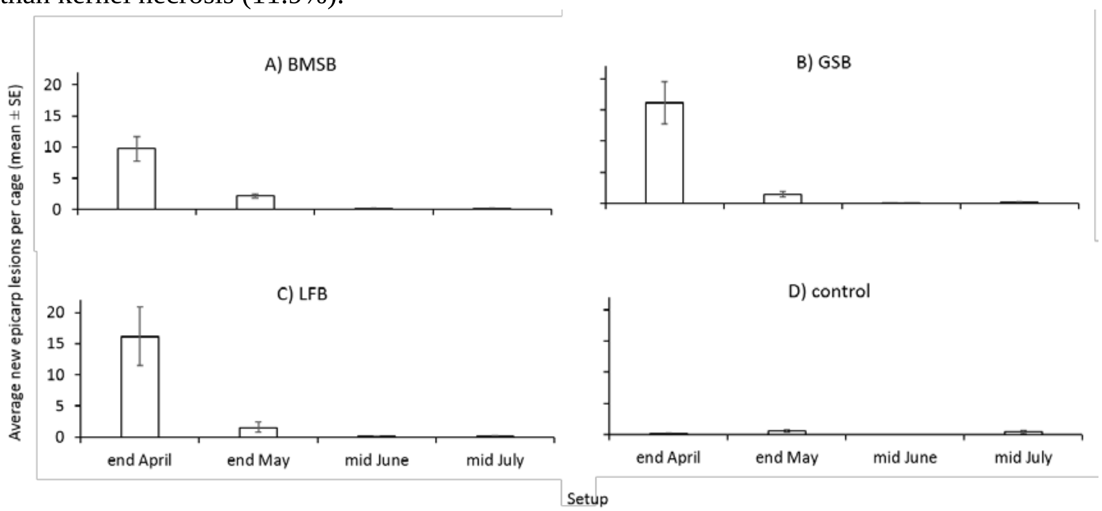

Figure 1 Insects were caged for 5-day feeding periods, from April to July and the amount of epicarp lesions were used as an indication of damage from (A) brown marmorated stink bug (BMSB) adults, (B) green stink bug (GSB) adults, (C) leaffooted bug (LFB) adults, (D) no insect control.

The results are comparable to earlier studies that showed that next to insect size, the key to determine hemipteran damage levels is at what time of the season the insect feeds. 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. This seems to be the case in our first inoculation period at the end of April. 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.

Conclusion

BMSB adults caused the same amount of feeding damage immediately after a 5-day feeding period, as measured by epicarp lesions, as GSB and LFB. Preliminary data from harvested nuts suggests kernel necrosis due to insect feeding is similarly caused by the different pest species in comparable levels. This work suggests that, overall, BMSB adult damage appears to be similar to LFB and GSB adult damage, if, taking last year’s data into account, not higher. However, BMSB nymphs were unlikely to survive to the adult stage on pistachio in California’s San Joaquin Valley conditions. This suggest that pistachios are an unlikely season-long host and that 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. There are further indications that the Central Valley climate is not ideal to support BMSB development, which might contribute to keeping BMSB pistachio damage at lower levels than crop damage in the mid-Atlantic states.

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

__**Authors**__**:** **Judith M. Stahl**, Postdoctoral Scholar, Department of Environmental Science, Policy and Management, UC Berkeley; **Houston Wilson**, Assistant Cooperative Extension Specialist, **Robert Straser**, Graduate Student, **Jessica Maccaro**, Staff Research Associate, Department of Entomology, UC Riverside; **Kent M. Daane**, Cooperative Extension Specialist; Department of Environmental Science, Policy and Management, UC Berkeley

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 continued a study of novel, irrigated strips of annual summer 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). Our 2019 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 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 May, the center 7 row-middles of the trap crop plots were seeded to a mix of alfalfa (*Medicago sativa*) and radish (*Raphanus sativus*). 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. Subplots were commercially harvested, and crop damage graded according to industry standards.

Trap crop   Control

Results and Discussion

1. Seasonal populations of small bugs, large bugs, and predators. Populations of small and large bugs on the trap crop were greater than on the resident weedy vegetation in control plots, even though this trend was not significant for the latter - at least for the sample date of August 23 (Figure 1). In both cases this did not necessarily lead to lower densities in the tree canopy of plots with the trap crop (Figure 1). Predators were in greater abundance in the trap crop plots. They were more abundant on the trap crop itself than on weedy vegetation (Figure 1). A pilot study of seven potential trap crop species sowed individually in a strip next to the pistachio orchard showed differences in small and large bug as well as predator populations but more in depth experiments are required.

2. Large bug oviposition and parasitization. While oviposition of the three most abundant large bugs *Chlorochroa uhleri*, *Chinavia hilare* and *Thyanta* sp. occurred in similar numbers over the season on pistachio trees in both trap crop and control plots, peak oviposition in July/August was less pronounced in the control plots (Figure 2). At the same time, parasitism of stink bug egg masses was higher on trees in plots with the trap crop (Figure 2). Despite the high number of parasitized natural egg masses, none of the 244 exposed *L. zonatus* and *C. hilare* egg masses from the laboratory yielded parasitoids, indicating that the exposure methods need to be improved if this trial is to be carried forward.

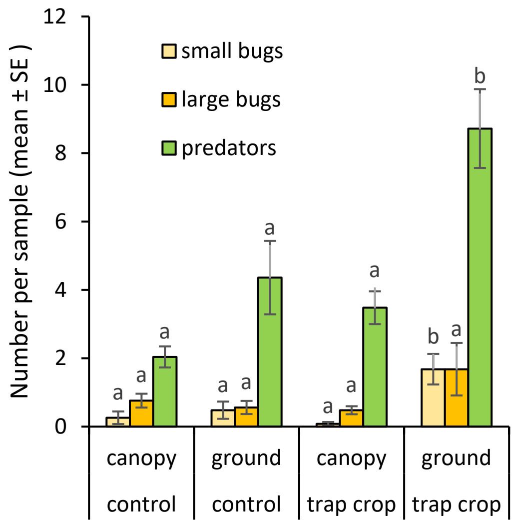

Figure 1 Abundance of small and large bug nymphs and adults as well as predators in canopy beat samples and ground sweeps August 23, 2019.

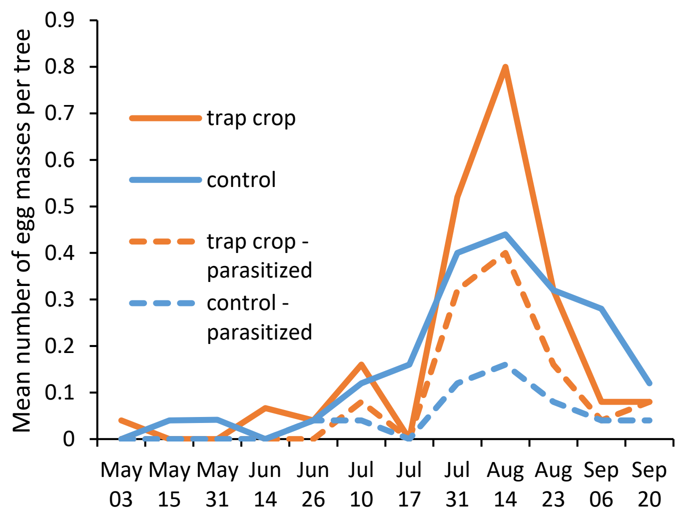

Figure 2 Freshly laid unparasitized (solid line) and parasitized (dashed line) egg masses of the three most abundant large bug species in the canopy in trap crop (red) and control (blue) plots visually assessed by searching five trees per plot for five minutes each.

3. Crop damage. There was less plant bug induced crop damage in nuts collected from the plots with the seeded trap crops (3.38%) than in control plots (6.05%).

<u>Conclusions and Practical Implications</u>

The development of a novel, center-line drip irrigation strategy has presented a unique opportunity to evaluate the use of trap crops to manipulate plant bug populations in pistachio orchards**.** We confirmed that trap crops can influence small and large plant bug densities as well as beneficial insect populations and despite the trap crop ground cover attracting large bugs, nut damage in the trap crop plots was lower than in control plots this year. Based on these results we will continue to refine the use of the current summer trap crops as well as begin to screen additional candidate trap crops for large bug attraction and suitability in the laboratory. This will ideally lead to the development of an improved IPM strategy that will decrease plant bug populations in the canopy when the pistachios are the most vulnerable, thereby reducing pesticide applications and crop damage.

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

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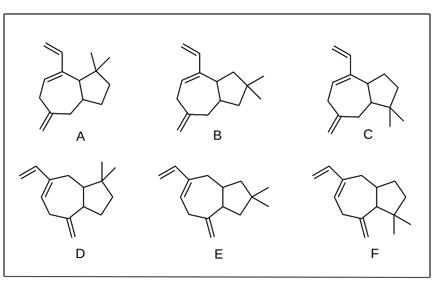
Introduction

Leaffooted bugs (LFB, primarily *Leptoglossus zonatus* LZ and *L. clypealis* LC) can cause major damage to 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 4th generation is possible in warmer years. Damage is unpredictable because bug populations immigrate rapidly and unpredictably into nut crops from surrounding crops or native vegetation. Because of these rapid buildups, and because bug damage may only become apparent after the bugs have moved on, continuous monitoring is a key factor in management. Current monitoring methods rely primarily on beat tray or sweep net sampling. Effective trapping systems would be immediately useful for monitoring. Thus, **our first goal is to carefully examine the pheromone-mediated behavior of leaffooted bugs, with the aim of identifying any insect-produced compounds that could be exploited in IPM programs**. In parallel, **our second goal is to develop effective methods of trapping LFB, with or without attractants**.

LFB could use at least four different types of pheromones. First, both sexes produce alarm pheromones and defensive secretions which are unlikely to be involved in sexual interactions. Second, male LFB produce compounds such as benzyl alcohol which are not attractants per se (Wang and Millar 2000) but seem to function as aphrodisiacs, rendering unwilling females receptive to mating. Third, we have shown that sexually mature male, summer-form LZ and LC produce a group of compounds that are likely to be aggregation pheromones that attract both sexes. 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. Our current work is focused on identifying, synthesizing, and field testing the latter two types of pheromones.

Results and Discussion

1. Identification of potential attractant pheromones from male *L. zonatus*. We have shown that sexually mature summerform male LZ consistently produce a group of nine compounds, eight of which we have identified. In June 2018, we isolated a sample of the 9th compound, which elicits the strongest responses from antennae of female bugs, but the amount was not sufficient to fully identify the compound. We were able to narrow the most likely structures down to six, shown below. We synthesized what we considered the most likely candidate, but it was not correct. Thus, since last summer, we have collected ~100 extracts from males, and repurified a larger amount of the compound, which will be reanalyzed by microbore NMR the first week of December. From the resulting spectra, we hope to conclusively identify the single structure which constitutes the unknown. It will then be synthesized for field testing. In addition, we have developed a new, more efficient synthesis of *cis*-□-bergamotene, one of the other major components in the blend produced by LZ males. That synthesis is currently being scaled up to produce several grams of the compound for field trials. To get at possible pheromone-mediated differences in the behaviors of summerform and winterform bugs, we also conducted a series of bioassays testing the mating responses of all four possible combinations of pairs of summerform and winterform males and females. However, responses of all the bugs, including the summerform



male/summerform female positive controls, were very low, likely because the glass dishes used for filming the bioassays were too small or otherwise unsuitable. These bioassays will be repeated with larger cages, to determine whether winterform females are essentially invisible to males of both types due to changes in the female's cuticular lipids, or whether winterform males are not responsive to sexually mature females, or both.

2. Optimizing trap characteristics, including color, with and without attractant lures.
Work in 2017 determined that an unbaited black hanging-panel trap was attractive to LFB and thus could be a useful tool to evaluate candidate pheromone lures as those were developed. Additional efforts in 2018 and 2019 attempted to see just how useful an unbaited trap could be by (1) comparing LFB capture across an array of different trap colors and (2) comparing LFB capture in traps at the orchard edge and interior with populations in the tree canopy. The idea was to see (1) if trap color increases attractiveness and (2) can unbaited traps at the orchard edge provide any information about the timing of orchard colonization and/or LFB populations in the crop canopy.

In 2018, LFB capture in unbaited hanging black panel traps positioned at the edge of multiple almond, pistachio, and pomegranate orchards did not reflect populations in the crop canopy. In 2019, pairs of unbaited hanging black panel traps were set up at the edge and 50 yards away from five almond orchards with persistent spring LFB populations. The idea here was to see if a trap 50 yards out from the edge (in an open field) could detect LFB as they moved towards the orchard edge. Trap capture was overall very low, even though there were notable LFB populations in the crop canopy at all sites. Thus, unbaited traps did not reflect the timing of LFB colonization of almond trees.

Trap color preference was first evaluated in a pomegranate orchard in fall 2018 (Oct. – Dec.). Here, LFB adults exhibited a strong preference for yellow traps, followed by blue and green traps. In 2019 the colored traps were set out sequentially in almond (Mar. – May), pistachio (Jun. – Sept.) and pomegranate (Sept. – Dec.) blocks. LFB capture was very low in almonds and so no preferences could be determined. Many more LFB adults were subsequently recovered in pistachio and pomegranate. Surprisingly, there was no clear color preference observed in the pistachio orchard. In the pomegranate block, LFB catch was numerically higher in yellow and blue traps, but there was not a statistically significant difference amongst the different trap colors.

3. Test attraction of LZ to infrared radiation
A previous study documented that *Leptoglossus occidentalis* responded to infrared (IR) cues and appeared to utilize them to locate feeding sites. As such, a preliminary trial was run to evaluate LZ response to IR cues. A modified two-choice chamber was constructed based on the experimental design used in the other study, and LZ adults were given the opportunity to choose between a low and high IR source. Response to IR was unclear, but this may be related to the design of the assay and what levels of IR are most biologically relevant to LZ. Additional work is now underway to better understand these factors, and follow-up experiments are planned for 2020.

<u>Conclusions and Practical Applications</u>

1. The complete blend of compounds produced by summerform male LZ should be available for testing this coming field season.
2. Testing of the effects of cuticular hydrocarbons from summer and winterforms will be repeated with better bioassays.
3. Unbaited hanging panel traps do not effectively provide information on LFB populations in orchards.
4. Yellow traps, and possibly blue or green traps, may be more effective than black traps.
5. While an unbaited hanging panel trap can attract LFB adults, practical applications appear limited without an effective lure or bait.
6. LFB attraction to IR cues remains unclear.

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

Authors: Christian Nansen, Associate Professor, Department of Entomology and Nematology, University of California - Davis; **Miles Dakin**, Doctoral Candidate, Department of Entomology and Nematology, University of California - Davis

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

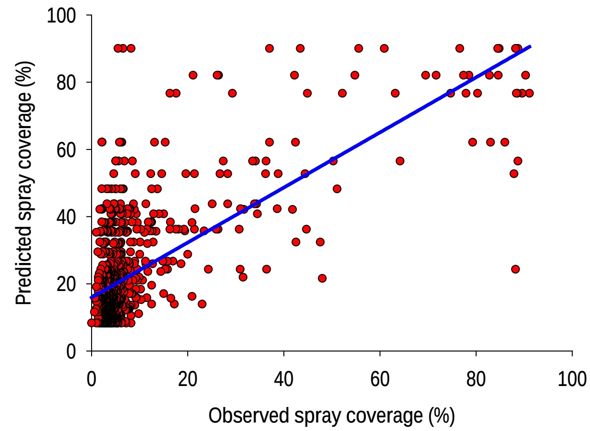
This project was initiated in 2018, and the project is composed of the following components: 1) Develop a damage model component describing the seasonal phenology of NOW population dynamics and of pistachio flower and fruit development. 2) Develop an insecticide spray application model component describing 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.

1. Develop a damage model component describing the seasonal phenology of NOW population dynamics and of pistachio flower and fruit development. We have established collaboration with Profs Ferguson, Zhang, and Laca, who have developed a comprehensive pistachio nut model (pistachio.plantsciences.ucdavis.edu). The model complements existing population models of NOW (Sanderson et al., 1989; Soderstrom et al., 1986). We can link this comprehensive temperature-driven model to temperature data we are currently collecting from vertical positions in pistachio canopies. Thus, we can, in detail, quantify possible temporal differences along vertical gradients within pistachio canopies. If temperature differences within pistachio canopies appear to cause vertical trends in pistachio nut development, especially if driving differences in timing of “nut slip”, then portions of the pistachio canopy may become at risk to navel NOW infestations at slightly different time points. We are therefore truly grateful to Profs Ferguson, Zhang, and Laca for their willingness to collaborate and make their modeling framework readily available to this project.

2) Develop an insecticide spray application model component describing the influence of operations spray settings and weather conditions on insecticide spray applications. Based on field trials conducted in both 2018 and 2019 growing seasons, a total of 1056 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. Spray coverage was quantified based on the water sensitive spray cards, and it was modeled (multi-regression analysis) as a function of:

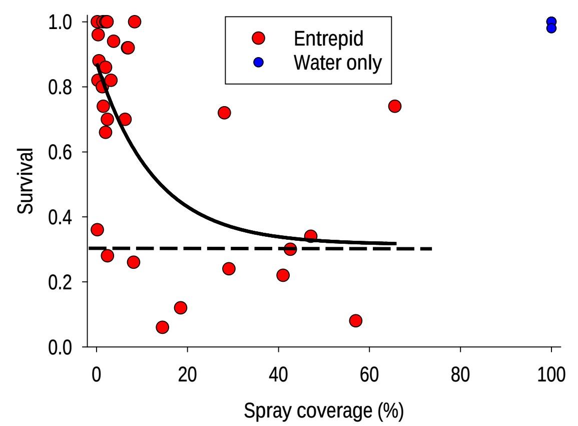
temperature (F), relative humidity (%), tractor speed (mph), tank pressure (PSI), and vertical position (bottom = 1, middle = 2, and top = 3) in pistachio canopies. Fig. 1 shows the highly significant correlation between observed and predicted spray coverages. Variance associated with the regression fit is likely linked to effects of cardinal directions (as pistachio trees were only sprayed on one side), differences in performance of among air blast sprayers, and heterogeneity in canopy structure and density (seasonal and varietal dynamics). Due to these important factors that were not accounted for, it is not surprising that the regression fit was associated with considerable variance. However, it is VERY encouraging that almost 50% of the variance could be explained in a data set comprising over 1000 data points. With this model, we can proceed towards development of a user-friendly spray optimization tool similar to "Smart Spray" (http://chrnansen.wixsite.com/nansen2/smartspray). Smart Spray was developed by Nansen as a freely available smartphone app to both optimize and to perform quality control of pesticide spray applications in strawberry fields.

Fig. 1. Prediction



We have initiated experimental bioassays to quantify the correlation between spray coverage and NOW survival (Fig. 2). Survival is determined 18 days after spray applications. So far, we have acquired preliminary data on entrepid (IGR, group 18) and identified an exponential decay response, in which an asymptote is reached at around 40% spray coverage. Although preliminary at this stage, this type of research is very important, because it implies that there would be limited benefits from attempting to achieve higher spray coverages than 40% when applying entrepid.

Fig. 2. Surviva



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Improved control of navel orangeworm: focus on increasing insecticide efficacy using adjuvants and improved timing (Part 1)

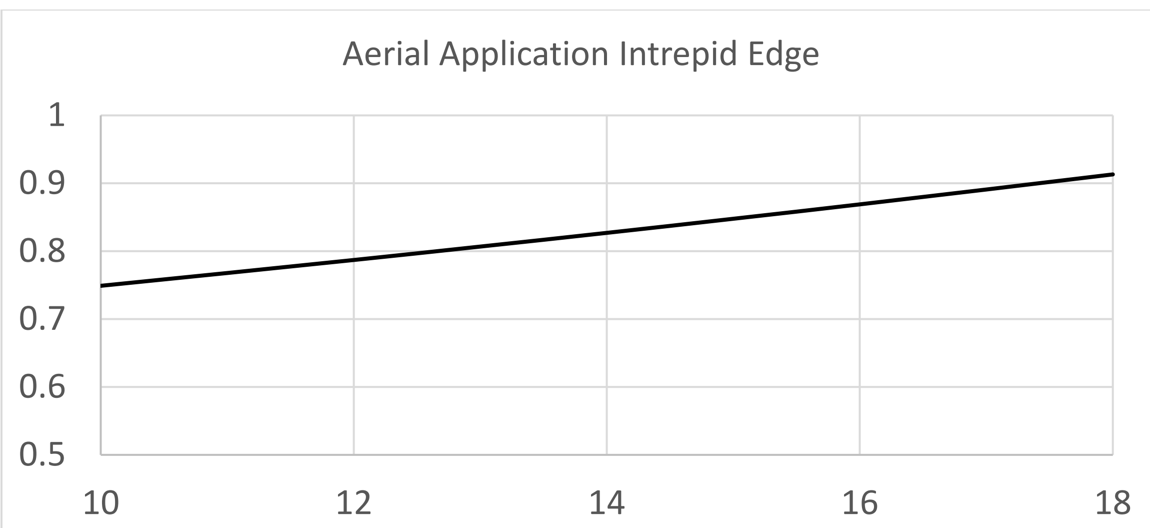
Authors: **Joel P. Siegel**, Research Entomologist, USDA-ARS, Parlier; **Carla Baker**, PCA, Weinberger, Fukoda and Associates, Madera; **Ryan Wylie**, Farm Manager, Agri-World Coop, Madera; **Devin Aviles**, Business Manager, Agri-World Coop, Madera

Introduction

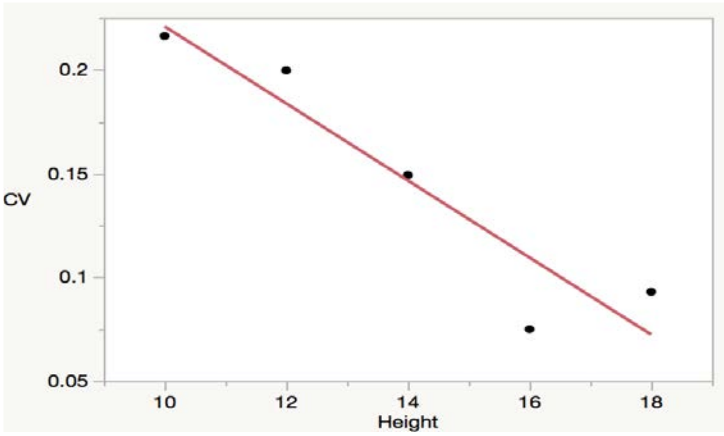
The purpose of this research is to improve control of navel orangeworm (NOW) by a combination of increased application efficacy and timing. The coverage provided by air application is being quantified because aerial application can cover an entire orchard overnight and air application is not affected by ground conditions. Any product named is for specific information purposes and does not constitute an endorsement by the USDA. The experiments reported are a follow-up to the studies conducted the previous two years (Summers 2017 and 2018) evaluating the ability of two organosilicone adjuvants (Hi-Wett and Kinetic) to reduce the amount of water needed for insecticide application and improve coverage in the upper canopy. In addition, the research conducted in 2019 continued our study of coverage following aerial application. Coverage was evaluated by contact toxicity bioassay. Filter papers were placed in the canopy using pvc poles, with the papers spaced at two foot intervals from 10-20 feet, or on hooks hung approximately 14 feet in the canopy. The filter papers were collected at selected intervals after commercial application, placed in petri dishes containing NOW wheat bran diet, and then challenged by placing egg masses in the center of the paper. Newly hatched larvae crawled over the filter paper to reach the diet and mortality was scored 18 days later. Control plates using unexposed filter paper were also used, and we also keep track of colony survival for every generation (records go back 5 years). The following graph reports the mortality from the filter paper placed at two foot intervals on pvc pipe. Intrepid Edge® was applied at 19 oz/ac by air (15 gal/ac) using Kinetic® as adjuvant at 2.4 oz/100 gal.

Results and Discussion

Maximum mortality was 92% at 18 feet and decreased to 75% at 10 feet; we regard this as successful.



A different way to evaluate the coverage is to focus on the variability in mortality. Where there is better coverage mortality is more uniform. As coverage breaks down the mortality is more hit or miss. The appropriate measure for variability is the statistic, Coefficient of Variation (CV), calculated as (Standard Deviation/Mean)*100. The following is a plot of the CV vs height



This graph confirms that the least variability occurs in the upper canopy and the variability doubles by the time we descend to 14 feet. Results were the most variable at 10 feet as expected.

The next portion of the analysis focuses on Duration of Control, determined by sampling the filter paper hung in hooks at 12-14 feet. In this analysis I am also contrasting the mortality in the upper hook vs. bottom hook, a distance of 9 inches on days 7 and 9 after application.

Day 7 after application Mortality, early September application
Upper Hook: 84.0% (630/750 eggs)
Lower Hook: 83.1% (623/750 eggs)

There is no difference in mortality between the filter paper from top and bottom of the hook. Combined there is 1253/1,500 eggs killed for 83.53% mortality.

Day 9 after application Mortality
Upper Hook: 68.8% (1,376/2,000 eggs)
Lower Hook: 62.6% (1,284/2,050 eggs)

There is a 9.8% difference in mortality between the filter paper from top and bottom of the hook and this difference is significant (P<0.001). This represents an 17.6% decrease at the top of the hook and a 25.1% decrease at the bottom of the hook. This pattern of differences between the top hook and bottom hook emerging after day 7 was observed in other aerial trials in pistachio this year. A difference was noted in other aerial applications as well.

Day 9 after application Mortality, late September application
Upper Hook: 85.3% (1,279/1,500 eggs)
Lower Hook: 80.0% (1,160/1,450 eggs)

There is a 6.61% difference in mortality between the filter paper from top and bottom of the hook and this difference is significant (P<0.001). This pattern of differences between the top hook and bottom hook was also observed in figs.

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 provided excellent coverage and mortality at 14 feet was consistent, approximately 83%. However, the insecticide began to break down after one week in the first application, and in both applications after one week a difference in canopy height as small as 9 inches was significantly different. This will be pursued in future studies.

Improved control of navel orangeworm: focus on increasing insecticide efficacy using adjuvants and improved timing (Part 2)

Authors: **Joel P. Siegel**, Research Entomologist, USDA-ARS, Parlier; **Carla Baker**, PCA, Weinberger, Fukoda and Associates, Madera; **Ryan Wylie**, Farm Manager, Agri-World Coop, Madera; **Devin Aviles**, Business Manager, Agri-World Coop, Madera

Introduction

The purpose of this research is to improve control of navel orangeworm (NOW) by a combination of increased application efficacy and timing. As part of this research different classes of adjuvants and insecticide are evaluated. Any product named is for specific information purposes only and does not constitute an endorsement by the USDA. The experiment reported below examines the effect of three classes of adjuvant, citrus oil alcohol ethoxylate (Vintre® 50 and 100 gal/ac; 16 oz/100 gal), organosilicone (Kinetic® 50 and 100 gal/ac; 4 oz/100 gal), and methylated seed oil + organosilicone (DyneAmic® 100 gal/ac; 6¨4 oz/100 gal) when the application volume is varied. The insecticide evaluated was the pyrethroid Mustang® (zeta cypermethrin) applied at 4.3 oz/ac. Filter paper was placed at 5 feet on a lower limb and on hooks hung at 14 feet above ground. The filter paper placed at 5 feet was collected on days 1 and 14 after application and the filter paper on hooks collected on days 1, 7, and 14 after application. Control filter papers were hung in an untreated portion of the orchard and collected at the same intervals.

Percent Mortality

Treatment	**Control**	**Vintre (0.125%) 50 gpa**	**Kinetic (0.031%) 50 gpa**	**Vintre at (0.125%) 100 gpa**	**Kinetic at (0.031%) 100 gpa**	**DyneAmic (0.05%) 100 gpa**
5 ft Day 1						
	224/550 (**40.73%**)	161/300 (**53.67%**)	173/300 (**57.67%**)	194/300 (**64.67%**)	184/300 (**61.33%**)	384/600 (**64.00%**)
5 ft Day 14						
	284/600 **(47.33%)**	178/300 **(59.33%)**	164/300 (**54.67%)**	209/300 **(69.67%)**	133/300 **(44.33%)**	272/600 **(44.50%)**

Note that mortality is not impressive for any treatment because the ground spray rig (AiroFan PTO GB36) is calibrated to maximize coverage in the upper canopy. However, by day 14 both the Kinetic at 100 gpa and DymeAmic at 100 gpa treatments had lost all contact toxicity. In contrast, toxicity with Vintre at 100 gpa and Kinetic at 50 gpa was unchanged. The differences noted for Kinetic and DyneAmic at 100 gpa were significant at $P < 0.001$.

Percent Mortality

Hook 14 feet Day 1	**Control**	**Vintre (0.125%) 50 gpa**	**Kinetic (0.031%) 50 gpa**	**Vintre at (0.125%) 100 gpa**	**Kinetic at (0.031%) 100 gpa**	**DyneAmic (0.05%) 100 gpa**
	224/550 (**40.73**%)	881/1,200 (**73.42**%)	993/1200 (**82.75**%)	955/1,200 (**79.58**%)	749/1,200 (**62.42**%)	1680/2,400 (**70.00%**)
Hook 14 feet Day 7						
	249/500 **49.80%**	780/1,200 (**65.00%**)	618/1,200 **(51.50%)**	775/1,200 (**64.58%**)	602/1,200 **(50.17%)**	1,348/2,350 (**57.36%**)
Hook 14 feet Day 14						
	284/600 (**47.33%**)	608/1,200 **(50.67%)**	590/1,200 **(49.17%)**	725/1,200 (**60.42%**)	533/1,200 **(44.42%)**	1,248/2,400 **(52.00%)**

On day 1 after application, mortality was highest for Kinetic (50 gpa) and Vintre (100 gpa) and lowest for Kinetic (100 gpa). Mortality dropped significantly for all adjuvants by day 7 ($P < 0.001$) and the treatments with Kinetic as adjuvant failed, with mortality similar to the controls. There was no difference in the two rates of Vintre, and DyneAmic also retained contact toxicity on day 7. On day 14 however, 4/5 of the treatments had failed and only Vintre at 100 gpa retained activity. However, the drop in contact toxicity between Vintre on days 1 and 14 was 31.7%, and this difference was significant at P < 0.001..

<u>Conclusion</u>

This experiment demonstrated that both application volume affected the duration of control of the pyrethroid insecticide Mustang. In this study Mustang broke down 7 days after application when the organosilicone adjuvant was used and retained activity when the citrus oil alcohol ethoxylate was used at 100 gpa. This result suggests that Vintre works better at 100 gpa, and the data for 5 feet are in agreement, because the mortality for Vintre at 100 gpa was 19.2% greater than for Vintre at 50 gpa on day 14, $P < 0.001$. This experiment needs to be repeated both for different families of insecticide as well as other adjuvants.

Chemical and physical characteristics of pistachio hull split associated with infestation by navel orangeworm (*Amyelois transitella*)

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Introduction

The California Pistachio Research Board (CPRB) has stated an interest this year in research aimed at insect pest control. Our research group has long focused its efforts on the study of navel orangeworm (NOW) ecology and resistance to chemical pesticides. Previously, we demonstrated that NOW has developed resistance to pyrethroids in almond populations in Kern County. NOW is the major insect pest of pistachios in California and infests pistachios at hull split or later. The primary means of NOW management is to spray pyrethroids multiple times throughout the growing season. In light of the challenges pyrethroid resistance may present to NOW control in pistachios in the future, this year we proposed to evaluate the chemical and physical profile of the pistachio at hull split and to explore an alternative method of control that may circumvent resistance problems.

Our work has revealed that pyrethroid resistance in NOW is associated with differences in the quantity and/or composition of cuticular hydrocarbons, which may reduce insecticide penetration of the cuticle. One method of insect control that targets the cuticle is topical application of kaolin, an inert, naturally occurring clay currently registered for use in a number of crops as Surround ®. In preliminary work, we exposed NOW larvae to kaolin, the diamide insecticide chlorantraniliprole, or both in order to determine whether kaolin acts as an insecticide itself or as a synergist of a chemical insecticide. Subsequently, we completed laboratory assays with chlorantraniliprole in combination with two concentrations of kaolin (using almonds for our first trials); we have also performed field contact toxicity experiments in an experimental pistachio field with a spray containing Surround and Altacor and compared results with the efficacy of commonly used adjuvants. In addition, we have begun to assess volatile profiles of pistachios before and during hull split, both with and without kaolin sprays.

Another possible use for kaolin is to suppress the release of volatiles during hull split used by NOW to locate oviposition sites. To identify chemical profiles attractive to NOW and to test a method for disrupting their detection by NOW, we proposed to collect volatiles from orchards in Kern County and evaluate them in our UIUC laboratory. In addition, we proposed to assess the effects of kaolin sprays on detection of pistachio volatiles.

Results and Discussion

In the kaolin and chlorantraniliprole laboratory trials, we exposed susceptible and resistant female moths to nuts treated with kaolin levels based on both ~40 lb/100 g and ~15 lb/100 g (at the high and low ends of likely field application rates) for one week. At 40 lb, we found that kaolin application reduced infestation rates of almonds by 15%-20% relative to the control treatment. In addition, sprays containing both kaolin and chlorantraniliprole resulted in lower infestation than either treatment alone, suggesting a synergistic effect. However, although both strains were adversely affected by kaolin sprays, the pyrethroid-resistant strain of NOW was less affected by all treatments than was the susceptible strain (in particular, displaying a 23% higher infestation rate than the susceptible strain after kaolin sprays), which we did not expect. This finding suggests that a difference in cuticular hydrocarbon concentrations or structure may indeed confer cross-resistance to other classes of chemical insecticides. We obtained similar results for the 15 lb kaolin treatment, although the differences between pyrethroid-resistant and –

susceptible strains were less pronounced. Kaolin still reduced infestation rates and appeared to synergize chlorantraniliprole.

In our first field trials with pistachios, we used ~15 lb/acre kaolin sprays to avoid clogging or other sprayer problems. In this experiment, there were four treatments: Surround with Altacor and the adjuvant Kinetic, Altacor alone, Altacor with Kinetic alone, and Altacor with the adjuvant Vintre alone. The addition of Surround not only increased larval mortality over Altacor alone (pooled across days 1, 7, and 12 after application), but Altacor + Kinetic + Surround also significantly outperformed Altacor + Vintre (resulting in a 6.7% decrease in mortality).

Work on volatile collection from the field has not yet begun. However, we have identified a method for using sorbent cartridges to capture and transport these volatiles to Illinois. We have also developed a method for testing volatiles produced by pistachios sprayed with water or various concentrations of kaolin. This method has been used successfully in almonds, where there is a decrease in detection of chemical signals by moths with increasing kaolin concentrations. Our effort to replicate this method with pistachios is underway. Adjustments to the experimental protocol are needed because the water-based kaolin spray does not appear to cling to the surface of the pistachio as it does to the surface of almonds; preliminary results showed that kaolin did not suppress the emission of pistachio volatile, which may have resulted from inadequate adherence to nut surfaces. We will repeat the experiment, replacing the water with another solvent (such as acetone).

Conclusion

Both our laboratory assays and our field contact toxicity trials provide evidence that kaolin is effective in reducing NOW infestations. Kaolin may act as a viable insecticide on its own in almonds, as it inhibited infestation by NOW larvae in our laboratory assays. As well, it synergized the chemical insecticides we used in both experiments. Both results are encouraging from the perspective of broader NOW control strategies. The efficacy of kaolin sprays may provide an alternative to chemical insecticides, a counter to the problem of resistance in Central Valley populations, and relief from the intense selection pressure being applied to NOW from heavy pyrethroid usage. Further studies may confirm that Surround could be a useful addition to tank mixes for growers to improve the efficacy of insecticide sprays in a cost-effective manner.

We did also uncover evidence of cross-resistance in a pyrethroid-resistant NOW population. Although we had hypothesized a role for cuticular hydrocarbons in resistance to pyrethroids and possibly kaolin, we did not foresee increased resistance to chlorantraniliprole, because is structurally and mechanistically unrelated to pyrethroids. The CHC profile in the resistant strain may reduce penetration of chemical insecticides in general and may even confer resistance to the disruption of the cuticle by kaolin. The potential spread of insecticide resistance in the Central Valley presents a major challenge for NOW control; although our results with kaolin are generally encouraging, Surround may be less effective against such populations. We are in the process of checking populations from counties north of the Central Valley to determine if pyrethroid resistance has evolved elsewhere.

Although we do not have full results for the pistachio volatile assays, a similar study on almonds has demonstrated the feasibility of our approach. Thus, there may be multiple dimensions of utility for Surround sprays: as a direct insecticide, as a synergist of chemical pesticides, and as a disruptor of chemical signals used for orientation by the navel orangeworm.

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

Authors: **Ramon Jaime, Victor Gabri, Ryan Puckett, Dan Felts, Lorene Doster, Alexander Tako, and Themis J. Michailides,** University of California-Davis, Kearney Agricultural Research and Extension Center.

Introduction

Mycotoxins are toxic metabolites produced by certain fungi, which occasionally contaminate Pistachio. The most toxic of the mycotoxins, and highly regulated worldwide, are the aflatoxins produced by fungi in the *Aspergillus* section *Flavi*, which include *A. flavus* and *A. parasiticus*, which are the common aflatoxin producing species found in pistachios in California. Even though aflatoxin contamination in pistachio nuts is rare, the risk of product being rejected by the market, due to strict regulations of aflatoxin contamination worldwide, constitutes a high economical treat to pistachio industry and makes aflatoxin contamination an issue that need to be addressed. Pistachio is a high value crop and any load that is not accepted in the market means a considerable loss by re-sorting costs, additional lab analyses costs, and/or in extreme case product destruction.

Aflatoxin contamination management based on the use of atoxigenic strains of *A. flavus* technology is the only proven method to reduce aflatoxin contamination in various crops at commercial settings. Atoxigenic strains of *A. flavus* have been successfully used to reshape the *A. flavus* communities associated with aflatoxin-susceptible crops, thus reducing incidence of aflatoxin producers and consequently the quantity of aflatoxins in several crops. Treating crops with the atoxigenic *A. flavus* AF36, reduces the aflatoxin-producing potential of the *Aspergillus* fungi. Currently, the only registered atoxigenic biocontrol for use in tree nuts, including pistachio and almond, in California is *A. flavus* AF36 Prevail®. *A. flavus* AF36 naturally occurs in California and it is widespread in all the major pistachio-growing areas in the Central Valley.

The overall goal of aflatoxin management with atoxigenic strain technology is to change the population structure of the aflatoxin producing fungi *Aspergillus* Section *Flavi* in the soil of all crops susceptible to aflatoxin contamination (including pistachio and almond). This change from a population dominated by toxin producers to a population dominated by atoxigenic fungi reduces the overall aflatoxin production potential of the population, and consequently, results in lower levels of aflatoxin that will not exceed the permissible levels in the market. To better achieve this goal the implementation of an area-wide, long-term program is the best option. The population structure of *Aspergillus* in applied soils will tend to reach an equilibrium, then if applications are discontinued the atoxigenic strains will decline reverting to its original levels while the toxigenic strains will increase.

Results and Discussion

The use of atoxigenic strains of *A. flavus* is a proven and registered method for aflatoxin control, but its efficacy depends on its timely sporulation and dispersal for toxigenic strains displacement. *A. flavus* AF36 Prevail® sporulates abundantly in Californian orchards when night temperatures are around 25°C (77 F), which occur during the months of July and August. Earlier applications could be more effective, but it is unknown if the product will sporulate sufficiently. The effect of soil moisture and low temperatures on sporulation of *A. flavus* AF36 Prevail® and Afla-Guard® were evaluated in pots with soil in a growth chamber. Soil moistures were based on field capacity (FC) and temperatures were set according to maximum and minimum temperatures from May to July in the Central Valley, CA. Sporulation was determined by the percent of sporulating grains and the amount of spores produce per grain by a sporulation index (0-4), where 0 is no sporulation and 4 product grains with over 75% covered with spores. Results indicate that sporulation of AF36 Prevail® is delayed under low soil moisture and low temperatures, while Afla-Guard® has good sporulation even at minimum temperatures of 15℃ (59 F). AF36 Prevail has a percent of grains sporulating over 80% with an index of sporulation >1.5 at 16% to FC (21%) soil moistures and minimum temperature ≥25℃ (77 F), while Afla-Guard® reached these levels of sporulation at the lowest temperatures. Good sporulation at low temperatures in earlier applications could be more effective on aflatoxin reduction.

A successful control of aflatoxins will require that the majority of the propagules reaching the almond fruit belong to the applied atoxigenic strain. An experiment to deliver the biocontrol directly to the canopy of the orchard was initiated and is underway. The first observations show that the product can reach the canopy where the fruit is located and is able to infect the susceptible nuts. Also, the quantity of the biocontrol on the debris from the applied canopy was evaluated as a possible source of inoculum for the next season. Results indicate that the debris on the surface under the trees treated with the atoxigenic strain amended with kaolin clay and a polymer had 20 times higher incidence of the fungus compared to the debris from the not treated control and 4 times higher than the debris from the trees treated on the ground.

The evaluation of the effects of commercial applications of the atoxigenic biocontrol *A. flavus* AF36 in pistachio has been an integral part of the aflatoxin project. Both soil samples to determine the percentage of displacement of toxigenic isolates and library samples for aflatoxins analysis from treated and not treated orchards are being analyzed every year. 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 on the incidence of samples contaminated with aflatoxin on the treated fields (5.3%) compared to the untreated control (11.6%) and an increase on the percentage of the AF36 in the treated fields (71%) compared to the untreated controls (40%). Increase in the percentage of AF36 in the controls every year and the treatments not reaching their full potential indicate cross effects occurring between the treated orchards and the not treated neighbor orchards, with the non-treated orchards benefiting from the treatments and the treated orchards being affected by toxigenic isolates moving from the not treated orchards. Therefore, implementing an area-wide management using atoxigenic strains to reduce aflatoxin contamination in crop nuts might be beneficial in the long term. The influences of area-wide treatments in tree nuts in California are being evaluated in an area where both pistachio and almond orchards are grown together. Part of this area both pistachio and almond were treated, while in the other part only pistachios were treated. Currently we are in the process of evaluating the effects of the treatments in both the treated and non-treated almond orchards.

Conclusion

Earlier applications could be more effective. However, it is necessary that the applied biocontrol sporulates sufficiently in a timely manner to be effective. For a good sporulation in the early stages of the crop when the temperatures are not sufficiently warm for a rapid sporulation of the fungus, it is necessary to have a good moisture level in the soil. Introduction of new formulations and atoxigenic strains with better sporulation under suboptimal conditions will improve the efficacy of the biocontrol. Results from this year indicate that the atoxigenic product Afla-Guard® manufactured by Syngenta Co.) is able to sporulate sufficiently under such conditions. We are also looking to develop other atoxigenic strains native to tree nut orchards from California that may have advantages under California environmental conditions and which will have advantage to be used in an area-wide, long-term program in the California tree nut industries.

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

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Introduction

Ochratoxin A, a naturally occurring mycotoxin, is widespread in certain foods and animal feed. Several fungi in the genera *Aspergillus* and *Penicillium* produce ochratoxin while these fungi are decaying the crops involved. Specifically, of the *Aspergillus*, section *Circumdati* (*A. ochraceus*, *A. melleus*, *A. alliaceus*, and *A. sclerotiorum*) and from section *Nigri*, *A. carbonarius*, are known to have isolates capable of producing Ochratoxin A (OTA). From our experience working with *Aspergillus* fungi in fig and nut orchards in California, we frequently find *Aspergillus* in the *Ochraceus* and *Nigri* groups in soil from pistachio and fig orchards and one would expect ochratoxin-producing fungi to occur in pistachio orchards in the San Joaquin Valley. The recent notifications by the EU on Ochratoxin detection in pistachio loads is problematic and one could expect that soon tolerances for OTA will be set, resulting in rejection of exported pistachios; the EU regulatory limit is 10 ppb (parts per billion) in dried vine fruits. Therefore, developing management measures to reduce OTA contamination in pistachios is urgent and the industry needs to be ready to combat this problem by the time regulations on Ochratoxins are established. Our initial studies of Ochratoxin in pistachio began in 2016 when we submitted sub-samples of pistachio library samples for analysis by an independent laboratory. By 2017 we were able to implement our own protocol for extraction and detection of OTA by HPLC. Of the 201 samples submitted in 2016, 57.2% were positive for OTA with 25.9 of the samples with levels greater than 15 ng/g. In 2017, we determined that 53.3% were positive for OTA and 24.4% had levels above 15 ng/g. The results from 2018 are summarized below (**Tables 1 & 2**) and the 2019 samples are currently being processed for OTA as well as for aflatoxins. Additionally, we proposed the following objectives for 2019-2021: identify which fungi in pistachio orchards produce Ochratoxin A and quantify the levels of the toxin they produce; define factors that may influence Ochratoxin A content in pistachios; determine whether the application of the atoxigenic AF36 Prevail® and Afla-Guard® GR contributes in displacing ochratoxigenic fungi; and investigate additional management approaches to reduce Ochratoxin A contamination.

Results and Discussion

Thus far, over 160 isolates of *Aspergillus* and *Penicillium* spp. that are potential producers of OTA have been collected, cleaned, stored, and are in the process of testing for OTA production. These isolates were recovered from soil in pistachio orchards, from washings of pistachio leaves and fruit, and collected from spore traps in pistachio orchards. When it became apparent that ochratoxigenic producing species were more rare than anticipated, we consulted with help from Dr. Jeffrey Palumbo at the ARS/USDA in Albany, CA, and were able to receive isolates that have previously been affirmed as OTA producers. With these isolates we were able to confirm that our methods for culturing and extracting OTA are effective. At this juncture, 60 of the 160 isolates have been tested in the HPLC and only one isolate, of species *A. ochraceous*, that we collected from pistachio orchards was positive for OTA. Among the isolates in the growing collection are *A. ochraceous*, *A. carbonarius*, *Penicillium* spp. and some *A. terreus*. We are continuing to build this collection and aim to screen a few hundred isolates and then quantify the level of production by the ochratoxigenic isolates in pistachio kernels that are incubated over several days. With the OTA producing isolates we will conduct further *in vitro* studies to determine how environmental conditions (temperature and humidity) affect infection and production of ochratoxin in pistachio kernels. Furthermore, healthy pistachios have been prepared for co-inoculation experiments using an ochratoxigenic fungus and AF36 strain or the Afla-Guard® GR atoxigenic strain to determine whether the atoxigenic *A.flavus* (of AF36 Prevail® or the strain of Afla-Guard® GR) have any effect in reducing the production of Ochratoxin A. These lab experiments are currently underway.

In 2019, we were unable to fulfill objectives to quantify the frequency of potential OTA producing species from early split pistachios or from pistachios damaged by navel orangeworm. During the next two seasons we will be in a better position to conduct those studies. With ochratoxigenic isolates on hand we are ready to proceed with the remaining two objectives. We will co-inoculate healthy pistachio kernels with OTA producing isolates and the AF36 strain that is used as a biocontrol for mitigating aflatoxins in pistachio orchards. The levels of ochratoxin produced will be analyzed by HPLC and TLC for multiple combinations of isolates and biocontrol strain. Furthermore, some of the OTA producing strains that we accumulate, among other *Aspergillus* strains, will be tested for resistance to triazole and other fungicide groups. We are ready to proceed with this objective. Eventually similar tests will include the Afla-Guard® GR strain, which is a different *A. flavus* atoxigenic strain. Afla-Guard® GR has been submitted by its manufacturer to EPA and to CDPR for registration in pistachios and almonds. The field efficacy of AF36 to potentially mitigate ochratoxin will continue to be examined by HPLC analysis of pistachio library samples. Another 200 library samples representing east and west Kern County are currently being processed for extraction. The results from the 2018 pistachio library samples yielded lower ochratoxin positive samples (26.3%) than the previous two years (>50%). Oddly enough, there were more ochratoxin contaminated samples in blocks treated with AF36, the biocontrol for aflatoxin. Overall, ochratoxin levels were low (median = 5.5 ng/g) while the frequency of contamination seems relatively high.

Conclusions

Thus far in our Ochratoxin research, we are learning that while potential OTA producing species may be abundant in pistachio orchards, the frequency of isolates that actually produce ochratoxin may be very low. However, the relative abundance of these species in the soil (*Ochraceous* group, *A. carbonarius*, *Penicillium* spp.) compared to aflatoxigenic species (*A. flavus*, *A. parasiticus*), which do have a high frequency of toxin producers, may explain why there is a higher percentage of ochratoxin contaminated samples than aflatoxin samples in the analyses we have conducted. We aim to complete our objectives by isolating and testing many more isolates from pistachio orchards and to gain a better understanding of the frequency of ochratoxin producers and how the environment, plant stress, and insect pressure may affect ochratoxin production.

Table 1. Results from HPLC analysis of 2018 pistachio library samples.

Harvest	Treatment	Ochratoxin Positive (%)	Samples tested
Normal	AF36	31.2	125
	Control	10.2	59
Re-shake	AF36	53.8	13
	Control	25	12

Table 2. Descriptive statistics for the 55 OTA positive samples out of 209 tested from 2018 pistachio library samples.

Mean (ng/g)	Median (ng/g)	Range (ng/g)	Samples with OTA >15 ng/g
20.7	5.5	1.1 - 357.9	17

Integrated Conventional and Genomic Approaches to Pistachio Rootstock Development

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Introduction

Soil borne diseases such as *Verticillium, Phytophthora, Macrophomina*, and nematodes (root-knot, lesion, and ring) seriously limit sustainable production of pistachio in California. With the recent restrictions on the use of fumigants, the industry is exploring the host mediated resistance to develop rootstocks with durable resistance to soil borne pathogens. Rootstock breeding programs involve production of genetically diverse interspecific hybrids, embryo rescue, propagation, and disease evaluations, which is expensive and time consuming. Further, it is a daunting task to recombine resistance to multiple diseases along with important horticultural traits such as vigor, precocity, scion control, productivity, longevity, graft compatibility, propagability, adaptability, anchorage, and so on. Evaluation of hybrids for multiple traits, especially soil borne diseases is laborious, resources intensive, time consuming and requires a multidisciplinary approach. Wild *Pistacia* species within the sect. *Eu Terbinthus* are excellent sources of genes for resistance to soil borne diseases, adaptation to wide range of growing conditions, and graft compatibility. Extensive disease evaluation of germplasm is critical to identify donor species and genotypes for resistance and to develop effective breeding strategies for rapid development of improved rootstocks. Rootstock breeding programs continue to depend on conventional breeding techniques and phenotypic selection through disease testing. On a quest to develop next generation horticulturally superior pistachio rootstocks with combined resistance to multiple diseases, we have been producing genetically diverse interspecific hybrids involving the cultivated and wild relatives of pistachio that are known to possess resistance to biotic and abiotic factors. Micropropgation of interspecific hybrids is a major challenge in pistachio, while it is easy to shoot multiply, the rootability is very poor and each hybrid responds differently to rooting treatment making the task very complicated.

The current project focuses on the following two objectives: (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.

Results and Discussion

Interspecific hybrids, embryo culture, and micropropgation.

During 2019 spring, we focused on cross combination involving *P. integerrima, P. khinjuk, P. vera, P. palaestina*, and UCB 1: *P. vera x P. atlantica, P. vera x P. integerrima, P. vera x P. terebinthus, P. vera x* UCB-1, *P. khinjuk x P. integerrima, P. khinjuk x* UCB-1, *P. palaestina x P. khinjuk, P. palaestina x P. integerrima, P. integerrima x P. khinjuk, P. integerrima x* UCB-1. Mature seeds were harvested and embryo extracted for culturing and micropropagation. Excess seeds after embryo culture were stored in the refrigerator for future propagations. The germinated embryos are in different stages of shoot multiplication and rooting.

Successfully embryos cultured and shoot multiplied *in vitro* plants from 2018 harvest of hybrids are being rooted at the Sierra Gold Nursery and are in acclimation tunnels (Table 1). These will be soon moved to a greenhouse for further growth and development for production of plants for disease testing. During the period under report, we have entered eight new hybrids (Table 2) for disease evaluation for *Verticillium, Phytophthora, Macrophomina*, and nematodes. They are being evaluated for resistance to soil borne diseases.

Disease resistance evaluation of hybrids: Four interspecific hybrids produced during spring, 2017 have been subjected to evaluation 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. The results so far indicate that the four hybrids, VA, VA1, VA14 and VA17 (all vera x atlantica) have shown to be more tolerant to *Phytophthora* and *Macrophomina* than the widely used UCB1. 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 and the experiments have not been concluded yet.

Table 1 (on left). 2019 Pistachio hybrids in acclimation at the Sierra Gold Nursery and
Table 2 (on right). 2018 Pistachio Hybrids under disease evaluation.

Hybrid	Parentage	# of plants
PA6	palaestina x atlantica	16
VA2	vera x atlantica	33
VP11B	vera x palaestina	27
VP17C	vera x palaestina	48
VP18C	vera x palaestina	64
VP8A	vera x palaestina	78
VT10	vera x terabinthus	51
VT2	vera x terabinthus	22
VU16	vera x UCB1	41

Hybrid	Parentage	# of plants
VA17	vara x atlantica	36
IK39	integerrima x khinjuk	20
VT16	vera x terabinthus	31
VA	vera x atlantica	36
PxK	palaestina x khinjuk	28
VA11	vera x atlantica	21
VA15	vera x atlantica	14
VA7	vera x atlantica	10

A) *P. integerrima* x *P. khinjuk*; B) *P. palaestina x P. khinjuk*; and C) *P. vera x P. terebinthus*

Long-term saline irrigation strategies for pistachios on PG1 rootstock

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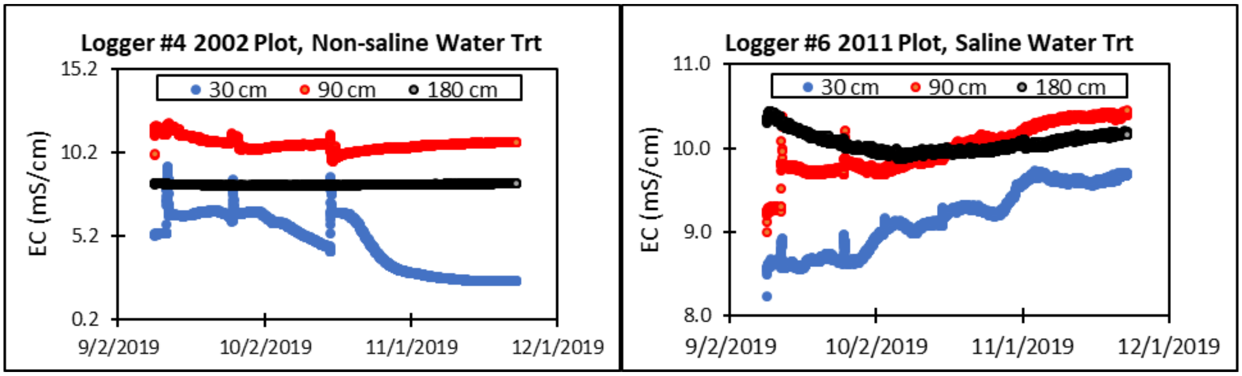
Introduction

Obtaining the recommended annual 44 inches of irrigation water (Goldhammer and Beede, 2004) has become a major problem for California pistachio growers. Consequently, growers are increasingly using poor-quality water groundwater with higher salinity (Cl and Na), boron (B) and selenium (Se) (Ayars et al. 1993). Due to pistachios' apparent Na and B tolerance, as well as the lack of dependable sources of good-quality water in central California, many pistachio growers have been irrigating their trees with water high in salinity, B, and Se. Eventually, the soil will, however, become saturated with Na, chloride (Cl) and B, and the soil's physical properties will deteriorate (Oster et al., 1999). Once the soil's osmotic threshold has been exceeded, the Na, Cl, and B concentrations can also exponentially accumulate in the tree and negatively affect yields and tree health. All three ions can be readily absorbed by roots, translocated to the scion, and concentrate in pistachios, thereby resulting in nut yield reductions (Karima et al., 2009). Hence, for the survival of irrigated pistachio production in California, utilizing lower-quality water with additional water management strategies will likely be a necessity during future drought conditions. However, this strategy requires obtaining new knowledge about managing the long-term effects of using poor-quality waters on soil quality. Recognizing the plant-based stress responses to soil quality changes with continuous saline irrigation will help determine the duration and quantity of saline water that can be safely applied while maintaining soil quality and tree growth and production (Pistachio Production Manual, 2016). Timing leaching of salinized root zones with good-quality water is of paramount importance for maintaining orchard viability under saline irrigation practices. Continuing the pioneering long-term salinity research of Sanden and Ferguson, we now can extend their efforts and examine the impact of continued long-term irrigation with saline water on salinizing the soil with Na, Cl, and B. In 2019, we installed Meter Group Teros 12 soil sensors from 0- to 120-cm depths at 10 different locations in pistachio fields irrigated with saline water in Panoche Drainage District since 2002 and 2011. With our sensors, we directly measured soil EC and soil moisture at different depths on saline soils receiving saline and non-saline irrigation water and measured biochemical and photosynthetic responses in healthy leaves and in leaves exhibiting the salt and B toxicity symptoms.

Results and Discussion

In the 2019 growing season, results from soil sampling at pre-growing season showed soil salinity and B concentrations ranging from 8.5 to 11.1 dS/m and 11 to 13 mg B L^{-1} at 0-45, 45-90, and 90-120 cm for 2002 planting, from 7.5 to 8.1 dS/m and 9 to 11 mg B L^{-1} for 2009 planting and from 6.1 to 7.3 dS/m and 8-10 mg B L^{-1} for 2011 planting. Saline water averaged 5 dS/m and 6 mg B L^{-1} and non-saline water contained <1 dS/m and <1 mg B L^{-1}. Irrigation water of both saline and non-saline quality occurred within the 5-acre plantings of 2002, 2009 and 2011, respectively. Our test sub-blocks consisted of 85 trees per water treatment, respectively. Water was applied via micro-sprayer, and irrigation scheduling and amounts were determined by JM Lord Consulting. Once the soil sensors for saline soils arrived from Washington, they were calibrated and installed at 30, 90, and 180 cm depths at both saline and non-saline irrigated treatments for 2002 and 2011 planting sites, respectively. Sensors have recorded continuous "real time" data every 15 minutes for changes in soil salinity and soil moisture at different depths (see Figures). Although sensors are still "settling in", direct measurements showed soil moisture initially ranged from 40-55%, 56-60% and 51-56% for the 30, 90, and 180 cm depths respectively in both saline and non-saline plots, while bulk soil EC ranged from 5.8-7.7, 8.9-11.2 and 8.3-11.1 dS/m for the 30, 90, and 180 cm depths respectively. During the last 3 months of the growing season, soil moisture has generally decreased depending on the sensor depth; 10-30% and 3-9 % at the 30 and 90 cm depths, respectively, and no decrease was measured at 180 cm. The soil bulk EC has ranged from a +7 to -14 % difference and a +7 to -1 % at the 30 and 90 cm depths respectively, and a +1 to 3% at 180 cm (an indicator that groundwater did not contribute to soil salinity). Overall, the saline irrigated plots are generally increasing in soil EC, while the non-saline irrigated plots are generally decreasing at the 30 and 90 cm depths. Our soil sensors allow us to immediately measure for salt movement and accumulation.



In the biochemical analyses, leaves were collected and separated into healthy and necrotic tissues from all planting sites. Physiological parameters in the leaves like quantum efficiency of photosystem 2, NPQ, leaf area, photosynthesis and chlorophyll were measured, as well as photosynthetic pigments, chlorophyll, carotenoids, total phenolics, proline and antioxidant capacity. These plant-based values will be used in conjunction with measured sensor data to help determine the soil and tree sustainability when irrigating pistachios with saline water (values will be presented in ‘final report’). All inorganic analyses were performed in our USDA Laboratory. Results showed that typical leaf B concentrations in healthy leaves ranged from 608-924 mg B/kg DM, leaf Na concentrations ranged from 1,831-5,250, and Cl concentrations from 6,983-10,650. In necrotic leaves, leaf B concentrations ranged from 2,732-3,576 mg B / kg DM, leaf Na concentration from 3,539-8,620 mg Na/kg DM, and Cl concentrations from 4,318-6,120 mg Cl/kg DM. In both leaf tissues, relationships were observed with Ca, K and Na concentrations.

Conclusion

The results of the first year effectively demonstrate the potential for using soil sensors to monitor “real time” salt movement and soil moisture in saline-irrigated pistachios. In just three months we were able to immediately monitor for changes in soil salinity and moisture after irrigation with saline or non-saline water on pistachio trees grown in salinized soils. These soil data in association with plant-response data collected in 2019 and data be collected in 2020 with other sources of “ARI pistachio funding” and Pistachio Board funding will provide growers with vital information needed to implement soil salinity management strategies for preserving the sustainability of using saline water on pistachios under field conditions.

Determining Pistachio Hull Susceptibility to Navel Orangeworm as a Function of Degree-day Accumulation

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Introduction

The navel orangeworm (NOW) is the main insect pest of Californian pistachios and causes significant crop losses every year. NOW adults deposit eggs on the surface of pistachio hulls, once the larvae hatch, they enter the shell and feed on the kernels. The resulting combination of damage to the nut and the presence of aflatoxins make the pistachio nuts unmarketable. Three interactive components promote NOW infestation: insect densities, conducive environmental conditions, and a susceptible host. Thus far, research has focused almost exclusively on the insect's interaction with the environment, while pistachio host susceptibility has been largely unexamined. Interestingly, it has been reported that high insect pressure does not necessarily result in high NOW infestation of pistachio hulls under similar field conditions. For instance, in 2010, 2011 and 2018, there were historically low infestation levels in various California pistachio growing areas even though the monitored insect populations were high. These observations suggest that there is an underlying reason why the insects were not displaying normal egg-laying behavior and this is likely related to changes in pistachio hull susceptibility. The main goal of this project was to create a baseline on pistachio nut growth and maturity in response to heat accumulation (i.e., degree days) in order to identify critical developmental points when pistachio hulls become susceptible to NOW oviposition.

Results

The study was conducted weekly starting at 120 heat units (April 25, 2019) and concluding at 2,740 heat units (October 11, 2019) using pistachio nut samples from ‘Kerman’ trees grafted on the UCBI rootstock located in Kearney Agricultural Research and Extension Center (Latitude 36°35'53.6"N, Longitude 119°30'24.0"W). This period included from fruit set to full maturity (> 70% shell split). We randomly selected 12 trees from four rows in the orchard and continuously sampled them throughout the season. About four clusters yielding about 50 nuts of uniform maturity were collected per tree.

We performed imaging of the whole nuts (size, shape, and color parameters) using the VideometerLab® and measured volume, fresh weigh and dry weight every week for the duration of the study. The results indicate that before 1,000 heat units there was the transition from stage 1 to stage 2 of nut development and around 2,000 heat units there was the transition between stage 2 and stage 3 together with the initiation of shell split. Remarkably, we identified specific parameters obtained from imaging data (e.g., area and the *L of color measurements) that correlated to more traditional measurements of nut growth (e.g., fresh weight and volume of the nuts). These results open the possibility of using non-destructive methods to assess nut development.

Starting at 865 heat units, we started measuring the strength of the hull, the shell, and the kernel using a TA.XT2i Texture Analyzer and a perforating probe. At about 1,500 heat units, the hull starts getting softer with a dramatic loss of firmness around 2,300 heat units, when the hull started to deteriorate. The shell started to harden before 1,000 heat units, reaching its maximum firmness at about 2,000 heat units when shell split started (3%). The kernel firmness started to be detected about 1,500 heat units and gained its maximum strength just after 2,200 heat units.

At 1,220 heat units, we initiated the analyses of kernel growth and development by imaging (same methods as whole nuts) and measurements of fresh and dry weight. At about 1,500 heat units is when the kernel started to rapidly grow, reaching its maximum size between 2,300-2,500 heat units. Although the size remained quite constant after 2,300 heat units, the kernel dry weight continued to increase until 2,600 heat units (~62% split). Also, at this time, we started measuring respiration measurements using a CO_2 gas analyzer and quantification of ethylene production using a gas chromatographer (GC) coupled with a Refractive Index Detector. These measurements were performed with gas samples collected from clusters in the field. The clusters were enclosed for 1.5 h in air-tight bags until a gas sample was retrieved using a syringe. On the same day of collection, all samples were injected into the instruments. The respiration of the whole nuts appears to continuously decrease after 2,000 heat units (when shell split was first recorded). Ethylene production seems to be steady, except for a decrease at about 2,300 heat units.

As part of the chemical analyses, we measured volatiles from detached nuts every week starting at 1,220 heat units. Whole nut and kernel samples were heated in an airtight container and used for solid-phase microextraction. The volatile profiling was performed on a gas chromatograph coupled with a 220-MS ion trap (Agilent Technologies). Whole nuts produced higher levels of volatiles than the separated kernels, yet the patterns of volatile emission throughout the study were similar among the two types of samples. Remarkably, the whole nuts and the kernels had a peak in volatile emission at 1,800 heat units, which precede the initiation of stage 3 of nut development. Volatile emission was still high until about 2,300 heat units. The four most abundant volatiles across all samples were: limonene, α-terpinolene, α-pinene, and myrcene. Moreover, we quantified and profiling kernel fatty acid content. Purified oil samples were obtained from oven-dried kernels and run on a GC with a Flame Ionization Detector (Agilent Technologies). The accumulation of fatty acids appeared to spike about 1,600 heat units and remained pretty much constant at about 2,100 heat units. Also, the ratio of mono-unsaturated/poly-unsaturated fatty acids in the kernel changed from 1.0 at 1,200 heat units to 2.2 at 2,100 heat units. Other chemical analyses, such as the chlorophyll content of hulls, shells, and kernels, are still being completed.

The oviposition of gravid females caged on pistachio clusters was monitored from 1,600 to 2,740 heat units. We did additional caging experiments passed the evaluation period (until Nov 4) to obtain complementary data for the late season. Each cohort of five gravid females was allowed to oviposit over 10 days, at which point cages were removed and all eggs were counted on the twigs, leaves, rachis, and pistachio nuts, as well as the mesh bag itself. Since the total amount of plant material in the cages varied over time, and between cages, egg deposition was measured as the total number of eggs per gram of substrate. About 2-5% of all eggs were consistently deposited each on the twigs, leaves, and rachis (i.e. about 10% total across all three areas). The mesh bag itself was highly preferred and received about 84-94% of all eggs. Nuts were initially less preferred, receiving only 0-2% of all eggs between 1,600-2,600 heat units. Starting at 2,650 heat units, NOW egg deposition on nuts increased dramatically to 5-19% (average 12%), which resulted in a decreased number of eggs on the mesh bags.

Conclusion and Practical Applications

We are still analyzing the physiological and biochemical data and finding correlations with the insect caging experiment. However, we are confident that the data provide a clear baseline of nut developmental processes as a function of heat units to subsequently study host factors associated with susceptibility to NOW oviposition. A better understanding of when the pistachio hull becomes susceptible to oviposition as a function of degree-days may help reduce ineffectively timed sprays and improve the efficiency of integrated pest management strategies. Also, characterizing pistachio nut maturity as a function of degree-days will facilitate accurate harvest timing for all pistachio growers to maintain the highest nut quality. We expect that this first study will be a stepping stone to subsequent research on host attractant factors to NOW oviposition (e.g., physiological and biochemical biomarker), which we are planning to conduct in the next 2 to 3 years of this research.

Efficacy trials of new dormancy-breaking treatments in pistachio

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Introduction

In our second year of exploratory research into dormancy-breaking agents (DBAs), we continued evaluating late-winter DBAs for pistachio (GA_3, ethephon) and comparing them with oil and unsprayed standards. We followed two experimental blocks, the "old block" established in 2018 (which also included AVG) from which we got a second year of yield data, and a larger "new block" established in 2019 with two different application times. Resources for indoor experiments were primarily devoted to a combined dose-response and application time trial of GA_3 on 'Kerman' cuttings. Our main objective this year was to identify a single most promising new DBA to put future effort towards registering for use in pistachio.

Results & Discussion

Chill accumulation contributes to the completion of endodormancy. By definition, a dormant cutting that breaks bud when transferred to warm, well-lit surroundings is no longer endodormant, but ecodormant.

We discovered that a large dose of GA_3 can break the endodormancy of 'Kerman' cuttings. This activity was previously known in other species, such as peaches, but we newly report this activity in pistachio. Cuttings taken in mid-January and transferred indoors required 200 ppm GA_3 to break bud; the minimum dose of GA_3 necessary to break cuttings' endodormancy decreased with time, roughly 3-fold per week, and cuttings taken in mid-February and transferred indoors required no added GA_3 to break bud, indicating that endodormancy had naturally been broken by that time.

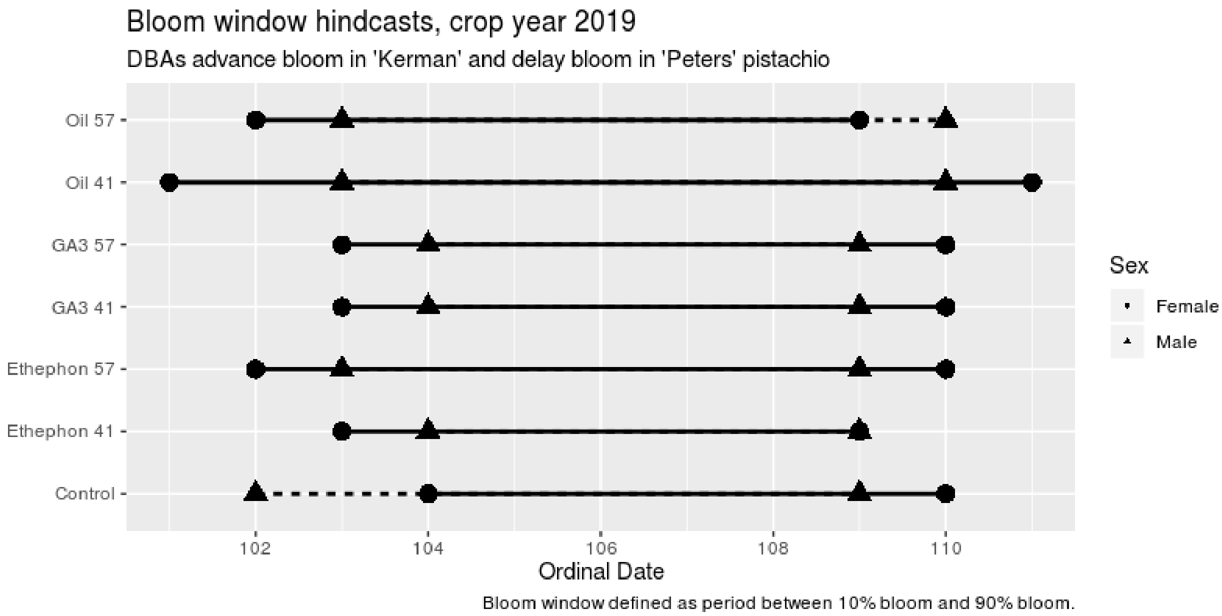
Ethephon was also included in the cuttings study. Ethephon was ineffective both alone and as an adjuvant with GA_3 to break endodormancy (that is, only the GA_3 was effective in the GA_3 + ethephon mixtures). These results suggest that low doses of GA_3 during endodormancy may substitute for marginal chill.

After the trees were confirmed to have entered ecodormancy, DBA spray treatments were conducted, and the trees were monitored for signs of having exited ecodormancy and resumed their growth. Growth initiation was first detected as an endogenous increase in the respiration rate of floral buds in mid-March. For a second consecutive year, DBA elevation of pre-bloom respiration was linked to DBA advancement of bloom. Using a novel method of carbohydrate quantitation, we demonstrated that twig starch content and bud non-hexose sugars content (which presumably consists mostly of pentose) increased rapidly following growth initiation. Bud starch increased throughout ecodormancy to a maximum at the time of growth initiation and then decreased thereafter. Increased respiration and carbohydrate accumulation signify the initiation of growth in pistachio floral buds.

The application of DBAs tends to increase carbohydrate content in treated tissue. DBAs can induce early bud starch peaks without endogenous analog.

All tested DBAs advanced bloom in 'Kerman' but delayed bloom in 'Peters'. DBAs' overall effect on bloom synchrony was equivocal. Overlap in mid-bloom was improved, but pollen coverage of the earliest female shoots was worsened.

DBA effects on bloom and synchrony are summarized in the following figure:



The second year's yield data from the old block showed that no DBA improved 2-year cumulative yields. The first year's yield data from the new block were compromised by a logistics mistake: trees in different treatments were shaken differently, preventing comparison between the new experimental treatments and the standard treatments. There was no difference in yield between oil and unsprayed treatments. Ethephon treatment yielded 13 pounds less per tree than GA_3 treatment, which is discouraging, but inconclusive.

Both test years, 2018 and 2019, had adequate chill to complete winter rest at our location. Thus, the DBAs' efficacy to mitigate yield losses due to insufficient chill could not be evaluated. Future trials of DBAs should be multi-year trials and should document DBA effects on yield-generating physiological processes, such as floral bud retention, fertilization, nut set, fruit abscission, and/or kernel fill.

Every tested chemical afforded higher (but not significantly higher) yields when applied in mid-February instead of late February. Mid-February application coincided with the transition from endodormancy to ecodormancy. Any late-winter DBAs that require complete winter rest to be effective (including oil) are likely to share this same effective time window.

Conclusions

GA_3 is the most promising candidate DBA for use during endodormancy (roughly December through January). As a DBA, GA_3 should only be used during endodormancy. Use of GA_3 during ecodormancy (roughly mid-February and later) risks inducing bud drop, reducing cluster counts and yields.

Single doses of GA_3 can break endodormancy in pistachio cuttings. The minimum dose of GA_3 needed to break endodormancy is a measure of endodormancy depth. Bioassays based on this principle may alert growers to impending endo-to-ecodormant transition, which is likely the optimum oil application time.

Oil remains the most effective DBA for use during ecodormancy (roughly mid-February and later). Claims about its efficacy in years with marginal chill remain unsupported.

Cracking the Black-Box of Pistachio Dormancy Break: Tracking biochemical changes in relation to oil spray timing for adequate chill accumulation

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Introduction

Pistachios require enough chilling temperatures to overcome winter dormancy and develop buds and leaves normally. Because of changing climate patterns, insufficient chill accumulation has become a significant concern for the California pistachio industry. Researchers have reported that application of horticultural oil helps in achieving uniform bloom and leafing, but the timing and underlying physiological mechanisms are poorly understood, particularly in relation to the chill accumulation curve during the dormant season. In order to apply the right kind of dormancy- or rest-breaking agents (RBAs) such as oil effectively, we need to first understand the underlying physiological mechanisms of how dormancy and bud break happens in pistachios. This study aims to unlock this black box, and focuses on the biochemical and metabolic nutritional changes within pistachio buds in relation to oil spray timing. This should improve production and better resource utilization. This project was started to understand more details about horticultural oil and other rest breaking chemical candidates as well as the best time of applying those. Overcoming erratic bloom and shortening the bloom and harvest windows not only improves production but also helps improve fruit quality via reduced pest infestation and better resource utilization. The main objectives of the study are to track changes in amino acids and plant growth regulators, particularly ABA and GA, to evaluate the correlation between biochemical changes in bud and chill accumulation in both oil sprayed and control trees and to investigate the effect of RBA application on growth and productivity.

During the 2018-19 dormant season, three sites were selected across the state: Strain Ranch in Arbuckle (Colusa County), S&J Ranch in Madera County, and Valley Orchard Management in Cantua Creek, in Westside of Fresno County. The bud sampling started at all sites, during first week of January 2019, before any RBA spray application. The RBA applications (Oil sprays at all 3 sites and Oil and Dormex treatments in Cantua Creek site) were done at particular Chill portion (CP) accumulation and not by the calendar. Dormex application was done at 59 CP accumulation in Cantua creek site. The complete treatment schedule is given in Table 1.

Results & Discussion

The bloom data showed that there were significant differences among treatments in terms of bloom advancement and length of bloom period. At the Cantua Creek site, the oil spray at 51 and 59 CP provided 3 and 2 days advancement in full bloom over the control treatment, respectively. Application of Dormex at 59 CP and oil at 48 CP did not advance full bloom in relation to control. At the Madera site, oil application at 48, 51, and 59 CP, showed advancement in full bloom over control by 2, 2, and 4 days, respectively. At the Arbuckle site, the oil sprays done at 53 and 69 chill portions showed advancement of only 1 day over the control, while oil spray at 50 CP did not show any advancement over control.

The length of bloom was measured in the number of days from bud break to full bloom. The length of bloom window for control treatment was 5 days in Cantua Creek site, 9 days at the Madera site and 14 days in Arbuckle site. RBA sprays in Cantua Creek and Madera sites had no significant effect on length of bloom window, while in Arbuckle site, oil spray at 69 CP shortened bloom window by 3 days on an average. Bud swell and full bloom data showed similar trends for male trees at all 3 sites.

Field weight data for 2019 harvest was analyzed for per tree weights and showed significant differences among treatments. At the Cantua Creek site Dormex treatment showed highest yield per tree (88 lbs/tree) and was significantly greater than control (65.8 lbs/tree) and all three oil sprays (50.88, 74.1 and 63 lbs/tree, respectively). Madera site showed that only oil spray treatment at 59 CP has significantly higher yield (70.75 lbs/tree) than all other treatments including control. Control (37.16) was not different from other 2 oil spray treatments at 48 and 51 CP (48.91 and 40.67 lbs/tree, respectively). At the Arbuckle site, oil spray at 50 CP had the highest yield (26.43 lbs/tree), followed by control (22.58 lbs/tree). The lowest yield was in trees with oil spray treatment at 69 CP (18.63 lbs/tree). The bud samples are currently being analyzed for amino acids and PGR content and those data will soon be reported.

Table 1: Treatment plan, spray dates and chip portions for the 3 study sites.

Treatments	**Rate**	**Spraying Dates and Chill Portion (CP)**		
		Cantua Creek	**Madera**	**Arbuckle**
Control	N/A	Dry Control	Dry Control	Dry Control
Dormex (AlzChem)	4%	7 Feb (57)	N/A	N/A
Oil S1 (IAP440)	6%	*Skipped	29 Jan (48)	23 Jan (50)
Oil S2 (IAP440)	6%	29 Jan (51)	7 Feb (51)	29 Jan (53)
Oil S3 (IAP440)	6%	6 Feb (56)	*Skipped	*Skipped
Oil S4 (IAP440)	6%	12 Feb (61)	19 Feb (59)	20 Feb (69)

No. Of Blocks= 4; 10 trees per treatment block- 8 female+2 male trees.
Statistical design= Randomized Complete Block Design (RCBD)
**Skipped: Spray timing had to be skipped due to very wet ground and field conditions not suitable.*

Conclusions

- Dormex application gave significantly higher yields than the control and any oil timing.
- Oil spray timed at certain CP provides some yield benefit, but we need data from multiple years before drawing any conclusions on which oil timing are better.
- Dormex application did not provide any bloom advancement or shortening of bloom window.
- Dormex spraying at 59 CP did not enhance bud break in Cantua Creek Site.
- Trees in Cantua Creek site were OFF and will be ON next year while Madera site were ON this year. Trees in Arbuckle were young and semi-OFF
- It seems that oil at CP >50 is more effective in bud break enhancement than CP<50.
- RBA timing aimed at certain CP accumulation has certainly proved to be a good strategy for answering this research question rather than timing based on calendar dates.

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

Authors: Patrick Brown, Professor, Department of Plant Sciences, University of California, Davis; **Louise Ferguson**, Cooperative Extension Specialist, Department of Plant Sciences, University of California, Davis; **Douglas Amaral**, Project Scientist, Department of Plant Sciences, University of California, Davis.

Introduction

In many fruit crops, such as kiwifruit, apple, cherry, and pistachio, winter dormancy is an essential part of plant development. During dormancy, a period of exposure to low temperatures (winter chilling) and subsequent spring warming, is needed to release flowering buds from dormancy and stimulate subsequent bud break and flowering. Irregular bud break and variable flowering may have a strong negative impact on production and therefore economic returns. Materials such as oil sprays, kaolin clays, and calcium carbonate have been shown to have beneficial effects on synchronized bud break and flowering, thereby improving yield. Irrespective of the cause, the threat of climate warming to pistachio in California is real. Recent research has demonstrated that Californian pistachio growing regions are becoming warmer, with less winter fog, and that low chilling will become a more frequent challenge for pistachio production in California. Research on strategies to mitigate the effects of marginal chill seasons is essential to the long-term productivity of pistachio in California.

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 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.

Results

Year 1 of this experiment involved site selection, bud collection during the accumulation of winter chill, rest-break applications and monitoring of rest-break, yield and quality. Buds collected will subsequently be used for molecular determination of a flowering signal for use as a diagnostic.

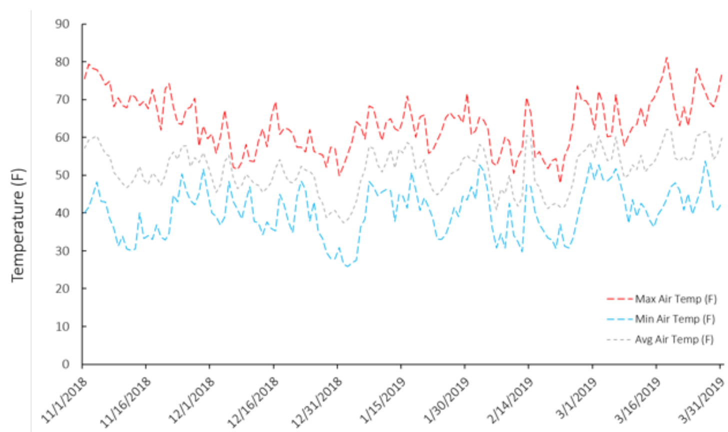

Figure 1*. Average, minimum and, maximum daily temperature (ºF) data – Kern County.*

Site location and climate data:

Three different high yielding commercial pistachio cultivar "Kerman" orchards interplanted with cultivar "Peters" near Wasco in Kern County, and Kettleman City in Kings County, California. Air temperature, humidity, and sunlight hours were measured with loggers placed at the top of two trees located in different quadrants of each pistachio orchard (Figure 1). Chilling accumulation was calculated as chill unit (CU) according to the Dynamic Model (Figure 2). 2018/2019 was a high chill year and by late January/early February chill portion requirement (58 CU) had been fulfilled in all three monitored sites.

<u>Spray and sampling</u>:
A randomized complete block design with five oil and one control treatment was established at each orchard. Five trees per treatment were selected and three replicated blocks were monitored for each treatment for a total of 18 plots per orchard. Treatments with bud-break enhancing compound (6% horticultural oil) includes: very early (45 CU), early (50 CU), optimal (55 CU), late (60 CU) and very late (65 CU) treatments. Pistachio bud samples were collected from untreated trees to subsequently identify genes correlated with the changing physiological status of the buds. In parallel, the effects of differently timed rest-breaking treatments on bud break, flowering and yield are being monitored.

The effects of horticultural oil treatments on bud break and flowering are shown in Figure 3. In general, the phenological observations revealed no effects of treatments on bud break and flowering of pistachio trees. All treatments reached full bloom by April 14th. Because the first year of this experiment was assigned to stablish the experiment and to collect samples, no data on gene activity has been analyzed yet.

Conclusion

During the first year of the project, field trials were executed. These trials were focused around monitoring the activity of dormancy related genes and applying bud-break enhancing agents to Pistachio trees at different moments. Data showed that application of horticultural oil (6%) at different chill accumulation stages (45, 50, 55, 60 or 65 CU) does not affect bud break when tree chill requirement is satisfied earlier in the season (early-mid February). However, rest-breaking agents may still play a major role in pistachio production in warm regions or during low chill years.

Unfortunately, this project has been abandoned as the company providing the proprietary molecular analyses and detection technologies has entered bankruptcy and the underlying technologies are now frozen until such a time as the bankruptcy is settled. Bud tissues collected from these trials will be maintained at -80ºC with the aim of continuing this analysis when new technologies become available.

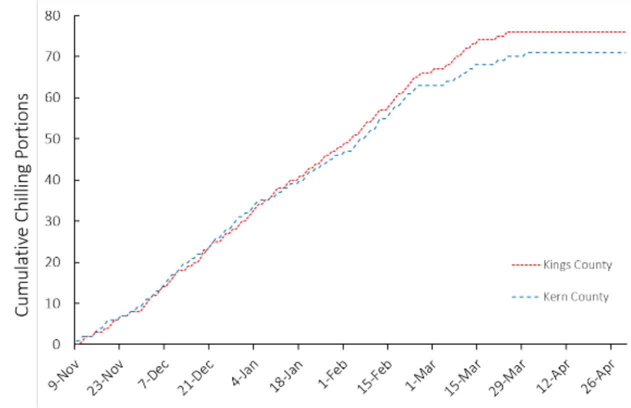

Figure 2. *Cumulative chilling portions in King and Kern counties*

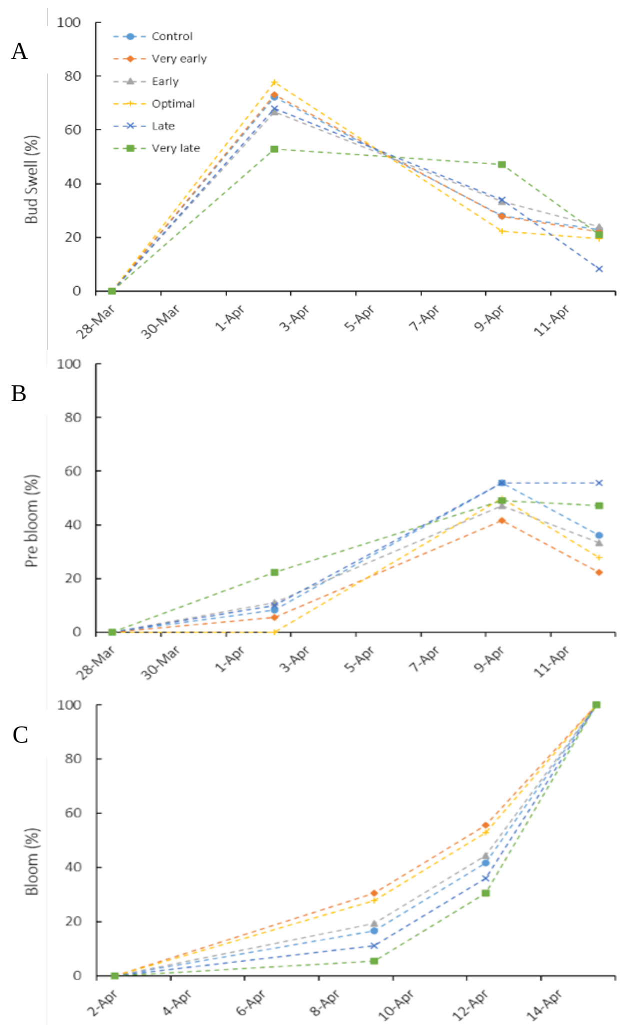

Figure 3. *Bud break and flowering rate in pistachio trees cv. Kerman in response to horticultural oil treatments.*

Pistachio Improvement Program – 2019

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 continue to develop 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 second year of the project are listed below.

Results and Discussion

1. Establishment of germplasm and scion breeding blocks: From mid-July through early September we continued budding into our germplasm blocks in Davis and Winters, which were planted in 2018. Last year we budded ~45% of trees, and this year we budded an additional ~45%. Almost all the trees in Winters are now budded, whereas the larger Davis block has additional trees available that need to be budded in 2020 as they are sizing up. On October 9, an additional 3 rows of UCB-1 rootstocks were planted at Wolfskill Experimental Orchard (WEO), expanding our existing germplasm block there. We plan to use these trees to graft wild *P. vera* from the USDA *Pistacia* C block, where an unidentified soil-borne pest or pathogen is killing *P. vera* trees on their own roots. *P. vera* trees on rootstocks are little affected. Many of these accessions are from areas of central Asia where further collecting will be difficult or impossible. *P. vera* seed from controlled crosses in 2018-2019 is scheduled to be planted in spring 2020 into two blocks at WEO. Potential additional sites for future scion seedling blocks include the West Side Research and Extension Center in Five Points and the Nickels Soil Lab in Arbuckle.

2a. Scion breeding crosses: Early elite females were crossed with early males, the rationale being that early leafing date is correlated with low chill requirement. In March-April, with assistance from Craig Kallsen and Dan Parfitt, the Buttonwillow site was used as a source of Gumdrop and Golden Hills females and Tejon and Zarand males which were crossed in a factorial design. Several early-leafing females from WEO were also pollinated with Tejon and Zarand. On August 30 several thousand nuts were harvested from controlled crosses at Buttonwillow, with great variation in pollination success that appears to be strongly related to pollen age and quality. Putting small pieces of male inflorescence directly onto bagged female inflorescences produced the most nuts, but this technique is only feasible when a male and female are flowering at the same time. Harvesting pollen from drying male inflorescences, storing it at -20C over a saturated solution of $MgCl_2$, and applying it later by syringe produced more variable results. Similar results were observed for the rootstock crosses. We suspect we were not prompt enough in moving harvested pollen to cold storage: male inflorescences were left to dry for 24-48 h before moving pollen to -20C. In spring 2020 we will reduce the drying time to 8-12 h and keep male inflorescences on ice during transport.

2b. Rootstock breeding crosses: To further explore the *P. atlantica x P. integerrima* heterotic combination that underlies the California pistachio rootstock industry, we used our genetic data to identify *P. atlantica* and *P. integerrima* individuals from the USDA and WEO collections, and performed controlled crosses between them. Most of these individuals were previously identified as belonging to *P. atlantica* and/or *P. integerrima* in the GRIN database. Our *P. atlantica* group also includes *P. terebinthus*, as we are unable to clearly separate these groups using genetic data. Poor seed yields were obtained from these crosses, likely due a combination of the pollen storage issues described above and the low canopy light levels in the USDA *Pistacia* blocks. Pollinations made onto trees along the edges of the block were more successful. To remedy this, we propose to begin hedging the collection each winter, and possibly in the summer as well, to bring more light into the canopy and bring down overall tree size. A spray program should also be implemented.

3. Marker-assisted selection: We are working to develop a SNP marker that can be used to predict sex across the genus *Pistacia*. A genome-wide association study (GWAS) was performed on 467 *Pistacia* individuals from the USDA and WEO collections using a dataset of 149,100 GBS markers and sex (male/female) as the phenotype. This analysis identified a single chromosomal region that strongly associates with sex. For the most significant SNP, 283/287 individuals identified as females using the genotype data were also phenotyped as females. The remaining 4 individuals probably represent phenotyping errors, as 2/4 of them contained the annotation “unidentified female” in the GRIN database. We are developing this GBS SNP marker into a cost-effective KASP assay.

In early 2020, all seed from scion breeding crosses will be planted, followed by tissue harvest, DNA extraction, and genotyping for the newly developed sex marker. Marker results will be used to cull males down to no more than 10% of the individuals from each family. This process would be more efficient if seeds, not seedlings, could be genotyped with the sex marker. To that end, we will test DNA extraction and genotyping from a small stab of embryo material harvested from the split portion of the nut. If successful, this technique would allow us to discard males before planting and reduce greenhouse expenses.

4. Tissue culture: We gained additional expertise in propagating and (especially) rooting microshoot cultures of clonal rootstocks from commercial sources, and we established new microshoot cultures of four UCB-1 seedling selections from a salinity screen. This year we extracted and cultured zygotic embryos from immature *P. vera* seed from cv. Kerman to initiate somatic embryo cultures –these cultures are now in progress but have not yet begun to produce somatic embryos. Future efforts will focus primarily on developing somatic embryo cultures from immature seed of UCB-1 or other rootstock crosses, as these could be used for generating gene-edited rootstocks. If successful, the same approach could then be expand for use in generating specific trait changes in established scion cultivars (eg: Golden Hills).

5. Synergistic activities: On May 13, 400 genotyped UCB-1 seedlings from a salinity screen were transplanted into a commercial orchard site being established in moderately saline soil near Mendota, CA. The seedlings were separated into 4 groups, each of which was planted in a separate row: 100 seedlings carrying the beneficial allele at both major salinity QTL (‘good-good’), 100 seedlings not carrying the beneficial at either QTL (‘bad-bad’), as well as 100 seedlings each of both other combinations (‘good-bad’ and ‘bad-good’). We continue to work on salinity tolerance in *Pistacia* using CDFA funding. Two recently completed greenhouse screens include a “diverse *Pistacia*” screen of 480 individuals from 6 *spp.*, and a “diverse *vera*” screen of 304 individuals. Both screens identified mother trees and germplasm pools that could be used as future sources of salinity tolerance, and both screens identified individuals that are far more salinity-tolerant than UCB-1. Data from the diverse *vera* screen are being used for a GWAS. CDFA funds were used to purchase a chloridometer, which can now be used to measure chloride concentration in plant samples with virtually no cost other than labor. Startup funds are being used to perform a transcriptome experiment in salt-stressed UCB-1 seedling roots. The experiment used roots tips from 12 UCB-1 seedlings (3 individuals each of the ‘good-good’, ‘good-bad’, ‘bad-good’, and ‘bad-bad’ groups described above) harvested before and after exposure to salt.

Conclusion

Our *P. vera* scion breeding program is very new but expanding rapidly. While this part of the program grows, we will continue to focus on screening rootstocks for useful traits, developing our tissue culture capacity, and preserving and characterizing germplasm.

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

Authors: **Gerald Dangl**, Plant Identification Lab Manager, Foundation Plant Services, UC Davis; **Judy Yang** , Staff Research Associate, Foundation Plant Services, UC Davis; **Deborah Golino**, Director / UCCE, Foundation Plant Services, UC Davis.

Introduction

This is the second and final year of this project. 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 pollinated manually 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, were not protected from outside pollen and were 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 (UCB-Female x X) from the open-pollinated UCB-Female trees. We examined flower and fruit development on the open-pollenated *P. atlantica* female trees and the phenotype of the resulting UCB-Female x X seedlings. Using DNA markers, we determined the identity and location of the male parent of many of the seedlings and investigated the species ancestry of the feral male *Pistacia spp*. trees growing within a 1.5-mile radius of the unprotected UCB-Females.

Results

With the additional year of maturity, the four unprotected UCB-Female trees produced tens of thousands of seeds, as opposed to less than 150 seeds produced in the first year of the study. From each of the four mother trees, 196 seeds entered our stratification and germination protocol. The germination rate was ~ 80%, which is greater than the germination rate for last year's UCB-Female x X seed (~ 45%) and less than the 90% or more typical of pure UCB-1 seed. Germinated seedlings were subsequently planted in individual containers. There were clear phenotypic differences among the UCB-Female x X seedlings. As a group, they were also very different from UCB-1 seedlings. The final report will include detailed photographs and address phenotypic differences among the UCB-Female x X seedling population and document how the UCB-Female x X population, as a whole, differs from UCB-1 seedlings. These differences could aid efforts to rogue non-UCB-1 seedlings in a nursery setting.

DNA was extracted from leaf samples of the open-pollinated (UCB-Female x X) seedlings and genotyped with 14 DNA markers. We obtained clear DNA profiles for 366 seedlings. These DNA markers show inheritance from the parent generation into the progeny. The DNA profiles obtained for all of the seedlings were consistent having UCB-Female as one parent. Removing the portion of the DNA profile inherited from the known mother allows us search for the male parent.

Only six of the 366 seedlings were standard UCB-1 hybrid siblings, with the *P. integerrima* UCB-Male as the male parent. This is in stark contrast to last year's result of 32 UCB-1 out of 59 seedlings. The unprotected UCB-Female trees are exposed to pollen from our adjacent UCB-Male pollen source trees and to UCB-Male pollen venting from the enclosures protecting the seed-production females. Though the UCB-Male trees typically shed pollen two to three weeks before the female flowers are mature, we speculate that in the spring of 2017, pollen shed from our UCB-Male males sufficiently overlapped with receptivity of the unprotected UCB-Female flowers to produce the larger percentage of UCB-1 seed.

To identify the male parent of the remaining UCB-Female x X seedlings, feral *Pistacia spp*. trees growing within ~1.5 miles of the UCB-Females were tagged in mid-March and early April 2019. Most of the trees were visually confirmed as males. Leaf samples were collected at a later date. Unique, unambiguous DNA profiles were generated for 109 trees, including retesting 51 samples collected in 2018. We conclusively identified the male parent of 237 of the 363 UCB-Female x X seedlings. Thirty-five feral males produced one or more seedlings. The majority of successful males, 32 of 35, produced ten or fewer seedlings, while the remaining three successful male trees produced 45, 42 and 29 seedlings.

It's logical to hypothesize that distance and direction to the unprotected UCB-Females would affect male pollination success. The most distant feral male was 1.3 miles (2104 meters) from the unprotected UCB-Females and actually fathered two seedlings. Four other feral males more than a mile from the unprotected UCB-Females were successful pollen parents. Two of the most successful males, producing 45 and 29 seedlings, were ~ 0.43 miles (689 meters) and 0.51 miles (826 meters), respectively, from the unprotected UCB-Females. The closest feral male was ~250 feet (76 meters) away from the UCB-Female trees and produced 42 seedlings. However, there were seven other males within 1000 feet (305 meters) of the UCB-Females; five of them combined produced only 16 seedlings, and two did not produce any seedlings. Clearly, distance is not the only factor affecting male success. We also considered wind direction and speed. Though there are essentially no feral males to the south, our UCB-Females did receive pollen from all directions in which there were feral males. The final report will elaborate.

Successful males must have shed pollen when the unprotected UCB-Females were receptive. Pollen shed phenology is at least partially genetically controlled. Based on analysis of the DNA profiles, we examined whether species ancestry of the feral males was related to successful pollination. Species references from the National Clonal Germplasm Repository, Davis (NCGR-D) were included as references. Preliminary results assigned the feral males to three species ancestry groups: *P. atlantica*, *P. chinensis* and hybrids of the two (*P. atlantica* x *chinensis*). With the doubled sample size, these results were more robust than those from the first year of the study. Successful males were found in all three groups. The final report will provide more detailed results on the genetics of the feral males as related to breeding success.

Conclusion and Practical Applications

In the second year of the study, the four unprotected *Pistacia atlantica* UCB-Female trees produced tens of thousands of seeds, as opposed to less than 150 seeds in the previous year. These female trees are potentially exposed, while receptive, to large amounts of UCB-Male pollen from our pollen source trees and from pollen venting from the enclosures protecting the seed-production females. However, only six of 366 seedlings examined were standard UCB-1 hybrid siblings, with the *P. integerrima* (UCB-Male) as the male parent. This is in stark contrast to last year's result of 32 UCB-1 out of 59 seedlings.

We identified the feral male parent for 237 UCB-Female x X seedlings. The successful feral males are of complex species ancestry, including P. atlantica, P. chinensis and *P. atlantica x chinensis* hybrids. Based solely on phenotype of the open-pollinated UCB-Female trees, it is clear that UCB-Females are reproductively compatible with the diverse feral males that are ubiquitous in much of California.

Some of the successful males were over a mile distant from the UCB-Females. It is clear from this two-year project that isolating UCB-Females by distance to male trees is not sufficient. UCB-Females, not isolated from exogenous pollen with a physical barrier will produce contaminated UCB-1 seed. Our tests have also shown 100% UCB-1 seedlings from physically isolated, hand-pollenated UCB-Females.

We have over 360 open-pollinated UCB-Female x X seedlings. For 237 of these seedlings, we know the location and species background of the male parent. These are potentially an interesting genetic resource. Anyone interested in further study of these UCB-Female x X plants should contact the authors.

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

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Introduction

Pistachio is one of most rapidly expanding crops in California and a major contributor to its economy; with its environmental adaptability affords expansion into marginal land and yield improvement on current acreage. 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 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 beneficial. Plant roots are the first contact to nutrients and microorganisms and are important for robust and sustainable growth. So far, most of the pistachio rootstock characterization is based on aerial part (leaves and shoots) while little is known about their root system under environmental stresses. Thus, it remains challenging to select for superior rootstocks without investigating their root system, and how it adapts to environmental stresses.

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. Endodermal cells undergo differentiation states consisting of deposition of lignin, forming the Casparian strips, and suberin lamellae. The Casparian strips correspond to localized lignin depositions sealing the apoplastic space. This endodermal layer is differentiated by suberin build up, 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.

Our project aims at the establishment of cellular and molecular methodologies to assess pistachio root adaptation under environmental stress including drought and salinity. Root apoplastic barriers are important for transport of water and ions to the vascular system of the plant. Characterization of cellular barriers in pistachio roots that are altered under stress response will allow the development of cellular and molecular markers for identification of sustainable genetic material and mechanisms of salt exclusion under salinity.

Results From this Review Period

During this review period we performed analysis of UCB-1 and *P. Integerrima* plants 1) grown in the green house, 2) UCB-1 plants grown in the field and 3) performed multiple repeats of tissue culture plants to obtain statistically significant data for publication.
Three different developmental zones (0-2) were excised from the root tip of active roots reflecting a developmental gradient and were analyzed via microscopy to detect sodium localization, root anatomy and development.

Results derived from experiments in a laboratory setting
A) The UCB-1 hybrid under salinity treatment showed sodium sequestration in plant vacuole in combination with a likely exclusion mechanism that inhibit the transport of sodium to the leaves.
B) Structural features of specialized cells were observed in leaves of UCB-1 as a potential sequestration mechanism that takes place in leaves and contributes to salinity tolerance. C) Increased suberin deposition both in endodermis and exodermis were observed for UCB-1 seedlings under salinity treatment. D) Interestingly, in *P. integerrima* roots, secondary xylem was initiated much earlier than to UCB-1 in response to salt treatment.

Greenhouse grown UCB-1 and P. integerrima plants
In preliminary work, UCB-1 seedlings and *P. integerrima* exposed to 200 mM NaCl were classified as "tolerant" or "moderate" based on their number of healthy leaves remaining at the end of the experiment, and samples from three zones in the root tip were sectioned and stained for suberin and sodium. The most susceptible individuals had stopped growing with complete leaf loss and were not sampled. Results suggest greater increase of suberization under salt stress in tolerant seedlings. This effect was particularly pronounced at the very tip of roots (zone 0). Sodium sequestration in the vacuole of root cortex cells was enhanced in zone 1 of tolerant seedlings. In contrast to UCB-1 seedlings, *P.integerrima* seedlings did not show sodium localization to the vacuole under salinity. Preliminary data suggests that there is a smaller increase in suberization in tolerant seedlings and less overall suberization when compared to UCB-1.

Field grown plants:UCB-1 plants budded to Kerman were grown in San Joaquin Valley with 8 dS/m NaCl and 6 ppm boron treatment. Diverse phenotypes of root maturation were observed in the UCB-1 population with early initiation of secondary growth of both the vascular cambium and phellogen and high amounts of suberization in both the endodermis and exodermis.

Conclusion and Practical Applications
The results suggest that both ion exclusion and sequestration are mechanism contributing to salinity tolerance. Importantly, structural differences observed in root tissues within the different genotypes can be related to halo-tolerance. The increased suberin deposition correlated with salt ion exclusion, suggests that these barriers contribute to halotolence 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. Currently we have excised and processed material for transcript analysis from UCB-1 and *P integerrima* plants with different degrees of salinity tolerance. These samples will be examined for altered gene expression of specific transporters and biosynthetic genes of suberin, allowing the identification of molecular markers for salinity tolerance.

So far, our results suggest that ion exclusion and sequestration are mechanism contributing to salinity tolerance, through different layers of cellular and anatomical responses. This includes differential accumulation of polymers such as suberin in the endodermis and exodermis. Thus, understanding the genetic and cellular mechanisms and the of endodermis and exodermis development in pistachio rootstocks, the overall root plasticity and correlating them with plant phenotypic responses can provide an effective way for the identification of the most suitable genotypes under stress response. Long term application of these discoveries include the resilience of pistachio plants as unique mechanisms of CO_2 sequestration in the form of suberin and its release in need. Thus, in the long term pistachio plants are the ideal crops for climate change resilience.

Evaluating new training systems for pistachio

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Introduction

This study was initiated to investigate alternative training systems for pistachio. 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. The conventional training method involves heading the trees at approximately 43 inches and then doing in-season tipping and dormant heading cuts to generate the desired tree structure. Some California growers have been using a modified central leader training system and the results of these orchards look promising with good tree structure and the first commercial harvest being moved up by 1 to 1.5 years. The unpruned treatment was untouched except for removing any branches that were too low.

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

Results and Discussion

Trial #1 Kings County- Midday stem water potential was measured approximately every month in 2017 and approximately every two weeks in 2018 and 2019. There were no significant treatment differences in midday stem water potential on any date in any year but the conventionally pruned treatment tended to be the most stressed on most dates. By the fall of 2019, although there were no differences in scion diameter among treatments, the unpruned trees were significantly taller compared to modified central leader or conventionally trained trees. The rootstock diameter was significantly smaller on the conventional compared to the other two treatments. Trees were hand harvested in 2019 with the conventional, modified central leader and unpruned treatments producing 0, 0.5, and 55.3 pounds of good nuts per acre respectively.

Trial #2 Kings County- This orchard was only flood irrigated on 3 dates in 2018 and on two dates in 2019 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. In 2019, there were no significant treatment differences in height, rootstock diameter, or scion diameter. Trees were hand harvested in 2019 and yields for conventional, modified central leader and unpruned treatments were 0, 0, and 16.2 pounds of good nuts per acre.

Trial #3 Yolo County- This trial utilized nursery grafted trees. There were more problems with leaning trees than at either of the other trials described above. This has been previously observed by others and likely these trees are more flexible due to having been raised in crowded conditions in the nursery. There was extensive cold damage from the nursery in these trees at planting. This did not impact the conventional or modified central leader trees since the damaged tips were pruned off during the dormant season. However, approximately 50% of the shoots on the unpruned trees were damaged and behaved and these shoots behaved like pruned shoots. Approximately 10 conventionally trained trees broke loose of ties to the wooden stakes on extreme north wind days and bent over towards the ground as if they were made of rubber. By November 2019, rootstock diameter was significantly larger on unpruned compared to either other treatment but scion diameter and tree height did not vary among pruning treatments. Trees trained to metal stakes were much stronger and were largely strong enough to have stakes removed by the end of the first year. There were no significant differences in rootstock diameter, scion diameter and tree height for the wood versus metal staked trees by Nov. 2019. There was no cropping at 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. Yields were significantly higher for the unpruned compared to either of the other treatments at both Kings County sites. Data collection will continue in all three of these trials in 2020.

Identification of Superior UCB-1 Rootstocks Using DNA Markers: Phase 3: 2019

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Introduction

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

We are currently completing the first year of Phase 3 of a multi-year project that started in 2013. 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. We have also genotyped all of these tress by sequencing. Simultaneously, we have been 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 commercial orchards that are planted with UCB-1 seedling rootstocks. We are separating out the effects of environment and genetics on vigor and providing molecular markers to allow the early identification of inferior seedlings that need rogueing prior to planting in orchards.

Results and Discussion

Since 2013, we have sequenced 1822 trees and collected rootstock and scion circumference measurements from almost 2,009 UCB-1 and 197 PG I trees from ten commercial orchards in the Central Valley. In 2019, we added two more orchards to our analysis to increase the age range of orchards studied. The rootstocks in these orchards now range in age from 3 to 18 years, are grafted with either *P. vera* cvs. Kerman or Golden Hills, and are located from Merced to Bakersfield. Phenotypic data from all of these orchards were collected again this year. In addition, we have extracted DNA samples from bark and are in the process of sequencing 384 trees from two additional commercial orchards located in Huron and Madera. Interestingly, trees in all UCB-1 orchards sampled have 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 circumference, in both UCB-1 and PG I orchards.

In 2019, we collected another round of multi-trait phenotyping data in the experimental orchard (including trunk caliper, tree height, and canopy diameter). This dataset confirms that tree height and trunk caliper in early years is are poor predictors of tree size in later years. We were also able to record the sex and flowering time for the 203 trees that reached sexual maturity.

From March 2018, we began regular drone flights over the experimental and commercial orchards to capture multispectral imagery. This year we also captured multispectral drone imagery for eight of the commercial orchards. In total, this has generated over 57.2 gigabases of data that we are now in the process of analyzing. These data are being used to demonstrate the relationships between canopy volume size, tree height, growth rate, and rootstock caliper.

Last year, we generated 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). This year, we completed ultra-long-read nanopore sequencing for all three species, allowing us to greatly improve the assemblies as well as generating a draft assembly for *P. vera* cv. Kerman. These provide high quality genomic resources for pistachio. Combined with genetic information, these allowed us to identify the regions of the genome associated with sex determination in all three species. This will allow us to develop molecular markers for sex. We also developed very high resolution genetic maps for both *P. atlantica* and *P. integerrima* using genotyping by sequencing data of the UCB-1 trees in the experimental orchard at Davis. These maps confirmed the contiguity and accuracy of our genome assemblies, as well as refined the location of the genomic regions determining trunk caliper. These maps also identified a large region of the sex chromosome supporting a ZW-sex determination system.

Conclusions

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

Over seven years, we have collected a large amount of phenotypic and genetic data from both experimental and commercial orchards and have now data for more than 3,000 trees. This year we continued our multi-year multi-trait phenotyping of the experimental orchard in Davis, as well as sampled four hundred new trees from young (3 years old) and old (18 years old) commercial orchards. Multispectral aerial imaging surveys of the orchards that we have sampled was conducted. This will accelerate and enhance our phenotyping efforts. We have completed chromosome-scale genome assemblies for three *Pistacia* species: *P. atlantica*, *P. integerrima*, and *P. vera* cvs. Kerman and Siirt and have deployed a novel high-throughput, low-cost, genotyping and trait association approach that allows us to handle data from thousands of trees and dozens of traits simultaneously. We are using these data to separate out the effects of environment and genetics on stunting, as well as better understand the growth characteristics of UCB-1 and how it relates to canopy volume.

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 based on phenotypic characteristics is unlikely to be effective. Furthermore, we have observed a bimodal size distribution of trees in all commercial orchards sampled. This reflects a genetic basis for the stunting phenomenon observed by growers. We have identified molecular markers for two chromosomal regions that determine trunk caliper and explain this size distribution in commercial orchards. 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.

Development of new, reliable, vigorous, clonal rootstocks

Authors: **John E. 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 the 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 establish in vitro and multiply material from proven trees in the field.

We surface sterilized seeds and established 120 clonal seedling lines in vitro. We have been studying the growth of these shoots in vitro to understand the variation. When molecular markers for vigor are available, these lines are in position to produce clonal trees to verify growth differences and the robustness of the markers.

Results and Discussion

Data were collected every 4 weeks at transfer on 120 seedling clones on shoot number and length, and callus volume of each clone, using 4 replicates/clone. At each transfer, shoots were excised to reduce each culture vessel to a single shoot. There was substantial variation for in vitro growth among the seedling lines. Most clonal lines (70/120) elongated a single shoot; however, 2 clones produced at least two shoots (one primary and one axillary shoot) each month, which doubles the production rate of most clones. Likewise, most shoots were shorter than 2 cm tall after each month in vitro; however, 8 clones were ≥ 2 cm tall and 1 clone consistently grew to be over 3 cm tall at the end of each month. Ability to micropropagate efficiently, as seen with some seedling clones, is important for efficient commercial micropropagation. Rooting successes have improved throughout the year. Some of the seedling lines have shown initial rooting successes of 75%. Many of our seedling lines seem to struggle to root, so rootability may be a strong selection factor.

Additionally, meristems have been excised new field materials have been introduced from the Russell Ranch planting that has been phenotyped. These are the most vigorous trees in the orchard. These have been tested repeatedly and show no sign if endophytes.

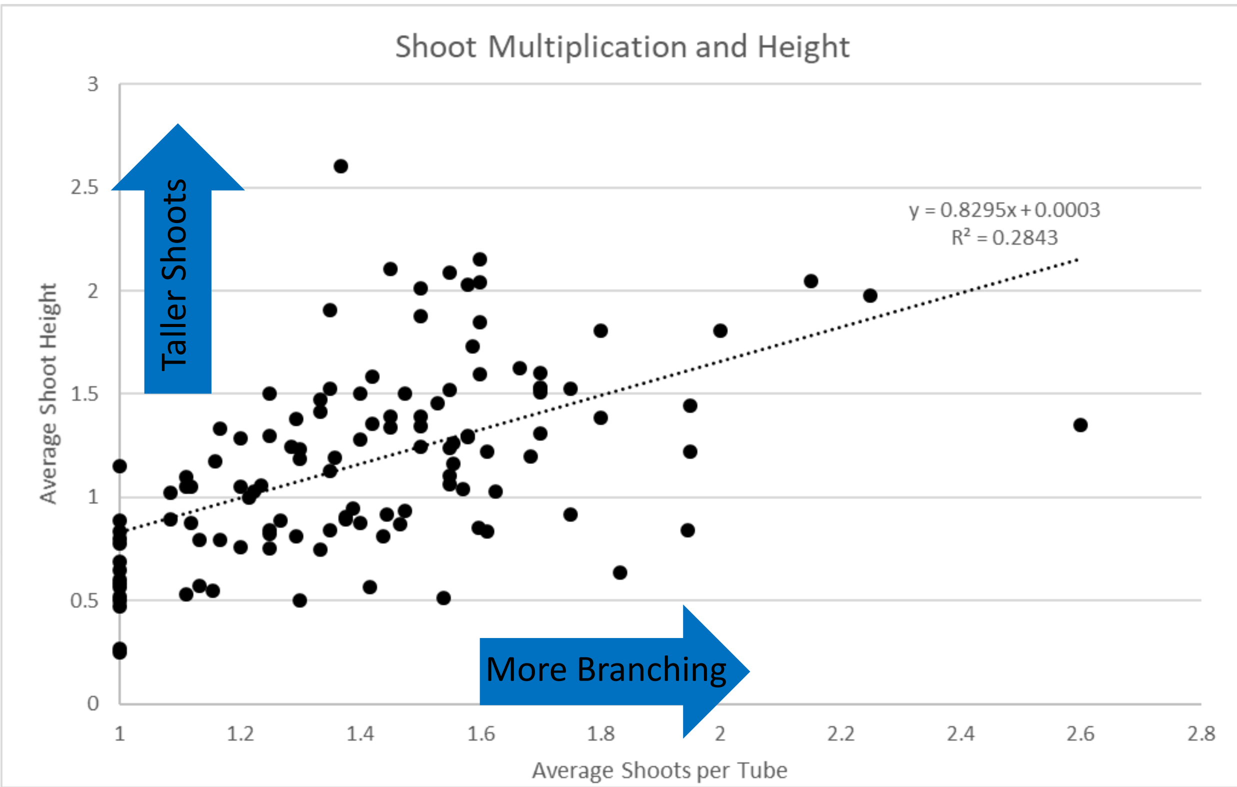
We have worked with seedling germination extensively and improved on our previous methods. Previously, we had worked with a more traditional route to scarify seeds in highly concentrated (18 Molar, 36 Normal) sulfuric acid. This worked to soften the seed coat and simultaneously sterilize the outer pericarp allowing us to implant clean seed into media.

We have since developed a method by which we can cut the seeds outside the sterile hood environment and sterilize the seed utilizing Sodium Dichlorocyanuric acid (NaDCC) in a 3-hour agitated soak. Utilizing NaDCC, we have improved our germination protocols and currently have 120 seedling lines with 4 tubes of each. Leaf samples of each line has been collected and distributed to the Michelmore lab. We hope to utilize the genetic markers they are developing to not only rapidly screen our seedling population for viable rootstock candidates, but also aid in confirming his markers.

Last year, three vigorous trees from a planting of 1,200 nongrafted UCB-1 seedlings were identified based on 4 years of field growth measurements. The meristem cultures excised from these trees have developed and have been multiplied. We currently have approximately 300 shoots between the three trees cultured in this way. We have had limited success in producing adventitious roots on these cultures.

With the 5th year of data available, 3 new trees have been identified and we plan to place similarly place these trees in culture as well.

Figure 1. Microshoot number and height vary greatly among UCB-1 seedling clonal lines.



Conclusion

All seedlings tested grow and produce rootable shoots in tissue culture; however, there is variation among the clonal seedling lines. Differences in micropropagation efficiency would be a valid selection criterion in addition to selection for vigor. A clonal rootstock with good vigor would have limited success if there are propagation barriers. If molecular markers for vigor can be developed, this population of UCB-1 clones is set to be tested and potentially produce plants that have good, moderate, or poor vigor for field testing. If there is a delay in marker development, using this technology, it will be easy to establish a new population of seedling clones in vitro.

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

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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, rootstock bark overgrowths at the nodes, and abnormal growth and cracking at some graft unions became known as Pistachio Bushy Top Syndrome (PBTS). These symptoms may be caused by *Rhodococcus fasciens*, 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. We obtained subclonal shoot cultures of the clonal UCB-1 line from 3 commercial labs. This has resulted in 7 subclonal lines that DNA tests confirmed all matched "Duarte Old Clone 1," and therefore can be traced back to the same female mother tree. Some of these labs had been producing plants that exhibited PBTS symptoms. The cultures were tested and are free from *Rhodococcus*. Shoots are being rooted and acclimatized to the ambient environment to be planted in a replicated study in 2020.

Results and Discussion

We are quantifying growth differences among the subclonal lines for growth in vitro. Some of the lines are more vigorous than others when in culture. An example of observable differences is that one of the lines (Z03) produces some shoots that are fasciated (fused), whereas this occurs with none of the other subclones. Our data are beginning to show that the putative "normal" type (Z02) appears weaker in vitro compared to the other subclones from the same lab. That may explain why technicians doing tissue culture transfers may have inadvertently selected those subclonal lines that grew better, but performed poorly in the field. The percentage of these in orchards increased in newer plantings, likely resulting from technicians selecting "quality" shoots over poor growing shoots when doing tissue culture transfers. These in vitro differences may be caused by bud sports that are common in fruit crops, or from somaclonal variation.

Table 1. In vitro growth differences of UCB-1 shoot cultures were received three commercial laboratories

	Laboratory 1			Laboratory 2		Laboratory 2	
	Subclonal line no.			Subclonal line no.		Subclonal line no.	
	Z01	Z02	Z03	X03	X04	Y01	Y25
Mean number new shoots per tube	4$\pm$1.91	2.7$\pm$0.78	4.18$\pm$3.95	3.22$\pm$1.2	3.25$\pm$2.05	3$\pm$1.13	3.22$\pm$1.09
Mean tallest shoot	3.06	1.875	3.33	1.8	1.73	2.12	3.2
Mean height of all shoots across all tubes	2.39	2.09	2.74	2.07	1.77	2.1	2.7

Rooting has been challenging with some of the subclonal lines. However, shoots continue to be rooted and acclimatized to the greenhouse environment in preparation for field comparisons in 2020.

Conclusion

Shoot culture from different labs and from within the same lab of all the same "clone" have growth differences in culture. This may explain why technicians may have inadvertently selected more and more good looking shoots that unfortunately produced off-type plants.

Some subclones have been rooted and acclimatized, whereas other subclones are being rooted and hardened to the greenhouse environment.

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

Author: Maciej Zwieniecki, Professor, Department of Plant Sciences, UC Davis

Introduction

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

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

Results and Discussion

Near real-time results of NSC content are presented via the Carbohydrate Observatory data website: http://zlab-carb-observatory.herokuapp.com/. Our website is freely accessible and allows participating growers to review and compare NSC content in their orchards with the overall values observed throughout the entire Central Valley. Carbohydrate content in the form of soluble sugars and starch can be analyzed across varying aspects, ranging from large spatial dimensions encompassing geographical parameters (county) down to individual tree components (rootstock, scion, age).We are currently completing the third full year of data collection.

(1) NSC and Yield

Using yield data from volunteering farmers, we were able to determine a relation between monthly levels of NSC and the following year's yield (Figure1).

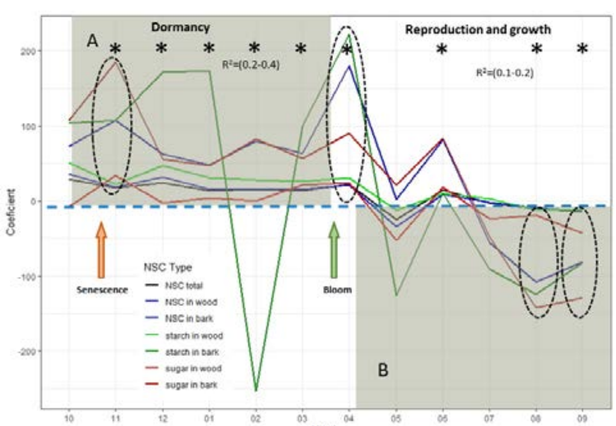


*Figure 1. Shown is the slope of correlation between carbohydrate content and yield (coefficient of linear function formulated as: yield= coefficient*NSC + offset. Region 'A' shows a generally positive correlation (coefficient>0) of winter NSC levels with following summer yield. Region 'B' shows a generally negative correlation (coefficient <0) between current summer yield and level of NSC. '*' denote significance with p<0.05 for the correlation.*

Overall, orchards with high levels of carbohydrates in the fall were more productive during the following season than those with high levels of NSC in the summer. Specifically, high levels of carbohydrates particularly in October and November provide high correlation values (Fig. 1). In addition, April NSC levels in bark were also a good predictor of high yields. Interestingly, high yields were associated with the exhaustion of NSC reserves in August and September, demonstrated by the strong negative correlation of current year yield and NSC content (Fig. 1). Late summer exhaustion may lead to low NSC levels in October and November (no time to recover NSC reserves during senescence) resulting in low yields the following season; and thus, predict and explain the alternate bearing character of pistachio orchards. Now we can ask the question if and how post-harvest management practices can overcome the natural NSC depletion during high yield years. We can also provide a focused goal for breeding programs that aim at reducing alternate bearing in pistachio in proposing breeding for either high NSC content or fast accumulation of NSC in fall.

(2) NSC based mechanism of dormancy

Thermal conditions during fall/winter/spring are major determinants of pistachio phenology. However, the biological principals governing a plant's internal clock which result in synchronous senescence and bloom remain unknown. Our proposed mechanism detailed in the recent publication (Sperling, et al., 2019) uses spring sugar 'starvation' as a trigger for bloom. Following analysis of the extensive data set generated by the Carbohydrate Observatory, a mechanism, which is applicable to most nut species, was proposed. It allows for the concurrent tracking of chill and heat hours over the dormancy period. The beta version for the project is currently available for almond at: http://zlab-chill-heat-model.herokuapp.com/. Analysis and test of bloom predictive model for Pistachio is being tested using available bloom data from pistachio trial.

(3) NSC manipulation influences bloom time and synchrony

Experimental manipulation of pistachio trees was performed to determine the role of early defoliation on the fall accumulation of NSC in combination with girdling, which affects the redistribution of NSC in dormant trees. Defoliation was applied in October and November, with girdling in October, December and March. October defoliation affected November content of NSC and if the redistribution of NSC was not impeded by girdling, branches recovered content by late December. Early girdling resulted in the maintenance of higher NSC content in the branches, but March girdling resulted in reduced content just prior to bloom. The combination of both treatments generally resulted in lower NSC content during bloom time. Apical buds' development on all defoliated and girdled branches were delayed significantly (over a week later than control branches). Similarly, lateral buds were delayed by defoliation and girdling with November defoliation in combination with March girdling almost completely halting bud growth. This finding suggests that senescence affects bloom time (by the potential inadequate accumulation of NSC resulting in bloom delay) an that spring conditions affect NSC redistribution and are important for synchronous bud breaking. I rewrote this sentence here: These findings suggest that the conditions surrounding senescence, specifically the accumulation of NSC, and spring, the redistribution of NSC, affect bloom time and play an important role in synchronous bud break.

Conclusion

- Fall NSC content in twigs between October and December as well as content of NSC during bloom (April) can be positively linked to yield. High yield results in NSC exhaustion in late summer where high yield results in low NSC content.
- Alternate bearing can be linked to the accumulation-exhaustion pattern of NSC and suggests that the development of management practices that reduce the exhaustion of NSC or rapidly restore reserves can lead to a reduction in alternate bearing.
- A predictive model that includes winter thermal conditions to evaluate the progress of dormancy in combination with tree NSC management can be formulated for Pistachio.
- Natural senescence and spring thermal conditions promote NSC redistribution across the tree crown result in synchronous early bloom. All conditions that reduce fall NSC content or affect spring redistribution of carbohydrates result in bloom delay and asynchronous bud break.

Pistachio irrigation training module & monitoring demo sites

Authors: **Blake Sanden**, UCCE Irrigation Management Farm Advisor (emeritus), Kern; **Daniele Zaccaria**, UCCE Agricultural Water Management Specialist, UCD; **Kristen Shapiro,** Staff Research Assoc., UCD; **Richard Snyder,** Biometeorology Specialist (emeritus), Dept. LAWR, UCD; **Khaled Bali**, UCCE Irrigation Management Specialist, Kearney Agricultural Center

Introduction

California pistachio acreage has expanded by more than ¼ million acres in the last 10 years. Most of this expansion has occurred on marginal soils unsuited to other permanent crops but manageable for pistachios due to the high degree of salt tolerance of these trees (Sanden et al., 2004, Ferguson et al., 2002). Production experience farming orchards on these soils and even non-saline soils has shown that real water use for these orchards is often less than that predicted by using the ET crop coefficients (Kc) developed by Dave Goldhamer (1995) from a study done on the Westside with Bob Beede in the mid 1980's. Using the average "normal year" potential ET for the SJV the Goldhamer numbers predict a 45-48" seasonal water use for pistachios. A recent Pistachio Research Board and CA DWR project by Daniele Zaccaria (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) using state of the art energy flux monitoring indicates a reduced water use by as much as 50% in mature pistachios as salinity increases. Combine the impact of salinity and soil structure on pistachio ET with the protracted juvenility of orchard development (taking 6 to 9 years to reach just 50-60% cover), recurring droughts and reduced groundwater pumping under the Sustainable Groundwater Management Act (SGMA), it is absolutely imperative that pistachio growers have an effective capability for real-time soil and tree water status monitoring to do optimal irrigation scheduling. Growers are aware of this and eager to find the best options to monitor their orchards to insure optimal growth, yield and efficiency. However, there is a dizzying and expensive array of technology out there that leaves most growers confused. This project is trying to clear the fog and help growers evaluated for themselves what technology would help most for their particular orchard setting. With this goal in mind, we are using state of the art soil and plant monitoring technology in three mature pistachio production fields (Kerman/UCB1) from eastside to westside with increasing salinity to provide real data for irrigation monitoring and training by attempting to achieve the following **objectives:**

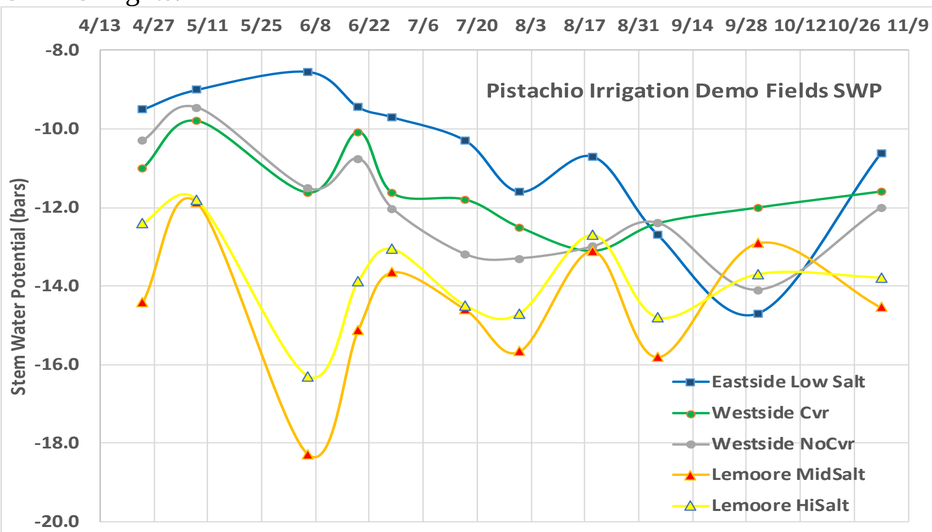
- Showing/comparing real-time web-based and field data for optimal irrigation scheduling
- Showing the impact of salinity on monitoring and ET
- Provide traditional workshops, field meetings, newsletters and an open-access website for training
- Provide an on-line real-time BLOG for comments/explanation/training

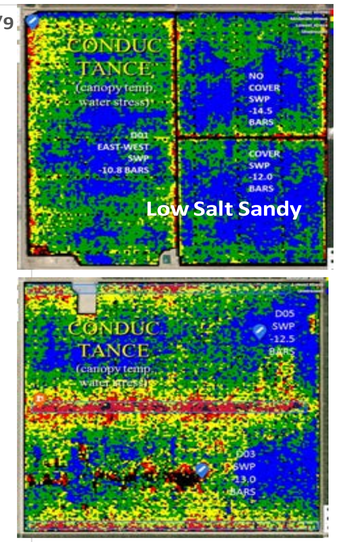
Results and Discussion

There was some initial confusion in approving and funding this project as it was originally linked to the Zaccaria ET project as both use common field sites. To have the real-time ET data generated from the Zaccaria project is an important confirmation of the optimal monitoring and scheduling that comes from this DEMO project. However, each project can structurally operate independently. This was finally made clear and funding of the DEMO project commenced June 1. Some field instrumentation had started earlier, but the late confirmation of the project has meant a lot of catch up on field data collection and website set-up meaning that there has been no major grower outreach during the 2019 season. We only just now have the website functioning with an explanation of the project using a narrated PowerPoint file and supporting data at a click with an understandable user interface. Currently, only a season-long summary of different data is available to growers. A truly interactive website will be available in 2020.

URL: https://fruitsandnuts.sf.ucdavis.edu/pistachio-irrigation-training-field-monitoring-demo

Monitoring equipment: Recognizing the need for side-by-side comparisons and clear examples/explanations for growers we have received support/contributions of equipment from commercial providers to instrument this project. **Pre-existing (from Zaccaria project):** WaterMark blocks and pressure transducer tensiometers at 18, 36 and 48 inch depths readings recorded hourly by field logger (not on-line), Phytech dendrometers measuring tree trunk shrink/swell (real-time web reporting), pressure chamber stem water potential measured using the wet rag technique. **New site equipment:** Sentek Drill & Drop capacitance probe with Jain Logic reporting and pressure sensors tracking run time (real-time web), CERES imagery biweekly (4/24, 5/8, 6/6, 6/19, 6/28, 7/17, 7/31, 8/19, 9/5), additional Phytech dendrometers, SWP & neutron probe monitoring to 9 feet corresponding to CERES flights.





		SATURATION %				EC (dS/m)				7/1/2019 - 10/2/2019					Metered	Inches/%	Inshell[2]
SITE	SOIL	7/14 2015	11/15 2016	2/10 2018	4/25 2019	7/14 2015	11/15 2016	2/10 2018	4/25 2019	Pressure Sensor Hrs	Calcultd Inches	Metrd Inches	Avg SWP (bars)	Canopy[1] Cover (%)	Irrigation 2019 (in)	Canopy Cover	Yield (lb/ac)
Eastside non-saline	Colpien L (Nord)	30	32	37	29	1.8	2.7	3.8	8.9	388	16.29	17.0	-10.6	68%	36.5	0.54	5,515
Westside non-saline	Cerrini SL (Excelsior)	25	--	26	21	--	--	2.8	4.3	870	18.26	19.8	-12.0	67%	36.1	0.54	*708
Lemoore semi-saline	Lethent CL	52	55	60	47	7.2	7.8	6.7	10.8	636	13.35	14.5	-14.5	46%	20.4	0.45	2,883
Lemoore saline	Lethent SiCL	59	62	79	54	8.2	8.2	7.4	11.2	502	10.54	11.4	-13.8	40%	17.3	0.44	1,615

[1]Bruce Lampenin mobile undertree survey of % photosynthetically active tree cover.

[2]Average plot yield for 90 trees centered around site instrumention. *Annual average yield for this field was 4,000-4,500 lb/ac previous 8 years.

Conclusion

There is no substitute for good dirt! Growers do a good job reducing irrigation when saline soils restrict root water uptake and increase tree stress (above figure for SWP), but the result is reduced tree canopy and yield. A combination of field monitoring technology checking soil and plant moisture status. We cannot yet say what is the optimal combination of these technologies at this time. Stay tuned in 2020.

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Evaluation of Pistachio Scion-Breeding Selections, 2019-20

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

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. Interested, gracious and generous private cooperating growers have made many of these trials possible with their donations of land, labor, equipment, time and long-term use of land.

In 2019, we continued evaluation of nine male and/or female advanced scion-selection trials. These trials were planted from 2007 to 2016. Five of these trials have the objective of identifying male advanced selections, 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. Several male selections in some of these trials are demonstrating better bloom synchrony and precocity with Kerman than existing cultivars. The other four scion selection trials compare novel advanced female scion selections with existing commercial cultivars such as Gumdrop, Golden Hills, Lost Hills or Kerman for yield, nut quality characteristics, bloom and harvest timing. These trials are located in areas with varying amounts of winter chill. We continued to evaluate the cultivar Gumdrop in the oldest trial containing this U.C. cultivar and in a demonstration trial within a Lost Hills orchard established in 2014.

In 2018, we identified several selections, from the seedling, breeding trial planted in 2012 that not only displayed harvest maturity equal to or in advance of Golden Hills, but showed nut characteristics that, based on the past work of other researchers, may make them less susceptible to navel orangeworm damage. The nuts of these trees showed these same potentially useful characteristics this season. In the summer of 2019, these trees were cloned by grafting buds on Platinum®, UCB-1 seedling and/or experimental seedling rootstocks into two separate randomized and replicated experiments. One of these experiments was established within a mature advanced-selection trial that is providing excellent habitat for navel orangeworm and should continue to do so once these experimental selections begin producing nuts.

The advanced selection called ‘KB25-78’, which is being evaluated in the Jasmin trial, has demonstrated characteristics not seen in other selections to date. The Jasmin trial is located in the ‘citrus belt’ of Kern County where winter chilling can be inadequate. The clean, open, inshell split nut percentage of KB25-78 has averaged a modest 67% over the past 4 years, similar to that of Kerman (Table 1). However, despite this relatively low split-nut percentage, cumulative edible yield is 2000 lbs. per acre higher than the next highest yielding cultivar ‘Lost Hills’ (Table 2). ‘KB25-78’ has two traits that may account for this enhanced yield performance. It blooms early, a week before Golden Hills, and harvests later with ‘Kerman’, so it takes full advantage of the available growing season (Table 2). In addition, it produces many branch spurs, especially early in its lifecycle, which provide locations for more flower buds. While these results are interesting, the question then becomes, for a selection such as ‘KB25-78’, would growers and processors be interested in producing pistachios using tree that has a moderate split nut percentage but a higher potential to produce edible yield? In short, is it worth continuing to collect data on this selection? Industry feedback related to this question is important for this breeding program and would be welcome. One reason for continuing to evaluate this selection is that split percentages in this trial have been lower for the existing cultivars Kerman, Golden Hills and Lost Hills than is normal, suggesting that split nut percentages for KB25-78 may be higher if grown at a different location. For now, we will continue to evaluate this selection at Jasmin, reexamine past data from an existing trial in Arizona, and begin to collect data from an additional trial just coming into bearing in 2020 (sixth leaf) on the valley floor in the southern San Joaquin Valley.

We continued to evaluate other seedling selections from our breeding program that are progeny from parents that have displayed greater tolerance to an inadequate winter rest period (i.e. less chilling), have displayed fewer leaf-canopy and flowering symptoms of inadequate chilling themselves or have genetics that should reduce their chilling requirement. Evaluation is being conducted in a trial planted in the Coachella Valley of California in 2017, where chill is dependably inadequate for current pistachio cultivars. The seedling trees and grafted trees, both experimental and existing scion and rootstock cultivars, are demonstrating a diversity of vegetative growth under low-chill conditions. This year we doubled the number of plant entries. This trial will become more interesting once the trees are old enough to bloom.

Table 1. Selected nut quality characteristics averaged from the first harvest (2016, 7th leaf) through 2019 (10th leaf) of the three highest-yielding commercial pistachio cultivars and the advanced selection KB25-78 in the Jasmin trial, Kern County

Variety	Inshell split nuts, % [A]	Edible closed shell, %	Harvested blank nuts, %	Loose shells and kernels, %	All Insect damage, %	Number of nuts per oz.
Kerman	66 a [B]	20.6 c	8.6 b	0.3 a	0.6 a	21.1 b
KB25-78	67 a	15.1 bc	13.8 c	0.2 a	0.7 a	20.0 a
Golden Hills	78 b	13.5 b	4.3 a	0.2 a	0.1 a	21.7 b
Lost Hills	84 b	4.9 a	5.1 a	0.8 b	1.2 a	19.1 a

[A] Percentages are expressed as percent of the total weight of harvested nuts, adjusted to 5% moisture, for the given nut quality characteristic in that column. Not all nut quality characteristics are tabulated.
[B] Values followed by different lower-case letters in the same column denote significant differences by Fisher's protected LSD test at $P \leq 0.05$.

Table 2. Average full bloom and harvest readiness date, edible yield percent by weight and cumulative edible yield in lbs. per acre from 2016 – 2019 (7th - 10th leaf) for pistachio cultivars and the advanced selection KB25-78 in the Jasmin trial. Kern County.

Variety	Year of first harvest	Ave. full-bloom date	Ave. harvest readiness date	Edible yield, % [A]	Cumulative edible yield, lbs./acre [B]
Kerman	2016	April 13	Sept. 15	31.1 a [C]	8818 b
KB25-78	2016	April 2	Sept. 14	30.9 a	13681 d
Golden Hills	2016	April 10	Sept. 1	33.7 a	10476 bc
Lost Hills	2016	April 11	Sept. 5	33.7 a	11245 c

[A] This category is the percent of the total green harvested material that is edible yield (i.e. payable weight) adjusted to 5% moisture.
[B] This category is the cumulative edible yield (i.e. payable weight), in lbs. per acre, adjusted for 5% moisture and corrected for the percentage of male trees per acre in the orchard.
[C] Values followed by different letters in the same column denote significant differences by Fisher's protected LSD test at $P \leq 0.05$.

Evaluation of Pistachio Rootstock-Breeding Selections, 2019-2020

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 U.C. Farm Advisor Joseph Maranto. Since 2009, the breeding program has included development of experimental rootstocks (please see a separate report on our scion breeding program).

As part of this breeding program, potential seedling rootstocks originating from breeding crosses made in 2009 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 UCB1 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 is the identification of breeding lines or individual rootstocks that produce higher early yields, have a reduced pruning requirement with a closer tree spacing, may confer greater cold and salt tolerance, a smoother graft-union and comparable Verticillium wilt and Phytophthora root and crown rot resistance to that possessed by existing commercial rootstocks. The first harvests of two of these rootstock trials occurred in 2017.

Results and Discussion

The rootstocks from the U.C. breeding program are novel in that the parentage is different from UCB1 or pure *P. integerrima* rootstocks. One of the seedling rootstock-selection trials was planted in a colder and saltier area of the SJV in 2013. Each of the experimental rootstocks in this trial originated from a seed and each was grafted to the Lost Hills scion cultivar. While each experimental rootstock is genetically distinct, often from crosses with different parents, they are somewhat similar based on the interspecific crosses in their parentage. Yield and nut-quality characteristics of the better performing individual trees in this trial were measured in 2017 and 2018. In 2019, yield was estimated visually on the tree. These data were compared to UCB1 seedling controls planted within the same trial. Cumulative yield per tree over the past three years has been better for the experimental rootstocks than for the UCB1 controls, although the UCB1 seedlings are catching up. These experimental rootstocks, generally, produce a more compact, less vegetatively vigorous 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 equivalent yields in the future. These experimental rootstocks, at this stage, suggest that they may be amenable to planting at a closer tree spacing with reduced future crowding than UCB1 seedling rootstocks. These less vigorous rootstocks may have a reduced pruning requirement per pound of nut produced. 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 good cold tolerance. These rootstocks demonstrate little suckering compared to UCB1 seedlings. 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 those in the rootstock trial 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). Almost from planting, the canopy of Golden Hills on UCB1 rootstock demonstrated large areas of leaf-tissue necrosis with early leaf drop, with few or no leaf symptoms on scions grafted to the novel experimental rootstocks. Replicated leaf-tissue analysis was undertaken in 2017 and yearly, and demonstrated that leaves of the UCB1 scion had roughly twice the boron concentration of the experimental rootstocks. Over the past three-year period, cumulative edible yields between UCB1 and the experimental rootstocks in this high boron soil have not been significantly different at the 5 percent level of significance. However, the highest yielding experimental rootstock averaged 2743 lbs./ acre of edible yield since 2017, compared to the UCB1 seedlings at 1746, which was significantly different at the 7% level. Additional evaluations are being made in replicated trials established using large pots, using both grafted seedling and cloned experimental and commercial rootstocks, to further document differences in boron uptake made in the field trials.

Conclusions

These rootstock trials are in the initial stages. What the potential differences in performances noted among our experimental and UCB1 seedling rootstocks might mean for future pistachio production 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.

Improving Pistachio Harvesting Machines Using a Tree-specific Feedback Loop

Authors: **Dr. Reza Ehsani**, Professor, Mechanical Engineering, University of California, Merced; **Dr. YangQuan Chen**, Professor, Mechanical Engineering, University of California, Merced; **Dr. Louise Ferguson**, Professor, Department of Plant Sciences, University of California, Davis.

Introduction

Nuts are one of the most suitable crops for mechanical harvesting because of their hard shell and the physical properties of the nut trees. Currently, trunk shakers are the most commonly used mechanical harvesting system for harvesting pistachios. Trunk shakers have not been significantly modified since the late 1970s. On most of the existing commercially available trunk shakers, the shaking frequency can be adjusted by the operator. The newer harvesting systems can create different shaking patterns, varying intensity vs. time. These patterns can be programmed into the computer that controls the shaking system. This ability is very useful and allows the operator to adjust the shaking pattern for different trees. However, determining the best shaking frequency and pattern for each size of tree is not very well known and is subjective. It relies heavily on operator experience and is done by trial-and-error. Determining the best shaking frequency and shaking pattern is challenging because the optimum choice is different for each tree and is a function of tree canopy size, mass, amount of leaves, and branch configuration.

Results and Discussion

A series of field tests were conducted at Wonderful Pistachio orchards in Lost Hills, CA, in September of 2019. Thirty trees were chosen for the experiment. Three categories of trees were chosed based on their trunk circumference size: 1- Small circumference less than 25″, 2- Medium circumference between 25″ and 37.7″, 3- Large circumference greater than 37.7″. These sizes were selected so that trunk diameter would be less than 8″ for small, between 8″ and 12″ for medium, and greater than 12″ for large trees. To find the best shaking frequency for each tree, a series of standard shaking pattern inputs has been designed, which includes a ramp input, a step input, as well as another pattern that has been used by the operators of the harvesting machines during the 2018 harvesting season. Medium and large trees were shaken four times and small trees were shaken two times, as shown in Table 1. Patterns are shown in Figure 1. First, each tree was shaken two times with the same pattern. This was done to study the effect of overall tree weight loss due to fruit removal.

Table 1. Shake sequence of each tree size

	SHAKE 1	SHAKE 2	SHAKE 3	SHAKE 4
SMALL	Pattern 4	Pattern 4		
MEDIUM	Pattern 1	Pattern 1	Pattern 2	Pattern 3
LARGE	Pattern 1	Pattern 1	Pattern 2	Pattern 3

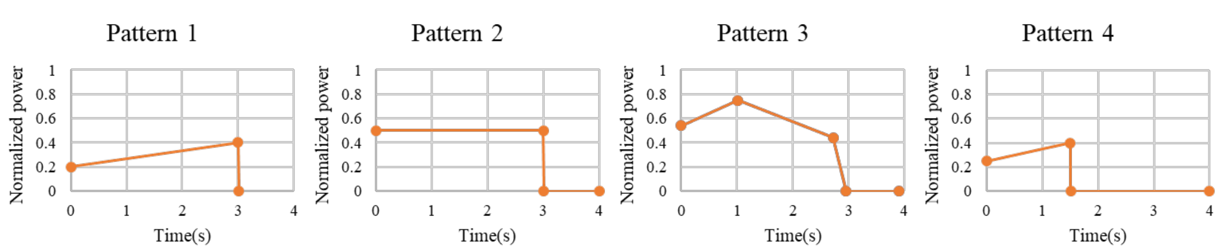

Figure 4. Four patterns used in the field experiment. Pattern 1 and 4 are ramp inputs, pattern 2 is a step input, and pattern 3 is what has been used by the operator during the 2018 harvest season.

Multiple wireless sensors were designed and built to record and monitor tree and machine movement during each shake. These sensors include an accelerometer, a wireless module, and a storage module. A wireless hub was built to inter connect these sensors and enable data recording. A high-speed camera was also used to record the movement of trees alongside wireless sensors. A sensor was attached on the shaker head and three sensors were attached to each tree. A red marker (Fig. 2) was also attached to each tree to be used for tracking via the high-speed camera.

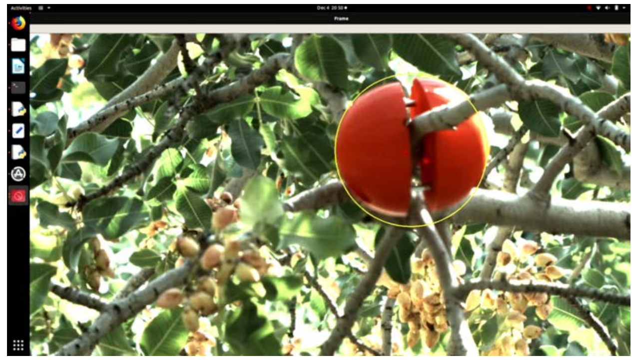

Figure 5. High-speed camera used to track a red marker on tree for shake monitoring

It was observed that the shake duration for most of the trials was about 30% longer than what was programmed into the shaker computer. Figure 3 shows the best shaking intensity for each tree, which was calculated based on the field test experiment. Using the trend line in this figure, the best shaking intensity for any tree can be found based on trunk circumference.

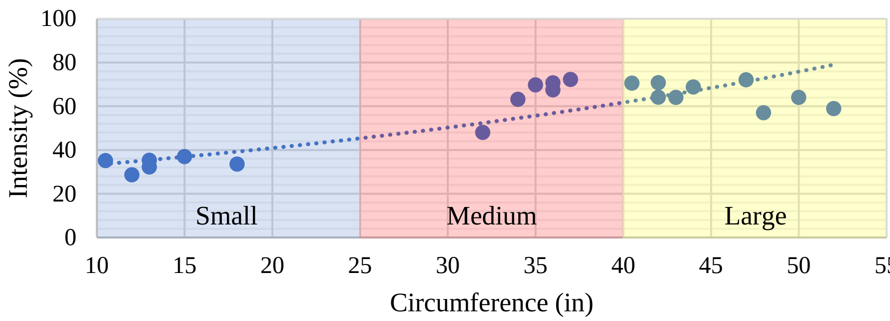

Figure 6. Best shaking intensity vs. tree circumference

Vision-based data showed a similar trend but needs to be fine tuned.

Conclusion

Effectiveness of a pattern for better harvesting efficiency is dependent on tree specifics, including trunk diameter (equivalent to trunk circumference), trunk height, and overall tree size. In this study, trees were categorized based on trunk size: small, medium and large. The effect of each pattern on each size was studied using wireless sensors and a high-speed camera system.

The vision-based system needs more tuning so it can be used independent of the wireless sensors. Then it can be used to monitor tree movement in real time as a tree is shaking, which can optimize the duration of the shake pattern.

A model was developed to find the best shaking intensity for any given trunk size. The best shaking intensity (nominal intensity) can be extracted using Figure 3. Based on this number, a shaking pattern similar to pattern 1 can be programmed into the shaker computer where the start and end points of the ramp would be 10% lower and 10% higher than the extracted number. For example, a tree with a circumference of 30″ would have a nominal shaking intensity of 50% (based on Figure 3). The shaking pattern for this specific tree size would start at 45% intensity and ends at 55%. The mass of a tree will decrease during a shaking session due to fruit removal resulting in an increase in the natural frequency. This increasing ramp pattern has been selected to adjust for this increase in the natural frequency.

Anthracnose of Pistachio in California: Distribution and Period of Sensitivity

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Introduction

The main pistachio production regions in Sacramento and San Joaquin Valleys were surveyed to quantify the extent of orchards affected by the anthracnose blight disease. In total, 24 commercial orchards located in seven different counties were visited to assess the presence of anthracnose symptoms in leaves, twigs and fruits. Cultivars surveyed included: Kerman, Red Aleppo, Golden Hills and an Iranian cv. not identified.

In a second study, we aimed to identify the period when pistachio is most susceptible to anthracnose infection. For that, monthly inoculations with *Colletotrichum fioriniae* (11K11) and *C. karstii* (3G24) started in Mid-April, using a suspension of 50,000 spores per ml of each isolate. For each combination of *Colletotrichum* species and inoculation month, we used 30 pistachio clusters of cv. Red Aleppo located at UC-KARE. Clusters were harvested in Mid-August to evaluate the symptoms of each single nut and determine the incidence (%) of the disease. Means were compared with ANOVA and separated with Fisher's LSD test at probability of 5%.

Results and Discussion

C. fioriniae was found on Butte and Glenn counties, two out of seven counties surveyed (Table 1). In Orland (orchards nº8 and nº22), *Colletotrichum* isolates were recovered from fruits, peduncle and leaves of Red Aleppo cultivar, while in Chico (orchard nº10), isolates were obtained only from the peduncle of Kerman cultivar (Table 1). In orchard nº8, where Red Aleppo is interplanted with Kerman, we were able to recover *C. fioriniae* from fruits twigs and leaves of the latter cultivar. The linear distances of orchard nº8 (where anthracnose was first reported in 2016) and orchard nº10 (Kerman) was 16 miles and orchard nº22 (Red Aleppo) was 5 miles. *Colletotrichum karstii* was reported in 2008 and 2010 in southern San Joaquin Valley but it was not found in 2019. We visited the same commercial orchard (nº13) where *C. karstii* was collected for the first time in the past.

Significant differences ($P = 2.2\times10^{-16}$) were observed among monthly inoculations made with *C. fioriniae*. Higher disease incidence was observed on clusters inoculated in mid-April (77.7% of fruits), resulting in severe cluster collapse as observed in the previous year (See Executive Summaries 2018). Month after month the incidence rates decreased progressively, reaching to 7% for inoculations made in July. Results are presented on Figure 1. *Colletotrichum karstii* did not caused the same disease levels as observed in the previous year and no differences ($P = 0.41$) were observed using this pathogen among the four inoculations made from April until July (Figure 1).

Conclusion

C. fioriniae was observed to occur in two new locations since its first report in 2016. The first new location was a Red Aleppo orchard (known to be very susceptible to anthracnose) and the second a Kerman orchard. The Kerman orchard presented only latent infection showing that this cultivar is mostly tolerant to pistachio anthracnose. On unsprayed Kerman trees 34% of surveyed nuts were free of symptoms (See Anthracnose fungicide trial 2019).

Early season infection by *C. fioriniae* can cause entire cluster to collapse if susceptible varieties are infected. A high *C. karstii* infection occurred in June with 12% of blighted nuts while this was not significantly different from other months.

Table 2. Orchards surveyed for the incidence of pistachio anthracnose in California 2019.

Nº	County	City	Cultivar	Fruit	Stem-end	Leaves
1	Sacramento	Sacramento	Kerman	-	-	-
2	Sacramento	Sacramento	Kerman	-	-	-
3	Yolo	Davis	Golden Hills	-	-	-
4	Yolo	Davis	Golden Hills	-	-	-
5	Yolo	Woodland	Kerman	-	-	-
6	Glenn	Orland	Kerman	-	-	-
7	Kern	McFarland	Kerman	-	-	-
8	Glenn	Orland	Red Aleppo	+	+	+
9	Butte	Chico	Kerman	-	-	-
10	Butte	Chico	Kerman	-	+	-
11	Butte	Chico	Kerman	-	-	-
12	Butte	Nord	Kerman	-	-	-
13	Tulare	Exeter	Kerman	-	-	-
14	Tulare	Tulare	Kerman	-	-	-
15	Tulare	Farmsville	Kerman	-	-	-
16	Colusa	Arbuckle	Kerman	-	-	-
17	Colusa	Arbuckle	Kerman	-	-	-
18	Colusa	Arbuckle	Kerman	-	-	-
19	Tulare	Ducor	Kerman	-	-	-
20	Tulare	Terra Bella	Kerman	-	-	-
21	Glenn	Orland	Kerman	-	-	-
22	Glenn	Orland	Red Aleppo	+	+	+
23	Glenn	Willows	Kerman	-	-	-
24	Yolo	Woodland	Iranian cvs.	-	-	-

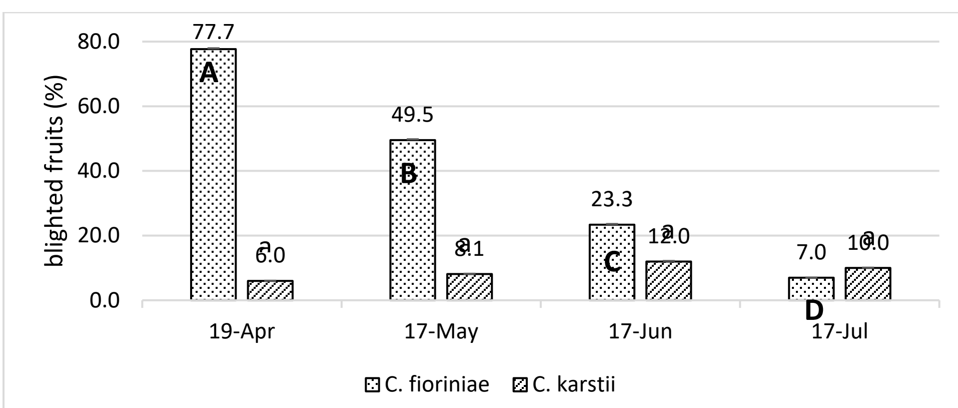

Figure 1. Seasonal susceptibility for *Colletotrichum fioriniae* and *C. karstii* infection of pistachio clusters. Bars with similar letters are not different at α=0.05 using the Fisher's LSD test. High and low case letter separates *C. fioriniae* and *C. karstii* means respectively.

Managing Anthracnose caused by *Colletotrichum fioriniae* on pistachio

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Introduction

In a Red Aleppo pistachio orchard in Glenn County, we evaluated three commercial fungicides registered for pistachio usage. They were: Merivon (a.i. fluxapyroxad and pyraclostrobin); Quash (a.i. metconazole); and Fontelis (a.i. penthiopyrad). All fungicides were applied with a handgun sprayer at maximum label rate. The surfactant (Dyne-Amic) was added to each fungicide mixture. Controls were untreated trees of cultivar Kerman and Red Aleppo. The trial included 7 trees per treatment. The calendar spray included four applications at monthly intervals, starting in mid-April and ending in mid-July, with evaluation of the diseases done in late August (two weeks before commercial harvest). Several pistachio clusters and leaves were randomly harvested from each replicated tree separately in the field and brought to the laboratory where 200 random fruit samples and 50 leaves per treatment were visually inspected for anthracnose lesions. A weather data logger monitored the orchard's temperature and relative humidity to support epidemiological conclusions. Statistical analysis was made with ANOVA and means were separated with Fisher's Least Significance Difference (LSD) at 5% probability.

Results and Discussion

The incidence of healthy pistachio nuts obtained in the 2019 fungicide trial revealed that none of the fungicides used could provide adequate anthracnose control. Treated and non-treated pistachio cv. Red Aleppo had more disease than the untreated pistachio cv. Kerman control. Even when we compared the incidence of fruits with anthracnose spots (one of the earliest disease symptoms), the fungicides Quash, Fontelis and Merivon did not provide statistically lower disease incidence than that of the untreated control of the Red Aleppo trees. Our trial also revealed a higher anthracnose tolerance by cv. Kerman (control), where only 8.1 and 16.8% of nuts were half- or full-blighted, respectively. On the other hand, the cv. Red Aleppo (control) was very susceptible to the anthracnose, with 11.6 and 67.1% of pistachio nuts being half- and full-blighted, respectively (Table 1).

The evaluation of pistachio leaves revealed similar trends as those presented for fruits, where the Kerman had higher frequency of healthy leaves than the Red Aleppo trees treated with Fontelis. The fungicides Quash and Merivon were statistically similar with Fontelis, but not different than Red Aleppo control (Table 2). Regarding the last category of leaves (Cat. 5, >16 lesions/leaf), Fontelis provided better control than Quash and Merivon when sprayed on Red Aleppo trees. However, for this same category, unsprayed Kerman trees obtained showed zero lesion counts, confirming the higher disease tolerance (Table 2).

Conclusion

The anthracnose fungicide trial suggests a failure of fungicides to control this disease in this specific orchard most likely because of multiple fungicide applications during the last 20 years to control the dominant disease of the orchard, the Botryosphaeria panicle and shoot blight might have resulted in selection of resistance among the *Colletotrichum* populations.

Fontelis has showed to be the most effective fungicide to control anthracnose on leves, as suggested by our trials *in-vitro* from 2017 (See Executive Summaries from 2017). However, it was not effective in controlling the fruit blight.

Climate conditions such as temperature (not showed) and relative humidity (Figure 1) reveals more conducive conditions for development of anthracnose disease in Glenn Co. in comparison with the conditions in Fresno Co. Considering this, orchards in northern SJV are at higher risk for anthracnose disease than orchards in central and southern SJV.

Table 1. Efficacy of fungicides against the anthracnose of pistachio fruit (K=Kerman; RA=Red Aleppo; Trial in Glenn County).

Fungicide[a]	n[b]	frequency of healthy (std)[c]		frequency of spotted (std)[c]		frequency of half blighted (std)[c]		frequency of full blighted (std)[c]	
K-Control	1400	34.2 (0.19)	a	40.9 (0.10)	a	8.1 (0.04)	b	16.8 (0.06)	c
Quash (4 fl. oz)	1400	0.4 (0.01)	b	25.3 (0.10)	b	14.6 (0.05)	a	59.7 (0.07)	ab
Merivon (6.5 fl. oz)	1400	0 (0)	b	27.6 (0.15)	ab	12.1 (0.03)	ab	60.4 (0.14)	ab
Fontelis (20 fl. oz)	1400	0 (0)	b	36.3 (0.25)	ab	12.9 (0.05)	ab	50.9 (0.21)	b
RA-Control	1400	0 (0)	b	21.4 (0.05)	b	11.6 (0.04)	ab	67.1 (0.05)	a

[a]Commercial fungicides and dosage used.
[b]Number of pistachio nuts evaluated per treatment.
[c]Incidence (%) and standard error of the mean.

Table 2 Efficacy of fungicides against the anthracnose of pistachio leaves (K=Kerman; RA=Red Aleppo; Trial in Glenn County).

Fungicide	n[a]	Frequency Cat. 1[b]		Frequency Cat. 2		Frequency Cat. 3		Frequency Cat. 4		Frequency Cat. 5	
K-Control	350	65.7	a	27.4	a	6.8	b	0	b	0	c
Quash	350	11.2	bc	27.5	a	19.9	a	14.2	a	26.9	a
Merivon	350	9.1	bc	22.5	a	18.2	a	17.7	a	32.5	a
Fontelis	350	16.6	b	36	a	20.3	a	12.6	a	14.3	b
RA-Control	350	8	c	27.1	a	14.6	a	13.7	a	36.6	a

[a]Number of pistachio leaves evaluated per treatment.
[b]Incidence (%) in each category in percentage and standard error of the mean. Cat. 1=Healthy leaves, Cat. 2=1-5 lesion/leaf, Cat. 3=6-10 lesion/leaf, Cat. 4=11-15 lesion/leaf and Cat. 5=>16 lesion/leaf.

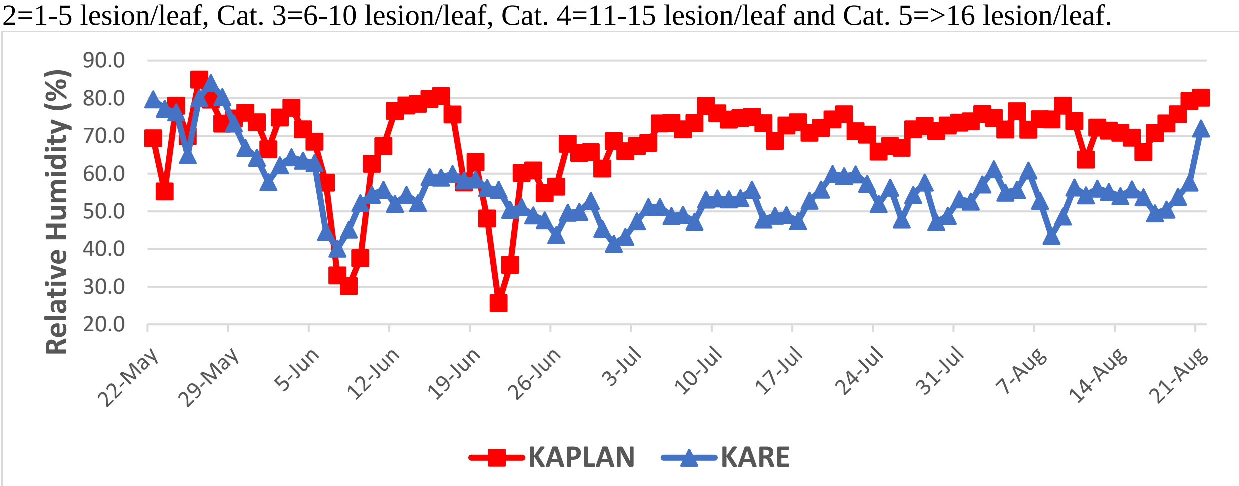

Figure 1. Average daily relative humidity in a Red Aleppo pistachio orchard in Orland (Glenn Co. ‑ Kaplan) and Parlier (Fresno Co. ‑ UC KARE) from May 22 until August 21, 2019.

Management of Botryosphaeriaceae Panicle and Shoot Blight of Pistachio (Fungicide Trial 2019)

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Introduction

In California, the 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 Sacramento Valley this disease continued to be a significant pistachio production problem. The risk of the Bot disease was higher in 2019 due to long rainy season in the spring. When the risk for infection by Botryosphaeria is high frequent fungicides are needed in order to control this disease. This disease could cause significant losses if weather and conditions were conducive in an orchard and the grower failed to follow an aggressive fungicide program. This situation was observed in the 2019 season, with orchards in the central and southern SJ Valley when during August trees started showing clusters and shoots collapsing because of Botryosphaeria panicle and shoot blight. Fortunately, with financial support by chemical companies, we continued testing the efficacy of registered fungicides and new (experimental) fungicide formulations. The objective of the 2019 spray trial was to determine the fungicide efficacy against Botryosphaeria blight of pistachio in a conducive for disease year such as 2019 was.

Four rows of a 36-years-old commercial pistachio orchard in Butte County, California, were used to determine the efficacy of nine fungicide programs and 23 sole fungicides applications, including 10 experimental formulations. Five replicated trees served as the untreated control. The orchard was irrigated using drip emitters. Each treatment consisted of five single-tree (cv. Kerman) replications using a randomized complete block design. All treatments consisted of four applications, performed on 23 April (full bloom), 20 May, 20 June, and 18 July. All sprays were applied with a handgun sprayer using 400 gallons of water per acre. No late dormant spray was made. Disease was recorded on 9 September by counting the blighted panicles among 100 random fruits panicles per replicated tree. The proportions of blighted fruits were statistically analyzed after square root transformation. Significant differences were subject to analysis of variance (ANOVA) and means were compared with Fisher's LSD test at $\alpha = 0.05$.

Results and Discussion

As expected the disease was high (57% blighted clusters in the untreated control trees). The best fungicide program in the 2019 trial was a mixture of Fontelis (a.i. penthiopyrad-FRAC#7) and Abound (a.i. azoxystrobin-FRAC#11) corresponding to 0.3% of infected clusters (Treat. 13, Table 1). Similar efficacy was observed for treatments 17 and 18, corresponding to the sole application of Merivon (pre mix of fluxapyroxad-FRAC#7 and pyraclostrobin-FRAC#11) and by the sole application of Pristine (pre mix of boscalid-FRAC#7 and pyraclostrobin-FRAC#11) with infection incidences of 1.4% and 2.3%, respectively. The next three best products were also formulations of SDHI-FRAC#7 fungicide (Treats. 11, 7 and 12), and statistically presented similar result to those of Treats. 17 and 18 (the two second best). The results presented this year, correspond well with the efficacy results obtained in 2018 (See Executive Summaries 2018) where products formulated with SDHI-FRAC#7 and QoI-FRAC#11 fungicides were the most effective products in reducing disease. It seems that fungicide programs of SDHI and QoI result in better disease inhibition than SDHI and DMIs. For instance, Treats. 15, 14 and 16 where the SDHI Fontelis was mixed or alternated with Quadris Top, Tebucon 45 and Quash (all presenting DMI on their formulations) resulted in 35-, 53-, and 66-time more incidence than our best result (Treat. 13).

Conclusions

Fungicide sprays by mixing or alternating SDHI and QoI fungicides results in more Botryosphaeria panicle and shoot blight control than other combinations of fungicide classes.

Although there is no evidence of selection of resistance among the Botryosphaeriaceae members, it is essential to still alternate fungicide formulations other than SDHI and QoI to avoid the selection of resistance among populations of *Alternaria alternata* (causing Alternaria late blight) or *Colletotrichum fioriniae* (causing anthracnose).

We are currently surveying the sensitivity of about 80 isolates of Botryosphaeriaceae members stored in our culture collection since 1984 up to date to check for any possible changes in sensitivity.

Table 3. Efficacy of fungicides against Botryosphaeriaceae panicle and shoot blight in a commercial pistachio orchard in Butte Co. 2019.

Treat.[1]	Fungicide	Rate	Spray Dates				Disease Incidence
			23-Apr	20-May	20-Jun	18-Jul	on tree (%)[2]
13	Fontelis + Abound (Ab)	20 fl oz / 12 fl oz	Fon+Ab	Fon+Ab	Fon+Ab	Fon+Ab	0.3 a[3]
17	Merivon (Mer)	6.5 fl oz	Mer	Mer	Mer	Mer	1.4 ab
18	Pristine (Pri)	14.5 oz	Pri	Pri	Pri	Pri	2.3 abc
11	Luna Sensation (LS)	5 fl oz	LS	LS	LS	LS	4.4 bcd
7	Serifel + Merivon	8 oz + 5.5 fl oz	Ser+Mer	Ser+Mer	Ser+Mer	Ser+Mer	4.8 bcde
12	Fontelis (Fon)	20 fl oz	Fon	Fon	Fon	Fon	6.5 bcdef
29	A20560E (AE)	9.1 fl oz	AE	AE	AE	AE	7.1 cdef
28	A20560E (AE)	6.84 fl oz	AE	AE	AE	AE	8.7 cdefg
27	A20259G (AG)	13.7 fl oz	AG	AG	AG	AG	9.5 defgh
15	Fontelis / Quadris Top (QT)	14 fl oz	Fon	QT	Fon	QT	10.7 defghi
21	Pyraziflumid (Pyr)	4.7 fl oz	Pyr	Pyr	Pyr	Pyr	12.3 defghij
9	LE + Baythroid (Bay)	6 fl oz + 2.8 fl oz	LE+Bay	LE+Bay	LE+Bay	LE+Bay	12.8 defghij
20	Pyraziflumid (Pyr)	3.1 fl oz	Pyr	Pyr	Pyr	Pyr	12.9 defghijk
8	Luna Experience (LE)	6 fl oz	LE	LE	LE	LE	13.7 efghijkl
14	Fontelis + Teb 45	20 fl oz / 8 oz	Fon+Teb	Fon+Teb	Fon+Teb	Fon+Teb	15.9 fghijklm
6	Serifel (Ser)	8 oz	Serifel	Serifel	Serifel	Serifel	17.9 ghijklmn
2	PhD + Tebucon 45 (Teb)	6.2 oz + 4 oz	PhD+Teb	PhD+Teb	PhD+Teb	PhD+Teb	18.6 ghijklmn
5	UC-2	7 fl oz	UC-2	UC-2	UC-2	UC-2	19.7 hijklmno
16	Fontelis / Quash	20 fl oz / 4 oz	Fontelis	Quash	Fontelis	Quash	19.9 hijklmno
4	UC-1	5 fl oz	UC-1	UC-1	UC-1	UC-1	19.9 hijklmno
22	SA0650001	55 fl oz	S1	S1	S1	S1	20.7 hijklmno
30	Quash	4 oz	Quash	Quash	Quash	Quash	20.8 ijklmno
10	LE + Serenade Opti (SO)	6 fl oz + 20 oz	LE + SO	LE + SO	LE + SO	LE + SO	21.5 ijklmno
23	SA0650004	28 fl oz	S4	S4	S4	S4	21.8 ijklmno
3	UC-1	4 fl oz	UC-1	UC-1	UC-1	UC-1	25.5 jklmno
31	V10424 (V24)	4 fl oz	V24	V24	V24	V24	25.9 klmno
1	PhD	6.2 oz	PhD	PhD	PhD	PhD	26.0 klmno
32	Dart	0.25% w/w	Dart	Dart	Dart	Dart	27.5 lmno
24	SA0650004 (S4)	42 fl oz	S4	S4	S4	S4	27.6 lmno
26	SA0670001 (S1)	80 oz	S1	S1	S1	S1	29.1 mno
33	Dart	0.35% w/w	Dart	Dart	Dart	Dart	30.9 no
25	SA0650004 + SA0670001	14 fl oz/ 40 oz	S4 + S1	S4 + S1	S4 + S1	S4 + S1	33.8 o
34	Control	unsprayed	-	-	-	-	57.2 p

[1]A non-ionic surfactant (NIS) was added at 0.0625% vol./vol. to all treatments, except Trts.31 and 32.

[2] Blighted fruit panicle / 100 panicles for each of 5 replicated trees were recorded on 9 September 2019.

[3] Numbers followed by different letters are significantly different according to Fisher's LSD test at 5% probability. Statistical analysis was done after square-root transformation of the data. Values presented here were back transformed from the square-root mean values.

Sensitivity of *Phoma fungicola* to several fungicides registered on pistachio

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Introduction

In total, three *Phoma fungicola* isolates (7G056, 7G057, 7G058) recovered from Arizona pistachio in 2012 were used to test their sensitivity (*in vitro*) to 13 commercial fungicides, representing active ingredients (a.i.) currently registered for use on pistachio. The fungicides used were: Topsin (a.i. thiophanate-methyl, FRAC#1), Quash (a.i. metconazole, FRAC#3), Score (a.i. difenoconazole, FRAC#3), Sercadis (a.i. fluxapyroxad, FRAC#7), Luna Privilege (a.i. fluopyram, FRAC#7), Fontelis (a.i. penthiopyrad, FRAC#7), Abound (a.i. azoxystrobin, FRAC#11), Cabrio (a.i. pyraclostrobin, FRAC#11), Gem (a.i. trifloxystrobin, FRAC#11), Vangard (a.i. cyprodinil, FRAC#9), Elevate (a.i. fenhexamid, FRAC#17), Ph-D (a.i. polyoxin-D zinc salt, FRAC#19) and K-Phite (a.i. mono and dipotassium salts of phosphorous acid, FRAC#P07). Fungicide formulations were diluted in water to make a stock solution of 100 mg a.i. per ml. Stock solution was added to potato dextrose agar media to produce two final discriminatory dosages of 1 and 10 µg/ml. A 4-mm mycelial plug from a 10-days-old culture was placed in the center of fungicide-amended and non-amended media. Plates were incubated at 25^{o}C for 10 days when measurement of two perpendicular diameters were taken. Four replicates per isolate:fungicide were prepared. Percentage relative growth (RG) was used to measure inhibitory effect among treatments. The fungicide Quash, Fontelis and Abound were only tested at 10 µg/ml concertation. The FRAC#7 (SDHI) and #11 (QoI) were also tested for effects on germination inhibition, considering their mode of action which is effective against respiration complexes.

Results and Discussion

The mycelial growth assay at 10 µg/ml revealed that DMI-FRAC#3 (Quash and Score) and SDHI-FRAC#7 (Sercadis and Luna Privilege) yielded less than 1% of colony size on non-amended control (Figure 1). The fungicide Fontelis (SDHI) also resulted in a small relative growth (RG) of approximately 10%. Among the QoIs (FRAC#11), Cabrio was the only product resulting in RG < 20%, for all other two QoI products (Abound and Gem), the RG ranged from 60-80% and 30-50%, respectively (Figure 1). Vangard (AP-FRAC#9) also resulted in good colony inhibition at 10 µg/ml, with RG around 15% (Figure 1). With less colony inhibition (from 55 to 85%) Elevate (Keto Reductase Inhibitor), PhD (Polyoxin) and K-Phite (phosphonate) fungicides resulted in 55 to 85% colony inhibition (Figure 1) while Topsin M showed 30% (7G056) to 67% (7G058) inhibition of growth. At 1 µg/ml dosage, the same trends among the fungicides were observed with a general decrease of RG (Figure 2). The mycelial assay revealed small variations among isolates. The isolate 7G058 of *P. fungicola* seemed to be less sensitive than the 7G056 isolate when tested in media amended with Topsin M or Gem.

The germination assay demonstrated the high susceptibility of all three *P. fungicola* isolates to QoIs (Cabrio and Gem) and SDHIs (Luna Privilege and Sercadis) (Table 1). With the exception of Luna Privilege resulting in less than 10% of germinated conidia at 1 µg/ml, all other products inhibit spore germination by 100% in these studies.

Conclusion

Phoma fungicola showed high sensitivity to fungicides formulated with DMI-FRAC#3 (RG < 10% at 10 µg/ml) and SDHI-FRAC#7 active ingredients (RG < 20% at 10 µg/ml).

Vangard, an AP-FRAC#9 fungicide, also presented a good mycelial inhibitory effect at 10 µg/ml. However, at 1 µg/ml its RG ranged from 34 to 43%.

Despite low mycelial inhibition effect achieved by the QoI-FRAC#3 fungicides, the germination assay demonstrated that QoI are a good option to manage Phoma blight.

The results presented here justify the selection of SDHI, QoI, DMI and AP fungicides to test their efficacy in the field.

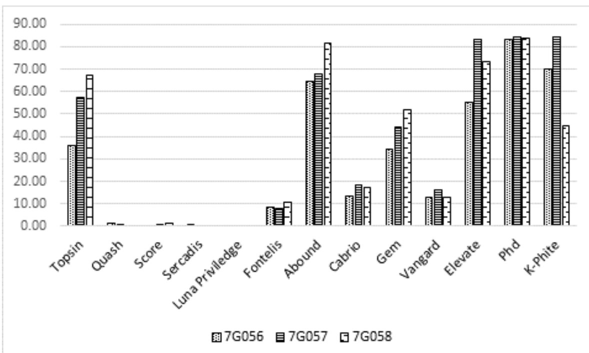

Figure 7. Percentage relative growth (% RG) of Phoma fungicola cultured on media amended with 10 µg/ml of 13 commercial fungicides.

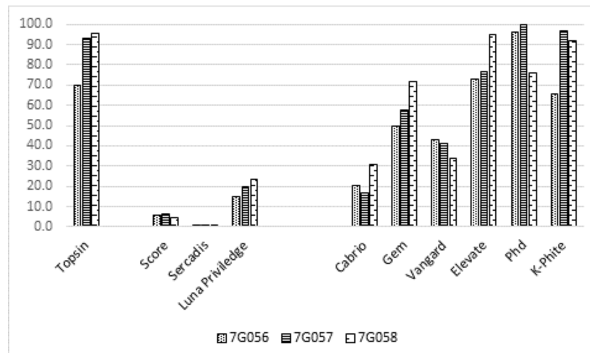

Figure 8. Percentage relative growth (% RG) of *Phoma fungicola* cultured on media amended with 1 µg/ml of 10 commercial fungicides.

Table 1. Sensitivity germination assay of *Phoma fungicola* on media amended with SDHI and QoI fungicides.

Fungicide	Group (FRAC#)	n of germinated conidia and percentage[a]		
		0 µg/ml	1 µg/ml	10 µg/ml
Cabrio	QoI #11	150 (100%)	0	0
Gem	QoI #11	150 (100%)	0	0
Sercadis	SDHI #7	150 (100%)	0	0
Luna Privilege	SDHI #7	150 (100%)	13 (8.6%)	0

[a] 50 conidias of each 7G056, 7G057 and 7G058 isolates of *P. fungicola* were pooled together to evaluate the germination rates at 1 and 10 µg/ml.

Managing *Phoma fungicola* causing Phoma blight in Arizona pistachio

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Introduction

Phoma fungicola is the cause of a severe blight of pistachio in Arizona that can cause significant yield losses when conditions are conducive to the disease. In 2013, when this disease was reported for the first time, 85% of buds were found to be infected. The amount of bud infection dropped to 35% in the following years when DMI sprays were used. In 2019, we established fungicide trials in two Kerman orchards – Cochise and Graham Co. – in Arizona. Two spray programs were tested, spray program 1, formulated with SDHI and QoI fungicides such as Merivon and Luna Sensation, and spray program 2, formulated with DMI and AP fungicides, like Quash and Inspire Super. In total, 9 acres were treated with each spray program and 40 unsprayed trees were used as control. Controls were not exposed to any fungicide application in 2019. Spray applications were done on 15-Apr, 15-May, 9-Jul and 15-Aug. The fungicide Topsin (MBC-FRAC#1) was applied on 15-May to alternate the above-mentioned fungicides. Two weeks after each spray application, 600 fruits were collected from pre-marked trees and tested for latent infection in artificial media. The frequencies of fruit infected with *Phoma fungicola* were quantified and subjected to ANOVA; mean comparisons were with the Fisher's LSD at 5% probability.

Results and Discussion

In March 2019, samples of 120 dormant buds were taken from each orchard and subjected to BUDMON assay. *Phoma fungicola* was found to infect 67.5% of buds in the Cochise Co. orchard and 75.8% in the Graham Co. orchard. Later in the season, the levels of *P. fungicola* isolates in fruit collected from Cochise Co. (n=66) and Graham Co. (n=70) were not different (P=0.87). Significant differences ($P=9.8\times10^{-10}$) for the period of sampling were observed, revealing that the incidence of *P. fungicola* was significantly higher in July and August samples than those in April and May (Table 1). The treatment main effect was significant ($P=1.0\times10^{-15}$), revealing that unsprayed trees had 7- and 3-times more *P. fungicola* incidence than trees treated with SDHI/QoI and DMI/AP fungicides, respectively (Table 2). No significant differences were observed between the spray programs 1 and 2 (Table 2). Our statistical analysis showed an interesting and significant ($P = 3.8\times10^{-8}$) interaction between the sampling period (month) and the fungicide treatment (spray program) used. April was the only period where the SDHI/QoI program resulted in higher pathogen inhibition, corresponding to 4- and 6-time less incidence than the DMI/AP program and the control treatments, respectively. In this same month (April), Merivon (SDHI and the QoI formulation) and Quash (DMI formulation) were used on treatments 1 and 2 respectively, but when this same fungicide combination was repeated in August no significant differences were observed. In May, it was recorded the smallest *P. fungicola* counts for the entire season. In July and August, the unsprayed trees yielded in average 8 and 5.5 higher incidence than the sprayed trees. For the same period, the SDHI/QoI program was only numerically superior in comparison with the DMI/AP fungicide program (Figure 1). The weather data retrieved from the Arizona Meteorological Network (AZMET) suggest a relationship between rainfall and increased incidence of *P. fungicola*. In both orchards, it was observed that incidence level of *P. fungicola* spikes when rains occurs (Figure 2).

Conclusion

The usage of fungicides significantly reduces the amount of *P. fungicola* incidence in comparison with untreated trees. However, the fungicide programs of SDHI/QoI or DMI/AP result in similar control levels of the disease.

High incidence of *P. fungicola* blight occurs during increased rainfall periods.

The low disease incidence found in untreated trees in May suggests that the May fungicide application could be skipped.

Table 1. Monthly incidence of *Phoma fungicola* on pistachio fruit from Arizona

Month[a]	n[b]	*P. fungicola* count[c]	*P. fungicola* frequency (%)	
July	2390	51	2.13	a
August	2400	49	2.04	a
April	2760	34	1.23	b
May	2560	2	0.08	c

[a]Month of fruit sampling, performed two weeks after fungicide application.
[b]Number of pistachio fruit pieces plated for both orchards.
[c]Count of *P. fungicola* colony forming units.

Table 2. Effect of fungicide programs on incidence of blight caused by *Phoma fungicola* on pistachio fruit in Arizona

Treatment[a]	n[b]	*P. fungicola* count[c]	*P. fungicola* frequency (%)	
Control	3520	97	2.76	a
DMI/AP	3190	26	0.82	b
SDHI/QOI	3400	13	0.38	b

[a]In each site for each fungicide program, the sprays were applied on 15-Apr, 15-May, 9-Jul and 15-Aug.
[b]Number of pistachio fruit pieces plated for both orchards.
[c]Count of *P. fungicola* colony forming units.

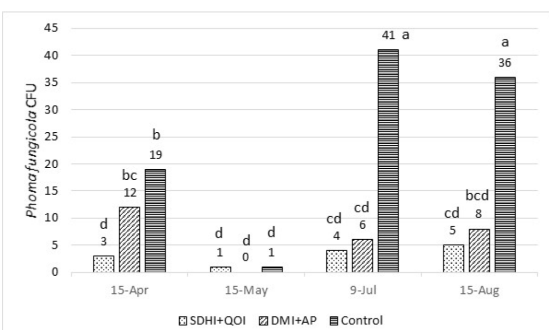

Figure 9. Number of Phoma fungicola colonies isolated from pistachio fruit in Arizona in 2019.

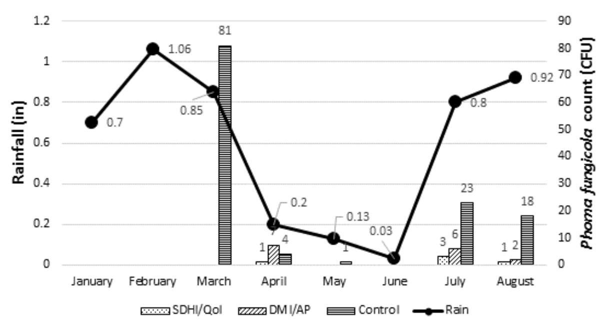

Figure 10. Relation between rainfall and *P. fungicola* count in Cochise Co. Arizona.

Management of Alternaria Late Blight of Pistachio (Fungicide Trial 2019)

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Introduction

In a Kerman experimental orchard planted in 2014 at Kearney Agricultural Research and Extension Center (KARE) in Parlier CA, we tested spray programs in addition to solo fungicide applications. Treatments consisted of three spray applications approximately four weeks apart (4 Jun, 3 Jul – critical time for spray – and 5 Aug). All fungicides were applied at rates mentioned in Table 1. Each treatment consisted of five-single trees replications. Sprays were applied with a handgun sprayer at 400 gallon per acre. The orchard was irrigated with fanjet micro-sprinklers. Symptoms of the disease developed only very late in the season and for this reason evaluation was performed in October 9th. Fungicide efficacy was evaluated using the whole tree evaluation method or efficacy score, where 1 = the least control, 5 = the best control, and 2, 3, and 4, are the intermediate levels of increasing ALB control. Significance levels were made with Kruskal-Wallis test at 5% of probability and means were separated with Fisher's LSD test.

Results and Discussion

The 2019 fungicide trial revealed that all treatments obtained very close scores and many fungicides programs were clustered under the same statistical significance. The 11-best results (treatments 3, 19, 2, 7, 21, 10, 9, 11, 8, 12, and 20) ranged with scores of 4.16 to 4.68 (where 5 is the best control). The very first 5 best sprays included the experimental fungicides UC-2, A20259G, UC-1, A20560E and the commercial fungicide Luna Experience (ranked in the 4th position). Following the five top products, Luna Experience was among the mixture and/or alternation with Movento, Serenade Opti and Baythroid. Luna Sensation and another experimental product were the last two fungicides of these eleven best results. In the 2018 trial (see Executive Summary 2018), other SDHI-FRAC#7 based fungicide (Fontelis, Luna Experience and Luna Sensation) made the top 6 products to control ALB disease. The SDHI products belong to a newer fungicide generation that, despite of reports on several mutations associated with a lower sensitivity of *Alternaria*, fluopyram (one of the active ingredients of Luna package) offers an efficient ALB inhibition (See California Agriculture vol.72(3): 170-178).

Other SDHI fungicides such as Fontelis (treat.13), Merivon (treat.26), Pristine (treat. 6) and Pyraziflumid (treat. 17 and 18) did not result in high efficacy against ALB, as observed for the Luna Package or the same fungicide products when compared with those used in the 2018 trial. As expected, the unsprayed trees (control) showed the highest ALB disease, but its score was not statistically different from the score of the seasonal applications of Serifel, Rhyme (injected by the irrigation line), Pristine, or the alternation of Fontelis with Quadris Top.

Conclusion

Luna Package products such as Experience (fluopyram FRAC#7 and tebuconazole FRAC#3) and Sensation (fluopyram FRAC#7 and trifloxystrobin FRAC#11) were among the first 10 best treatments to control ALB disease.

The clustering of so many products under the same significance levels (see letters in the last column of table 1) may be caused by the low conducive conditions of our research station for ALB resulting also in a long delay of the disease development.

Table 4. Efficacy of fungicides against Alternaria Late Blight in an experimental pistachio orchard in Fresno Co. (KARE) 2019.

Treat.	Fungicides	Rate	Spray Dates 4-Jun	Spray Dates 3-Jul	Spray Dates 5-Aug	Score	
3	UC-2	7 fl oz	UC-2	UC-2	UC-2	4.68	a
19	A20259G	13.7 fl oz	A20259G	A20259G	A20259G	4.53	ab
2	UC-1	5 fl oz	UC-1	UC-1	UC-1	4.56	ab
7	Luna Experience (LE)	6 fl oz	LE	LE	LE	4.55	ab
21	A20560E	9.1 fl oz	A20560E	A20560E	A20560E	4.47	abc
10	LE + Movento 240 SC (Mov)	10 fl oz + 9 fl oz	LE + Mov	LE + Mov	LE + Mov	4.38	abcd
9	LE + Serenade Opti (SO)	6 fl oz + 20 oz	LE + SO	LE + SO	LE + SO	4.30	abcde
11	LE + SO / Mov	10 fl oz + 20 oz / 9 fl oz	LE + SO	MOV	LE + SO	4.25	abcdef
8	LE + Baythroid (Bay)	6 fl oz + 6.8 fl oz	LE + Bay	LE + Bay	LE + Bay	4.25	abcdef
12	Luna Sensation (LS)	5 fl oz	LS	LS	LS	4.16	abcdefg
20	A20560E	6.84 fl oz	A20560E	A20560E	A20560E	4.16	abcdefg
23	V-10424	4 fl oz	V-10424	V-10424	V-10424	4.01	bcdefgh
24	Dart	0.25% w/w	Dart	Dart	Dart	3.94	bcdefghi
25	Dart	0.35% w/w	Dart	Dart	Dart	3.87	cdefghij
26	Merivon (Mer)	6.5 fl oz	Mer	Mer	Mer	3.84	cdefghij
13	Fontelis	20 fl oz	Fontelis	Fontelis	Fontelis	3.74	defghij
15	Fontelis / Quash	20 fl oz / 4 oz	Fontelis	Quash	Fontelis	3.75	defghij
5	Serifel (Ser) + Mer	8 oz + 5.5 fl oz	Ser + Mer	Ser + Mer	Ser + Mer	3.63	efghij
22	Quash	4 oz	Quash	Quash	Quash	3.57	fghij
1	Ph-D	6.2 oz	Ph-D	Ph-D	Ph-D	3.51	ghij
17	Pyraziflumid	3.1 fl oz	Pyraziflumid	Pyraziflumid	Pyraziflumid	3.53	ghij
18	Pyraziflumid	4.7 fl oz	Pyraziflumid	Pyraziflumid	Pyraziflumid	3.46	ghij
4	Serifel	8 oz	Serifel	Serifel	Serifel	3.31	hijk
16	Rhyme-drip	7 fl oz	Rhyme-drip	Rhyme-drip	Rhyme-drip	3.20	ijk
6	Pristine	14.5 oz	Pristine	Pristine	Pristine	3.20	ijk
14	Fontelis / Quadris Top	20 fl oz / 14 fl oz	Fontelis	Quadris Top	Fontelis	3.16	jk
27	Control	untreated	-	-	-	2.50	k

Taxonomy, biology and ecology of *Rhodococcus* bacteria occurring in California pistachio and other woody hosts as revealed by genome sequence analyses

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Introduction

In 2011, Pistachio Bushy Top Syndrome (PBTS) emerged as a major problem affecting the clonal UCB1 pistachio rootstock in orchards in California, Arizona, and New Mexico (Savory et al, 2017). A second outbreak occurred in trees planted in 2015 and 2016. It was recently postulated that PBTS is an infectious disease caused by two bacterial species belonging to the genus *Rhodococcus*; *R. fascians* and *R. corynebacterioides*. Based on these findings, the detection of *Rhodococcus* in pistachio nurseries and orchards has led to the prophylactic removal and replacement of millions of dollars of pistachio trees and planting stocks with the hope of mitigating the spread of PBTS (Stamler et al, 2015). Importantly, it also implicated the two nurseries associated with the dissemination of the affected rootstocks as culpable in spreading the putative causal agent of PBTS.

The conclusion that *R. fascians* and *R. corynebacterioides* cause PBTS has been challenged by groups unable to replicate the results reported by Stamler *et al.* (Savory et al, 2017). The conflicting data and interpretation have led to a debate as to whether or not *R. fascians* and *R. corynebacterioides* cause PBTS and how the detection of *Rhodococcus* sp. in PBTS afflicted pistachio orchards should guide growers (Chang et al, 2018; Randall et al, 2018; Vereecke et al 2018). Given the substantial legal and economic implications of these findings for both the pistachio producers and nurseries that supply them, it is essential to determine what role, if any, *R. fascians* plays in PBTS. A clear understanding of the relationship between the *Rhodococcus* populations in nurseries and orchards with those in the wild may provide insight into the association between *Rhodococcus* and the recent PBTS outbreaks.

Results and Discussion

To characterize the *Rhodococcus* populations within California pistachio nurseries and orchards, we sampled leaves from both symptomatic and asymptomatic pistachio trees from these locations. Additional isolates were collected from symptomatic bark tissues to potentially enrich for the causal agent of PBTS in those individuals. We further explored the *Rhodococcus* host range by sampling diverse plant species from nurseries and orchards, and various native and ornamental woody species. Whole genome sequencing was carried out for each of the bacterial isolates and 233 draft genomes were assembled.

Multi-locus phylogenetic analysis using approximately 400 universally conserved genes resolved the taxonomic relationships among the 233 strains and previously characterized reference genomes. Whole genome alignments were used to calculate the Average Nucleotide Identity (ANI) between the different genomes, an important metric for determining membership in a species. The results of the two independent analyses were congruent. These analyses indicate that six different *Rhodococcus* species are present in our collection representing >98% of all isolates. The vast majority of the isolates are *R. fascians* (n=150). Two species constitute the bulk of the remaining isolates, *R. kyotonensis* (n=31) and an uncharacterized species resembling *Rhodococcus* sp. Leaf225 (n=38). Neither of these species are known phytopathogens. Four of the remaining isolates resemble PBTS1, one of the two seminal species reported to cause PBTS (the other being *R. fascians* isolate PBTS2). Based on phylogenomic and ANI analyses, we find that PBTS1 and the four strains we isolated are *R. kroppenstedii* rather than *R. corynebacteroides* as originally reported. We did however isolate two *bona fide R. corynebacteroides* strains with genomes

>95% identical to a *R. corynebacteroides* reference. The last two isolates represent a novel *Rhodococcus* species. These results indicate that diverse *Rhodococcus* species populate California's pistachio nurseries and orchards.

R. fascians constituted the majority of our isolates and this species has been implicated in causing PBTS. In order to further characterize this subset of our collection, we performed phylogenetic, ANI, and pan-genome analysis on all *R. fascians* genomes. All three analyses independently partitioned the strains into the same 11 homogeneous lineages. We used these 11 groupings to investigate the relationships among strains that were identified in different nurseries, orchards, and on the native flora.

Since 2011, there have been two reported outbreaks of PBTS attributed to two nurseries distributing *Rhodococcus* infected trees to growers. The first outbreak, occurring in pistachio trees planted between 2011 to 2014, was attributed to Nursery 1, and the second outbreak in trees planted in 2015 and 2016 was attributed to Nursery 2. We compared the genotypes of strains isolated from Nursery 1 or Nursery 2 with those of strains isolated from orchards that received trees from either Nursery 1 or Nursery 2. We also examined *Rhodococcus* genotypes present in native woody flora and mature fruit and nut trees planted prior to the 2011 PBTS outbreak.

Several *Rhodococcus* genotypes isolated on site at Nursery 1 resemble those found in the orchards they sold trees to. However, these orchards also contained numerous additional *Rhodococcus* genotypes not found at Nursery 1. This suggests an alternative source of these orchard specific bacteria. Of the genotypes co-occurring in Nursery 1 and the Nursery 1 associated orchards, these were also found on native woody flora and on mature fruit and nut trees planted prior to Outbreak 1. While Nursery 1 may be the source of bacteria found in these orchards, we cannot preclude the possibility that the bacteria were acquired from the surrounding environment. In contrast, we only identified two *Rhodococcus* genotypes on-site at Nursery 2 and neither of these genotypes were found on any of the orchard trees that were acquired from Nursery 2. This again suggests that many of the *Rhodococcus* isolates populating the trees in these orchards were acquired from a source other than Nursery 2 from which they were derived.

We sampled *Rhodococcus* from both symptomatic and asymptomatic trees. However, we did not observe any relationship between bacterial species and the health of the tree from which they were isolated. Virulence in phytopathogenic *Rhodococcus* species requires specific *fas* and *att* genes present on a linear virulence plasmid. In the absence of the virulence plasmid, *Rhodococcus* is not only avirulent, but may be beneficial to the host. We queried all genomes using BLASTP to determine if virulence genes were present, finding that none of the 233 genomes in our collection encoded the requisite virulence proteins. Similar analysis of the previously reported reference genomes did identify *fas* and *att* homologs, validating the sensitivity of the BLASTP method. Furthermore, aligning genomes of a representative subset of our genomes with that of the virulent type strain *R. fascians* D188 failed to identify sequence contigs resembling the linear plasmid.

Conclusion

Significant controversy surrounds the finding of *R. fascians* and *R. corynebacteroides* as causal agents of PBTS. We can neither implicate nor exonerate *R. fascians* in the PBTS disease outbreak responsible for the removal of millions of dollars-worth of trees. However, we can conclusively state that a significant *Rhodococcus* population resides outside of PBTS afflicted areas with identical *Rhodococcus* and *R. fascians* genotypes found in both pistachio orchards and nurseries. None of the strains that we isolated contain either a virulence gene or the virulence plasmid. Although we cannot preclude the existence of a small population of *Rhodococcus* harboring the virulence plasmid, our data supports the occurrence of an endemic, broadly distributed and avirulent *R. fascians* population in the San Joaquin valley.

Evaluating pistachio rootstock tolerance to soil-borne diseases

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Introduction

Our laboratory recently identified new soil-borne diseases affecting pistachio trees in California. Phytophthora root and crown rots appeared as emerging new threats to pistachio, particularly in areas where soil conditions or cultural practices promote prolonged soil wetness. Similarly, the fungus *Macrophomina phaseolina* was detected as an emerging soil-borne pathogen causing crown rot in UCBI rootstocks. *Fusarium* species also were found occasionally in association with crown rots in UCBI rootstocks. In the last two years, we have conducted experiments to determine the relative tolerance of UCBI, PGI and Platinum rootstocks to these new soil-borne pathogens. This work aims to identify tolerant/resistant rootstocks that can be used to sustainably managed soil-borne diseases of pistachio.

2018 results suggested that Platinum is the most tolerant rootstock to crown rot diseases when compared to PGI and clonal UCBI rootstocks. However, the susceptibility of Platinum, a single decent of a PGII seed, to Verticillium wilt is unknown, although PGII had shown substantial levels of susceptibility to Verticillium in past experiments (Epstein et *al*. 2004).

This year, we continued the evaluation of the susceptibility/tolerance of clonal UCBI, PGI and Platinum rootstocks against soil-borne pathogens. Two large-scale experiments were conducted at the Kearney Agricultural Research and Extension Center on potted trees (2-year-old) as well as trees planted in the field (3-year-old). Stems of pistachio rootstocks were inoculated using mycelium plugs of different isolates of *Phytophthora niederhauserii*, *Phytophthora* taxon walnut, *Phytophthora cinnamomi*, *Macrophomina phaseolina* and *Fusarium proliferatum.* Experiments using *Verticillium* are ongoing and no data are yet available to indicate the susceptibility of Platinum vs. clonal UCBI rootstocks to this disease.

Results and Discussion

This year results revealed significant differences in the susceptibility (expressed as the lesion length produced in stems inoculated with mycelium plugs) among clonal UCBI, PGI and Platinum rootstocks to the various soil-borne pathogens. Overall, Platinum was most tolerant rootstock to all pathogens combined. The following figures illustrates averaged lesion lengths produced in the various rootstocks approximately 10 months after inoculation of various *Phytophthora* spp., which represent the most common and widespread pathogens of pistachio in California. In the gravel bed experiment, using potted plants, Platinum appeared as the least susceptible rootstocks to *Phytophthora* (30.5 mm average lesion length), followed by PGI (32.75 mm average lesion length) and clonal UCBI (44.72 mm average lesion length) (Fig. 1). In the field experiment, Platinum also appeared as the least susceptible rootstocks to *Phytophthora* (24.38 mm average lesion length), followed by clonal UCBI (34.08 mm average lesion length) and PGI (49.55 mm average lesion length) (Fig. 2). In November 2019, we obtained new Platinum and clonal UCBI plantlets that will be root-inoculated with *Phytophthora* and *Verticillium* in the spring of 2020.

Conclusion

This study identifies significant differences in the tolerance of clonal UCBI, PGI and Platinum (a PGII decent) rootstocks to soil-borne pathogens including *Phytophthora*, *Macrophomina* and *Fusarium* species. Platinum appeared as the most tolerant rootstock overall against all soil-borne pathogens. On the other hand, UCBI and PGI alternately appeared as most susceptible rootstocks.

This year data (with two trials) confirm last year findings (one trial) suggesting Platinum is the most tolerant rootstock to soil-borne pathogens. Hence, Platinum might be a preferred rootstock in areas at risks for Phytophthora diseases. Nevertheless, our results remain preliminary and multiple years experiments including root inoculation trials will need to be conducted before validating these findings. Caution should be taken also before planting Platinum in area that have been exposed to the Verticillium wilt pathogens. Previous research and recent field observations have indicated the possibility of a higher susceptibility of Platinum rootstock to Verticillium.

Research continues in our laboratory to provide the California pistachio industry with knowledge of the tolerance of commercial rootstocks to soil-borne pathogens and to identify most tolerant rootstocks as a sustainable management strategy against soil-borne diseases.

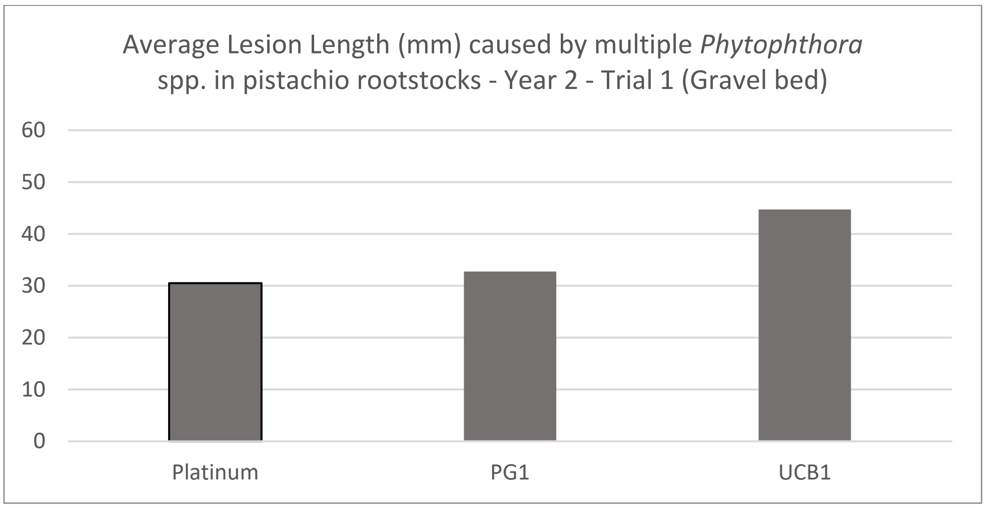

Fig. 1. Average lesion length (mm) produced in stems of Platinum, PGI and UCBI rootstocks inoculated with mycelial plugs colonized with *Phytophthora* isolates. Lesion sizes were averaged for 4 *Phytophthora* isolates representing 2 *Phytophthora* spp. (*P. niederhauserii* and *P. cinnamomi*) and for each rootstock. Data indicate higher tolerance of Platinum rootstock to *Phytophthora* pathogens.

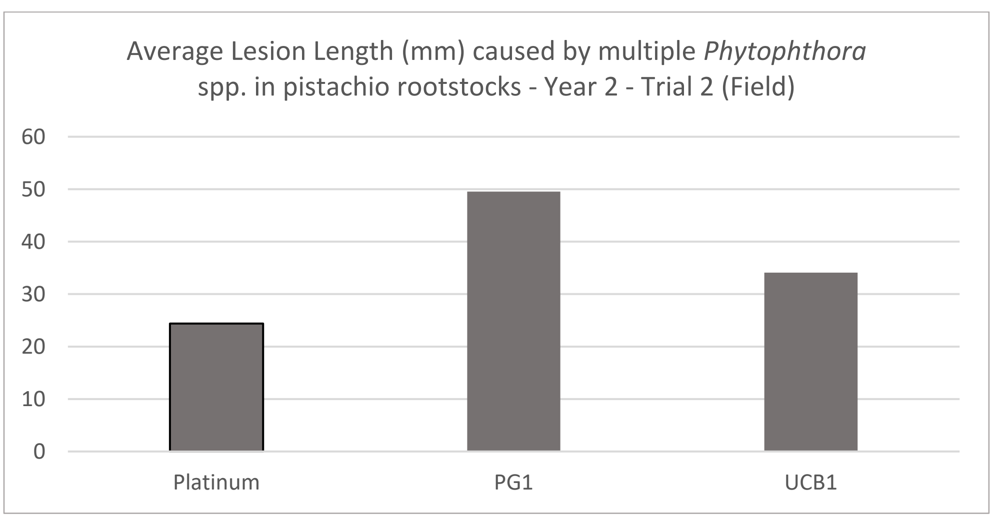

Fig. 2. Average lesion length (mm) produced in stems of Platinum, PGI and UCBI rootstocks inoculated with mycelial plugs colonized with *Phytophthora* isolates. Lesion sizes were averaged for 6 *Phytophthora* isolates representing 3 *Phytophthora* spp. (*P. niederhauserii*, *P.* taxon walnut, *P. cinnamomi*) and for each rootstock. Data indicate higher tolerance of Platinum rootstock to *Phytophthora* pathogens.

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

In preliminary screens of UCB1 clones, large differences between defined clones of this cross were identified (McKenry, unpublished). In recent work, interaction of *Pratylenchus vulnus* with *Mesocriconema xenoplax* (ring nematode) on pistachio illustrated the susceptible host status of one clone of UCB1 (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, and in 2019 four more genotypes provided by Dr. Mallikarjuna Kuma Aradhya (USDA-ARS Davis). Controls were *Prunus* rootstocks with known susceptibility to the respective nematodes or *Juglans* rootstocks. Three sets of rootstocks were planted into sandy loam field plots (first planting 2017, second planting 2018, third planting 2019) for inoculations with *Pratylenchus vulnus* and *Meloidogyne* sp. Three sets were planted to sandy soil in contained plots for inoculation experiments with *Mesocriconema xenoplax* (first planting 2017, second planting 2018, third planting 2019).

__Results and Discussion__

__The field screen for susceptibility to RLN and RKN planted in 2017__ included nine clonal UCB1, one seed derived UCB1, two *Pistacia atlantica*, one *P. terebinthus*, one *P. integerima* and one hybrid of *P. atlantica* and *P. integerima* (15 genotypes total). In 2017, plants had been planted in pairs of two per genotype in four replicate plots. In 2018, all plants were measured for height and diameter. For each genotype, one of the plant pair was removed, and root systems were rated for nematode-induced galling before fine roots for nematode extraction were excised. These results were reported previously. These trees are scheduled for repeated evaluation in the 2019-2020 dormant season.

The corresponding plantings in 2018 and 2019 are scheduled for their first and second year evaluation. One screen for susceptibility to *Mesocriconema xenoplax* was planted in single-plant plots in sand tanks in 2017, a second one in 2018, and the third in 2019. In 2019, soil cores were taken from the root zone of the test plants of the 2017 and 2018 plantings, and nematodes extracted with a sugar flotation-centrifugation method that aims at nematode stages in the soil matrix. In the sampling in 2018, 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. In 2019, ring nematode numbers had increased compared to the 2018 sampling. There still were no significant differences among the different test lines but numbers under pistachio were on a similar level as under peach rootstock 'Lovell'. A longer incubation will be necessary for obtaining a comprehensive assessment.

The later plantings (2018 and 2019) included experimental lines provided by Dr. Malli Aradhya (USDA-ARS).

Conclusion

Overall, root lesion and root-knot nematode numbers had been lower than in susceptible standards in the 2018 evaluations. In the first- and second-year evaluations of ring nematodes numbers were comparable to prunus rootstocks. The typical slow development of pistachio will require continued monitoring of nematode population development.

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