Executive Summaries 2017

California Pistachio Research Board
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2017 Manager's Report

Bob Klein

Annual report messages are a time for reflection on the past year and an expectant look to the new year. Reflection on 2017 reveals a year starting with a bang and ending with a fizzle.

We came into 2017 with the legacy of the record 2016 crop (largest in history at nearly 900 million pounds), but with disappointingly high levels of defects including insect damage. It is sobering to think that the quantity of insect damaged nuts (>20 million pounds) was larger than the entire crop of 1981 (14 million pounds) and similar to the size of the 1983 crop (26 million pounds). The 2016-2017 winter and water supplies were looking good due to above normal rainfall, chill was ample, and the wet cool weather drove down navel orangeworm populations. Bloom weather was good and the crop potential, even after the prior on-year, was about 700 million pounds. Ah, the heady days of spring!

Then came a hot summer with near record numbers of days over 100°F and warm nights that decreased photosynthesis and increased insect pressure - and 100 million pounds were lost. The crop numbers came in at 605.7 million pounds with as much insect damage as 2016, averaging over 2%. The size of the crop may have disappointed many, but it still was the second largest in industry history. The current winter looks more like drought with little precipitation and cold nights, and warm sunny days without fog. The number of hours under 45°F looks good, but the quantity of the chill is offset by the sunny days, leading to concerns about adequate quality of chill. We still have time to accumulate quality chill – hope still exists.

Climatic conditions over the last five years and the expanded acreage of navel orangeworm (NOW) sensitive crops (almonds, walnuts, figs and pomegranates in addition to pistachios) have conspired to increase the insect damage in pistachio crops. This increased damage has in turn led to increased levels of aflatoxin. Damage levels over about 0.5% make meeting aflatoxin tolerances in some markets particularly problematic. The use of AF36 has not been the game changer we had hoped for and research is underway to determine how it could be used more effectively. While exporting processors routinely test the exported lots, this does not eliminate rejections of lots upon arrival at destination. The rejection levels have become of great concern to the European Union (EU). This past fall, the US pistachio industry's aflatoxin controls were audited by the EU. The pistachio industry, through the Administrative Committee for Pistachios, will be developing a formal voluntary aflatoxin testing and reporting program in response to this audit. This will not solve the problem due to statistical reasons related to the distribution and sampling of contaminated nuts, but will add expense to the marketing of pistachios. We hope that the program will reduce the mandatory testing levels on US pistachios, but the long-term solution lies in producing pistachios with lower levels of insect damage.

Regulators in the EU have become concerned about levels of another mycotoxin, ochratoxin A (OTA). OTA is produced by fungi related to the ones that produce aflatoxin, but we know relatively little about OTA because it has not been a concern in the past. With the increased testing of pistachios for aflatoxin, there has been increased testing for OTA as well and the number of rejections for OTA have increased over the past few years. We suspect that the conditions that increase aflatoxin also increase OTA but only research will tell. We are working diligently to provide data to the EU to avert the establishment of OTA tolerances in the EU and we expect to discuss this more during the coming year.

Reducing aflatoxin and OTA are significantly dependent on reducing NOW damage. The CPRB is funding research on the use of sterile NOW to control NOW populations in the field. This research is made possible because pistachio growers approved an increase in the maximum assessment rate for the CPRB. We have great hopes for the sterile insect technology but are unlikely to have any firm conclusions on its value for 3-4 years. It is not an immediate solution to NOW and related problems. In the meantime, growers will need to rely on more established methods of NOW control including scrupulous sanitation done early in the dormant season, monitoring of NOW populations and generational timing, prompt spraying, efficient spraying techniques, supplemented with mating confusion, and early harvest. Diligent use of these methods allowed some growers to reduce their insect damage in 2017 relative to 2016 so it is not impossible. But be prepared for a more aggressive pesticide program!

After several years of warnings, Food Safety Modernization Act (FSMA) compliance is upon us. The first group of growers, those with gross receipts >$500,000, will need to become compliant with the Produce Safety Rule in 2018 with smaller growers required in 2019 and 2020. There are aspects of the Produce Safety Rule that won't require compliance for several years, but it is critical to start the process earlier. The California Department of Food and Agriculture will be conducting compliance inspections under the authority of the US Food and Drug Administration and are currently hiring and training inspectors. Their inspection priorities have not yet been established, but they will be conducting training inspections in 2018 and compliance inspections in 2019. When the Produce Safety Rule was first drafted, we were told that farm compliance inspections were not being contemplated and now they are a reality – any vision for the future of these inspections is murky at best.

Looking forward into 2018, we can see regulatory challenges in the EU with aflatoxin and OTA, plus FSMA compliance in the domestic market. In the on/off cycle of pistachio, we are "scheduled" for an on-year but we don't have a good handle on the potential production based on flower bud counts. The early winter weather has not been consistent with great on-year production, but we have some time to catch up with both chill and precipitation.

Thank you all for your efforts in producing the highest quality pistachios in the world and best wishes for the 2018 crop year.

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Pathology

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

Authors: Jocelyn Millar, Professor, Dept. of Entomology, UC Riverside; **Kent Daane**, Cooperative Extension Specialist, Environmental Science, Policy, and Management, UC Berkeley; **Houston Wilson**, Asst. Cooperative Extension Specialist, Dept. of Entomology/Kearney Ag. Center, UC Riverside; Dr. Sean Halloran, SRA, Dept. of Entomology, UCR.

Introduction

Epicarp lesion, nut abortion, kernel necrosis, and stigmatomycosis (pistachio) associated with the feeding of a suite of true bug species is a major source of yield losses in California nut crops. Among these species, leaffooted bugs (LFB, *Leptoglossus* spp.) are some of the most damaging pests. In California, LFB overwinter primarily as adults, then move to feeding sites and start to oviposit in the spring. There are typically 3 generations per year, although a partial or complete 4th generation is possible in some regions. Damage is unpredictable because bug populations can immigrate into nut crops within a few days from surrounding crops or native vegetation. In the congeneric species *L. australis*, there is evidence that males move into a crop first and begin producing an aggregation pheromone that accelerates the rapid buildup of bugs in the crop (Yasuda and Tsurumachi 1994). Because of these rapid buildups, and because symptoms of bug damage may only appear some time after the bugs have moved through the crop, continuous monitoring of bug populations is a key factor in timing treatments. Current bug monitoring methods rely primarily on beat tray or sweep net sampling. Trapping systems based on pheromonal or related attractants would be of great value for monitoring purposes. Thus, our overall goal is to reexamine the pheromone-mediated behavior of leaffooted bugs, with the goal of identifying any insect-produced compounds that could be exploited in IPM programs.

LFB could use at least four different types of pheromones. First, both sexes produce alarm pheromones and defensive secretions which are not species specific, and so are unlikely to be involved in sexual interactions. Second, male LFB have a pair of glands whose contents are species-specific. These compounds are aphrodisiacs, rendering females receptive to mating, but they do not attract adult bugs into traps. Third, fragmentary evidence suggests that male, summer-form LFB may produce aggregation pheromones that attract both sexes into orchards. Finally, LFB form overwintering aggregations in sheltered spots, and it is likely that pheromonal signals assist in the formation and maintenance of these overwintering aggregations. These signals are likely to be different from the signals used to attract bugs into a crop for feeding and mating. The possible source and structures of these pheromone compounds are not known.

Our project objectives are to identify and verify the function of each type of pheromone, with the goal of developing practical applications of one or more of these pheromones for LFB management.

Results and Discussion

Because we initially had difficulty in establishing colonies of LFB to work with, we did not request additional funding for our second year of work. Thus, this report covers work for the period March 2016-November-2017. In addition, a new hire at Kearney Agricultural Research and Extension Center (KARE), Dr. Houston Wilson, has joined the team to carry out field trials of traps and test lures.

Summer-form colonies of *Leptoglossus zonatus* (LZ) were established in late summer of 2016, and they transitioned into the nonreproductive, overwintering form. The colonies were refreshed with field-collected insects through 2017. We were not able to collect our second target, *L. clypealis* (LC), until recently because for unknown reasons, this species has become less common in central California. We finally located an overwintering site near UCR in October 2017, and collected several hundred adults. Through the winter, we will try and revert them to the reproductive summer-form so that we can establish a colony. Specimens have also been sent to Daane to start a colony at KARE.

Other work conducted at UC Riverside to date includes:

1. Extraction and identification of the alarm and defensive compounds produced by immature and adult LZ.
2. Collection and analysis of odors released by sexually immature and sexually mature LZ adults, from both summer- and winter-form individuals. The analyses showed that sexually mature summer-form males specifically produce several compounds, which are not produced by adults of any other type or sex. These compounds are strong candidates for attractant pheromones used to attract large numbers of conspecifics into nut orchards. All of these compounds have been identified, and the key compound has been synthesized in small amounts for testing. We are currently assessing whether the synthesis can be improved, or whether we can instead isolate the key compound from a commercially available plant oil.
3. Analysis of cuticular hydrocarbons from summer- and winter-form LZ adults of both sexes, to assess whether these compounds might be involved in forming or holding overwintering aggregations together. We have found clear differences between the sexes, and between summer- and winter-forms, so it is likely that these compounds are indeed overwintering signals.

Work conducted by Daane and Wilson to date includes:

1. Providing LZ starter colonies to the UCR team.
2. Field trials attempting to demonstrate attraction of adults to caged summer-form males and females. This experiment was not successful due to the difficulty in keeping bugs alive in cages.
3. Testing of different trap types, which showed that cross-vane panel traps are far superior to other types of traps for catching LFB, even with no attractant lure.
4. A quick-and-dirty field trial of the impure synthetic reconstruction of the sex-specific compounds produced by summer-form male LZ, showing some evidence of attraction to the synthetic lure.

Conclusions

This project is now on a firm footing with colonies of the two target species at both UCR and KARE, and we are making good progress. Specifically, we have strong leads for a male-produced aggregation pheromone for LZ, as well as leads for compounds involved in overwintering aggregation formation. For the coming year, we anticipate further field tests of the synthetic pheromone candidates, and doing the analyses described above on our newly established colonies of LC.

Understanding aggregation behavior of the leaffooted bug, *Leptoglossus zonatus*

Authors: **Andrea L. Joyce,** Assistant Professor, Entomology University of California, Merced.

Introduction

The leaffooted bug species, *Leptoglossus zonatus*, is a large seed-feeding insect found on many host plants including pistachio (McPherson et al. 1990). This species has been collected in California from Butte County to Kern County in the Central Valley and can be damaging to pistachios (Michailides 1989, Joyce et al 2014, 2017). Although there are at least two species of leaffooted bugs which occur in pistachios, *Leptoglossus clypealis* and *Leptoglossus zonatus*, *L. zonatus* is substantially larger than *L. clypealis*. A field-cage study of feeding damage from *L. zonatus* and *L. clypealis* on almonds found that *L. zonatus* was more damaging (Joyce et al. 2015). *L. zonatus* appears to have become more abundant in the last few years in California (Haviland 2007, Joyce et al. 2014). There is a need to develop traps or monitoring devices to detect the presence of this insect in orchards, before the damage from bug feeding is observed and before it causes economic damage. Although leaffooted bugs are large insects, they are difficult to monitor as they are elusive. Currently there is no pheromone or attractant available for monitoring or trapping this insect. The research in this ongoing study will contribute to understanding the biology and behavior of *L. zonatus* and the ability to trap or monitor this insect.

Attractants for leaffooted bugs could include pheromones, host plant volatiles or plant volatiles associated with bug feeding (Aldrich et al. 1979, Yasuda 1998, Wang and Miller 2000). A notable behavior of leaffooted bugs is the overwintering aggregations formed by adults, which then disperse the spring as temperatures warm and day length increases (Daane et al. 2008). Semiochemicals such as aggregation pheromones and sex pheromones, or host plant volatiles might eventually be manipulated for detection, monitoring or trapping tools and included as components of an IPM program. However, the aggregation behavior of *Leptoglossus* spp. in the Central Valley has been difficult to manipulate and may involve environmental or seasonal cues which need to be further studied (Daane 2007). We will investigate the relative attraction of volatiles to *L. zonatus*, and the environmental conditions that promote formation of aggregations and contribute to dispersal from aggregations as well. The ability to attract, detect and quantify adult *Leptoglossus* spp. in the field before damage is observed in pistachios or in almonds could improve timing of controls and could also reduce the use of preventative insecticide applications.

Results and Discussion

We continued to raise the insect *Leptoglossus zonatus* in the lab during the year, so that insects were available for lab bioassays. Large numbers of insects were produced to conduct the studies described below, which were 1) to examine the age of sexually maturity of adult *L. zonatus*, and 2) to examine attraction to volatile odor sources in the wind tunnel and 3) to investigate dispersal from aggregations.
Before experiments began, we had prepared cages of adult females and separate cages of males that were 1 week old, 2 weeks old, 3 weeks old, 4 weeks old, 5 weeks old, and 6 weeks old.

The age of sexually maturity of adult *L. zonatus* was investigated. We previously had an estimate of the age of the insects' sexually maturity of approximately 2 weeks. This year we ran experiments to pinpoint the age when *L. zonatus* adults would mate most rapidly. We compared the mating frequency of unmated adult male and female *L. zonatus,* for three different age categories. We setup cages with 2 week old males and females, 4 week old males and females and 6 week old male and female insects. These insects were all observed at the same time each day, to determine the mating frequency for 2 wk, 4wk, and 6 wk old adults. We experimentally determined the age of sexual maturity for *L. zonatus* We found that 4- week old and 6-week old male/female pairs would mate most rapidly. For all experiments described for 2017, we used 4-week old unmated male and female adult *L. zonatus* for investigating attraction to odor sources.

Adult insects which were 4 weeks old and unmated were used in behavioral bioassays in the wind tunnel. Six different experiments were run to examine whether males, females or combinations of males and females were more or less attractive than plant odors. Insects were only used for one behavioral trial to avoid pseudo-replication. Dual choice trials were run to compare the following 1) the attraction of females to either 10 males vs. a control, 2) the attraction of females to either 10 females vs. a control, 3) the attraction of males to either 10 females vs. 10 males, 4) the attraction of females to either 10 females vs. 10 males. The next set of experiments involved dual choice trials again, but one choice consisted of mating pairs of *L. zonatus*. Experiment (#5) compared the following 5) The attraction of males to either 5 mating female/male pairs vs. attraction to a control, and finally the last experiment was the following; 6) the attraction of males to 5 mating pairs of male/females vs. 10 females. Data recorded included the number of landings on each odor choice, the time spent on each odor, and the time from the start of the experiment until a test insect landed on an odor. At least 25 replicates were run for each of these experiments. Insects were observed for at least 15 minutes in experiments. In the wind tunnel, behavioral trials were continued to examine attraction of males and females to volatile odor sources. The results found that males were most attracted to females, which is consistent with our preliminary data reported last year. In experiment 2, females landed equally on either males or on the control. When males or females were offered a choice of males vs. females (Exp. 3&4), there was no significant difference in landings on males vs. females. Finally, the attraction of males was tested to odors of females vs. mating pairs, and there was no significant difference in attraction. However, when males were tested to determine their response to odors associated with mating pairs vs. a control, males had nearly twice the number of landings on mating pairs as they did on the control. Odors associated with mating pairs will be further investigated as attractants.

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

Conclusion

There are many potential volatile attractants for adult *L. zonatus*. This year, experiments included both insect odors and host plant odors. Results suggest that the most attractive odor source is associated with mating pairs of *L. zonatus.* This is being further investigated to pinpoint the most attractive age of mating pairs and the associated odors. Volatiles will be collected and attraction to volatiles will be tested. Traps using insect mating pairs as lures will also be tested in the field this spring.

Aggregation behavior is being monitored in the field this spring to relate dispersal to temperature. Information on temperature might be used in a model to predict when leaffooted bugs in aggregations would disperse and arrive in adjoining fields. This research will contribute to an early detection system such as a trap for *Leptoglossus zonatus,* which is needed to develop an IPM program for this species.

Population dynamics and epidemiology of navel orangeworm damage to pistachios: weekly damage

Authors: **Joel P. Siegel,** Research Entomologist, USDA-ARS, San Joaquin Valley Agricultural Sciences Center, Parlier**; Carla Baker,** Weinberger, Fukuda and Associates, Tulare**; Chris Wiley,** Agri-world Coop, Madera**; Devin Aviles,** Agri-world Coop, Madera**, Joe Coelho,** Valley Orchard Management, Fresno**, Matt Strmiska,** Adaptiv, Fresno**, James Nichols,** Nichols Farms, Tulare, **Todd Fukuda**, Weinberger, Fukuda and Associates, Tulare

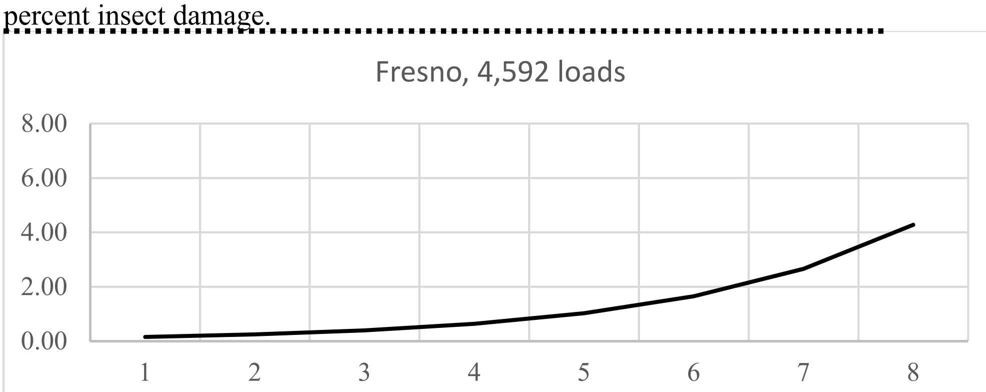
Introduction

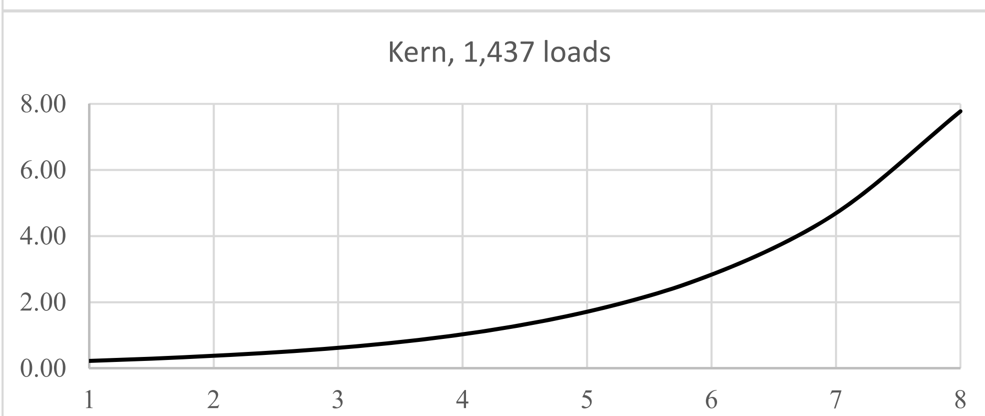
The purpose of my research is to improve control of navel orangeworm by a combination of improved spray timing, increased application efficacy, proper insecticide choice and rotation, and the integration of mating disruption with existing control strategy. Additionally, I monitor the pattern of harvest damage, using gradesheets supplied by individual growers and processors, with the goal of improving navel orangeworm control.

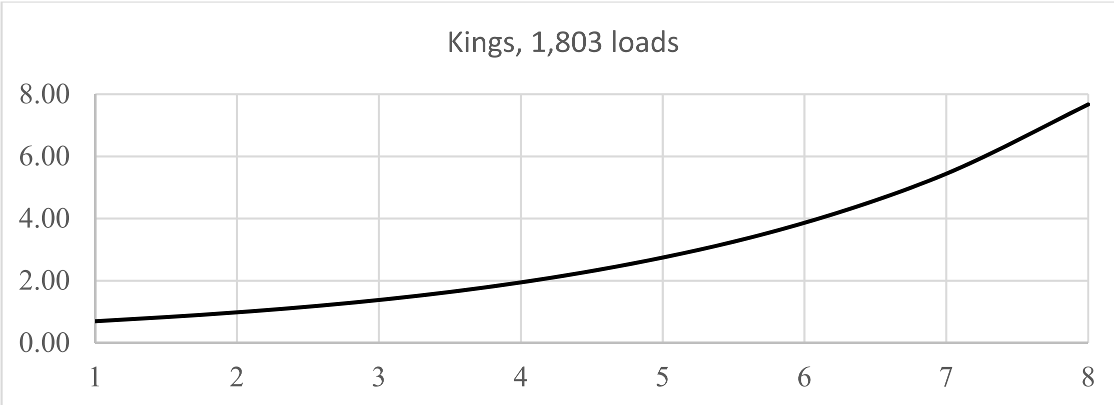
Results

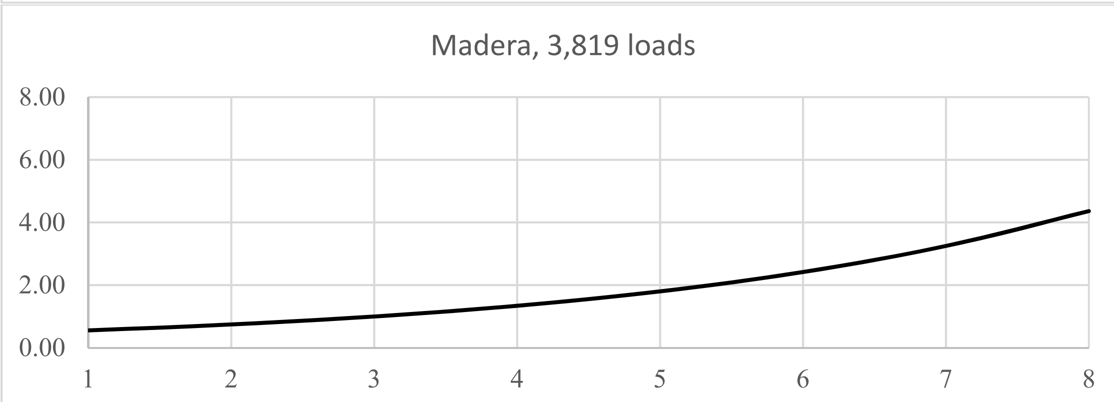
In 2016, I calculated the pattern of damage in the loads received by the processor in a spreadsheet format, using the variables days after August 21, percent split, percent dark stain, and percent shell defect. You can request a copy of the spreadsheet by contacting me at: joel.siegel@ars.usda.gov.

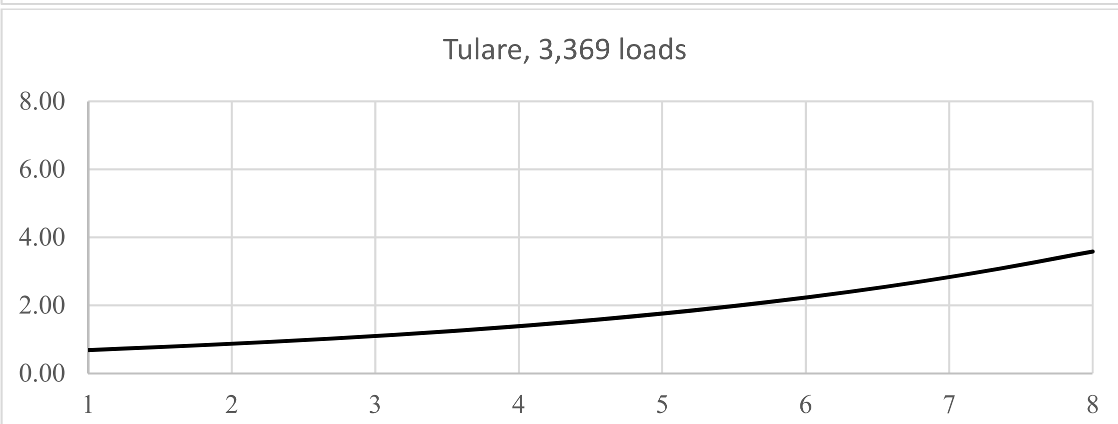
The following graphs report weekly damage for the San Joaquin Valley counties of Madera, Fresno, Tulare, Kings and Kern. The X-axis is week of harvest, beginning August 21. Y-axis is percent insect damage.











Conclusion and Practical Applications

Damage increased exponentially, with a doubling time just under 2 weeks/14 days. Damage was highest in Kern and Kings county. The 2017 harvest will be analyzed to confirm that the doubling time for damage is 2-weeks. These results underscore the importance of early harvest. It is essential to plan ahead so that your crop does not linger in the field because damage is accelerating over time. These data underscore the need for new strategies to protect the late harvested crop.

Population dynamics and epidemiology of navel orangeworm damage to pistachios: influence of mating disruption on trap capture

Authors: **Joel P. Siegel,** Research Entomologist, USDA-ARS, San Joaquin Valley Agricultural Sciences Center, Parlier**; Carla Baker,** Weinberger, Fukuda and Associates, Tulare**; Chris Wiley,** Agri-world Coop, Madera**; Devin Aviles,** Agri-world Coop, Madera**, Joe Coelho,** Valley Orchard Management, Fresno**, Matt Strmiska,** Adaptiv, Fresno**, James Nichols,** Nichols Farms, Tulare, **Todd Fukuda**, Weinberger, Fukuda and Associates, Tulare

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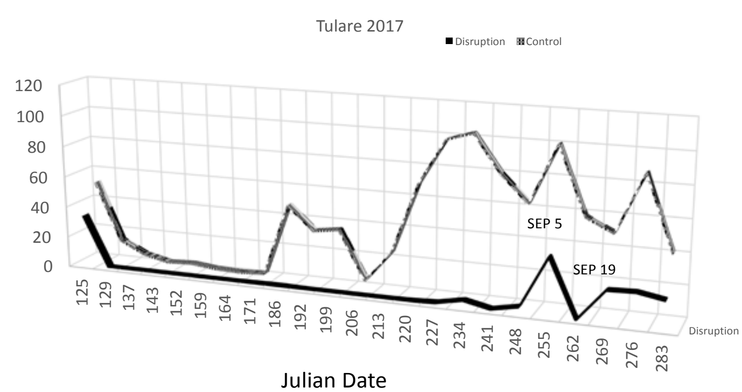
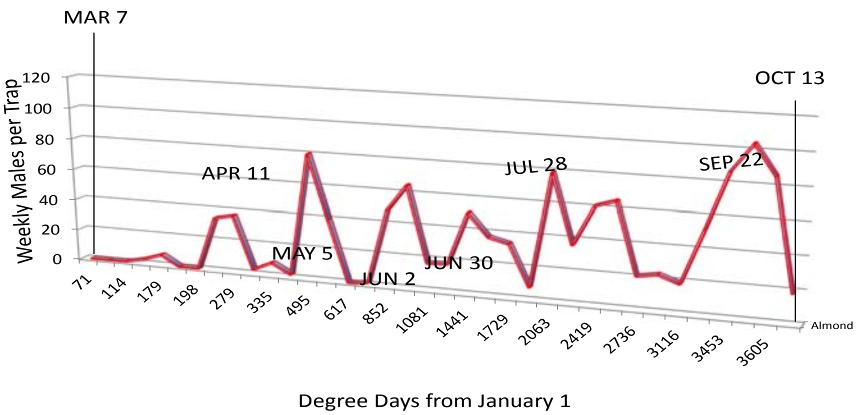
Results

In 2017, I ran a series of pheromone traps both within mating disruption blocks of pistachios and in almonds adjacent to mating disruption. These graphs illustrate that although mating disruption shuts down traps for long periods, trap capture spikes during periods of mass NOW movement. By the time a peak is recognized it is often too late to prevent damage. Consequently, it is better to use mating disruption and a full spray schedule for the first year of implementation until the standing population is reduced.

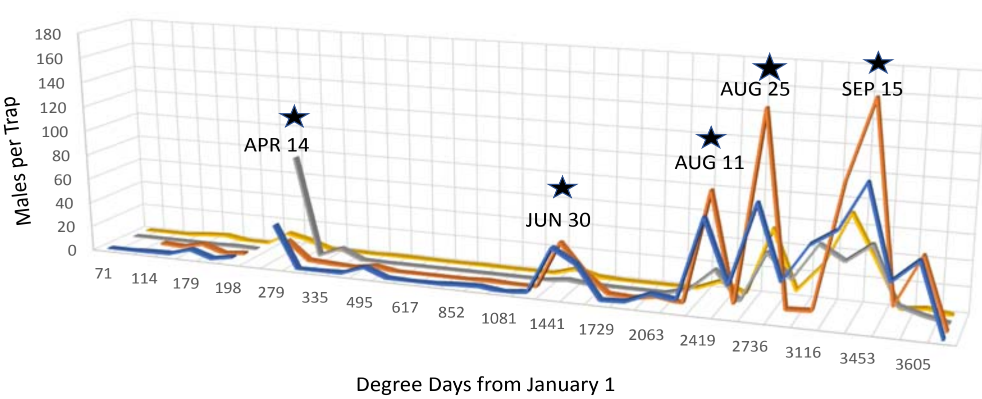
Mating disruption block compared to nearby control block, Tulare County



The following graph illustrates the pattern of activity in Madera County, using traps located in a 1,000 acre almond block that is not using mating disruption.



The next graph reports the pattern in a Madera pistachio block with partial mating disruption. Stars mark shared activity peaks with almonds.



Conclusion and Practical Applications

Despite mating disruption, pheromone lure traps caught moths during peaks in flight activity (measured in almonds) throughout the season. In Tulare county, trap suppression was most effective from early May through early September, while in Madera county the intervals were shorter; mid-April through late June and basically the month of July. These data underscore that huge populations are moving into pistachio orchards after Nonpareil almond harvest and it is essential to stick with the pre mating disruption spray program, even when no moths are captured, until the standing population is reduced. These late August –late September capture peaks coincide with higher pistachio damage On the bright side, these data demonstrate that traps placed several miles from mating disruption blocks can successfully indicate NOW pressure..

Population dynamics and epidemiology of navel orangeworm damage to pistachios: comparing application efficacy: ground vs air

Authors: **Joel P. Siegel,** Research Entomologist, USDA-ARS, San Joaquin Valley Agricultural Sciences Center, Parlier**; Carla Baker,** Weinberger, Fukuda and Associates, Tulare**; Chris Wiley,** Agri-world Coop, Madera**; Devin Aviles,** Agri-world Coop, Madera**, Joe Coelho,** Valley Orchard Management, Fresno**, Matt Strmiska,** Adaptiv, Fresno**, James Nichols,** Nichols Farms, Tulare, **Todd Fukuda**, Weinberger, Fukuda and Associates, Tulare

Introduction

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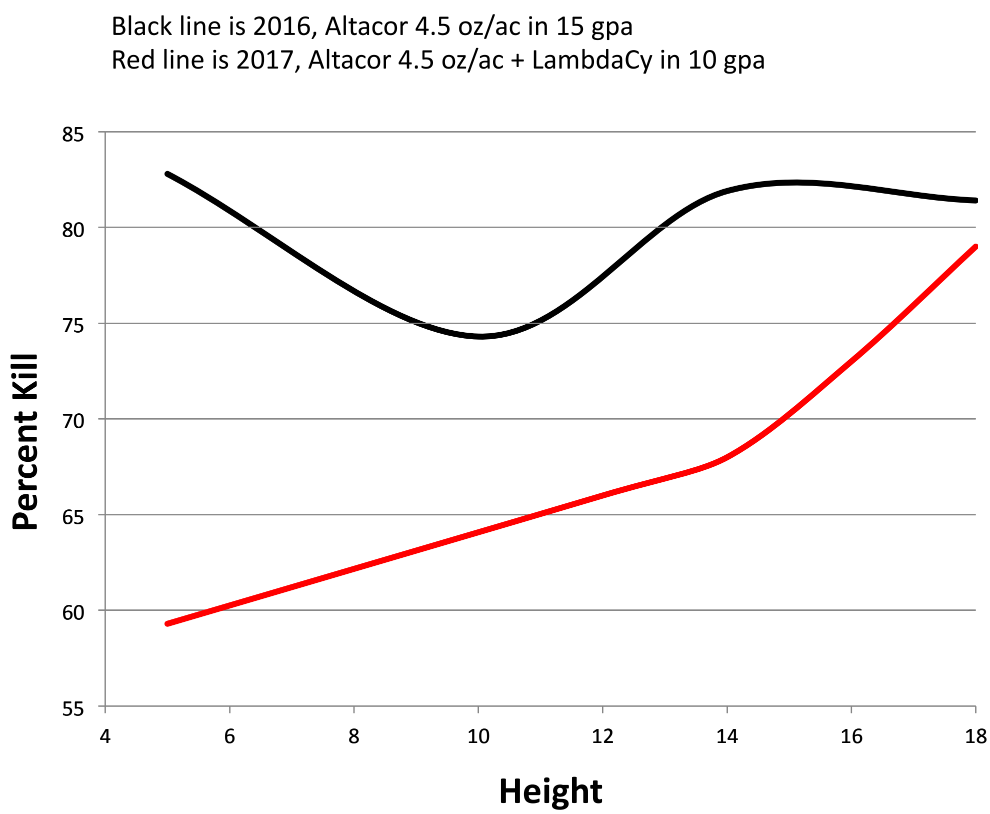
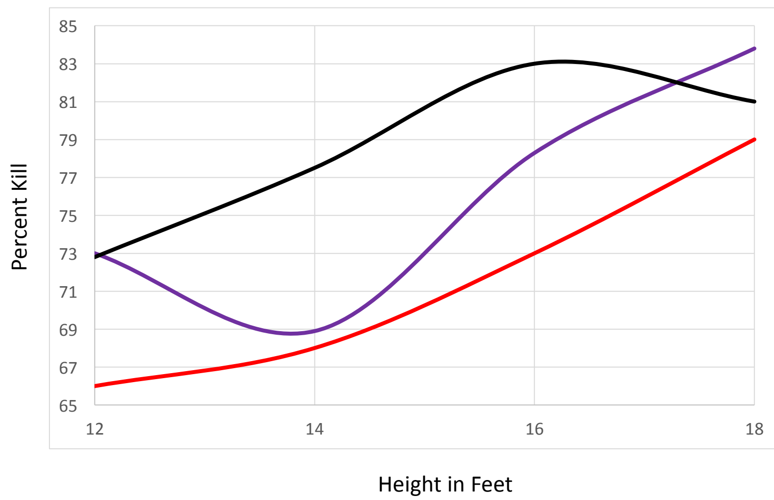
Results

In 2016, I monitored an aerial application of Altacor applied at 15 gpa (4.5 oz Altacor/ac). Using my filter paper bioassay, percent kill was assessed at 10-18 ft with additional monitors placed at 5 feet above ground. Coverage was even throughout this range, and the study was repeated in 2017 using a lower volume, 10 gpa, with Altacor at 4.5 oz/ac+LambdaCy at 5.12 oz/ac. At the lower volume of 10 gpa, coverage was not even and dropped precipitiously from 18 feet to 5 feet.



I also compared ground application at 50 gpa using multinozzles to the aerial application at 10 gpa. The adjuvant used in the aerial application was Cohere, while the ground applications used Kienetic and HiWett. Despite some variation at 14 feet, both ground applications were the same and both were better than the aerial application. At 12 feet, the 10% difference in percent kill between ground and air is most pronounced, (Chi Square of 6.69, $0.01 > P > 0.005$).



Conclusion and Practical Applications

My past research has demonstrated that application efficacy increases with spray volume (200 gap was better than 100 gpa+multinozzles). If spray volume can be decreased to 50 gpa, using a silicone-based adjuvant, considerable time will be saved for an application. However, I did not compare the efficacy of ground sprays at 50 gpa to applications made at 100 and 200 gpa. This will be the focus of my 2018 pistachio research, as well as conducting further assessment of aerial application. If the research demonstrates that there is no loss in efficacy, growers may wish to consider spraying with a reduced volume.

Automated solid state canopy delivery (SSCD) system to deliver mist-cooling to increase winter chill for dormancy and bud break

Authors: Yiannis Ampatzidis, Associate Professor, Department of Physics and Engineering, California State University, Bakersfield (CSUB); **Louise Ferguson**, Extension Specialist, UCCE, UC Davis; **Lu Zhang**, Postdoctoral Scholar, UCCE, UC Davis; **Reza Abdolee**, Assistant Professor, Electrical Engineering Department, CSUB; **Emerson Argueta,** Research Assistant**,** CSUB**.**

Introduction

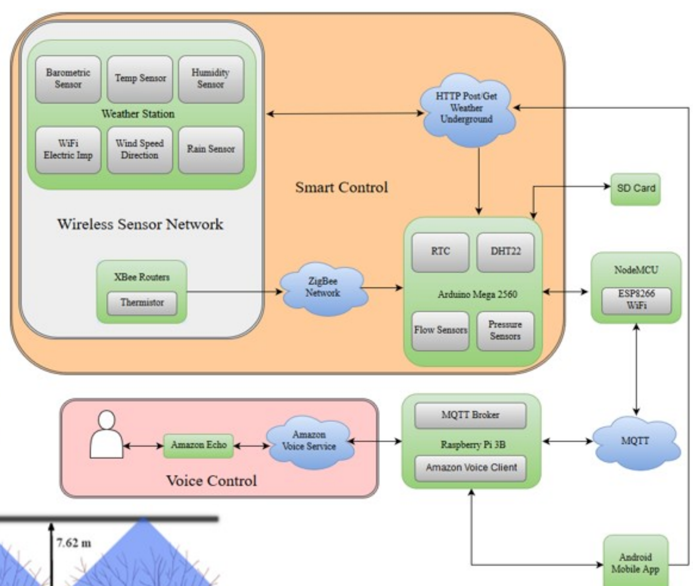
As the Central Valley fog disappears, the chill accumulation required for dormancy breaking is decreased resulting in abnormal flowers, poor bloom synchrony, high rates of blanks and lowered yields. Kern County has been experiencing higher than average winter temperatures and is predicted to undergo an average increase of 1.8 °C to 4.5 °C annually (Cal-Adapt, 2017). It appears this warmer climate was the primary factor in the drop in California's 2015 pistachio production; 47% from 2014 to 2015 (California Department of Food and Agriculture, 2016). We examined the ability of the user-friendly low-cost wireless Solid Set Canopy Delivery (SSCD) system to cool down bud temperatures on sunny (winter) days.

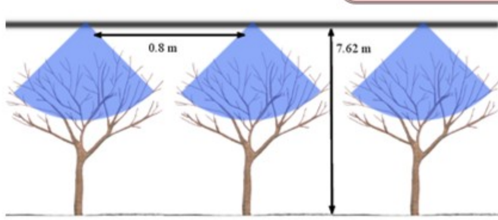
Materials and Methods

The proposed Voice-Controlled and Wireless Solid Set Canopy Delivery (VCW-SSCD) system includes (Fig. 1):

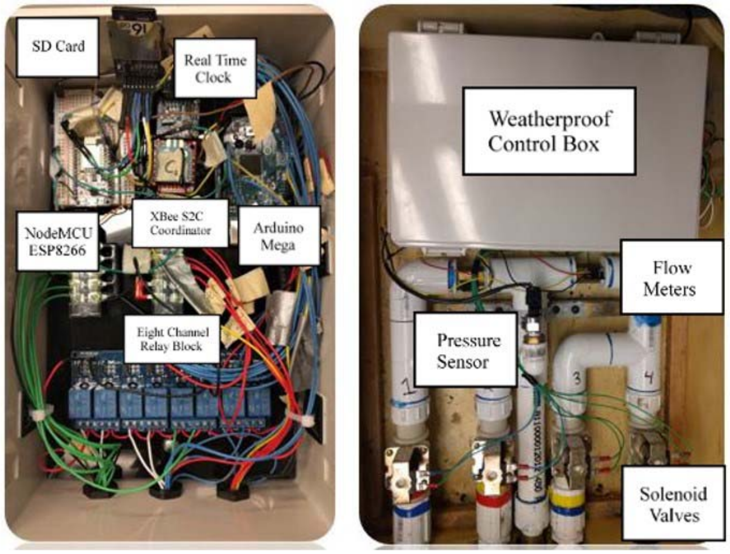
i. A SSCD system (Fig. 2) to apply mist and cool down the bud temperature.
ii. A Wireless Sensor Network (WSN) and a Weather Station (WS) to collect weather- and crop-related data from the field (several zones).
iii. A Voice-Controlled (VC) system using Amazon Alexa (Amazon Echo) and a server.
iv. A mobile app (Android; Fig. 3) to visualize the collected data and control the SSCD system.
v. A Smart Control (SC) system (Fig. 4) to collect the data from the WSN, receive commands from the VC and control the SSCD system. It communicates with the mobile app too.

Right: Figure 1. System Block Diagram. The Smart Control (SC) system collects data from the Wireless Sensor Network (WSN) and the weather station, receives voice commands from the Voice-Controlled (VC) system, communicates with the mobile app, and wirelessly control the SSCD.

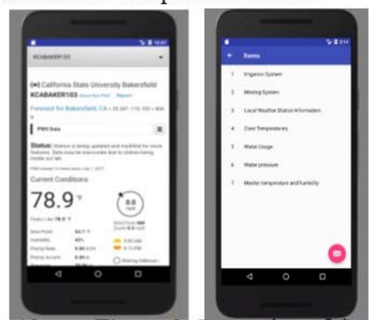




Left: Figure 2. Mounting emitter configuration of the pilot SSCD system.



Left: Figure 4. The Smart Control (SC) System includes an Arduino Mega 2560 microcontroller, a node MCU Wifi module, a XBee S2C, a real time clock, 8-channel relay block, solenoid valves, flow meters and pressure sensor.



Above: Figure 3. Screenshot of the mobile app: weather station data.

The Android application was developed to retrieve the collected data from our server (Raspberry Pi 3; data from the WS and WSN), visualize them and display all the information in graphical format (using the Androidplot software), and provide real-time data to the users. It can also control the SSCD system remotely (turn on/off the water valves). The Android application includes a user friendly graphical interface (Fig. 3); JSON parsing (in Android Studio) was used to retrieve data from the Weather Underground web-software and our server. The mobile application is a module-based, allowing users to access real-time data, control water valves, and perform analysis. The user is able to view reports from certain periods of time; hourly, daily, weekly, monthly, and yearly.

Results and Discussion

We deployed this system in an orchard (Bakersfield, CA) that is divided into 8 zones. Each zone includes a different type of sprinkling nozzle to evaluate the effectiveness of different nozzles in wetting the trees and the effect of them on the tree's evapotranspiration. Each zone also is assigned a valve that is controlled by the SC.

We will evaluate this system during 2017-2018 season and measure:

- The duration of misting (hours/treatment) at different temperatures.
- The intervals between applications to conserve water (hours/days); and determine when evaporative cooling ceases at different temperatures.
- Droplet size for maximum evaporative cooling duration.
- Amount of water applied (volume, ha-cm) per application.
- Volume of mist and energy consumption per treatment and per tree.
- Flower quality evaluation (bloom synchrony, pollen and ovule viabilities).
- Postharvest fruit quality data (tree yields, nuts grading by processing factory).
- Investigate potential salinity and toxicity problems (water, soil and plant analysis).

Conclusion and Practical Applications.

The main goal of this project is to develop a novel Wireless Solid Set Canopy Delivery system to deliver mist-cooling, and to evaluate the effectiveness of the misting system (different types of nozzles, system configuration etc.). We developed and deployed the SSCD system in a commercial pistachio orchard in Bakersfield, CA; we will evaluate it during 2017-2018 season. This system will help California growers to further understand, and perhaps manipulate the chilling requirements for pistachio.

Evaluation of rootstocks for pistachio production

Authors: **Robert H. Beede**, UCCE Farm Advisor, Emeritus; **Louise Ferguson**, UCCE Pomology Specialist, UC Davis.

Introduction

In 1989, Dr. Louise Ferguson wisely initiated five rootstock trials distributed throughout the state. They were located in western Kern County, the UC Westside Field Station (western Fresno County), the Kearney Agricultural Center (eastern Fresno County), eastern Madera County, and Shasta County (near Anderson). Each trial included P. *atlantica*, P. *integerrima*, (Pioneer Gold I), P. *integerrima* x P. *atlantica* (Pioneer Gold II), and P. *atlantica* x P. *integerrima* (UCB-1). Each trial consisted of 400 trees (cv. Kerman) which were divided into 100, four-tree plots, containing one of each rootstock. Ten of these four-tree plots where then grouped into a single 40-tree irrigation set to facilitate irrigation and nutrition studies. Male trees (cv. Peters) were also replicated on each rootstock and placed every third tree within the row and every third row.

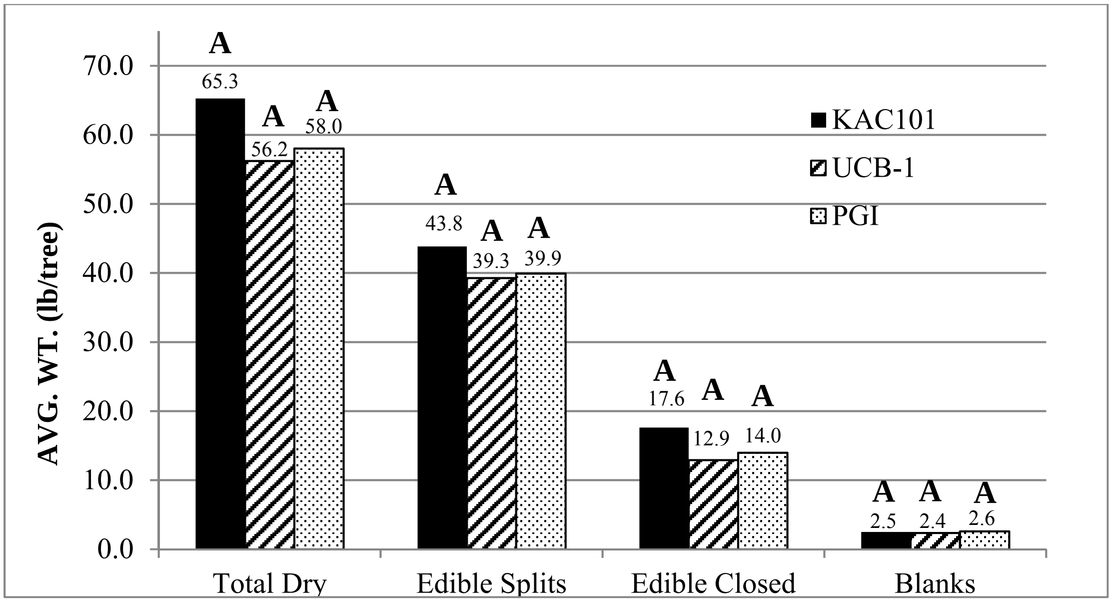
These trials provided milestone information about cold (Shasta County), verticillium (Westside Field Station) and salinity tolerance (western Kern County). The Kern County (prior to its use as a salinity experiment), Madera, and Kearney Agricultural Center (KAC) trials served to establish production differences among the four rootstocks. Cumulative yields from the first five bearing years showed UCB-1 significantly more productive than PG II or PG I, the latter two being very similar in yield. P. *atlantica* was significantly less productive than the other three rootstocks.

The lead author also assessed the relative productivity of these four rootstocks at orchard maturity. Yield data was collected in 2009 and 2011, when the trees were 20 and 22 years old, respectively. The orchard in both years was heavily cropped, which provided an excellent opportunity to contrast rootstock performance at maturity to that recorded during their first five bearing years. Yield data collected from 74 individual trees per rootstock showed that UCB-1 and PGII had become very similar in total dry weight and split nut production, which was significantly greater than that of PGI and P. *atlantica*. P. *atlantica* produced the least. UCB-1 and PGII produced a two-year average of 7.5 pounds more split nuts than PGI, and 14 more pounds than P. *atlantica*.

Individual tree yields from the KAC trial also revealed significant production differences in seedling rootstocks within a given species or hybrid. This spurred great interest in clonal development procedures and monitoring commercial fields for "superior seedlings". In 2003, the authors identified one such UCB-1 seedling, and a long term, replicated trial was established by the lead author to compare its performance as a vegetatively propagated clone (named KAC101) to standard UCB-1 and P. *integerrima* seedlings. Yield data collection began in 2008 (sixth leaf), and has continued annually. Each of the 16 trees per rootstock is commercially shaken. Dry weight and nut quality are derived from samples submitted to Wonderful Farms.

Results

Chart 1 provides the 2017 yield summary of key production categories for the clonal rootstock experiment. Field weight data was converted to inshell split, edible closed inshell and shelling stock, and blank nut weights from two 20 pound composite samples taken at harvest for each rootstock and submitted for standard evaluation by Wonderful Farms. The results show that KAC101 continues to yield very similarly to UCB-1 and PGI, based upon total dry, edible split, closed inshell, and blank nut weights.



Now 15 years old, the cumulative total yield data from the sixth leaf is reported in Table 1. Over ten years, KAC101 has produced 36.3 pounds more total dry weight than UCB-1, and 44.1 more pounds than PGI. This represents an average of only three to four pounds per year. This is not statistically significant.

Rootstock	Cumulative Total Dry Wt. 2008-2017 (lb/tree)	2017 Nut Quality (percent)			
		Dry weight	Split inshell nuts	Closed shell nuts	Blank nuts
KAC101	327.3	42.10	67.09	27.01	3.86
UCB-1	291.5	42.54	69.79	23.03	4.23
PGI	283.2	41.88	68.81	24.04	4.47

Conclusions and Practical Applications

KAC101, a seedling of UCB-1 selected from the main trial and vegetatively propagated by hardwood cuttings, has performed similarly to randomly selected UCB-1 and PGI seedlings. Now 15 years old, the total dry yield of KAC101 averages 3.6 pounds per tree more than UCB-1, and 4.4 pounds more than PGI. This amount is not statistically significant, hence annual yield comparison with existing commercial rootstocks is concluded. Yield records have not suggested justification for verticillium, nematode, salinity, and cold tolerance screening. Ease of cloning using commercial tissue culture procedures might justify its release. Previous denutting experiments performed on individual, mature trees in the old block during two on-years strongly indicates that "superior" rootstock performance (relative to yield only) is strictly a function of tree size, and **NOT** greater fruiting density. Canopy measurements within the **mature** rootstock trial support this conclusion, because the average UCB-1 and PGII tree is larger than either PGI or P. a*tlantica*. The release of an unproven UCB-1 sibling also subjects the pistachio industry to unnecessary risk.

Effect of hedging and topping on pistachio alternate bearing: 2012-2017

Authors: Robert H. Beede, UCCE Farm Advisor, Emeritus; **Louise Ferguson**, UCCE Pomology Specialist, UC Davis; **Bruce Lampinen**, UCCE Pomology Specialist, UC Davis; **Sam Metcalf**, Staff Research Associate, UC Davis.

Introduction

Although mechanical side hedging of pistachios is common, questions persist about whether alternate bearing (AB) can be mitigated by hedging prior to an off-year, rather than an on-year. This seems counter intuitive to the bearing habit of pistachio, since the yield loss from heavy pruning prior to an on-year is partially compensated for by a higher set percentage in the remaining flower clusters. The capacity for compensation is also dependent upon the severity of pruning. The higher crop load associated with on-trees also appears to reduce the total seasonal growth compared to trees severely pruned prior to an off-year.

To address this question, a randomized complete block, split-plot experiment was established in the rootstock trial (UCB-1, PGII, PGI, and P. *atlantica*) at the Kearney Agricultural Center in Parlier during the winter of 2011-12, an off-year for this orchard. This east-west planted orchard was divided from north to south to create two identically designed experimental halves. The **east half** was mechanically side hedged and topped in 2011-12, and the west half was hand pruned. Side hedging consisted of cutting **every other middle** at six feet from the tree trunk. Topping was then performed at 14.5 feet. Hedging and topping (H&T) severity varied by rootstock due its effect on tree size. UCB-1 and PGII were thus more severely pruned than PGI and P. *atlantica.* Up to five feet of growth was removed from topping the largest trees. Hand pruning was performed on the non-hedged side to insure efficient nut removal and duplicate best commercial cultural practices.

During the winter of 2012-13, the same H&T methods were applied to the **west half** of the trial (hand pruned the previous year), using the same tree middles. Observations confirmed that this half of the orchard was going into a pronounced on-year. During the 2013-14 winter, the alternate rows not yet mechanically pruned on the **east half** of the trial were side hedged.Thus, by delaying hedging of the alternate rows one season, both sides of the east half of the trial were hedged prior to an off-bearing year. This same procedure was applied to the west half of the trial during the 2014-15 winter to complete the mechanical pruning of the on-year treatment.

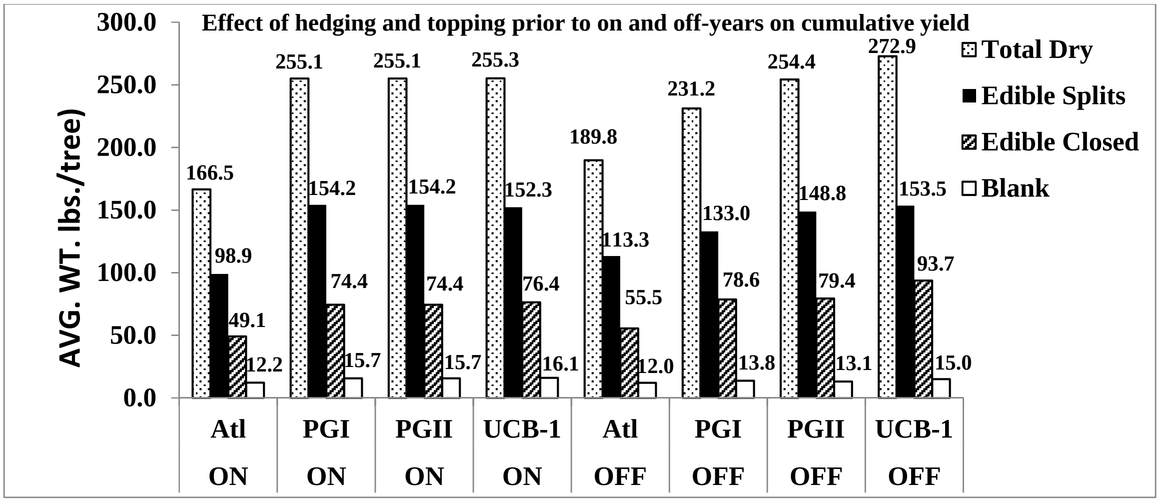
Hand pruning was performed annually on rows not freshly hedged. In addition, tipping of the one-year-old growth on the sides and tops of the trees mechanically pruned in previous years was also performed according to commercial practices.

Individual tree yields were collected annually using tarps and a shaker. Twenty pound, composite samples were taken by rootstock in each of the five replications for each pruning treatment. The 40 samples were then commercially evaluated. The grade sheet data was then used to calculate the total dry yield, as well as the pounds of split nuts, edible closed shell nuts, and blank nuts.

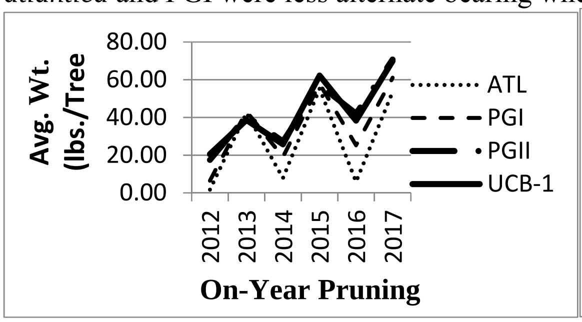
Results

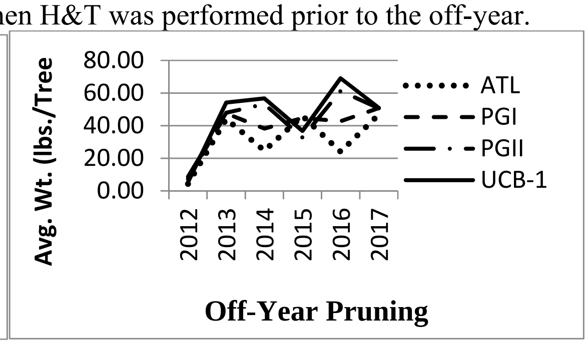
As reported in previous summaries, aerial thermal imagery documented that the trees on UCB-1 and PGII were very similar in size, and significantly larger than P. *atlantica* and PGI at the beginning of the experiment. Consequently, the trees on UCB-1 and PGII were more severely pruned than the trees on the other two rootstocks.

The chart below is the cumulative yield (2012-17) by rootstock and nut categories affecting yield.



Yield of Kerman on the rootstocks of highest vigor (UBC-1 and PGII), was not affected by the year in which hedging and topping was performed. PGI was slightly less productive when pruned prior to the off-year, but not significantly so. Being the smallest tree, P. *atlantica* yielded the least, but slightly more when pruned in the off-year. The next three charts show the bearing pattern by rootstock following H&T prior to the on and off-bearing year, and the AB index. P. *atlantica* and PGI were less alternate bearing when H&T was performed prior to the off-year.





Effect of hedging and topping on the Alternate Bearing (AB) index. Three complete AB cycles.

Rootstock	Year Topped and Hedged	Alternate Bearing Index	Rootstock	Year Topped and Hedged	Alternate Bearing Index
Atlantica	On	0.79	PGII	On	0.27
Atlantica	Off	0.41	PGII	Off	0.27
PGI	On	0.55	UCB-1	On	0.29
PGI	Off	0.21	UCB-1	Off	0.30

Conclusion and Practical Applications

The more vigorous rootstocks UCB-1 and PGII yield equally, and their bearing habit does not appear affected by whether they are hedged and topped prior to on or off-year. The AB index of rootstocks P. *atlantica* and PGI benefited from off-year reconstructive pruning. The yield depression from pruning prior to the off-year depends upon its severity. Except for P. *atlantica*, the yield of the other three rootstocks was not significantly different in 2017, presumably due to there being no difference in shaded area (PAR) after two years of not hedging. There were essentially no differences in nut quality attributable to hedging and topping during the trial.

Evaluation of mechanical and chemical strategies to enhance winter chill accumulation in pistachios

Authors: Gurreet Brar, Assistant Professor, Department of Plant Science, CSU Fresno

Introduction

Lack of chill accumulation in pistachios has been a growing production issue in the California for the past several years. Growers have been facing crop losses as a consequence of these changes and there is a dire need to address this very important issue. The current study is being conducted with the following **objectives** for this project:

1. To evaluate the effect of various chemical and mechanical strategies like netting, Kaolin clay and horticultural oils on bud temperatures and bloom of pistachios.
2. To study the effect of these strategies on nut development and yield.

This report presents summary of results from year 1 of 2. This study hypothesized that some mechanical and chemical methods could be beneficial to compensate for lack of chill or to induce physiological changes in the trees so as to have a normal bloom and crop load. Fifteen-year-old pistachio trees of Kerman variety on UCB-1 rootstock were selected for this study at the California State University Campus in Fresno. The treatments included covering the trees with two type of shade netting (Black and Gray), spray of horticultural oils and kaolin clay dust. These treatments were compared with an unsprayed control over two years. Trees were covered by November 1 and the shade nets removed in late February both years. In the kaolin clay treatments, trees were sprayed with clay material throughout the winter months as and when need depending on rain events. The idea was to keep the trees covered throughout the chill accumulation period. Temperature and light intensity data were collected both inside and outside the shade netting using dataloggers. Chill accumulation was calculated using dynamic chill accumulation model as described by Glozer (2009). Data on bloom progression, nut growth, nut weight and volume, shell strength progression, yield and number of blanks were collected.

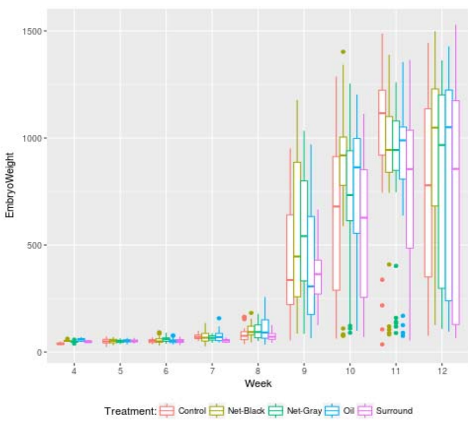
Results and Discussion

The experiment included 4 test treatments plus an unsprayed control. Four treatments were: Oil spray, Kaolin clay (Surround) spray, Black Net and Gray Net. Experiment was designed as a Randomized Complete Block Design with 6 blocks. There were 5 replications in each treatment unit. Results and discussion of the research project. Horticultural oil was applied in late January of 2017, while Surround spray was first applied during first week of December after leaf fall and then reapplied at intervals depending on rains, to cover the trees throughout dormant season. The experimental block accumulated 65 chill portions as of March 1 which denotes that it was a good chill year. Bloom progression evaluation data showed that oil spray trees were ahead in blooming as compared to other treatments and control. The Netting- both Black and Gray were not significantly different from the control but were significantly different from oil treatment in terms of progression of bloom. The oiled trees were earliest to bloom (full bloom around March 27), Surround treatment was next to bloom while most of the Netting and control trees were the last among treatments. The nut phenology data showed that during initial weeks, oil treatment trees were ahead in nut size and shell strength progression. However, when all season data were pooled at the end of the season, no significant effect of treatment was observed in terms of nut development (Fig. 1) or shell strength. Although yield was not significantly different among treatments, control trees had the least variability in yield and on an average yielded higher (Fig. 2). Both black and gray shade netting trees had large variability in yield. Surprisingly, oil and Surround treatments had higher number of blanks as compared to both black and gray netting as well as control trees. In case of split in-shell percentage, treatment differences were not statistically significant. However, control treatment had the higher percentage of split in-shell nuts.

The results from year 1 suggest that there were no significant differences between treatments in terms of yield, nut development or percent split in-shell nuts at harvest. The oil spray and Surround treatments had significantly higher number of blanks as compared to black and gray netting treatments and control trees. This suggests that in a good chill accumulation year, any chemical or mechanical strategies do not play any significant role in altering the production or yield characteristics in pistachios. This work will be repeated in 2018 growing season as well.

Conclusion

Since 2016-17 winter had sufficient chill accumulation, therefore the control trees did not have any physiological issues related to lack of chill. The year 1 results from current study show that in a good chill year, any oil or kaolin spray or shade netting do not have significant effect in improving nut growth & development, yield or quality in pistachios.



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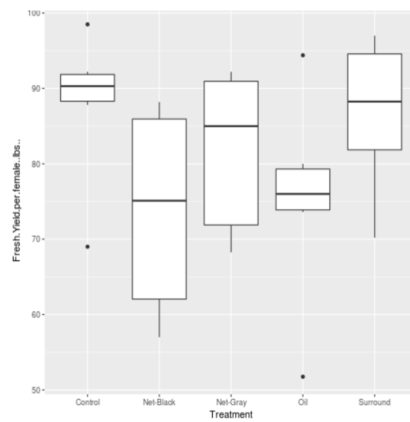

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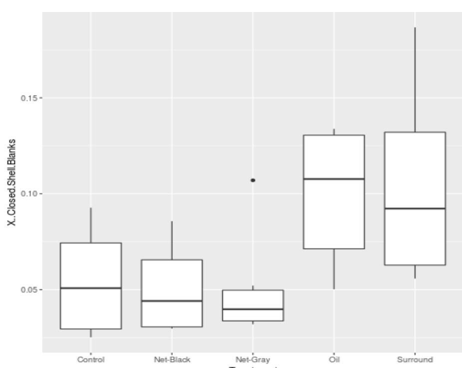

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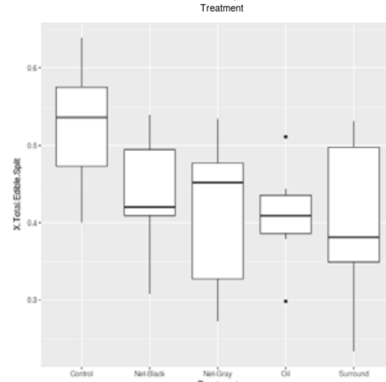

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Figures 1-4. Plots showing 1. development of nut weight during season, 2. yield data, 3. Percent of blank nuts and 4. Percent of split in-shell nuts, among different treatments in pistachios

Winter applied spray amendment impact on winter chill accumulation, carbohydrate levels, flowering, leaf out and yield

Authors: Mae Culumber, UCCE Nut Crop Advisor for Fresno County; **Bruce Lampinen**, UCCE Integrated Orchard Management Specialist, UC Davis

Introduction

Increasingly warm winters in the Central Valley in recent years has prompted research to identify methods that mitigate the impacts of decreased chill accumulation. Kaolin clay and calcium carbonate based products are typically used to guard against sunburn during the summer months. However, preliminary research suggests these materials may have some potential to reduce the adverse impacts of warm winter days. Kaolin treated trees resulted in higher cluster counts, lower blanking, higher yields, and an increase of 1 chill portion when temperature was monitored over a two-week period in 2015 (Doll, unpublished data). A calcium carbonate based spray applied to pistachio trees in Fresno County the same year, resulted in a 5-6°C decrease in bud temperature compared to untreated trees (Beede, unpublished data). Bud break responses varied with the timing and frequency of applications, suggesting there may be different critical stages within the dormancy period where chilling plays a more or less important role in bud break timing. This summary describes preliminary bud break data collected during the 2016-2017 winter trial in response to kaolin clay and calcium carbonate spray amendments. Further research needs to be conducted spanning multiple years to better assess what impact these sun blocking/refracting amendments have during years where winter temperatures fall short of chill requirements.

Results

The intent of the project was to measure the effects of two different commercially available sun reflecting and refracting amendments on pistachio tree phenological responses and winter chill accumulation. The trial took place from 2016 to 2017 in a commercial pistachio (*P. integerrima,* PG1) orchard located in Kerman, CA. (36.699772, -120.171268) on a coarse-loamy, mixed, superactive, thermic soils. The rootstocks were planted and budded with Kerman (*Pistacia vera* L. cv. Kerman*)* in 2006. Male pollinizers (*Pistacia vera x P. integerrima* L. v. Peters) were budded to every fifth tree in every fifth row. Harvest production began in 2013. The experiment included three treatments arranged in a random complete block design with five treatment replicates. Each treatment plot was five acres in size with five rows per treatment plot. The tested treatments include: 1) a kaolin clay based product (Surround®) applied at a 25 lb/ac rate 2) (Microcal®) applied at a rate of 2 gal or 28lbs, and 3) an untreated control. Application rates for the two amendments were based on the label recommendation for each product and applied in the late December. An abundance of rain and saturated conditions in the orchard persisted throughout the 2016-2017 winter preventing additional applications. It was intended that one of the data trees in each treatment would be equipped with temperature sensors as described below in December of 2016, however we were unable to secure the equipment and install them before the first treatment application.

Tree phenological responses included ratings of the treatments for timing of bud break. Preliminary data was collected in late March 2017 to evaluate any effect of treatment applications from December 2016. Ten buds were evaluated for bud length at bud break for five trees in each five-replicate block for all three treatments for a total of (10 x 5 x 5 x 3 = 750 buds evaluated). The average bud length in the third week of March 2017 ranged 0.6 to 5 cm with no differences ($p<0.4736$) between treatments in terms of bud emergence.

Conclusion and Practical Applications

Excessive precipitation, favorable chill accumulation temperatures, and difficulties with securing equipment and materials constrained the evaluation of sun blocking materials to increase chill portion accumulation in pistachio in 2016-2017. According to data from the closest CIMIS weather station at Five Points, approximately 7.9 inches of precipitation fell between November 2016 and March 2017, an inch more than the seasonal norm. An estimated 70 chill portions (Dynamic Model) accumulated, satisfying the minimum threshold for adequate chill portions. There was no difference in the progression of bud-break between different materials compared to the control. Although this trial did not yield much empirical data to evaluate the effects of spray amendments on chill and bud break, difficulties applying the materials for purposes of the trial raises questions about the practical application of these products during a wet dormant season.

Cellular, subcellular and molecular characterization of salinity tolerance in pistachio with novel tools

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Introduction

Soil salinization in California is increasing. Pistachio plants with relative high saline tolerance afford the possibility to use marginal land and improve yield on current acreage. So far, little is known about the cellular uptake and translocation of nutrients and salts throughout the pistachio plant under salt stress, and more importantly how to systematically and economically select for elite cultivars. Pistachio trees are strongly contributing to California's economy. Their relative saline tolerance provides great potential for future expansion into marginal land with elevated salt levels.
Roots play a key role in the salt tolerance of plants, for they represent the first organs to control the uptake and translocation of nutrients and salts. Accumulation of Na^+ in the roots is an adaptive response used by several woody species to minimize its toxicological effects on shoots. Accordingly, the control of the root-to-shoot transport of salt can serve as a criterion for tolerance. We focus on the phenotypical, genetic and molecular characterization of this hitherto ill understood mechanism. Understanding the mechanism of sodium uptake, transport and sequestration at the cellular and molecular level is valuable and can provide a convenient and economical way of identifying desired plant "characteristics" to be selected for in rootstocks, scions or their combinations in order to achieve optimal plant performance and composition. Insights gained will be assisting in the development of better agricultural practices under saline conditions.

Our work aims at the establishment of cellular and molecular methodologies to identify sodium, potassium and chloride uptake, ion sequestration and its effect on cellular morphology and viability for various rootstock scion combinations. Our working hypothesis is that sodium and chloride transport and sequestration in pistachio cells is an important and identifiable trait for salinity tolerance and that it is mediated by the activity of specific transporters. By observing sodium, potassium and chloride localization in live plants at the subcellular level with non-invasive fluorescence microscopy and saline induced structural/morphological cell and cell wall changes, we are aiming at the in depth characterization of salinity tolerance. Thus, understanding the cellular ion sequestration in combination of root structural characterization, can provide evaluation criteria for the identification of most suitable rootstocks.

Results and Discussion

We developed and adopted methodologies for the precise tissue staining of sodium, potassium and chloride in root and leaves of pistachio seedlings. The developed approaches allow *live, in vivo* imaging at the cellular and subcellular level. In order for the indicator dyes to be effective, a number of custom steps in the staining process had to be developed. Towards establishment of the subcellular distribution co-staining methods were developed with markers for subcellular compartments and membrane structures, while circumventing signal form the auto fluorescence from plant tissue, in particular chlorophyll. This was achieved by taking advantage of the difference in the time that a fluorophore spends in the exited state before it emits light, which can differ by 0.5- 4 nano seconds depending on the fluorophore. The Leica SP8 microscope used in our studies, allows a signal selection via gating for collecting time dependent fluorescence.

Phenotypic analysis of salt treatment The salinity treatment led to an accumulation of pigments in leaves manifesting itself in a "red-brown" leaf color. This effect was observed after one week of 100 mM of sodium application. Differences in leaf browning was observed between UCB-1, *P. atlantica* and *P. integerrima* and with *P. integerrima* being the most severely affected one. Further, the overall "robustness" and root architecture was considerably different between the various genotypes, suggesting that this characteristic of the rootstocks may play a role in mechanisms of salinity tolerance. Notably *P. integerrima* showed significantly different root development and overall root system architecture compared to UCB-1 and *P. atlantica*. It is likely that observed differences in the overall root system architecture contribute to differences in the response of these genotypes to salt stress.

Cellular imaging of sodium and potassium Localization of sodium under salinity treatment was observed in root cells of UCB-1, *P atlantica* and *P. integerrima*. Sequestration of sodium under salinity treatment was observed in root parenchyma cells of UCB-1 and was also present in *P. integerrima*, while this effect was not pronounced in *P. atlantica*. Characteristic cell damage was observed during prolonged salt treatment manifesting itself in sodium dispersing throughout the cytoplasm. Overall these data showed sodium uptake under salinity treatment which is enhanced in *P. integerrima.* Further, examination of leaf tissue in UCB-1 using an SP8 confocal microscope with gating imaging, revealed a specialized cell type accumulation of sodium compared to non-treated plants. These specialized cells in UCB-1 may contribute in the overall salinity tolerance.

On the basis of these identified ion localization patterns, we hypothesize that both sodium uptake via the roots and its subsequent specific distribution, determines cytotoxic effects during NaCl stress. This is most likely effected by a mechanism related to ion balances that is not yet fully explored and understood. The mechanism appears to control transport of sodium to the leaf tissue with an observable reduction in plants exhibiting less cytotoxic effects compared to the plants with "red-brown rendered leaves". In agreement with our subcellular data, ion content analysis showed increased sodium and chloride in roots and leaves of *P. integerrima* compared to UCB-1 and *P. atlantica*. Altogether these data suggest that increased uptake and transport of sodium and chloride takes place in *P. integerrima* compared to the other two genotypes tested that leads to an increased cytotoxicity and leaf damage.

Further, root cellular structures were analyzed, by staining root sections with aniline-berberine, a vital stain for suberin, lignin, and callose, showing a characteristic staining of the root endodermis. With the methodology developed the role of endodermis under stress conditions will be determined in continuing studies to examine its role in salt stress response. The root structural differences observable in tissues within the different genotypes can be related to halo-tolerance.

Conclusion The overall results suggest that both ions uptake and sequestration are mechanism contributing to salinity tolerance. Further, structural differences observed in tissues within the different genotypes can be related to halo-tolerance. With our established methodologies, we will conclude the evaluation of different rootstock genotypes and contribute to the understanding of mechanisms contributing to salinity tolerance. Application of these methods in genotype characterization efforts affords a unique opportunity to assess rootstocks and elite cultivars in an efficient and cost effective way. Genotypes, with these phenotypic characteristics, will be examined for altered gene expression of specific transporters, allowing the identification of molecular markers for salinity tolerance.

Field dust: how it affects pistachio pollination

Authors: **Lu Zhang**, Postdoctoral Scholar, UC Davis; **Bob Beede**, UCCE Farm Advisor, Emeritus; **Alan Scroggs**, President, Westside Farm Management, Inc.; **Narges Mehvelati**, Junior Specialist, UC Davis; **Leigh Francis Archer**, International Agricultural Development MS Candidate, UC Davis; **Eden Aurelia Lange**: Student Intern, UC Davis; **Mateen Sajid**, Research Associate, UC Davis; **Emre Bilen**, Researcher, Department of Pomology, Atatürk Horticultural Central Research Institute, Turkey; Louise Ferguson, Extension Specialist, Plant Sciences Department, UC Davis.

Introduction

In 2016, we conducted the preliminary research on the effect of dust on pollination and fruit set. Our preliminary study indicated field dust could interfere with pollination and reduce the fruit set and kernel weight per cluster. This year we addressed the following questions:

How does the dust influence the pollination? As a wind pollinated species, the pollen and stigmas of pistachio (pollen receptor of female floret) are exposed to air and could be contaminated by dust. There are two ways dust can harm pollination and fertilization. First, the dust could decrease both pollen density and activity on the stigma. Second, the dust could directly destroy the stigma or initiate parthenocarpy. Parthenocarpy is fruit set without successful fertilization. With parthenocarpy the dust stimulates the pistil to produce gibberellic acid (GA) for ovary growth resulting in premature nut drop or a blank.

How does dust influence nut growth and split rate? The final economic value in pistachio is a function of the percentage of split nuts. It has been demonstrated that nut split rate is a function of embryo growth. Could the pollen and dust mixture will further influence kernel growth?

Do herbicide residues harm pollination and yield as a direct effect of the herbicide harming the stigma and pollen?

Methods

Pollen of Peters pistachio were collected in early blooming orchards. The pollen activity of pollen and dust mixtures at different volume ratios of 1:0, 0:1, 1:1, 1:4, 1:16, 1-1 and 1:1 toxic were tested by AmpaZ30 by Amphasys AG. The 1-1 was dust was applied first and pollen 4 hours later. The 1:1 toxic was pollen and dust mixture with dust was pre-treated with GlyStar Plus and Treevix herbicides. Clusters with flowers at the green tip stage were bagged and hand-pollinated with the seven pollen and dust mixtures at the different ratios on five successive days. The flower stigma structures were observed by scanning electron microscope (SEM). Florets of the pollen, dust trials, and the bagged control were sampled and tested for GA concentration by using HPLC-MS. Fruit set, blanking, nut drop, nut split percentage, and nut and embryo size of the cluster unit were recorded.

Results and Discussion

Herbicide Residues: Compared to the pollen and dust mixtures with volume ratios at 1:0, 0:1, 1:1, 1:4 and 1:16, our results showed that the sample of 1:1 toxic mixture with herbicide pre-treated dust destroyed pollen viability (sample size: 4500 particles). This demonstrates that herbicide residues introduced during pollination through dust can destroy pollen viability.

Dust and Yield: Dust alone, and mixed with pollen, decreased fruit set, pollination and initiated parthenocarpy. Collectively, these results suggested that at pollination dust can affect pollination, fruit set, blanks and split percentage and yield.

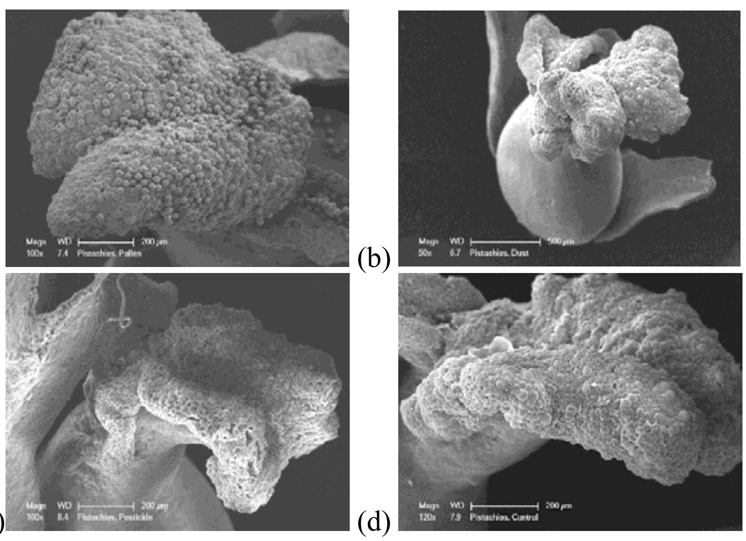
Stigma: the stigma wilted after the dust application and the papilla cells of stigmas disappeared with the toxic 1:1 treatment, suggesting again that dust could influence the effective pollination period and that herbicide residues in the dust were harmful to both pollen and the stigma.

Parthenocarpy: the GA3 content in flowers of both the pollen and dust treatments was higher than in non-pollinated flowers. The increase in GA3 level suggested dust could stimulate parthenocarpy, and therefore it produced the unexpectedly high percentages of blanks we observed.

Conclusion and Practical Applications

Our 2017 results further corroborated preliminary 2016 results; dust during pollination decreased fruit set, and increased the blank, and decreased split nuts, percentages. Pollen viability and stigma quality are damaged by dust, particularly if contaminated with herbicide residues. Collectively, this data indicates anything that can be done to decrease dust in the orchard during pollination is recommended; this includes windbreaks if spring winds are a perennial problem, not mowing during pollination, and applying preemergent herbicides as early as possible.

We propose to conduct further trials to study the dust dynamics *in vivo* in polluted flowers and explore the mechanism of pistachio parthenocarpy in 2018.



(a) (b) (c) (d)

Fig 1. Scanning Electron Microscope (SEM) pictures of stigma structures with (a) pollen applied, (b) dust applied, (c) 1:1 toxic with dust polluted by herbicides applied and (d) control without any application. The female stigma functions as the receptor of pollen and its diameter is approximately 500 μm. The pollen is spherical and the diameter is 20 μm (a). With the dust treatment (b), the stigma wilted after the application. With the toxic 1:1 treatment (c), the papilla cells of stigmas disintegrated. These results demonstrated that dust can decrease the effective pollination period and that herbicide residues in the dust are harmful to pollen and the stigma.

Determining the effect of Acadian LSC seaweed extract on pistachio inflorescence bud abscission

__Authors__: **Leigh Archer***, Graduate Student Researcher; **Narges Mahvelati*,** Junior Specialist; **Lu Zhang***, Postdoctoral Researcher; **Emre Bilen*, Mohammad Raza*, Mateen Sajid*, Abdollatif Sheikhi*, Ahmad Tajabadipour*,** Visiting Scientists; **Eden Lange***, Student Intern, **Louise Ferguson***, Extension Specialist
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__Introduction__

This trial examined the potential for biostimulants, produced by Acadian Seaplants LLC, and the currently registered growth regulator, MaxCel®, and low-biuret (LB) urea, to mitigate the visible mechanism of alternate bearing in pistachios. Specifically, the trial examined the ability of these products to prevent the abscission of the lateral fruiting buds produced on current year's shoot growth distal to the one-year-old bearing shoots. Buds on current year's growth produce the following year's crop. In addition to monitoring bud drop this trial examined production the following year to determine if the treatments increased productivity of treated trees. The trial was conducted in 2016 and 2017 at the Strain Ranch in Arbuckle, Colusa County, California (Latitude 39.012868, Longitude -122.035319). The orchard is 9-year-old Kerman scion on PG1 rootstocks planted at 17 feet in-row and 19 feet between rows, with a Peters pollinizer planted at a 1:25 male to female ratio. Eight trees were selected from 8 replicate rows to receive the 8 spray applications both seasons. A matching set of 8 replications was also sprayed in 2017 only. Treatments were applied at 1085 and 1380 accumulated temperature units to correlate with the initiation and midpoint of embryo growth respectively. The objective was to apply treatments before and at the time of bud abscission initiation. Yield and grading data from the 2017 season was completed on the 64 trees sprayed with the same treatments over consecutive years. Bud abscission data was collected for all 128 trees in both blocks.

The 8 treatments being compared were Acadian A LSC®, Acadian B (an unregistered Acadian product), MaxCel® (6-benzyladenine (6BA) 1.9% AI; currently registered for pistachio to increase yield and decrease alternate bearing), an untreated water control, each of these four treatments were also sprayed in combination with LB urea (Total Nitrogen Analysis 46.0 minimum). The Acadian and MaxCel® treatments are a biostimulant and growth regulator, respectively, formulated to alleviate alternate bearing by decreasing the visible mechanism, fruit bud abscission, on the current year's shoot growth of bearing branches. The Acadian treatments are seaweed extracts derived from *Ascophyllum nodosum*. The suggested mechanism of the product is an increase in shoot nitrogen levels that in turn promote chlorophyll development to enhance photosynthesis and carbohydrate status of the shoot. This would allow the leaves on the current year's shoot growth to better support both nut fill and developing buds simultaneously. These biostimulants also possess a cytokinin-like action that protects chlorophyll. The growth regulator, Maxcel®, is suggested to cause a shift in the relationship between competition for carbohydrates between the developing embryos on one-year-old wood and vegetative growth on current year wood. In combination with LB urea, the growth regulator spray changes the sink and source interaction between carbohydrate demand for reproductive and vegetative growth allowing for increased bud retention.

__Results and Discussion__

All the experimental trees in the 2016 season had equal crop loads ranging from 20-25 pounds of edible yield per tree, or 2300-2800 pounds per acre. The bud drop percentage from the

2016 growing season demonstrated that none of the eight foliar sprays had any consistent significant effect on the percentage of abscised buds on bearing or nonbearing shoots. In 2016 abscission ranged from 57-87%, with the control treatments exhibiting the lowest percent loss. These preliminary results indicated that the biostimulants were ineffective at reducing bud abscission on shoots with clusters. The mean percent bud drop compared across all treatments of branches without clusters was statistically insignificant, ranging from 10-18% across treatments. These results supported the conclusion that bud abscission is a within-shoot phenomenon related to the cropping load of an individual branch.

The 2017 growing season crop loads were higher than the previous year. Average yields across the 8 treatments ranged from 31-36 pounds dry weight per tree versus 20-25 pounds dry weight in 2016. The yields across treatments were not significantly different from one another, again suggesting that the within-branch crop load is the primary factor in bud abscission. The 2017 season average percent bud drop was far greater and more consistent than in 2016, indicating that the trees are reaching full production potential and the alternate bearing tendency of the tree will manifest even more sharply in 2018 and beyond. The average percent bud drop ranged from 86-95% in trees that were selected during the first year of the trial and sprayed with the biostimulant and growth regulator + LB urea treatments for two growing seasons. The average percent bud drop ranged from 86-92% in trees that were selected only for the second year of the trial. The averages between the two blocks were not significantly different, suggesting that the biostimulant or growth regulator + LB urea sprays will not impact crop load in subsequent growing seasons.

Conclusion

Improving the photosynthetic capacity of individual branches through the use of biostimulants does not appear to target the visible mechanism of alternate bearing in pistachio. Nor were the growth regulator + LB urea treatments tested during this trial successful in decreasing the rate of bud abscission. Growth regulator and biostimulant treatments are purportedly useful for increasing the photosynthetic capacity resulting in increased carbohydrates available to the branch and altering the sink/source relationship between embryo development and vegetative growth. The results did not show that an increase in potential photosynthetic capacity translated into an increase in carbohydrates available for current season's developing nut clusters and inflorescence buds, nor a reduction in bud abscission rates.

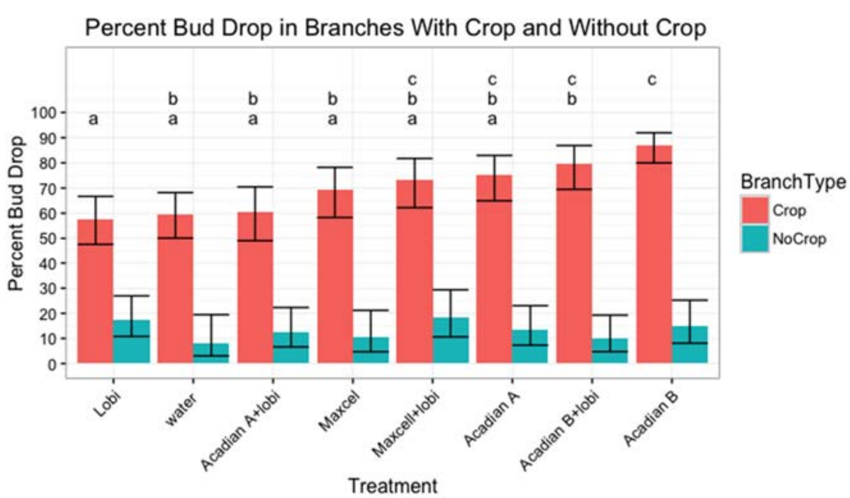

Figure 1: 2016 results of means comparison at p<.05 showing significant difference in the percent bud drop between branches with and without crop.

Model comparisons of pistachio nut growth and the development of web applications

Authors: Lu Zhang, Postdoctoral Scholar, UC Davis; **Emilio Laca**, Professor, Plant Sciences Department, UC Davis; **Liyu Ying**, Software Developer, Intel Corperation; **Ruoyi Gao**, Applications Programmer, Plant Sciences Department, UC Davis; **Louise Ferguson**, Extension Specialist, Plant Sciences Department, UC Davis

Introduction

Knowing a plant's seasonal growth and development as a function of temperature accumulation can facilitate production. The ability to predict the three nut growth stages, or biomarkers: 1) hull and shell (pericarp) expansion; 2) endocarp (shell) hardening; 3) embryo development, can aid in irrigation, pest management, and harvest timing.

Nut growth is a function of heat, or physiological time. Plants require a specific amount of heat to develop through their seasonal growth and crop development. We plotted the correlations between degree-days and calendar days in multiple pistachio cultivars in multiple locations for three years. The final results demonstrated that at different locations the growth stages can differ by almost a month within a season. To accurately predict when a specific growth stage might occur requires developing a model; a prediction equation which a grower can use by inputting local factors to generate predicted growth.

We compared several models: Asymptotic Regression, Michaelis – Menten, 3-parameter Logistic and Gompertz models, to select the one that provides the most accurate prediction for pistachio nut growth.
The predictions from the best model were then converted to mathematical formulas to build a web application; a website where growers can input their location, cultivar and local temperature accumulation to date. The website can then calculate when a growth stage, by heat unit accumulation and date, will be achieved for that location and cultivar. The final output will give also information on nut size, shell firmness, kernel size, nut split rate and optimal harvest date.

Methods

The nut growth of five cultivars, i.e. Kerman, Lost Hills, Golden Hills, Kaleghouchi and Pete 1 from six locations was monitored through the 2014, 2016 and 2017 growing seasons. Bloom date, endocarp and embryo growth, shell hardness, nut split, fresh and dry weight and yield and CPC grade were measured and recorded. Hourly temperatures were collected by data loggers (Onset HOBO® U23-001 Pro v2 Temperature/ RH Data Loggers) installed at each location. The thermal units were determined by taking daily average and removing the base temperature of 7 ℃.

Model Comparisons: the full theoretical model for the year and site sources of variation was: total deviation e = e_year + e_site + e_yr-st, where the combination of site and year as a random effect that combines the variation. The comparisons among the Asymptotic Regression, Michaelis – Menten, 3-parameter Logistic and Gompertz models were coded in the R Statistics program.

Web Application: the interface was powered by Spring Framework, running on an AWS Server.

Results and Discussion

Four growth models fit the three stages of pistachio nut growth well. For all the models the coefficient of determination values, (R^2), were more than 0.86; R^2 shows how much of the variance is explained by the model. Akaike's Information Criterion (AIC) demonstrated that embryo length was better fitted by the four models than the other two growth stages. AIC is based on maximum likelihood and reflects the penalty for each parameters of models. The Gompertz model did not require random effects and had a high R^2 of 0.95. Both the R^2 and AIC indicated that the Gompertz model was the best models for all the variables.

Conclusion and Practical Applications

The most consistent model was the Gompertz. Compared to the other three models, it best predicted pistachio nut growth at both low and high heat unit accumulation. Now, using local historical and current temperatures in the Gompertz model the pistachio nut growth stages, including split, can be predicted. Fig 1 shows a screenshot of the web interface of 'Report and Summary', where pistachio growers can input the local temperature data and receive a prediction of current nut growth and the optimum harvest date for their orchard.

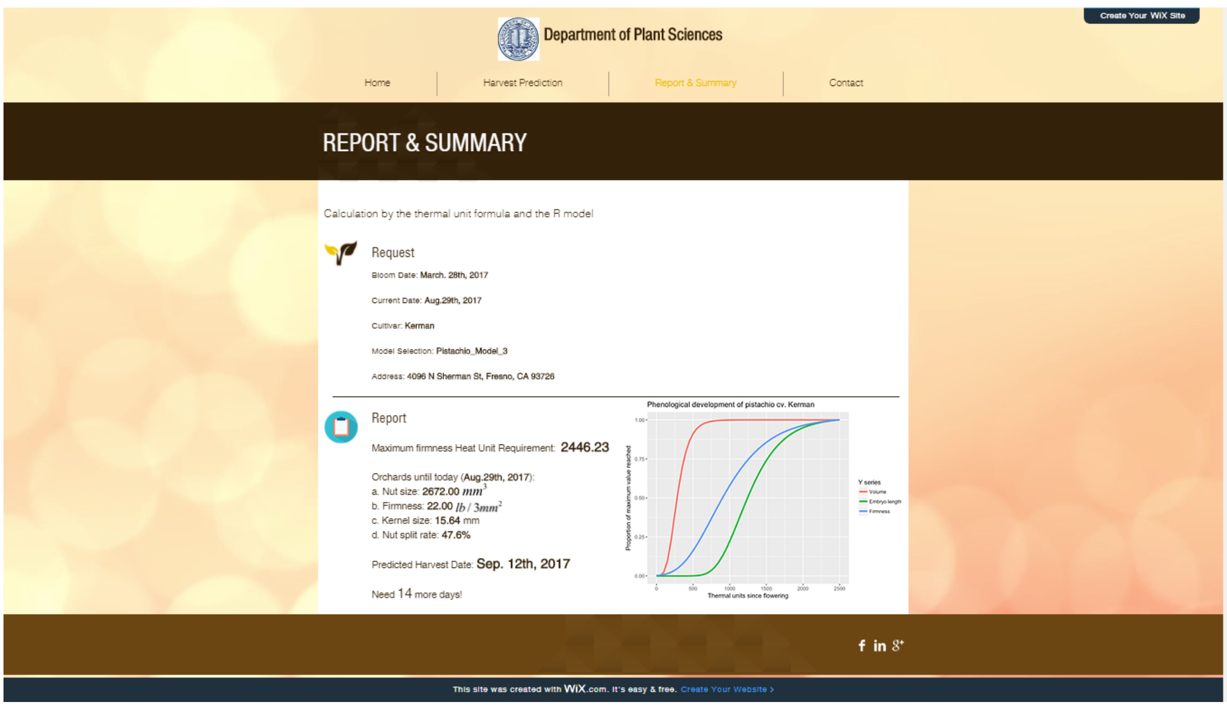

Fig 1. This website screenshot demonstrates predicted pistachio nut growth and harvest date. Growers enter bloom date, current date, cultivar and orchard location. The report predicts nut size, shell hardness, kernel size, nut split rate and optimum harvest date. The graph in the lower right corner is the prediction of the nut growth (red color: nut volume; green color: embryo length; blue color: shell firmness); the Y-axis is the Proportion of Maximum Value Reached, the X-axis is the Thermal Unit accumulation with a lower threshold of 45°F since bloom.

Evaluation of postharvest quality changes of fresh hulled pistachios during cold storage

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Carlos Crisosto and Louise Ferguson: Extension Specialists, Department of Plant Sciences, UC Davis

Introduction

Pistachios can be consumed fresh, dried and roasted. There is a growing market for fresh pistachio nuts, because of their essential micro-nutrients and bioactive constituents. The quantity of these health-promoting phytochemicals are influenced by various factors and practices; genetics (cultivar), environment (soil, radiation, precipitation, humidity), cultivation practices (irrigation, fertilization), and harvest and postharvest processing; time of harvest time, drying, roasting. Three days of sun drying has been demonstrated to decrease anthocyanins by 60% and vitamin E by 38% as well as phenolics, flavonoids. This demonstrates fresh pistachios are nutritionally superior.

Pistachio nuts are traditionally consumed fresh in Iran. In Australia, most pistachios are marketed dried but a small portion are marketed fresh. This is an option for small growers far from a processing facility, when the trees are young, and production is low. Marketing pistachios as a fresh fruit is a value added alternative. Fresh pistachio nuts are very perishable as metabolic activities, respiration and ethylene production, continue after harvest, producing senescence and changes in pigments, texture, aroma, carbohydrates, amino acids, fatty acids, bioactive compounds, secondary metabolites and other quality components. Nuts, with their high polyunsaturated lipid content, are particularly susceptible to the oxidation that produces off-flavors. Due to all these factors, postharvest storage of fresh pistachios is challenging. Despite the economic importance of pistachios little is known about the senescence and quality changes of fresh pistachios during postharvest storage.

The immediate objective of this study was to develop baseline information for evaluating quality changes in fresh pistachios under two different postharvest conditions; cold storage with and without <u>M</u>odified <u>A</u>tmospheric <u>P</u>ackaging; MAP. The long term objective is developing an alternative marketing outlet for California pistachios.

Plant Materials and Methods:

Fresh hulled “Kerman” pistachios from a commercial California orchard were harvested September 18th 2017. This experimental design was a Factorial based on a Completely Randomized Design (CRD) of 4 replications evaluating the effects of cold storage (60 days) on a non-packaged control and modified atmosphere packaging (MAP) on fresh hulled pistachio quality. The fruit quality parameters of firmness, decay and weight loss were analyzed during first 30 and 60 days at 0 °C and 90 ± 4% relative humidity. This trial is continuing and will terminate with an organoleptic taste and sensory evaluation at 90 days.

Results and Discussion

The freshly harvested kernels lost both firmness and weight during the 60 days of storage in the control versus the MAP treatment. The firmness dropped from 7.62 to 6.05 N, and weight loss was 5.34% and 0.43% for the control and MAP treatments after 60 days of storage, respectively.

In fresh pistachios, microbial growth on the shell and kernel surface are major indicators of decay. Decay in the control treatment was significantly higher than in the MAP treatments: 90.44% of the control treatments exhibited visible mold versus the MAP treatment's 0.35%;see Fig. 1 below

Conclusion and Practical Applications

In conclusion, fruit decay and mold growth, and firmness and weight loss are the most important quality factors which should be considered in fresh pistachio quality postharvest. These results demonstrate kernel firmness and weight declined and decay increased during 60 days of postharvest storage at 0 °C and 90 ± 4% relative humidity. The MAP treatment decreased weight loss and fruit decay, significantly (Fig 1). At 90 days the physiological and metabolic changes, and organoleptic quality will be determined.

Fig 1. Effect of MAP (left) and control (right) on decay and mold growth of fresh hulled pistachios after 60 days of storage at 0 °C and 90 ± 4% relative humidity

An investigation of nut blanking and shell splitting of pistachio based on thermal unit

Authors: Lu Zhang, Postdoctoral Scholar, UC Davis; **Narges Mehvelati**, Junior Specialist, UC Davis; **Leigh Francis Archer**: International Agricultural Development MS Candidate, UC Davis; **Eden Aurelia Lange**: Student Intern, UC Davis; **Mateen Sajid**, Research Associate, UC Davis; **Louise Ferguson**, Extension Specialist, Plant Sciences Department, UC Davis

Introduction

Pistacia vera is the genus and species in which endocarp dehiscence occurs with fruit maturation. Earlier research has provided evidence that the mechanism of shell split is that the physical forces exerted on shell suture by seeds. In developing our fruit growth phenology model we also measured the volumes of both nuts and embryos and used the calculated embryo:nut volume ratios (ENR) to determine the correlations between nut split, ENR and heat units. The results are presented here.

Methods

The cultivar samples and locations were collected weekly from mid-August through harvest. Nut clusters were collected from the south, north, east and west canopy. Hourly temperatures were monitored in each locations and thermal unit accumulation w calculated with a 7℃ (45°F) base temperature. In the lab we measured 20 tip nuts per trial for nut split and blank percentage, and nut and embryo volume using the water displacement method.

Results and Discussion

Blank, Split and Cultivars: in all locations, the nut split percentage of Kerman and Pete1 were consistently lower than that of Golden Hills, Kaleghouchi and Lost Hills. The nut split rate in our research was evaluated using only tip nuts to eliminate the possibility that the resource distribution to nuts lower on the clusters as not an issue. Therefore our results suggest that the weak split rate of Kerman and Pete1 could be induced by cultivar's potential non-split characteristics.

We also observed the Lost Hills blanking percentages were consistently statistically higher than that of the other 4 cultivars. Because we excluded the possibility of pollen density and lower nuts factors, this suggests that cross-incompatibility and embryo abortion could be the factors causing blanks in the Lost Hills cultivar.

Split and Heat: the coefficient of determination, R^2=0.822, not surprisingly, showed a strong correlation between heat accumulation caused nut split. In Fig 1, the result also demonstrated that nut split in Kerman initiated at 2000 heat units and the value of split rate quickly increased to 60% with an additional 600 heat units.

Embryo and Split: The average embryo:nut ratios (ENR) were summarized based on the measurements of 1600 individual nuts. The average ENRs of non-split nuts was 35.6 and 44.1 for split nuts. For the Kerman cultivar, the ENR values (non-split 34.44, split 43.03) and embryo volumes (non-split 1.15cm^3, split 1.63cm^3) between split and un-split nuts were both significantly different. However, the nut volumes of Kerman did not vary with locations or nut types (split/non-split). Collectively, these results suggest that nut split is a result of embryo growth that can be predicted using a phenology model.

Conclusion and Practical Applications

The results show Kerman and Pete1 have naturally low split nuts compared to Golden Hills, Kaleghouchi and Lost Hills. And that Lost Hills consistently have higher blank percentages. The results also demonstrate that nut split is a function of heat unit and the embryo:nut volume ratios. Using this information we can develop a model to predict optimal harvest date based on the correlations between ENR and embryo growth.

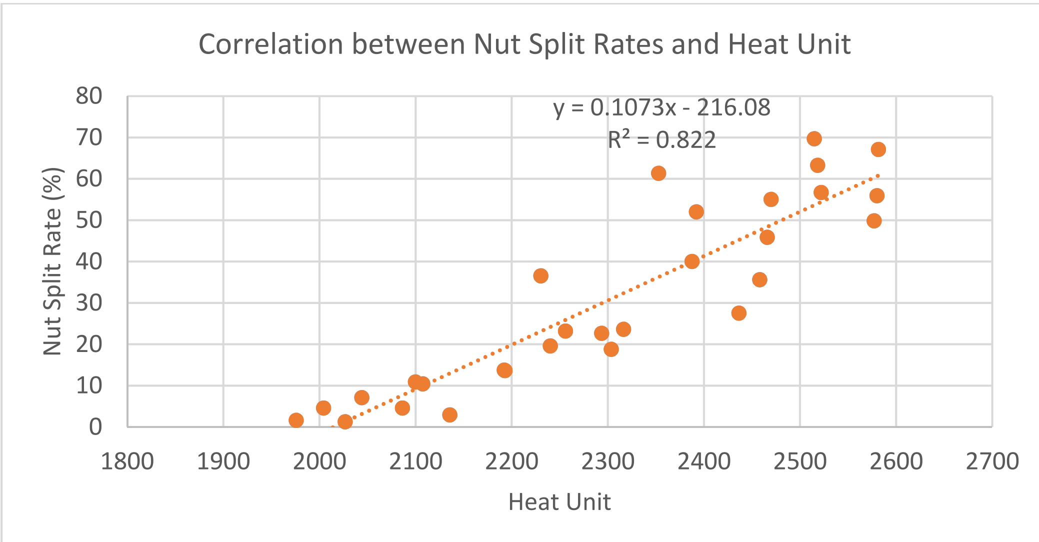

Fig 1. Correlation between the nut split rate and heat unit of nuts collected from six orchards from mid-August to harvest date in Kerman cultivar. The coefficient of determination R^2=0.822 reveals a strong relationship that nut split is caused by heat.

Bud abscission dynamics in pistachio as a function of branch carbohydrate status and embryo growth

Authors: Leigh Archer, Graduate Student Researcher, Department of Plant Sciences, UC Davis; **Narges Mahvelati,** Junior Specialist, Department of Plant Sciences, UC Davis; **Lu Zhang**, Postdoctoral Researcher, Department of Plant Sciences, UC Davis; **Louise Ferguson**, Specialist, Department of Plant Sciences, UC Davis

Introduction

This trial is an attempt to better understand the physiological mechanism of bud abscission, the visible cause of alternate bearing in pistachios. This investigation was conducted within a complementary study of bud abscission funded in part by Acadian Seaplants Limited. As part of the Acadian trial, 16 individual trees were selected as the "control" treatment and received control spray applications of water. These trees became the basis for this carbohydrate analysis experiment. Experimental work included biweekly calculations of percent bud abscission and individual branch carbohydrate analysis.

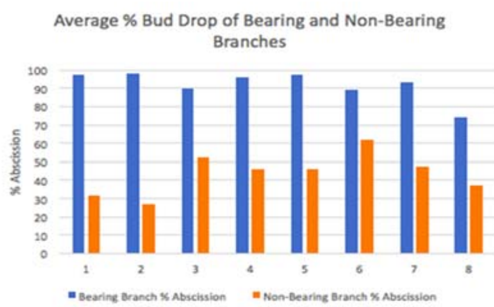

Figure 1. Average percent bud abscission of 8 untreated trees comparing bearing and non-bearing branches.

Results from the first year of the Acadian trial indicated that there were consistent and significant differences in bud abscission of bearing and nonbearing shoots within trees. This suggests that bud abscission is not directly correlated with total tree cropping status. Rather, abscission is a within-shoot phenomenon correlated the crop load on the one-year-old growth. The working hypothesis is that the developing nut kernels deplete the carbohydrates from current year's growth, where photosynthesis is more active due to the presence of leaves, while the carbohydrate status of last year's wood remains relatively unchanged.

Due to the process by which shoots grow and develop new buds, individual branches within a tree are simultaneously producing the current year's crop and generating buds for the following year. This concurrent growth and development places a high demand on available carbohydrates, and initiates the abscission of the basal inflorescence buds. The buds will generally drop sequentially from the base of the shoot to the tip as the kernels begin to develop. Attempts to better understand the mechanism behind the cause of bud abscission have been inconclusive. It is not established whether the competition for carbohydrates between current clusters and developing buds causes a hormonal signal initiating bud abscission, or if other factors are at play. This experiment was implemented to understand where, within a single branch, nut clusters obtain carbohydrates for growth and development. Much of the research to date has focused on whole tree cropping status and carbohydrate differences between "on" and "off" trees. This trial focused on shoots with and without crop loads to determine the mechanism for abscission at the individual branch level.

Results and Discussion

Inflorescences on 1-year-old wood develop into nut clusters beginning in mid-April, reaching full size by the end of May. The nut shells of individual pistachios then begin to harden and the inner kernel begins to enlarge. As the kernel begins to grow the basal inflorescence buds on new growth, shoots that have developed beyond the nut clusters, begin to abscise starting from the shoot base and moving sequentially to the tip. Previous studies have shown that greater percent abscission can be seen in shoots with a smaller leaf area to crop load ratio.

This trial affirmed that the second, more severe, phase of bud drop on fruiting branches is significantly related to embryo (seed) development. Previous work has found that the presence of fruit will not impact the uptake of carbohydrates by leaves, but fruit clusters will act on the sink/source relationship of carbohydrate distribution between developing embryos and flower buds.

Bud abscission data were analyzed using a linear regression, R^2, to determine the effect of embryo development on the degree of bud drop. The 2016 preliminary results demonstrated that there was a significant linear regression, R^2=.94, between nut growth and bud drop. Similar results were found during the 2017 growing season. An R^2 value of .97 indicates that bud drop is strongly associated with nut growth and is the result of crop load on the same branch.

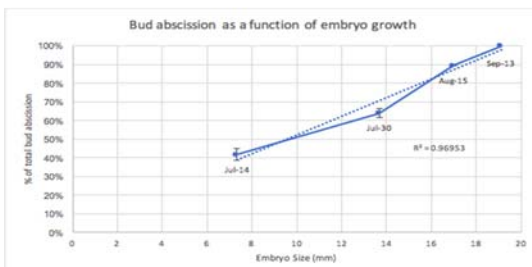

Figure 2: Percent bud drop as a function of embryo length from four sampling dates showing significant correlation between seed growth and bud abscission.

Conclusion and Practical Implications

Alternate bearing is not harmful to the tree; however alternating years of significant yield decreases is disruptive to commercial operations and marketing. Branches, trees, and orchards all tend toward synchrony in alternate bearing resulting in significant economic losses. Determining the mechanism behind alternate bearing may allow researchers to establish guidelines for appropriately managing bud abscission.

Results from this analysis, and previous research, suggest that the temporary competition for stored carbohydrates in the new wood of fruit-bearing shoots will initiate a hormonal signal that instigates bud abscission on current year's shoot growth. Removal of carbohydrates from current year's growth in order to meet demand for nutrients by developing kernels leaves very little available for inflorescence buds for the following season's crop.

Instead of a continued prominent focus on whole tree fruiting status, results from this study may allow researchers to develop new techniques for mitigating bud abscission based on individual branches within a tree. Characterizing the optimal shoot length, width, and leaf area for regular crop production and determining analytical tools for shoot carbohydrate status may be a useful. This research could benefit growers by providing a better understanding of alternate bearing and potential mitigation techniques.

Development of new, reliable, vigorous, clonal rootstocks

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

Introduction

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

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

Results and Discussion

Seeds are free from endophytes, but to overcome dormancy, require moist, cool stratification. This also causes microbial contamination of tissue cultures, making it necessary to use non-stratified, dry seeds. These seeds have a hard shell and it is necessary to cut through the shell to expose the cotyledons for the seeds to germinate. This shell is too hard to cut through and requires weakening. We found that it was necessary to soften the shell through acid scarification. UCB-1 seeds were placed in concentrated sulfuric acid (36 Normal), and required a 7-hour soak to weaken the shell to allow for them to be easily cut.

The seeds are surface-sterilized during their 7 hours in the acid. This is followed by three 5-minute rinses in sterile deionized water. About ¼ to 1/3 of the seed is excised opposite the hilum to avoid cutting the embryonic axis. This cut removes the sides of the cotyledons and allows germination of non-stratified seeds. This has resulted in about 100 new seedlings growing in culture. Other experiments are planned or underway to increase the efficiency of the process.

An effort was also made on established trees. From a planting of 1,200 nongrafted UCB-1 seedlings, after 4 years of growth measurements, the largest three trees were selected. In an effort to escape endophytes, apical meristematic regions were excised and placed in culture. These meristems have established in vitro and as of yet, assays have shown no endophytes. There are more than 60 shoots from these meristems at this writing. This approach is superior to using seeds because there are field performance data. However, seeds have great juvenility and therefore, typically have much better propagation vigor and are easier to multiply and root than more mature-sourced materials.

Conclusion

Research will continue to increase in vitro seed germination efficiency and to determine if endophyte-free cultures can be established from apical meristems.

Clonal *UCB-1* pistachio rootstock micropropagation: is pistachio bushy top syndrome a variant that occurred in tissue culture?

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

Introduction

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

Results and Discussion

Clonal UCB-1 shoot cultures were obtained from three commercial micropropagation laboratories. One lab had created three subclonal lines, and we obtained two of them. The third line had low numbers and once there are sufficient numbers, we will also receive cultures of this line. We received about 75 microshoots from the two subclonal lines and have multiplied these 150 shoots into about 1000 shoots. These will be used for rooting and greenhouse acclimatization experiments.

A second nursery provided two containers of their two preselected subclonal lines, totaling 34 shoots. We have created 15 subclonal lines from these based on microshoot morphology and currently have a total of 150 microshoots in culture.

A third nursery provided 14 containers totaling about 200 microshoots. Selections have been made based on growth and morphology and color of leaves. We currently have 34 selected subclonal lines from these 200 microshoots.

Conclusion

Progress will continue with rooting and acclimatization as we produce *Rhodococcus*-free plants for field testing for phenotype.

Identification of superior *UCB-1* rootstocks using DNA markers

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Introduction

There is now a variety of biotechnology and genomic approaches available for crop improvement. We are developing and adapting such approaches for pistachio to ensure that pistachio does not lag behind in benefitting from these technological advances. Pistachio has a small genome and application of these approaches provides several opportunities for rapid and significant improvement of the crop, particularly with regard to determining the basis of the stunting problem and increasing the reliability of the performance of the UCB-1 rootstock.

Results and Discussion

We are using high-throughput DNA sequencing and genotyping technologies to identify and manipulate natural genetic variation that influence the performance of the UCB-1 rootstock and stunting of scions. In the first two years of this project, libraries of many genes transcribed (expressed) in leaves of pistachio were generated, sequenced, assembled, and analyzed to determine the level of variation within and between each of the parents of UCB-1 (*Pistacia atlantica* female x *P. integerrima* male). Genotyping by sequencing of a random set of 95 UCB-1 trees plus the parental trees was made as well as reduced representation sequencing of 957 UCB-1 trees growing in Davis. This is the foundation for generating ultra-dense, gene-based genetic maps of both parental species that is currently underway. This will assign many genes to genetic bins ordered along each chromosome of pistachio. Measurements of phenotypes including height and trunk caliper have been made every year to provide data on the performance of UCB-1 trees. The genetic basis of the phenotypic variation will be analyzed to reveal the number and positions of chromosomal regions responsible for each trait as well as markers for them. The data will be displayed on a website to make genetic and horticultural information publicly accessible over the internet. This year additionally, DNA was extracted from 1032 grafted UCB-1 trees from commercial orchards in Central Valley and GBS libraries were sequenced. Performance in commercial orchards will be further analyzed in subsequent years to dissect the genetic and environmental basis of the stunting phenomenon.

Conclusion

The genomics data and the phenotypic data will be combined to find molecular markers for growth. This will enable early detection of UCB-1 seedlings that will grow into low vigor trees so that they can be rogued and not used in orchards.

Evaluation of pistachio breeding selections, 2017-18

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Introduction

The U.C. breeding program began with the original crosses made in 1989 by Dr. Dan Parfitt and Joseph Maranto. As of 2009, the program continues with the breeding and evaluation of novel scions, but also, experimental rootstocks. As new male and female pistachio cultivars are released to the industry, the focus of the program shifts to the evaluation of novel U.C. breeding crosses and other potential cultivars of interest to the industry. Older trials are abandoned and new trials created, often with the indispensable and long-term donation of land, labor, equipment and time by interested and generous private, cooperating growers. Currently, we are evaluating eight advanced scion selection trials. These trials were planted from 2007 to 2016. Four of these trials have the objective of identifying male cultivars that demonstrated robust flower development and close bloom synchrony with Kerman or Golden Hills in years with insufficient winter chill/excessive winter heat. Two of these four are located near Inyokern, east of the Sierra Mountains in the high desert. Two of these eight trials are focused on the long-term performance of the recently released U.C. cultivar Gumdrop compared to Golden Hills and/or Kerman on UCB-1 seedling rootstock and on experimental rootstocks from the U.C. breeding program. One of these trials includes additional experimental male selections as pollinizers for Gumdrop. The other two trials compare U.C. advanced breeding selections with existing commercial cultivars for yield, nut quality characteristics, and bloom and harvest timing. In addition, we have a seedling selection trial containing diverse breeding crosses which may contain individual trees with potentially novel combinations of useful traits for the industry, such as greener kernel color, low chill/high winter heat tolerance, earlier or later bloom and harvest timing and others. This trial was planted in 2012 and the most precocious trees began blooming in 2016.

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

Results and Discussion

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

U.C. cultivar Gumdrop: Gumdrop's most noteworthy characteristics is its early harvest (approximately 10 days earlier than Golden Hills and 20 days earlier than Kerman), while maintaining acceptable commercial nut quality characteristics. The earlier harvest of Gumdrop should reduce navel orangeworm infestation as pressure from this insect increases later in the harvest season. The earlier flowering data, and some observations made during leaf-out and bloom suggest that this cultivar may be more tolerant of 'insufficient winter chill/high winter heat' conditions. As research continues data and observations suggest the following regarding producing pistachios with Gumdrop:

1. Gumdrop, currently, is ready for harvest before nut processing plants are open. Gumdrop should only be planted by grower operations having access to a plant that is capable of processing the nuts of this cultivar when they are ready for harvest.
2. Later in the season (July) some nuts produce a drop of gum on the hull (hence its name). A Gumdrop harvest is 'stickier' than a Kerman harvest.
3. Gumdrop nuts to not hold well on the tree and air temperatures can be hot when Gumdrop is ready for harvest. A timely 'double shake' harvest is suggested. In the San Joaquin Valley, depending on season and location, the first harvest will occur in early August with a second shake a week to ten days later. Gumdrop is an alternate bearer like Kerman.
4. Gumdrop will perform best on well-drained soils where water 'ponding' does not occur.
5. Gumdrop has shown more growth variability on UCB-1 seedling rootstock. Growth among Gumdrop trees has been much more uniform on Platinum® clonal rootstock in an observation trial planted in 2014.
6. Gumdrop grows faster than Golden Hills or Kerman. A minimum spacing of 20' x 20' within the orchard may be advisable on productive agricultural soils.
7. A male cultivar, named Tejon was released to the industry as a principal pollinizer for Gumdrop. It may be advisable to plant a second pollinizer with Tejon to assist with Gumdrop pollination in years of low-chill. A non-proprietary cultivar, called Zarand, appears to meet this requirement. Zarand will be available from some commercial nurseries.

Seedling selection trial: The trees are being evaluated for leaf-out, bloom timing, and nut quantity/quality characteristics. Early leaf out/flowering dates, in addition to parentage information, are being used as selection tools for moving germplasm from this trial to the 'low-chill/high winter heat' evaluation trial in the Coachella Valley established in 2017.

Novel rootstocks: The rootstocks from the U.C. breeding program are novel in that the parentage is different from UCB1 or pure *P. integerrima* rootstocks. Two rootstock trials had enough yield present to harvest in 2017. In one of the rootstock selection trials, at 5th leaf, 12 selected rootstocks (grafted to Lost Hills) averaged 166 lbs. /acre of edible yield compared to 13 lbs. /acre for the UCB1 seedling controls. Contrary to what usually occurs with UCB1 or pure *P. integerrima* rootstock, the scion was of smaller circumference than the rootstock.

In another trial, two novel UC experimental seedling rootstocks are being compared to UCB1 seedlings. The scion is Golden Hills. Both the soil and irrigation water (up to 5 ppm) are high in boron. Average edible yields were not significantly different among the rootstocks at this the first commercial harvest. However, the canopy of Golden Hills on UCB1 rootstock demonstrated large areas of leaf-tissue necrosis with leaf drop, with few or no leaf symptoms on scions grafted to the novel rootstocks. Replicated leaf tissue analysis showed that leaves on UCB1 rootstock had average dry tissue levels of 1337 ppm, compared to 550 and 597 for the two novel rootstocks. Other differences in nutrient uptake were observed in these two trials. These rootstock trials are in the initial stages and what the differences in performance noted this year might mean are not clear. However, currently, the commercial rootstocks available to the industry is limited. Exploring new interspecific genetic combinations for rootstocks appears to be a useful exercise, both in general knowledge, and nut production possibilities inherent in diversity.

High density planting investigations in pistachio

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Introduction

The goal of this project was to evaluate existing high density plantings in pistachio to assess their efficiency. Growers are interested in increasing early production and one way to potentially do this is to plant at higher densities. However, this often has tradeoffs later in orchard life.

A survey was done among industry representatives, farm advisors and growers to locate any existing high-density plantings in pistachio. A number of higher than normal density orchards were found and surveyed.

Orchard 1. The first orchard was near Kettleman City in western Kings County. The orchard is Kerman planted on Pioneer rootstock in 1996 at a density of 11' x 17' (233 trees/acre). The grower planted a block nearby at the same time (also Kerman on Pioneer rootstock) at a spacing of 17' x 19' (135 trees/acre). The grower has observed that production in the high versus more normal density blocks have been similar over the life of the orchard. We installed timelapse cameras to measure the orchard floor shadows over the course of the summer and also ran our mobile platform lightbar to measure midday canopy photosynthetically active radiation interception in mid-summer. Because the closer row spacing and higher tree density require more trees, more passes through the orchard for maintenance operations, more passes for hedging, and more trees to harvest, the yields would need to be significantly higher to justify the increased costs of establishment and operations in the higher density orchard.

Orchard 2. The second orchard studied was a high density planting in southern Tulare County. This was a small planting inside of a larger conventional block and was planted at a spacing of 5'4" down the tree row and 20' across the drive row (411 trees per acre). The purpose of the planting is to evaluate tree training strategies for these high density plantings and also to determine the feasibility of using over the row harvesters in this orchard configuration. The orchard is only 3 years old so these evaluations have not been done yet. The idea would be to eventually plant the trees closer across the drive row as well as down the tree row after tree training and harvest details have been worked out.

Orchard 3. The third orchard is in a very high density configuration with a planting spacing at 9 feet down the row and 12 feet across. The rootstocks were just planted in 2017. The eventual plan is to harvest with a modified pistachio harvester that will likely fit around 3 trees at a time (but only shake the middle tree).

Results and Discussion

Orchard 1. The high density orchard had about 70% midday canopy PAR interception compared to 59% in the standard density planting measured with the mobile platform lightbar. This would suggest that the production should be higher in the high density orchard since the interception in the standard density was 85% of that in the high density orchard. However, according to the grower, the production has been similar in the normal and high density orchards. Since both of the orchards are hedged in both directions to keep the trees separate so that nuts are not shaken off adjacent trees which would miss the catch frame at harvest, it is possible that there is more disturbed canopy that would take several years to return to productivity following hedging in the high density orchard. This would result in higher production per unit PAR intercepted in the wider spaced orchard and could thus result in similar yields. This would suggest that under these circumstances, the extra cost of trees and the increased amount of hedging cuts required per acre due to the closer tree spacing would not be cost effective.

Orchard 2. At this point trees are growing well and will fill in down the tree row within a year or two.

Orchard 3. Trees were only planted in the spring of 2017 so it is too early to draw any conclusions.

Conclusions

Further investigation of high-density plantings is required before evaluating whether or not they are worthwhile. Preliminary data based on Orchard 1 results suggests that the increased costs of establishment, increased passes through the orchard for orchard floor maintenance, sprays, harvests, hedging, etc. are unlikely to be offset by significantly higher returns.

Irrigation investigations in pistachio

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Introduction

It is difficult to measure midday stem water potential on pistachio due to the bleeding of latex that occurs as you begin to pressurize the leaf in the pressure chamber. Our goal was to improve the accuracy of measuring water potential in pistachio and to investigate the relationship between current season water status, internode production and shoot growth. The plan is to look at the shoot growth in 2018 as it relates to the water potential that was present on the shoot at different points in 2017.

In order to investigate this, we installed a planting of nursery grafted Kerman trees from Sierra Gold Nursery on May 1, 2017 on campus at UC Davis. The trees had 9-10 leaves that had emerged by the time of planting. Four irrigation treatments were set up, 50% ET, 100% ET, 150% ET and 200% ET. The levels of irrigation were determined by trying to maintain the 100 ET treatment near the fully watered baseline, using the fully watered baseline for almonds as the reference.

We also did additional water potential and shoot growth characteristics measurements in a commercial pistachio orchard in Kings County (Golden Hills on PG1 rootstock).

Results and Discussion

UC Davis trial on campus. The midday stem water potentials were not very different among the different irrigation treatments averaging -9.7, -8.8, -9.2 and -8.8 bars among the 50, 100, 150 and 200% ET treatments respectively. This is despite putting on over three times as much water on the 200 compared to 100% ET treatments. Water on the 50 and 100% ET treatments were ceased during September 2017 yet they only fell to 2 and 1 bars below the baseline through late fall.

In terms of scion length, the greatest amount of growth occurred on the 100% ET treatment with all other treatments putting on about 90% as much growth. The pattern of trunk growth and scion diameter again were similar with the greatest growth in the 100% ET treatment but no significant differences. In terms of the number of nodes produced, the 100% ET treatment had the most (about 80) while the 150% ET treatment had significantly less (about 60) and the others were in between. The 100% ET treatment was also the tallest (although not significantly taller). The production of 80 leaves in the 100% ET treatment means that one leaf was produced about every 2 days (a little faster than the 3 days per leaf production rate we have observed in walnut).

In 2018 we will observe how many shoots emerge, how many leaves there are per shoot, and how many leaflets there are per leaf and try to relate this to the previous seasons water relations. Our goal is to relate these characteristics to the water potential the previous year when this section of shoot was emerging. We have found that in walnut, the number of leaves per shoot and the number of leaflets per leaf can be impacted by the previous year water status during the time that section of shoot was forming. We have also found that shoots that are excessively wet during elongation produce blank nodes in that zone the following year. We also plan to investigate the effect of in season tipping and dormant heading on the rate of shoot emergence the following year.

Kings County grower trial. Less intensive midday stem water potential readings were done in the Kings County grower orchard trial but results were quite similar to those at the UC Davis trial. In general the trees ran at 1-3 bars below the fully watered baseline. However, there was enough variation from tree to tree that we will be able to assess the impacts of previous year water relations on current year shoot emergence and growth.

Conclusions

There is a lot of work needed on understanding impacts of midday stem water potential on pistachio tree growth characteristics. In particular, we plan to look at how current season water relations during shoot development impact the following year shoot emergence.

Determination of soil-plant-water dynamics of mature pistachio orchards grown under saline conditions

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Introduction

A significant percentage of pistachio acreage in California is grown in areas with naturally high and induced soil salinity. Irrigation management in these areas is based on information (Kc values) developed for non-salt affected soils and water supplies, and for infrequent irrigation methods, such as surface and sprinkler irrigation.

Since 2015, our team has been measuring the evapotranspiration (ET) of micro-irrigated mature pistachio orchards grown on soils with increasing salinity levels (ECe from 1-2 up to 8-10 dS/m). The field data we collected during the first three crop seasons (2015, 2016 and 2017) show that salt affected orchards have 10-30% lower water use, reduced canopy cover and light interception by up to 45% and significantly increased plant stress than non-saline orchards. While differences between non salt-affected and salt-affected orchards are evident, the mechanisms for how different levels of soil-water salinity affect water productivity are complex and less clear. Salinity has multiple impacts on plant water use: a direct effect on ET due to lower soil water potential, which results in less water uptake, and an indirect effect on ET due to reduced plant growth and canopy size, resulting in less light (energy) interception that leads to reduced crop transpiration. Additionally, the high variability of soil structure induced by salinity further complicates the estimation of orchard water use in saline growing environments.

Research activities conducted in 2017

Within this project funded by the California Pistachio Research Board, besides continuing the field measurement of actual evapotranspiration of three well-watered mature pistachio orchards, our team investigated the specific effects of physical (canopy size) and physiological (soil osmotic effect on stomatal conductance) limitations to water uptake and crop evapotranspiration under saline conditions, with the aim to improve the accuracy and reliability of information on ET and Kc of mature pistachio orchards as a function of increasing soil-water salinity. To this aim, our team instrumented with a full-flux ET station an additional non salt-affected mature pistachio orchard with similar canopy size (45-50% canopy cover) and structure to a salt-affected orchard that is currently under ET monitoring within our on-going study. Both the orchards were well watered and not subjected to water supply limitations over the entire season 2017. At the additional non salt-affected orchards, our team conducted a soil survey in collaboration with scientists from the USDA Salinity Lab in Riverside, CA using the electromagnetic induction (EMI) technique followed by soil sampling and laboratory analysis to determine relevant physical and chemical parameters to characterize soil-water salinity. Also, an irrigation system evaluation was conducted at the orchard by the Mobile Lab of the North-western Kern County Resources Conservation District to determine the average water application rate and distribution uniformity. In addition, we installed trunk dendrometers at three individual trees within each of the footprint areas of the different ET stations over the four study orchards, and collected weekly measurements of the midday stem water potential to estimate the plant water status over the course of the entire crop season, and to correlate the values of maximum daily trunk shrinkage from dendrometers with values of midday stem water potential. Finally, at the high saline orchard we set up two experimental sub canopy ET stations, one within an area with high canopy cover area and the other in a low canopy cover area, respectively, to estimate the soil evaporation (E) and its relative contribution to the total ET fluxes.

Results and Discussion

The ET and Kc values our team measured over the 2017 crop season from the three study orchards are consistent with the values measured in 2016. Increasing level of salinity had the effect of reducing the actual ET by 10 to 40% and this reduction was observed along with a progressive reduction of canopy cover from 35 to 65%.

Measurement of ET at the additional non salt-affected orchard with 45-50% canopy cover (CC) enabled to estimate the effect of canopy size and of soil osmotic potential due to salinity on ET. Specifically, over the 2017 season, the 50% CC non-saline orchard used 11% less water than the 75 % CC non-saline orchard. In both these non-salt affected orchards the stem water potential and the dendrometers did not evidenced any water stress, suggesting that trees were transpiring water at their maximum physiological potential. Under such circumstances, we can say that the observed 11% difference in ET is due to the different canopy size and resulting different light interception of the two orchards.

Comparing the seasonal ET of the non salt-affected and salt-affected orchards both with 45-50% CC, we found a 13% difference in ET, meaning that the soil osmotic potential due to salinity reduces water uptake and ET.

Measurements from the two sub-canopy ET stations show that the estimated soil evaporation counts for about 5-15% of total ET in the areas with 65-75% CC up to 30-40% in the areas where canopy cover was strongly reduced by salinity.

Conclusions

Canopy cover and light interception are the main drivers of crop ET. These parameters are significantly affected by soil-water salinity, and such influence must be taken in due consideration for accurate irrigation scheduling in orchards grown under saline conditions. Salinity has a direct effect on ET, but we also think that salinity may not be the only factor affecting tree performance, the other being sodium absorption ratio (SAR), root asphyxia, and specific ions toxicity, such as sodium, boron and chloride. Root asphyxia due to excessive water application in degraded structure in deflocculated (sodic) soils may have a predominant adverse effect on tree performance on the long term.

Development of physiology based methods for sustainable management of pistachios under changing Central Valley climatic conditions

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Introduction

The overarching goal of this research is to characterize the physiological responses of pistachio trees to abiotic stresses with the aim of using this knowledge to improve production and guiding pistachio plant improvement - phase one carbohydrate management.

Currently, evaluating the physiological status of trees for guiding orchard management decisions are limited to analyses of tree water status, leaf nutrient levels, and visual observations. Until recently, these methods were adequate and produced dramatic improvement in pistachio yields. However, as the climate becomes more erratic and the abiotic stresses more severe, these proven approaches may become less effective. Research effort described here focuses on the development of new methodologies to measure trees physiological status that complement the currently used methods. Specifically, we aim at mechanistic understanding of tree non-structural carbohydrates (NSCs) management in the context of dormancy, chilling requirements and yield performance. We have employed large-scale approach to gain knowledge on carbohydrates seasonal dynamics across entire California to determine pistachio carbohydrate management in relation to climate, tree age, and geographic distribution. Specifically we want to determine if pre-dormancy carbohydrate status of the orchards can be managed and if it is correlated with following year, growth, heath and yield of the orchard.

Results and Discussion

Analysis of the carbohydrate content revealed very strong dependence of NSCs dynamics on tree phenology and growth (Figure 1; points 1-6 and a-b). We have noticed that there is large change of carbohydrates content in twigs during 'dormancy' and early spring (1) . This dynamic is characterized by accumulation of NSCs near buds (a) and their release during flowering (2) followed by accumulation (3) and reduction during vegetative growth (4). Low levels of NSCs are maintained thought the summer until near-end of stem radial growth (b). Carbohydrates accumulation (5) was completed in October - November (6). This pattern reflects yearly cycle of NSCs management by pistachio tree.

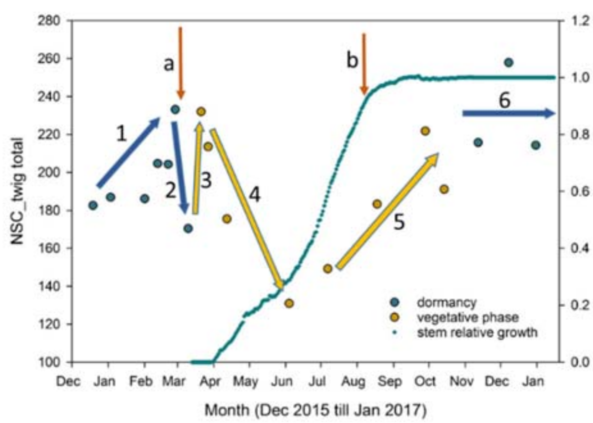

Figure 1. Change in carbohydrate content in pistachio twigs and relative radial growth of stem. For 1-6 and a-b denominators see text.

During fall of 2016, we have initiated a large-scale "citizen research" project to gain needed knowledge on carbohydrate seasonal dynamics in twigs of Pistachio trees across age groups, climate conditions and management practice to provide the proof of concept. Already over 40 orchards are supplying us with twig samples from across Central Valley, CA. The initial results allow us to link the dynamics of non-structural carbohydrates in twigs to phenology (Figure 2), and tree age. We are waiting for 2017 yield reports to address the issue if carbohydrate status and their season dynamics can be associate to orchards performance. We also expect to determine levels of carbohydrates for healthy trees to facilitated carbohydrate based management for specific locations. The description of the citizen research project can be found at: http://www.plantsciences.ucdavis.edu/plantsciences_faculty/zwieniecki/CR/cr.html.

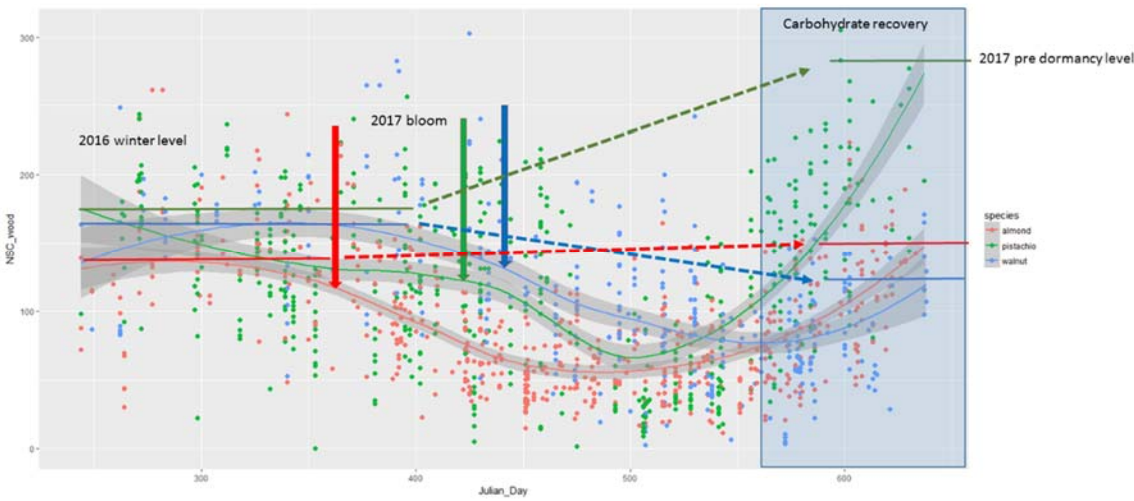

Figure 2. Seasonal pattern of soluble sugars and starch concentration (NSC) in three major tree crop species Almond, Walnut and Pistachio during 2016-2017 season. Each data point represent single orchard determined from averaging three twig samples. Gray lines are running average content of the total NSCs concentration in wood. Higher levels of NSCs in 2016/17 winter allow for the energy support of the bloom. During summer, all species reduced reserves to low levels reflecting demand for NSCs to support yield and tree growth that exceeds photosynthetic supply. In the fall, NSCs level is expected to recover to accumulate reserves for the spring of 2018. Interestingly, pistachio (green) accumulated almost twice as much NSCs 2017 fall, potentially reflecting its strong alternating crop behavior – 2017 was considered an OFF year, next year is expected to be an ON year potentially supported by an increased accumulation of NSCs.

Conclusion

Seasonal dynamics and levels of non-structural carbohydrates are closely linked to phenology of pistachio. NSCs level during dormancy and the pattern of their release to sustain spring growth might be a predictor of current year yield as NSCs reserves are necessary for formation of healthy flowers and early growth rate. The observed large accumulation of NSCs after an OFF (2017) year might predict the presence of the ON year in 2018, although with disclaimer that high level of NSCs might be necessary but not sufficient to guarantee the high yield as other factors might influence the outcome. In other words, low levels of winter NSCs might always result in low yield despite good orchard management during summer but high levels win not guarantee the outcome without proper management and climate. Analysis of carbohydrate in the fall can provide growers with information if post-harvest management reached the goal of orchard preparedness for dormancy, i.e. if NSCs level in woody tissue recover or exceed prior year winter levels and if it is consistent with local regional levels. This knowledge provides potential for precision post-harvest management leading to induction of dormancy and may lead to saving water and fertilizer treatment in the fall.

Physiology of sodium management in pistachio: stem adaptations

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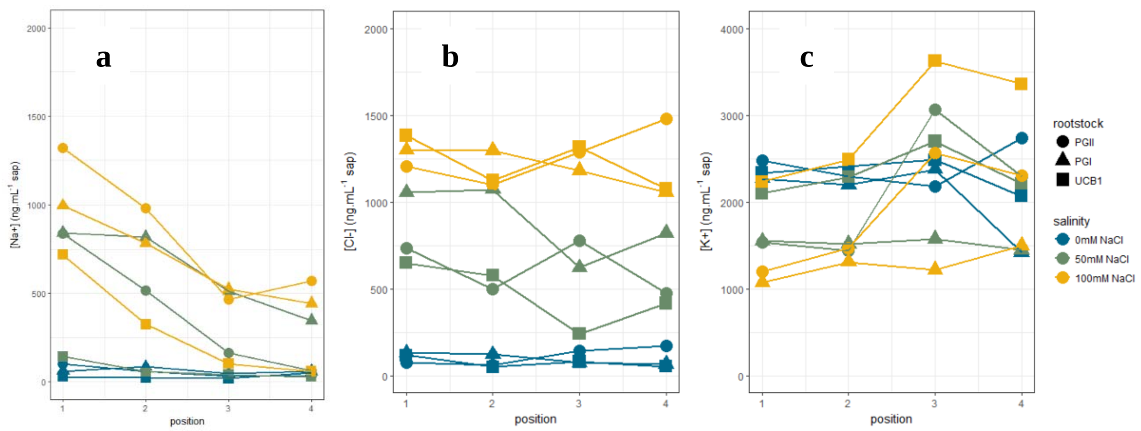
Introduction

Tree crops must contend not only with the water quality available each season, but also with the salts that may build in their tissues over decades of growth and production. While in any given year, salt tolerant trees like pistachio might be expected to rely on strategies that parallel those of annuals in order to manage nutrient deficiencies, osmotically-induced water stress, or ion-specific toxicity in roots or leaves, the longevity and significant volume of their trunks—up to 80 or 90% of total biomass—may provide an additional level of salt management and protection specific to woody perennials. Total exclusion of salts is not necessarily a viable short- or long-term tolerance strategy. Some salt inclusion via uptake from soil solution and xylem loading of inorganic osmolytes allows for the maintenance of an energetically efficient osmotic gradient that favors water movement from root to transpiring tissues despite low soil water potentials, relieving plants of exclusive reliance on compatible carbon solutes to achieve the same effect. However, without a strategy for storage or removal of salt, leaves at the end of the transpiration stream tend to disproportionately accumulate and concentrate any included ions as the water carrying them arrives and evaporates. Because many of a cell's metabolic functions are impaired by high concentrations of environmentally pervasive and minimally essential ions like sodium (Na+) or chloride (Cl-) this accumulation has potentially lethal implications for ill-adapted leaves and, by association, attached plants. Focusing on *stem* adaptations that might be exploited in woody perennials in particular, are there scenarios for achieving salt tolerance which both include uptake of salts, to manage osmotically induced water stress, but also minimize rates of leaf salt accumulation to toxic levels? This is the central question which has driven my PhD dissertation and work over the last five years for the CPRB.

Results and Discussion

One stem adaptation shown to contribute to a salt-inclusive tolerance scenario in even short-lived crop plants, is xylem retrieval, the ability to remove the salts from xylem sap before they arrive at transpiring leaves by dispersing them in adjacent living cells called xylem parenchyma. We have found that pistachios do indeed extract Na+ from the transpiration stream. Additionally, when we compared the Na+ retrieval strategies of three young rootstocks (UCB1, PGII, and PGI) we found that we cannot separate xylem retrieval from xylem exclusion as the concentration entering the stem impacts the rate of retrieval (Figure 1a). At moderate (50mM NaCl) levels of applied salinity, UCB1 excludes Na+ so effectively that there is no significant difference between its values just above the root crown (position 1) and the values of any rootstock's control treatments (0mM NaCl) at the same position. Thus, no significant xylem retrieval of Na+ is needed to achieve low concentrations at the apical meristem (position 4). At high levels of applied salinity (100mM NaCl), UCB1 continues to exclude a high percentage of applied Na+ and significantly increases retrieval so that, still, no Na+ arrives at the apical meristem. In contrast, we observed that PGI's strategy of moderate and high exclusion for moderate and high treatments, respectively, but no bump in retrieval from one level of salinity to the next resulted in in lesser protection of the apical meristem in even the moderate salt treatment. PGII's strategy of moderate exclusion and moderate retrieval at both moderate and high levels of applied salinity is enough to protect the apical meristem from Na+ only in the moderate salinity treatment. There was no significant difference among the tested rootstocks in either chloride (Cl-, Figure 1b) exclusion or retrieval. UCB1's success with Na+ may be explained, at least in part, by potassium (K+) selection. In accordance with significantly less concentrated Na+ at both base (position 1) and apical meristem (position 4) of UCB1s treated with 100mM NaCl, K+ is more concentrated at the same positions (Figure 1c).

Figure 1. Average measured sap sodium (a), chloride (b), and potassium (c) concentrations along the stem axis of 2-year old PGII, PGI, and UCB1 rootstocks taken after 10 weeks of treatment with 0, 50, and 100mM sodium chloride. Positions of sap extraction indicate a 2-3cm segment starting just above the root crown (1), just below the start of the current year's growth (2), just above the start of the current year's growth (3), and just below the apical meristem (4). Statistical significance referenced in the main text was determined by linear mixed effects models considering the fixed effects of rootstock, salinity, and position and the random effect of individual trees.



Although UCB1 demonstrates apparent advantages in a saline environment, analyzing the non-structural carbohydrates (NSCs) of another set of young (1-year-old) clonal UCB1 rootstocks after two months of treatment with 50 and 100mM NaCl, we found significant reductions in the starch concentrations of developing tissues (fine roots, all bark, and young wood). Starch reductions translated to significant reductions in the total NSC pool despite significant increases in bark sugar concentrations. Taking all of this into consideration, a year of recovery after salinity application and NSC pools in surviving salt-treated trees (6 of 7 in the 50mM treatment and 3 of 7 in the 100mM treatment) were indistinguishable from controls.

<u>Conclusion</u>

Despite UCB1's Na+ safe strategy, which may protect leaves from the rapid accumulation of one toxic ion even under conditions of high salinity, it demonstrates no protection from Cl- accumulation. UCB1 also demonstrates significant immediate energy costs at the end of a saline growing season, which may have implications for the survival of dormancy and spring leaf-out the following year. Further analysis is required to study whether PGII, which excludes less Na+ than UCB1, but still manages Na+ accumulation to some degree, is able to maintain higher NSC levels immediately following salinity treatment. This would indicate a short-term advantage to some Na+ inclusion in the absence of K+ selectivity, at least when NaCl application is moderate. In the long-term, however, one year of salinity stress does not demonstrate irreversible NSC consequences.

In addition to the data discussed in this year's report, my forthcoming dissertation will draw conclusions about the influence of the graft union in stem salt transport, long-term consequences of salt storage (is it permanent?), and the mechanism of xylem Na+ retrieval.

Real-time, in-situ detection of volatile profiles for the prevention of aflatoxin fungal contamination in pistachios

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Introduction

The objective is to demonstrate the feasibility of a Raman, in-situ warning system for detecting and removing developing fungal hot spots from pistachio stockpiles and transit containers, thus decreasing health risks and product loss because of contamination. The proposed project has the following goals: to calibrate the Raman fingerprinting of biomarkers, standalone and in premixed samples, to build a database with the vibrational profiles distinctive to the signatures of the bouquet emitted by the (contaminated or not) pistachios; to test the improvement in the detection of the detectable markers with enhanced Raman on a small probe. Specifically, for this first year we have been focusing on providing: 1. Raman system calibration; single and mixed gas-samples signatures.

Results and Discussion

The project has practically started in June 2017 when DoE approvals were received. The progress reported is thus for the period June-September 2017. We have worked closely with our collaborator, Dr. John Beck at USDA-ARS who has developed volatile emission profiles for pistachios and almonds in different humidity conditions, which evolution could be associated to fungal growth and therefore provide early-warning detection signals. We have selected the most significant components of the bouquet gathered by Dr. Beck's team, i.e. the most significant markers of growth conditions, and investigated their Raman signatures. We initially started our measurements with samples provided by Dr. Beck's team and subsequently procured our own. We have examined isobutyraldehyde, methyl salicylate, ocimene, limonene, pentanal, specifically and also analyzed 5-methyl-furfural, isobutanol, heptanal for almonds as both nuts suffer from similar diseases and commonalities could help support any conclusions.

We have at first taken Raman measurement of all the analytes in liquid phase to retrieve and identify the basic spectrum signature as the signal would be stronger; this was done for each analyte separately. For these measurements we have used a portable Raman system, a Delta-Nu Inspector Raman that was positioned on a stand in our lab for the tests but is designed to be used also as a battery-powered hand-held system for in-situ real-time measurements. Power required for the operation is <30mW. The wavelength of operation is 785nm, selected for reducing the fluorescence background which is also removed by postprocessing during which careful selection of the averaging parameters must be chosen to avoid removing the signal itself or on the contrary including too much noise. In the typical Raman plots that we analyze, the reported spectrum intensity vs. Raman shifts represent the peculiar molecules excited roto-vibrational modes, scattering the incident light back inelastically and thus with an inherent slight wavelength shift.

Once we have verified we have signals for the samples under consideration in liquid phase, we proceeded to test 2uL droplets on metallic nanostructured substrates (by Silmeco) for Surface Enhanced Raman Spectroscopy (SERS) signal. These samples provide enhancement of *both* the incident and scattered electric field *amplitude* providing a power of 4 light *intensity* increase. All SERS measurements were between 0.5 and 5 secs long (10x-100x shorter than in normal Raman) and averaged over >5 regions of each Silmeco slide and several repetitions. We have taken measurements at various time interval, from hours to days to monitor temporal evolution of the signal. All substrates presented a strong signal after they are exposed to the chemicals ('initial reading') as expected since the droplet provide a large volume contributing to the bulk-like Raman signal. However, we observed some peak intensity increase, most likely to be attributed to the enhancement of the signals from the molecules in proximity of the nanostructure (SERS). Five out of ight chemicals, methylsalicylate, ocimine, heptanal , 5-methylfurfaral and isobutylaldehyde, (only 4 in Fig. 1) show a strong signal after several days, indicating a strong affinity of the molecules to the substrate and suggesting the potential of the technique for gas phase detection. We were also able to measure nixed signal, specifically methylsalicylate on a isobutylaldheyde coated sample indicating that the former has a higher vapor pressure. Thus, we have performed a very simple experiment to prove gas-phase detection of the methylsalicylate. We have filled a vial and taped the substrate on its cap so that only the vapor from the analyte could reach the substrate once the vial was capped (Fig. 2). The signal appears very strong after 1hr (first measurements) and increases until it reaches some saturation at about 3days -afterwards the sample is moved away from the vial and the signal decays as expected. We postulate that the first hour a partial monolayer is created and then it continues to rearrange, even in multiple layers until the molecules are too far removed from the surface and cannot exploit the enhancement induced by nanostructures. Afterwards, the molecules tend to diffuse away since the adsorption is only based on VanderWaal forces (physisorption) and not covalent ones (chemisorption).

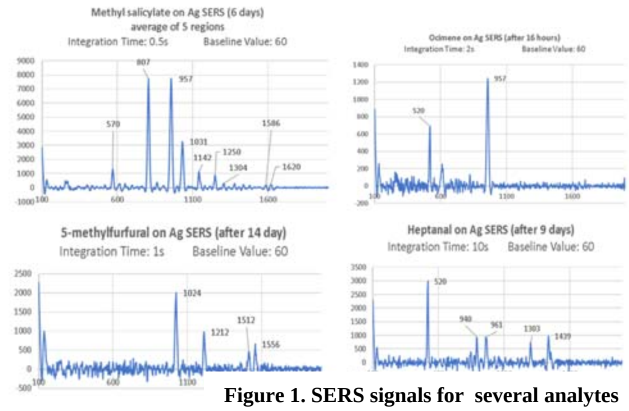

Figure 1. SERS signals for several analytes

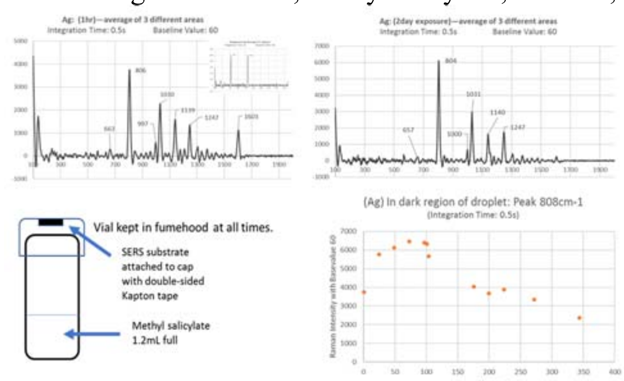

Figure 2 Gas-phase metylsalycilate

Conclusion

We verified both Raman and SERS signatures of various analytes representative of the pistachios (and almonds) breaths and fungi growth markers when related to humidity conditions. For the rest of the funding period we are planning to provide gas-phase analysis for the same group of chemicals. Preliminary results for methylsalicylate vapors provide confidence on the technique. We will aim at controlling temperature and humidity and provide data with enough spatial and temporal granularity to understand the evolution of the signal, critical information for technology development for any field application. For the analytes not providing strong. SERS signals we postulate that the equilibrium vapor pressure is very high and leading to quick evaporation or the affinity between the metallic nanopillars and the molecules is low. We will further investigate these cases since a higher volatility could be beneficial in the gas phase detection.

Soil survival, root infectivity, and management of *Rhodococcus* spp. causing pistachio bushy top syndrome

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Introduction

Pistachio bushy top syndrome (PBTS) has emerged as a new problem affecting commercial pistachio orchards planted between 2011 and 2016 in California, Arizona, and New Mexico. The etiology of PBTS has been associated with two species of gram positive bacteria that are closely related to *Rhodococcus fascians* (*Rf*) and *Rhodococcus corynebacterioides* (Rc). *Rf* is a known plant pathogenic bacterium that causes plant disease by producing a suite of novel cytokinins that influence plant growth and development. To our knowledge, PBTS is the first association of *Rc* with plant disease. Symptoms of PBTS have largely been observed in clonally propagated UCB-1 rootstock and include stunting, shortened internode length, swollen nodes, gall formation, and failure to bud. Because of the unprecedented nature of PBTS, no research-based data are available to predict the long-term productivity of PBTS-affected trees in orchards. As a consequence, most growers and land managers opted to either remove entire orchards or individual trees, depending on the level of disease incidence in affected orchards.

The soil survival potential of PBTS isolates of *Rhodococcus* spp. and potential for infectivity of roots of replants in former PBTS sites are two factors that may affect the health and productivity of replants. Additionally, the risk of reuse of tree stakes from PBTS sites is of concern to growers undergoing rogueing and replant operations. Soil survival studies conducted in 2016 indicated short term (weeks-months) survival potential of both isolates, with longevity varying based on time of year, soil moisture, and soil type. Infested soilless potting medium has also been observed to support short term (several months) survival of the bacteria and support infectivity of roots, particularly when both isolates are present.

The observed PBTS isolate survival in soilless potting medium and the potential for roots to serve as infection courts for these phytopathogenic bacteria suggest the ease of transmission and persistence in greenhouse propagation systems. Currently, there are no products registered for the suppression of populations of plant pathogenic *Rhodococcus* spp. on the phylloplane, and the emergence of PBTS has incited commercial interest in product development for this arena.

In an effort to address land management considerations and risk analyses of growers affected by PBTS, the following objectives were addressed in 2017: i) comparative analysis of the phenotype (suckering, trunk and scaffold diameters, bark texture, and yield) of PBTS trees compared to asymptomatic trees entering maturity, ii) determination of isolate presence on tree stakes, iii) assessment of survival potential of both *Rf* and *Rc* in field soils, including the influence of depth of inoculum, time of year, soil type, and soil moisture on survival, iv) investigation of root infectivity in soilless medium, and v) influence of a foliar-applied calcium silicate and silicon dioxide (Mainstay Si, Redox Chemical, LLC) .

Results and Discussion

Tree phenotype data was collected from 109 trees in a 7th leaf PBTS-affected ‘Golden Hills’ block on clonal ‘UCB-1’ rootstock in southwestern Tulare County. PBTS incidence in the block was 17.4%. Trees symptomatic of PBTS had significantly smaller trunk circumference

(P≤0.0001), more suckers (P≤0.0001), more suckers per cm trunk circumference (P≤0.0001), and smaller total scaffold circumference (P≤0.0001). Additionally mean trunk circumference, sucker number, suckers per cm circumference, and total scaffold circumference were significantly more variable then those of asymptomatic trees (P≤0.0001). Yield data was collected at harvest from symptomatic and asymptomatic trees (N=19) chosen at random within the block. Average yield was 13.1 and 48.9 lbs/tree in symptomatic and asymptomatic trees, respectively (P≤0.0001), but the variance in yield was similar between symptomatic and asymptomatic trees (P≤0.08). Last, the rootstock bark morphology was rated to assess the degree of "alligator skin" appearance. The "alligator skin" bark morphology was significantly correlated with reduced trunk circumference (P≤0.0001), and asymptomatic trees all exhibited the same bark morphology (no alligator skin). Symptomatic trees clumped into two different groups, indicating variability of bark morphology within the population of PBTS-symptomatic trees.

Tree stakes and plastic tree wraps on symptomatic trees in two PBTS affected orchards (Kern County and Tulare County) were assessed for infestation with *Rhodococcus* spp. in December 2016. Actinomycetes were routinely detected on stakes (N=14) and plastic wraps (N=14); however, stakes and tree wraps only yielded two and one *Rhodococcus* colonies, respectively, indicating low levels of contamination.

Soil survival studies initiated in February 2017 indicate an interactive influence of soil series and irrigation treatment (P≤0.002) and isolate and irrigation (P≤0.01) on pathogen survival over time. In general, populations of either bacterium decreased by at least 99% by April 2017 and were only detectable at trace levels by August. Soil survival studies initiated in August 2017 indicate limited survival potential of the bacteria in dry soil during summer. Neither *Rf* nor *Rc* were detectable 1 week after *in situ* incubation of inoculum in a commercial orchard. Similarly, in a screenhouse study, neither isolate was recovered after one week incubation in soils maintained dry. Moistening soil weekly to container capacity (mimicking irrigation events) extended survival of *Rf* to one week post-introduction, but only in the Vanguard soil series. *Rf* was not recovered beyond 1 week post-introduction, regardless of soil type or moisture treatment. Only trace levels of *Rc* were detected 14 weeks after introduction to the Gambogy-Biggriz soil, indicating potential for trace-level persistence.

The potential for foliar application of Mainstay Si to suppress epiphytic populations of *Rhodococcus* spp. on clonal UCB-1 pistachio rootstock in a commercial orchard was investigated. In a randomized complete block design, Mainstay Si- treated plants exhibited a 90 %, 80%, and 87% reduction of epiphytic *Rhodococcu*s spp. on the adaxial (P≤0.02), abaxial (P≤0.01), and total (P≤0.01) foliar populations, respectively.

Conclusion

The results demonstrate the long-term survival potential and root-infectivity of PBTS isolates of *Rhodococcus* spp. in greenhouse systems, and provide preliminary data on the use of Mainstay Si (Redox Chemical, LLC) in suppressing epiphytic pathogen populations. Soil survival studies indicate limited survival potential of the bacteria under field conditions and trace recovery of *Rhodococcus* spp. on stakes and plastic tree wraps suggest minimal risk in transmission of disease in reuse or recycling of these materials. Last, PBTS-symptomatic trees entering maturity exhibit more variability than healthy trees in the same block, and symptomatic trees exhibit a 73% reduction in yield.

(Re-)examining the role of *Rhodococcus* in Pistachio Bushy Top Syndrome

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Introduction

This project seeks independent validation of the claim that Pistachio Bushy Top Syndrome (PBTS) is caused by bacteria belonging to the genus *Rhodococcus*, as reported in a scientific article from the group of Dr. Jennifer Randall at New Mexico State University (Stamler et al 2015 Plant Disease 99:1468–1476). The article documents the results of a greenhouse study showing development of PBTS-like symptoms on clonal UCB-1 pistachio trees following inoculation with *R. fascians* Isolate 2 and/or *R. corynebacterioides* Isolate 1, originally isolated from PBTS-impacted trees. As explained in our original proposal, one of the reasons for a re-examination of this claim involves an inconsistency following a second publication by the same group (Stamler et al 2016 Genome Announcements 4:e00495–16) which presents the genome sequences of *R. fascians* Isolate 2 (renamed *R. fascians* PBTS2) and *R. corynebacterioides* Isolate 1 (renamed *R. corynebacterioides* PBTS1). The genomes were published along that of the model plant-pathogenic bacterium *R. fascians* D188, which harbors a linear plasmid pFiD188 carrying the so-called *fas* locus. It has been established by other researchers that without this locus (or the plasmid), strain D188 does not cause disease. This also is the basis for using *fas* genes as diagnostic markers for rhodococcal pathogenicity. Closer inspection revealed that the published PBTS2 and PBTS1 genomes do not contain a pFiD188-like plasmid or *fas*-like genes, which directly contradicts the Stamler 2015 paper by the same group.

The objectives of the project are as follows: 1) reproduce PBTS symptoms on clonal UCB1 trees by inoculation with *R. fascians* Isolate 2/PBTS2 and/or *R. corynebacterioides* Isolate 1/PBTS1, following the published 2015 Stamler et al protocol; 2) compare the impact of Isolate 2/PBTS2 and Isolate 1/PBTS1 on pistachio to that of other bacteria, including *R. fascians* D188 and its plasmid-cured derivative, and/or a selection of genome-sequenced rhodococcal and non-rhodococcal isolates from pistachio trees in California; 3) test the effect of tree rootstock, tree age, and tree tissue type on symptom formation by *Rhodococcus*; and 4) develop DNA-based methods to profile the microbial communities (including *Rhodococcus*) that associate with naturally or artificially contaminated pistachio trees and tissue types.

Results and Discussion

Objectives 1, 2, and 3 are dependent on the availability of the original *R. fascians* Isolate 2 and *R. corynebacterioides* Isolate 1. We have requested both isolates from Dr. Randall at New Mexico State University (NMSU) but to this date (December 1st, 2017) have not received them. We are currently negotiating with NMSU over the language describing the isolates in a Material Transfer Agreement (MTA) that would allow us to receive the *Rhodococcus* strains from Dr. Randall. We remain hopeful that we can work out an agreement with NMSU to receive the strains. However, we are now also entertaining the possibility that we never might, which frustrates not only our project but also undercuts the need that was expressed and recorded in the notes of the PBTS meeting that was held on May 23rd to "perform Koch's postulates with the original isolates'. It also means that the claim that *Rhodococcus* causes PBTS remains unchallenged.

Uncertain about when we would receive the original isolates from NMSU, we postponed hiring personnel on the project until now. We have requested a no-cost extension of our 2017 funds into 2018 and we will be hiring a postdoc to work and expand on Objective 4, for which we will use protocols now available in the Leveau and Trouillas labs to extract and interrogate by DNA-based means the microbial communities that associate with healthy and PBTS-symptomatic pistachio trees. More specifically, we will be seeking culture-independent, consistent association of *fas* genes and other microbial gene markers with symptomatic tissue from PBTS trees. The discovery of such markers will be guided by the CPRB-funded *Rhodococcus* genome project (PI Trouillas).

Conclusion

Progress on this project was delayed because we did not obtain the biological material that is needed for Objectives 1-3. We are re-aligning the project in anticipation of not receiving this material. We have asked CPRB for a no-cost extension on the project, which will allow us to develop original Objective 4 into a culture-independent approach to Koch's postulate of consistent association, seeking microbial gene markers that are specific to symptomatic tissues on PBTS trees.

Fungicide sensitivity of *Colletotrichum fioriniae* causing anthracnose on pistachio in California

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Introduction

In 2016, pistachios (*Pistacia vera*) of cv. Red Aleppo showing severe anthracnose symptoms (black, sunken and circular lesions) in fruit, leaves and rachises were observed in Glenn County, CA. By harvest time in September, lesions on fruit and rachises became pink due to mature conidia oozing from the acervuli of the pathogen. The isolates were identified as *Colletotrichum fioriniae*. In the same year, pathogenicity tests were performed, revealing their capacity to reproduce the symptoms observed in the field on fruits and leaves of Sirora, Joley, Golden Hills and Kerman cultivars. To develop appropriate control strategies for this disease, the sensitivity of *C. fioriniae* isolates were assessed for different fungicide groups already registered for controlling the major diseases (Botryosphaeria panicle and shoot blight and Alternaria late blight) of pistachio in California.

In total, 39 isolates of *C. fioriniae* were used to determine their sensitivity to the following commercial fungicides: Abound®, Syngenta (a.i. azoxystrobin), Gem®500SC, Bayer USA LLC. (a.i. trifloxystrobin), Quash®, Valent Chemical Com. (a.i. metconazole), Tilt®, Syngenta (a.i. propiconazole), Luna® Privilege, Bayer Crop Science (a.i. fluopyram), Fontelis®, Du Pont (a.i. penthiopyrad), Vangard®WG, Syngenta (a.i. cyprodinil), and Scala®SC (a.i. pyrimethanil). Sensitivity assays were performed to determine the effective fungicide concentration that inhibits mycelial growth by 50% in relation to their growth on potato dextrose agar without fungicide. This value is known as EC_{50}, and is used to monitor pathogen resistance to fungicide. Molecular mutations associated with resistance to quinone outside inhibitor (QoI) fungicide (azoxystrobin and trifloxystrobin) were identified by sequencing the 516-bp of the cytochrome *b* (cyt *b*) gene and making comparisons with sequences retrieved from the GenBank (NCBI).

Results and Discussion

Among the surveyed fungicide groups, the demethylation inhibitors (DMI) consistently inhibited mycelial growth at low dosages such as 0.37 µg/ml (ranging from 0.10 to 0.71 µg/ml) to metconazole (a.i. of Quash) and 0.79 µg/ml (ranging from 0.34 to 1.41 µg/ml) to propiconazole (a.i. of Tilt) (Fig. 1A). The succinate dehydrogenase inhibitors (SDHI) had the greatest variation between the two tested products, where penthiopyrad (a.i. of Fontelis) had a mean EC_{50} value of 0.09 µg/ml (ranging from 0.04 to 0.28 µg/ml) (Fig. 1B) and fluopyram (a.i. of Luna Privilege) did not inhibit mycelial growth (data not shown). As observed with Luna Privilege, the two anilino-pyrimidine (AP) fungicides cyprodinil (a.i. of Vangard) and pyrimethanil (a.i. of Scala) were also not able to inhibit growth (data not shown). Quinone outside inhibitor (QoI) fungicide sensitivity was tested by using the highest stock solution dosage, and as a result, mean EC_{50} values for azoxystrobin (a.i. of Abound) and trifloxystrobin (a.i. of Gem) were 647.82 µg/ml (ranging from 83.81 to 1,000.88 µg/ml) and 609.13 µg/ml (ranging from 108.35 to 956.39 µg/ml), respectively (Fig. 1C). Based on the QoI results, the sequencing analysis of the cyt *b* gene revealed that all 37 isolates had the mutation G143A, corresponding to high QoI resistance levels (data not shown).

The results obtained for QoI fungicides (a.i. azoxystrobin and trifloxystrobin) are mostly explained by the pressure these isolates have been exposed to fungicide sprays over the years. In the orchard where samples were collected from, the grower reported up to seven fungicide applications annually, including five QoIs alone or in mixed formulations to control Botryosphaeria panicle and shoot blight. The number of spray applications performed is justified by the need to control Botryosphaeria panicle and shoot blight caused by *Botryosphaeria dothidea* because this disease can be severe in this orchard annually. However, the overuse of these chemical groups has affected the sensitivity of *Colletotrichum* populations that cause anthracnose in pistachio. The sensitivity variability within the SDHI fungicides is in accordance with other *Colletotrichum* studies performed in different crops. Penthiopyrad (a.i. of Fontelis) and fluopyram (a.i. of Luna Privilege) belong to different SDHI chemical groups, and this may cause the difference of inhibition performance between products. The UC IPM website also reports limited anthracnose control in almonds when using Luna Privilege and no data is available for AP fungicides such as Scala and Vanguard. The highest *C. fioriniae* sensitivity levels were obtained for the DMI fungicides, and no DMI resistant isolates were observed. To reduce the risk of a pistachio anthracnose outbreak, as well as to manage the resistance build-up of *Alternaria* populations in Glenn and other counties, it is important to reduce the number of fungicide applications by following models that predict the most efficient fungicide usage. These models incorporate wetness duration and temperature and are readily available and proven to reduce the number of sprays. However, a weather logger needed in the orchard to monitor the critical weather parameters.

Conclusion

The current pistachio registered fungicides Fontelis (a.i. penthiopyrad, SDHI), Quash (a.i. metconazole, DMI), and Tilt (a.i. propiconazole, DMI) exhibit the highest inhibition capacity of *C. fioriniae* mycelial growth *in vitro*. However, recommendation for controlling pistachio anthracnose will depend on fungicide efficacy trials in commercial orchards. The G143A mutation conferring resistance to Gem (a.i. trifloxystrobin, QoI) and Abound (a.i. azoxystrobin, QoI) was present in all 37 sequenced isolates and its overuse should be avoided to prevent field failure in controlling anthracnose. Determining the predicted (isolate growth and reproduction characteristics) and realized fitness, the mechanism of resistance to multiple fungicides, are suggested for further studies in order to propose anti-resistance strategies for this pistachio pathogen.

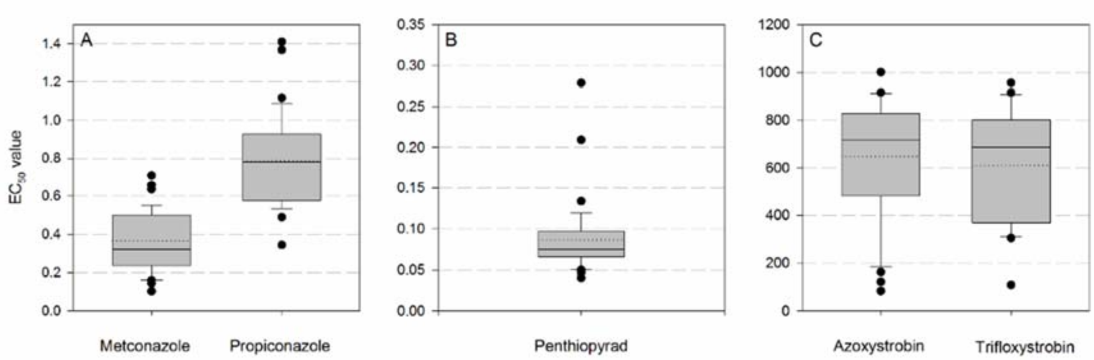

Figure 1. Fungicide sensitivity values of *Colletotrichum fioriniae* to DMI fungicides (A), SDHI fungicide (B) and QoI fungicides (C).

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

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

Introduction

Aflatoxins are toxic metabolites produced by *Aspergillus flavus* and *A. parasiticus* in several crops including pistachio. Pistachio is occasionally contaminated with aflatoxin, but this poses a high risk to international trade due to strict regulations of aflatoxin contamination in food and feeds. Aflatoxin control in crops is difficult and the only reliable method of control is using atoxigenic (unable to produce toxins) strains of *A. flavus* to displace or exclude the aflatoxin-producing strains of both *A. flavus* and *A. parasiticus*. Currently the only atoxigenic strain registered for use in pistachios in California is *A. flavus* AF36. The *A. flavus* AF36 strain is naturally widespread in California, occurring in all of the major pistachio-growing counties (Doster et al., 2014), but at too low a rate to significantly reduce the aflatoxin contamination potential of the natural population of *Aspergillus*. However, when the biocontrol *Aspergillus flavus* AF36 is applied on the pistachio orchards, the rate of the atoxigenic strain AF36 is increased and is able to compete and displace the toxin producing strains and consequently reducing the aflatoxin producing potential of the population.

Experimental applications of the atoxigenic biocontrol agent *A. flavus* AF36 to reduce aflatoxin contamination in pistachios in California started in 2002. The application of the AF36 product was successful in substantially increasing the proportion of the atoxigenic strain AF36 within the population of *A. flavus*/*A. parasiticus* fungi (Doster et al., 2014). In addition, the nuts from the orchards treated with the AF36 product did not have a higher incidence of kernel decay by *A. flavus* than nuts from untreated areas, suggesting that applying AF36 will not increase the number of moldy nuts. Nut samples from the orchards treated with the AF36 product were also less likely to be contaminated with aflatoxin than those from untreated orchards. Previously, the biocontrol was formulated in sterilized wheat inoculated with the atoxigenic strain. Currently a new formulation consisting of roasted sorghum grain coated with the atoxigenic strain has been developed and approved for its use in crop nuts, including pistachio, almond, and fig in California. The new formulation, called *Aspergillus flavus* AF36 Prevail® has been successful in applications on cotton in Arizona and we believe that it will also be successful in applications on pistachios in California. However, to better protect crops from becoming contaminated with aflatoxins, more research is required to optimize the application of the biocontrol and ensure more displacement of toxigenic strains. Information about optimum conditions for sporulation and dispersal of the biocontrol product can help facilitate the displacement of toxigenic strains and ultimately can reduce aflatoxin in pistachios.

Results and Discussion

The vertical mobility, measured by spore density, of *A. flavus* AF36 was studied under field conditions in an orchard at the Kearney Agricultural Research and Extension Center. Spore traps, consisting of eight 9-cm Petri dishes with 50 ml *Aspergillus* Differentiation Agar medium (Fluka, AFPA) per plate, were placed at four different heights from 30 to 230 cm. The biocontrol product *A. flavus* AF36 Prevail® was applied on the soil under the spore traps. In general, results indicate that *A. flavus* spores decreased exponentially as a function of height while the density of *A. niger* spores increased. Although further experiments need to be conducted, this situation could be explained by the fact that the tree canopy could work as a natural source of inoculum for *A. niger*.

It is important to consider that *A. niger* does not produce aflatoxins, but some isolates can produce ochratoxins, another important concern for the nut industries in California.

To determine the best placement, with respect to irrigation, for maximizing the sporulation of the atoxigenic *A. flavus* biocontrol product, AF36 Prevail® grains were placed at different distances (from 25 to 250 cm) from the irrigation micro-sprinklers under field conditions. Both the sporulation of the *A. flavus* AF36 product grains and the soil water content were periodically evaluated. In this experiment, we observed that sporulation of the AF36 product grains was optimal where soil moisture was between 13 and 18%. Conversely, AF36 sporulation was practically nonexistent in soil where there was excess (> 24% water content) or limited amount (<6% water content) soil water content.

To determine the distance that the atoxigenic biocontrol fungus *A. flavus* AF36 is able to disperse from the source of inoculum in nut orchards, we applied the AF36 product, at 10 times the normal rate on the soil around one tree in the center of an orchard at Kearney Agricultural Research and Extension Center. Preliminary results indicate that the fungus is able to move in all directions and that the spore density of total *Aspergillus flavus* decreased exponentially with increased distance from the source.

The impact of different arthropods as a potential cause of atoxigenic biocontrol product predation and loss was evaluated under field conditions. A video camera (BirdCam, Wingscapes) was placed to monitor feeding behavior of arthropods on soils where the biocontrol product was applied. Results indicate that in nontilled soils, *Oniscidea* species (roly polies or pill bugs) and different ant species can feed on the sorghum grain applied onto the soil. Conversely, the impact of these arthropods is minimal in frequently tilled soils.

Additionally, we will continue to analyze pistachio library samples for aflatoxins and evaluate the effect of commercial application of AF36 Prevail®. These samples originate from commercial blocks that have been part of AF36 biocontrol research efforts since 2008. Some of the blocks have been left untreated since the start of the project. Other blocks have been treated with 5 or 10 lbs. per acre of the product annually. Following harvest, samples are recovered from the Wonderful Orchards processing facility and after sorting they are ground and mixed up, then aflatoxins are extracted and run through special immunoaffinity columns that bind aflatoxin and are then run through high performance liquid chromatography for aflatoxin detection. The results are analyzed to determine the effect of different rates of treatment with the *A. flavus* AF36 Prevail® product.

Conclusion

The results presented here will be useful to optimize application of the biocontrol product *Aspergillus flavus* AF36 Prevail® and to devise aflatoxin reduction management strategies in pistachio crops in California. These studies indicate that when conditions are optimal, the product will readily sporulate and disperse. If the spores of the biocontrol strain are able to spread throughout the orchard and into the tree canopy, the potential to displace toxigenic isolates increases while the probability of aflatoxin contamination decreases. Our findings indicate that it is important to consider product placement with respect to the irrigation source and to consider the potential for product loss due to arthropods. Future research will focus on application strategies to deliver the biocontrol agent to the orchard environment in a timely matter even when conditions for sporulation of the product are suboptimal. New formulations and the optimal timing of application will be investigated. Also, we will look for other atoxigenic strains that can be developed which may have advantages in different environmental conditions.

Survival and resistance stability of SDHI fungicides on different *Alternaria alternata* mutants causing Alternaria late blight in pistachio (second year report)

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Introduction

Alternaria late blight (ALB), mainly caused by *Alternaria alternata,* is an important disease of California pistachio, and its control relies on multiple fungicide applications including the succinate dehydrogenase inhibitors (SDHI; FRAC 7) group. Increased ALB resistance levels were observed just a few years after the registration of Pristine (a.i. boscalid), accompanied with moderate to high cross-resistance with other registered SDHI fungicides. Molecular studies of resistant isolates of *A. alternata* revealed the presence of several single point mutations in different locations of the succinate dehydrogenase gene, contributing to different levels of SDHI resistance. For effective resistance management, it is necessary to understand the stability of fungicide resistance and to predict the survival in the orchard of individual mutants in California pistachio. If resistant mutants in a fungal population have low survival than the wild-type, their frequency will decline in the absence of the fungicide pressure. The objectives of this study were (i) to assess the resistance stability of *A. alternata* mutants to SDHI fungicides *in vitro*, (ii) to evaluate the resistance stability of *A. alternata* populations in the field by removing the SDHI fungicides from the spray program, and (iii) to evaluate the survival of *A. alternata* mutants after successive transfers *in vitro*.

In our study, fungicide resistance levels were determined throughout the effective concentration that inhibit mycelial growth by 50% (EC_{50}) in comparison to growth in the absence of fungicide. In total, four SDHI fungicides were tested: the boscalid (bo, a.i. of Pristine, BASF), fluopyram (fp, a.i. of Luna Experience, Sensation, and Privilege, Bayer), fluxapyroxad, (fd, a.i. of Merivon, BASF) and penthiopyrad (pe, a.i. of Fontelis, DuPont). The stability of fungicide resistance and mutant survival were evaluated for 15 isolates, three of each belonging to the following mutants: $SdhC^{H134R}$, $SdhB^{H277Y}$, $SdhB^{H277L}$, and $SdhB^{H277R}$ (the superscript indicates the mutation of these isolates), and the non-mutant or wild-type isolate group. These isolates were cultured on potato dextrose agar (PDA) without fungicide, and subjected to 12 successive transfers in PDA media to imitate different pathogen life cycles. The survival components we evaluated were: sporulation, germination rate, and area under mycelial growth curve. Evaluations occurred after the 1st and 12th transfer cycles. The results presented here include data from the first and second year experiments.

Results and Discussion

Resistance instability after 12 successive transfers were observed only in the $SdhB^{H277L}$ mutant, where the resistance levels to boscalid, fluxapyroxad, and penthiopyrad were significantly reduced to ranges corresponding to reduced and low sensitivity levels (Table 1). The $SdhB^{H277R}$ showed a significant instability to fluxapyroxad but not to boscalid, penthiopyrad and fluopyram (Table 1). Stable EC_{50} values were observed for the $SdhB^{H277Y}$, $SdhC^{H134R}$ and the wild-type isolates (Table 1). These results suggest that the mutants H277L and H277R may increase their sensitivity to SDHI fungicides such as boscalid, penthiopyrad and fluopyram after a certain period in the absence of SDHI fungicides use. But this is not true for the other two resistant mutants, H134R and H277Y, which account for 51.2% of the current population (surveyed in

2015). Despite the resistance of mutants H134R and H277Y that has been shown to be stable, their survival parameters suffered a penalty after 12 successive transfers in PDA media. It was observed in our results that both H134R and H277Y decreased their spore production and germination (only the H134R mutant) while the wild-type had only numerical and not statistical reductions for the same parameters (data not shown). These results suggest that wild-type isolates may have competitive advantages and eventually displace mutants in an orchard where the use of SDHI fungicides has discontinued. However, specific experiments need to be performed to test this hypothesis.

In our field experiment, the SDHI fungicides were discontinued from the spray program of three commercial pistachio orchards. The resistance levels were observed to decrease at the Orchard 1 (1st experiment year) for boscalid and fluopyram, but not for fluxapyroxad and penthiopyrad (data not shown) At the Orchard 2, the penthiopyrad resistance was affected by the proposed management, but not by the other three fungicides. In the second-year experiment only numerical reductions were observed (data not shown). Additionally, the mutant frequencies from the first population (early 2016) were compared with the latest population (late 2017). The mutant comparison after two successive years without SDHI applications was shown to be affected by the field spray management. In Orchard 1, the mutations such as S135R, H277Y/L/R and D123E found in 2016 were not observed in 2017. In Orchard 2, the H134R remained stable and the wild-type frequency was consequently increased (data not shown). In the same Orchard 2, the H134R and H277Y initially observed in 2016 were not found in 2017, but sensitivity values were not changed. Finally, in Orchard 3, we observed an increased frequency of H134R which resulted in a reduction of wild-type isolates.

Conclusion

In conclusion, the experiments presented here demonstrate that *A. alternata* isolates carrying the mutations H134R and H277Y have stable resistance levels when tested under laboratory conditions, but their predicted survival suggest that they may suffer a penalty which could lead to a competition disadvantage against the wild-type isolates in the absence of fungicide. The removal of SDHI fungicides from the pistachio spray program showed some effect on the mutant composition of *A. alternata* populations, but these changes did not reflect reduced resistance levels. A third year of SDHI removal is suggested to continue the monitoring of resistance in these three selected orchards.

Table 1. The effect of successive transfer on the sensitivity to SDHIs *in vitro* of *Alternaria alternata* causing late blight of pistachio.

Mutants	Transfer	n	Boscalid		Fluxapyroxad		Fluopyram		Penthiopyrad	
			EC_{50}		EC_{50}		EC_{50}		EC_{50}	
$SdhB^{H277L}$	1st	3	122.88	a	110.72	a	13.27	a	8.50	a
	12th	3	8.67	b	6.50	b	6.96	a	2.36	b
$SdhB^{H277R}$	1st	3	99.29	a	0.95	a	0.21	a	0.69	a
	12th	3	53.18	a	0.10	b	0.11	a	0.44	a
$SdhB^{H277Y}$	1st	3	122.88	a	6.49	a	0.52	a	9.39	a
	12th	3	84.51	a	1.26	a	0.46	a	5.58	a
$SdhC^{H134R}$	1st	3	122.88	a	57.59	a	4.42	a	30.74	a
	12th	3	109.16	a	68.94	a	7.73	a	9.54	a
wild-type	1st	3	0.24	a	0.02	a	0.45	a	0.49	a
	12th	3	0.57	a	0.10	a	0.54	a	0.26	a

Baseline sensitivity to benzovindiflupyr of *Alternaria alternata* isolates exposed to SDHI fungicides in pistachio with Alternaria late blight disease

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Introduction

Alternaria late blight (ALB), mainly caused by *Alternaria alternata,* is one of the most devastating diseases in pistachio produced in the Central San Joaquin Valley, in California. The succinate dehydrogenase inhibitors (SDHI) fungicides represent a broad-spectrum fungicide group with the fastest growth in terms of new compounds produced and launched into the market. Currently, 20 SDHI active ingredients (a.i.) are listed by the Fungicide Resistance Action Committee (FRAC), with six new a.i. listed in the last five years. In California, ALB can be controlled with the following SDHIs: boscalid (a.i of Pristine), penthiopyrad (a.i. of Fontelis), fluxapyroxad (a.i. of Merivon), and fluopyram (a.i. of Luna Products). However, different resistance levels for the mentioned a.i. were observed to increase in the last few years. In the Unites States, the a.i. benzovindiflupyr is reported to control many pathogens in wheat, corn, cucurbits, fruit, vegetables, grapevines, peanuts, pome fruit, potato, and soybean. Excellent intrinsic activity of benzovindiflupyr was reported against species of *Colletotrichum* and *Venturia inaequalis,* with *Colletotrichum* exhibiting resistance to the same SDHI used in pistachio.

In this study, 80 isolates of *A. alternata* from California [not exposed (baseline), n=32; exposed, n=48] were used to determine their sensitivity to fluopyram (98.13%, The Bayer Chemical Company) and benzovindiflupyr (Solatenol 97%, Syngenta Crop Protection) fungicides throughout the mycelium growth assay (EC_{50}). The baseline isolates were never exposed to SDHIs, while the exposed isolates were collected from orchards where SDHIs have been sprayed for longer than the 3 last years. The sensitivity density distribution of the isolates tested for fluopyram and benzovindiflupyr was analyzed by plotting the cumulative frequencies of the log-transformed EC_{50} values. Pearson correlation analysis was used to determine cross-resistance between fluopyram and benzivindiflupyr isolate populations. Fungicide efficacy tests were performed on detached pistachio leaves (cv. Kerman) to demonstrate the potential of each a.i. to inhibit six each SDHI-resistant mutants and wild-type isolates.

Results and Discussion

In this study, the mean EC_{50} value of the baseline isolates of *A. alternata* to benzovindiflupyr in California corresponds to 0.63 µg/ml (min=0.03, CI95%=0.45-0.88, max=7.13 µg/ml, Fig. 1). This was close to the 0.23 µg/ml EC_{50} value reported for fluopyram in previous studies (not statistically analyzed). This information will certainly support the monitoring of *A. alternata* sensitivity for SDHI fungicides in California pistachios and for quantifying changes to EC_{50} values. The sensitivity density distribution analysis revealed similar (*P*-value = 0.21) benzovindiflupyr and fluopyram curves within the SDHI exposed *A. alternata* and no differences (*P*-value = 0.21) between the two isolate populations tested for benzovindiflupyr were found (Fig. 1). Pearson correlation analysis showed that, for all baseline isolates, a significant, positive, and moderate cross-resistance between fluopyram and benzovindiflupyr EC_{50} (*P*-value= 5.3×10^{-6}, r = 0.57) (data not shown). The cross-resistance between the two a.i. tested increased to high (*P*-value = 2×10^{-16}, r = 0.76) when testing all SDHI exposed isolates (data not shown). Similar cross-resistance between fluopyram and benzovindiflupyr were observed in baseline isolates of *Venturia inaequalis* in apple. Similar a.i. intrinsic activity was observed within isolates (data not shown). Similarities between a.i. ingredients suggest that both fungicides are selecting for the same mutation, considering that the isolates carrying the SdhC-H134R mutation were mostly moderate resistant (MR) for both benzovindiflupyr and fluopyram; but SdhB-H277Y/L isolates with high fluopyram EC_{50} value were inhibited to sensitive levels with benzovindiflupyr (data not shown).

The fungicide efficacy test performed at the recommended label rate of each Luna Privilege (a.i. fluopyram), Aprovia (a.i. benzovindiflupyr), and a second Aprovia treatment at same a.i. concentration as Luna Privilege were performed in detached pistachio leaves (cv. Kerman). The results showed best lesion control achieved by protective applications of Luna Privilege for either H134R mutant and wild-type genotypes (data not shown). No differences between the two Aprovia (a.i. benzovindiflupyr) dosages were observed in the experiment. The results obtained in this study revealed that benzovindiflupyr and fluopyram present similar intrinsic activity *in vitro*, but benzovindiflupyr does not inhibit mutant and wild-type isolates as effectively as the fluopyram in the detached leaves assay. Differences may be associated with different binding capacities and arrangements of the SDHI molecule in the binding pocket.

<u>Conclusion</u>

The benzovindiflupyr active ingredient could not properly control SdhC-H134R mutants (commonly selected by the usage of fluopyram), but high intrinsic values were observed in the inhibition of all other SDHI mutant genotypes found in *A. alternata* from California pistachio. Benzovindyflupyr may be effective for control of ALB when rotating or mixing with appropriate fungicides. Sensitivity studies of other SDHI a.i. listed by FRAC are suggested in order to find a fungicide capable of inhibiting those isolate mutants that are mostly selected for by the use of boscalid and fluopyram. Aprovia fungicide is not yet registered for use in pistachio.

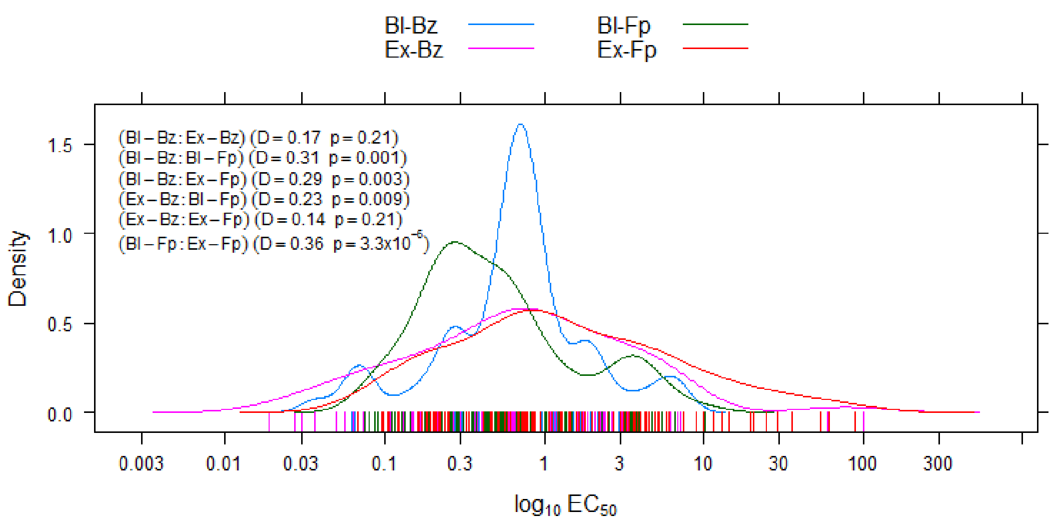

Figure 2. Sensitivity density distribution between benzovindiflupyr (bz) and fluopyram (fp) tested for not exposed (baseline, bl) and SDHI-exposed (ex) isolates of *Alternaria alternata.*

Management of Alternaria Late Blight of Pistachio

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Introduction

Alternaria late blight caused by *Alternaria alternata*, *A. tenuissima*, and *A. arborescens*, continues to be a major disease in California-grown pistachios. Favored by high relative humidity and dew, the disease is worse in late summer because periods with dew and relative humidity above 95% are usually longer during August and September. The disease can cause severe premature defoliation and staining of nutshells, resulting in reduced fruit quality.

The objectives of the 2017 spray trial was to determine fungicide efficacy against Alternaria late blight.

Results and Discussion

Fungicide efficacy trial. In a pistachio orchard at the Kearney Agricultural Research and Extension Center (Kearney), we tested spray programs in addition to evaluating single fungicide compounds. Treatments consisted of three sprays approximately four weeks apart [May 31, July 2 (critical time for spray), and August 2]. The trade names, active ingredients, and class of the fungicides used in these trials are listed in Table 1 of the full report. All the fungicides were applied at recommended label rates or at rates recommended by the manufacturer. Each treatment consisted of five single-tree replications. Sprays were applied with a handgun sprayer using 400 gallons of water per acre. The orchard was irrigated using fanjet micro-sprinklers. Symptoms of disease in this orchard developed only very late in the season, in fact they did not develop until after the normal harvest date. Although the crop was considered mature on about September 12, we did not evaluate the plot until October 9, 2017. On this day, the fungicide efficacy was rated using the whole tree evaluation method ("efficacy score" with 1 = the least control, 5 = the best control, and 2, 3, and 4, intermediate levels of increasing levels of control). Two people rated the trees and their scores were averaged to arrive at the final value.

Fungicide Trial – whole tree evaluation at Kearney. As mentioned earlier, disease symptoms developed late in the season for the efficacy trial at the Kearney Agric Res. & Ext. Center, forcing us to delay the evaluation until October 9, 2017. A mix of Luna® Experience and Baythroid® (Trt 13) had the highest score, 3.95 (Table 2). Indar® (Trt 11) was next higest at 3.85. The highest sixteen treatments were not statistically different from each other, ranging from 3.95 down to 3.25. All treatments were shown to be better than the untreated control which had a value of 1.95. (refer to the full report for the numbered treatments).

Conclusions

Though the disease was late in appearing and not as severe as in some past years, there is evidence that spray treatments significantly reduce visible disease symptoms in the orchard.

Acknowledgments
We thank the field crew of the Kearney Agricultural Research and Extension Center for taking care of the cultural practices of our pistachio orchards and the various fungicide companies for providing unrestricted gifts to support our research.

Early detection of pistachio Botryosphaeria panicle blight disease using high-throughput plant phenotyping

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Introduction

Botryosphaeria panicle blight (caused by *Botryosphearia dothidea*), a major disease of pistachios in California, first became a serious problem in Sacramento Valley in the late 1980s, but a couple of decades later, the disease caused a significant yield reduction in San Joaquim Valley as well. Botryosphaeria is known as the major threat to the California pistachios. Unfortunately, Botryosphaeria disease has a long latent period and the earliest symptoms can appear in late April to early May, if the temperature is warm enough. Dead or partially infected buds can show symptoms such as dead areas as early as mid-summer. However, a significant portion of potentially infected (or infested) buds remain non-symptomatic. According to a previous study, more than half of non-symptomatic (healthy looking) buds collected from an orchard in Butte County (December 1986) contained spores of *Botryosphaeria dothidea*. Some of these contaminated buds most likely develop infected shoots in spring. Conventional early detection practice suggests collecting up to 100 buds from random locations throughout the orchard and processing them using the BUDMON (bud monitoring) technique. The BUDMON technique (developed by Dr. Michailides) is highly sensitive and capable of diagnosing the samples with little or no visual symptoms. However, it requires sample collection/preparation, laboratory effort/cost, and it takes at least one week to have the diagnosis results. Although, the BUDMON technique is highly accurate and a reliable diagnosis method, it is not free and it requires lab labor. Therefore, the number of samples to be collected for BUDMON assay could be limited by the available budget for disease monitoring; while, an optimum Botryosphaeria management requires a maximized monitoring in which high special and temporal resolution data must be available.

For the current project, pistachio buds were collected from Botryosphaeria infected pistachio trees as well as from healthy trees in an experimental pistachio orchard at UC Kearney REC. After spectral measurements were conducted, the buds were tested by using the BUDMON method to determine the incidence of Botryosphaeria infection. Spectral analyses were conducted to identify the wavelengths that are relevant to the identification of Botryosphaeria positive buds while they were in non-symptomatic conditions.

An in-field spectral measurement tool (KOBIN Proximity) was designed and developed for in-field bud spectral data collection in the range of ultraviolet (UV), visible, and near-infrared (NIR) (300-1650 nm). KOBIN Proximity will be used in step 2 of this project to conduct a non-destructive time-series spectral measurement of pistachio buds.

Results and Discussion

A total number of 508 healthy and infected pistachio buds (in non-symptomatic condition) were collected in two datasets (223 buds in dataset 1 and 285 buds in dataset 2). Spectral reflectance of all the buds was measured in the range of 186 nm to 1031 nm with the resolution of ~0.3 nm in the Precision Agriculture Lab at UC Kearney REC. Then, the buds were given to Dr. Michailides Lab at UC Kearney REC to be diagnosed for *Botryosphaeria* and *Phomopsis* species using the BUDMON assay. Based on the BUDMON results, 316 out of 508 bud samples were heathy and they were categorized as the control class. 186 buds were diagnosed with *Botryosphaeria,* 14 buds were diagnosed with *Phomopsis* and only 8 buds had both *Botryosphaeria* and *Phomopsis*. Since the number of bud samples in the *Phomopsis* class was limited, compared to the other classes, Phomopsis class was not considered for the data analysis. A preliminarily spectral analysis was conducted in MATLAB software (version R2017a, MathWorks, Natick, MA) to determine the relevant wavebands for Botryosphaeria identification in the non-symptomatic stage. The rankfeatures function was used with a t-test evaluation criterion, in which reflectance in all wavelengths was employed as input features. The output of this process was a vector of cross-correlation coefficients (corresponding to all wavelengths) that indicates the relevance of wavelengths to Botryosphaeria identification. The results showed that the wavelengths in the visible range (400-700 nm) had minimum relevance to Botryosphaeria identification compared to the wavelengths in UV (below 400 nm) and NIR (above 700 nm). These results confirmed that the Botryosphaeria infected buds did not have any obvious symptom in the visible range, in other words, they were in a non-symptomatic condition during the time of the experiment. However, a growing trend in cross-correlation coefficients was observed in the NIR region as the wavelength increased between 800-1000 nm. In the next step of this project, the spectral reflectance of pistachio buds will be analyzed in an expanded NIR band up to 1650 nm using the KOBIN Proximity device.

KOBIN Proximity is a handheld field data collection device that includes a light source, batteries (14.8V for the light source, and 7.4V for other components), two spectrometers, a geographical positioning system, a microcontroller, a LCD screen, and a customized fiber optic probe head that was developed for bud spectral measurement. KOBIN Proximity is able to conduct an in-field spectral measurement in a nondestructive manner, log the location where the measurement is done, illustrate the spectral data on a LCD screen, and store the spectral data on a flash memory. This device will be used for collecting time-series spectral data from the same pistachio buds during the winter and spring of 2018. After the last round of spectral data measurement is conducted, the bud samples will be collected and processed with the BUDMON assay to validate the disease status of the samples. A time-series spectral analysis will be conducted to determine the accuracy of Botryosphaeria identification at different stages of the growth season.

Conclusion

Pistachio buds with Botryosphaeria infection remain non-symptomatic during the winter and early spring. The earliest symptoms appear in late April to early May, if the temperature is warm enough. Dead or partially infected buds can show symptoms such as dead areas as early as mid-summer. Still, a significant portion of potentially infected buds remain non-symptomatic. The bud samples used for this project were collected between March 20 to 27, 2017. The preliminarily results showed that pistachio buds with Botryosphaeria infection illustrate spectral characteristics similar to healthy buds in the visible band; however, the non-visible bands of UV and NIR demonstrated the potential for Botryosphaeria identification using spectral analysis.

By the end of this project in spring 2019, we expect to develop a practical in-field diagnosis methodology for the detection of Botryosphaeria infected buds before the appearance of symptoms. The methodology will involve the KOBIN Proximity sensor as well as a Botryosphaeria diagnosis software that will run on the KOBIN Proximity device.

Investigating canker diseases of pistachio in California

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Introduction

During recent surveys, we detected several new canker pathogens of pistachio in California. Symptoms included wood discoloration and branch dieback. Canker diseases occurred mainly in older pistachio orchards but also were found on occasion in young orchards. Main fungal pathogens identified from cankers included six *Cytospora* species*, Neofusicoccum mediterraneum, Diaporthe ambigua (=Phomopsis ambigua)*, *Schizophyllum commune*, *Phaeoacremonium mortoniae, Phoma* sp.*, Colletotrichum karstii* and *Phytophthora* species. The objectives of this study were to investigate the biology of these newly detected pathogens, assess their severity and evaluate the threat they may pose to pistachios. In 2017, we conducted experiments to determine the ability of these fungi to cause canker diseases. Additional goal was to determine and compare the susceptibility of the three main pistachio cultivars Golden Hills, Lost Hills and Kerman to canker pathogens. Pathogenicity studies were conducted in the field at the Kearney Agricultural Research and Extension center (KARE) using a selection of fungal species previously isolated from diseased trees and inoculated in twigs of 10-year-old pistachio trees. This year we completed a three-year study that aimed to identify and characterized fungal pathogens associated with canker diseases of pistachio.

Results and Discussion

Results of pathogenicity studies showed that all species tested were able to produce lesions and vascular discolorations in the wood of pistachio just a few months after infection. All fungal treatments produced cankers that were significantly longer than the control, suggesting that all fungi tested are pathogenic to pistachio. This work revealed also that all three scion cultivars including Golden Hills, Lost Hills and Kerman were equally susceptible to canker pathogens. All fungal isolates were recovered at a significant distance from the inoculation point showing their ability to colonize and infect the wood of pistachio. *Diaporthe (Phomopsis) ambigua*, *Schizophyllum commune*, *Phaeoacremonium mortoniae, Phoma* sp. and *Colletotrichum karstii,* tested in this study, appeared moderately virulent in all 3 pistachio cultivars and were only detected sporadically in pistachio in California. Therefore, these fungi are not considered major threat to pistachio. On the other hand, *Cytospora eucalypticola* and *Neofusicoccum mediterraneum* were particularly virulent in pistachio, causing substantial cankers and dieback symptoms. *N. mediterraneum* and *Cytospora spp.* were also the most frequent canker pathogens isolated in our study, and were rather common in mature pistachio orchards. *Cytopora* species have been largely recognized as severe canker pathogens worldwide and can be devastating to perennial crops. *N. mediterraneum* was reported as an aggressive canker pathogen of English walnut, almond and olive in California causing twig and branch dieback. Results from this study revealed also that inoculation of pistachio twigs with *Phytophthora* species resulted in severe aerial or perennial canker, killing rapidly entire scaffold branches. All *Phytophthora* species appeared pathogenic in Golden Hills, Lost Hills and Kerman scion cultivars. Although sporadic, canker diseases of pistachio must be monitored carefully in California as stress caused by drought can contribute to an exacerbation of canker diseases. It has been also common knowledge that trees subjected to any type of stress become more susceptible to infection by canker pathogens. The occurrence of similar fungal pathogens in almond and walnut in California have been on increasing concern in recent years and specific control measures are being investigated in these crops to mitigate the impact of canker diseases.

Conclusion

Several new canker pathogens of pistachio were detected and identified in California.

Canker diseases were mainly restricted to older pistachio orchards but occasionally occurred in young orchards.

All fungal species tested were pathogenic to pistachio, causing extensive lesions and vascular discolorations just a few months after inoculation.

Species of *Cytospora* and *Neofusicoccum mediterraneum* were the most common canker pathogens isolated from pistachio and appeared highly virulent to this host, causing substantial cankers and dieback symptoms.

Phytophthora species can cause aerial or perennial cankers in pistachio.

All three scion cultivars including Golden Hills, Lost Hills and Kerman were equally susceptible to canker pathogens.

This work exposed the occurrence of new and aggressive fungal pathogens of pistachio in California and we recommend careful monitoring of orchards for the detection of possible spread and amplification of these new diseases.

Investigating soil borne diseases of pistachio in California

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Introduction

In recent years, we detected various declines of pistachio trees caused by soil borne pathogens. Declining trees were usually characterized by chlorotic foliage as well as wilting, defoliation and eventual tree death. Trunks often expressed gumming together with crown rot symptoms. Investigations of the causes of tree decline in pistachio revealed the occurrence of several *Phytophthora* species including *P. niederhauserii*, *P. cinnamomi* and *P.* taxon walnut associated with crown or root rots. The fungus *Macrophomina phaseolina* was also commonly isolated from young declining pistachio trees and rootstocks expressing root and crown rot symptoms. Species of *Fusarium oxysporum*, *F. solani* and *F. proliferatum* were also detected on occasion from unusual crown rot symptoms. To date, little is known about soil borne pathogens affecting pistachio in California. Therefore, the objectives of this study were to characterize the newly discovered soil borne pathogens, investigate their pathogenicity and evaluate commercially available pistachio rootstocks for disease tolerance. In 2017, several pathogenicity and rootstocks susceptibility studies were conducted at the Kearney Agricultural Research and Extension center (KARE) using clonal UCB-1, PGI and/or PGII (Platinum) rootstocks.

Results and Discussion

Results of pathogenicity studies showed that *Phytophthora niederhauserii*, *P. cinnamomi, P.* taxon walnut and *M. phaseolina* can cause root and crown rots in both UCB-1 clonal and PGII (Platinum) rootstocks. Inoculation of young potted plants with *Phytophthora* spp. resulted in complete wilting and death of plants three weeks after inoculation. *Phytophthora* species were re-isolated from root and crown rot symptoms, thus completing Koch's postulates. In one experiment, PGII (Platinum) rootstocks appeared more tolerant to *Phytophthora* pathogens than clonal UCB-1 rootstocks, however these findings were not reproduced in a second experiment where both rootstocks were affected similarly by these pathogens. *Phytophthora* species affecting pistachio trees in California have been poorly investigated overall until the present study. This study is the first to report *Phytophthora niederhauserii*, *P. cinnamomic* and *P.* taxon walnut causing root and crown rots in pistachio in California. *P. niederhauserii* and *P. cinnamomi* causing crown rots were recently reported as emerging pathogens of almond in California. Recently, *P. taxon walnut* has been shown to be pathogenic on non-woody stems of *P. vera* in California.

Macrophomina phaseolina affected both UCB-1 clonal and PGII (Platinum) rootstocks equally, resulting in the killing of all inoculated plants. The occurrence of *M. phaseolina* in pistachio in California has not been reported prior to the present study. This year, additional cases of declining pistachio trees associated with *M. phaseolina* were detected. *M. phaseolina* has been known as the causal agent of Charcoal rot of many field crops including corn, cotton, sunflower, potato and sorghum. In California, charcoal rot appears to be the most important current concern for the strawberry industry due to its steady increase over the past 10 years. The fungus is known to produce microsclerotia, which production increases under low water potentials that occurs during drought. Despite little reports of *M. phaseolina* affecting perennial woody crops, we have isolated it also from declining table grapes and sweet cherry trees in California.

Fusarium solani and *F. proliferatum* appeared to diminish the growth of pistachio plants following soil inoculations, but did not cause wilting or death of plants. In the fruit and nut crops, *Fusarium* species often have been regarded as secondary invaders of diseased tissues and generally have received little interest for biological studies. This work is one of the first to investigate the taxonomy and biology of *Fusarium* species in pistachio. Isolates of *F. solani*, *F. oxysporum*, and *F. proliferatum* appeared to cause rot symptoms in stems of UCB-1 rootstocks. These fungi also appeared to affect the growth of rootstock plants. More studies are on-going to fully understand the role of *Fusarium* species in pistachio but a recent study in Tunisia showed that *Fusarium solani* can cause root rot of pistachio. The same fungus also can cause dry root rot of citrus in California, causing crown rot and girdling of the trunk base of citrus trees. *Fusarium oxysporum* is generally associated with vascular wilt and root diseases on a wide range of plants.

Another experiment was established at KARE in the fall of 2017 to compare the susceptibility of the main pistachio rootstocks including UCB-1, PGI and PGII (Platinum) to all the various soil borne pathogens isolated during this study. This experiment is on-going and aims to identify most tolerant rootstocks to improve management of soil borne diseases. Last fall, 144 plants of each of UCB-1, PGI and PGII (Platinum) rootstocks were inoculated using 2-3 isolates of all species under investigation.

Conclusion

Phytophthora species including *P. niederhauserii*, *P. cinnamomi* and *P.* taxon walnut were isolated from diseased pistachio trees in commercial orchards and all species were shown to cause root and crown rot diseases in pistachio.

Macrophomina phaseolina has been found repeatedly from declining pistachio trees and was generally associated with root and crown rots. The pathogenicity of this fungus was confirmed.

Fusarium oxysporum, *F. solani* and *F. proliferatum* also are found associated with pistachio crown and root rots. The biology of these fungi is still under investigation, but preliminary findings suggest they are pathogens of pistachio.

Experiments are on-going to identify most tolerant rootstocks to improve management of soil borne diseases.

Taxonomy, host range, genetic structure and diversity of *Rhodococcus* isolates from California pistachio

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Introduction

In the last six years, the Pistachio Bushy Top Syndrome (PBTS) has affected nearly 40,000 acres of pistachio in California with an estimated economic loss to the industry of at least $100 million. Stamler et al. (2015) have suggested an association of the disease with *Rhodococcus fascians* and a close relative of *R. corynebacterioides*. *Rhodococcus* is a genus of bacteria with members that are exceptionally plastic, and that can occupy a variety of ecological niches. Some, but not all, members of *R. fascians* and closely related species, are demonstrably capable of causing disease (i.e. are pathogenic). *R. fascians* can infect a wide range of plants, however, woody plants are rarely affected. The genetic basis of pathogenicity of *R. fascians* include three virulence loci (*att*, *fasR*, or *fas*) located on a plasmid, a mobile genetic element, which is not essential for the survival of the bacteria. Strains that lack the plasmid do not cause disease. Yet, the claim that *Rhodococcus* spp. are the causative agents of PBTS has had extraordinary consequences. In short, the mere detection of *Rhodococcus* within a pistachio orchard or at the nursery, regardless of whether the *Rhodococcus* isolate is pathogenic, has resulted in the removal of orchards and destruction of trees. During the 2015-2016 growing seasons, my lab conducted surveys in California to re-examine the cause of PBTS and determine the occurrence, distribution and host range of *Rhodococcus* bacteria in the main pistachio producing counties in California. Results of field observations and sampling raised questions about the validity of the current hypothesis attributing PBTS to *Rhodococcus* bacteria. The present project aimed to clarify the role of *Rhodococcus* bacteria associated with pistachio using a combination of biological, molecular and genome-based studies.

Results and Discussion

Seventy orchards affected with PBTS were visited and sampled for this study. Native and ornamental plants as well as other fruit and nut crops were also examined. Overall, 800 plant specimens including diseased and asymptomatic pistachio rootstocks were sampled and tested for the presence of *Rhodococcus* bacteria. Results of sampling revealed that *R. fascians* is relatively common on the cultivated, ornamental as well as native vegetation of California. In several occasions, *R. fascians* was isolated from leaves of asymptomatic pistachio trees, asymptomatic walnut, almond and stone fruit trees, asymptomatic native plant species in riparian areas, and asymptomatic ornamental plant species in city landscapes. *R. fascians* was detected inconsistently from pistachio plants exhibiting symptoms of PBTS and the presence of the bacterium did not correlate strictly with the occurrence of PBTS symptoms. Moreover, *Rhodococcus corynebacterioides*, previously shown to act synergistically with *R. fascians* to stimulate the development and expression of PBTS symptoms, was rarely recovered from plants sampled in our study. Our surveys also revealed no sign of spread of the disease between neighboring orchards as well as within orchards replanted following removal of PBTS trees. The inconsistency of isolation of *Rhodococcus* bacteria from plants expressing PBTS, the indication that the disease is not spreading, the occurrence of these bacteria in healthy pistachio trees and their broad host range suggest they are not pathogenic despite the previous claim. Instead, the occurrence of *R. fascians* on numerous healthy host plants including cultivated, ornamentals and native plant species suggest it may act a as an epiphyte. Moreover, we were not able to reproduce PBTS symptoms despite multiple inoculations of *Rhodococcus* bacteria onto young UCB-1 clonal rootstocks.

In this study, we also use whole genome sequence analyses to investigate the taxonomy, diversity and biology of *Rhodococcus* species associated with pistachio. Surveys and the broad sampling permitted the assembly of an extensive collection of *Rhodococcus* strains from different hosts in California, including diseased and asymptomatic clonal UCB-1 rootstocks, other tree crops as well as native plants and ornamentals. Overall, 230 *Rhodococcus* isolates were obtained and submitted for genome sequencing. Results of genome sequence analyses showed that isolates from multiple clades (at least 4 species) of *Rhodococcus* can be found living in association with Pistachio. More interestingly, no evidence of linear plasmids or virulence loci (*att*, *fasR*, or *fas*), based on multiple bioinformatics analyses, was found among the 230 isolates collected during this study including isolates from trees expressing PBTS. Whole genome sequence phylogenies also were inconsistent with a single source outbreak or association of genotypes with disease. *Rhodococcus* isolates from pistachio were genetically identical to isolates collected on the native vegetation suggesting these bacteria are most likely endemic to California, occurring naturally in orchards and natural ecosystems. Last, our results were inconsistent with previous conclusions that *Rhodococcus* species are responsible for Pistachio Bushy Top Syndrome, suggesting that the disease was misdiagnosed.

Conclusion

The presence of *Rhodoccus* bacteria on pistachio plants do not correlate with the occurrence of PBTS symptoms. *R. fascians* was isolated from asymptomatic pistachio trees. There was also no evidence that the disease is spreading.

Rhodococcus bacteria including *R. fascians* have a broad host range in CA, including other perennial crops, native and ornamental plant species.

Genome sequence analyses showed that 230 isolates of *Rhodococcus* including *R. fascians* isolates collected from pistachio trees expressing PBTS lacked linear plasmids or virulence loci. Analyses also revealed the occurrence of multiple species in pistachio.

Data generated from this study are inconsistent with previous conclusions that *Rhodococcus* bacteria are responsible for Pistachio Bushy Top Syndrome.

The cause of PBTS remained to be determined.

Characterizing pistachio rootstocks for host status to plant-parasitic nematodes

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Introduction

Pistachio (*Pistacia* spp.) is a major crop in the US. In California, growers often use the female cultivar 'Kerman' and the pollinating male 'Peters'. In the beginning of the pistachio industry in California, theses scions were grafted onto rootstocks of *Pistacia atlantica* and *P. terebinthus.* These rootstocks susceptible to Verticillium wilt appeared resistant to *Meloidogyne* spp. and *Pratylenchus vulnus* (root lesion nematode, RLN; Michailides and Teviotdale, 2014; Crane and Maranto 1988; McKenry and Kretsch, 1984). 'UCB 1', a hybrid of *P. atlantica* x *P. integerrima* was developed to combat increasing challenges with Verticillium. Overall, nematode problems in pistachio are considered minimal because in a California survey, only low population densities of plant-parasitic nematodes were found (McKenry and Kretsch, 1984). Susceptibility to *Meloidogyne* spp. (root-knot nematode, RKN) is generally reported as low (Westerdahl, 2015). *Xiphinema index* was found to infect *Pistacia vera* and *P. mutica* (Weiner and Raski, 1966).

Culver and coworkers (1989) chose *P. atlantica* as a resistant standard when screening woody perennials. In preliminary screens of UCB1 clones, large differences between defined clones of this cross have been identified (McKenry, unpublished). In recent work, interaction of RLN 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 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*.

It is the aim of this project to examine 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.

Results

Two experiments were initiated in 2017.

One field experiment has been established to investigate plant responses to root-lesion nematode (RLN, *Pratylenchus vulnus*) and root-knot nematode (RKN, *Meloidogyne incognita*) in sandy loam soil at a location with previous history of walnut cultivation. This experiment contains nine different commercial UCB1 clones, one seedling UCB1, two *Pistacia atlantica*, one *P. terebinthus*, one *P. integerrima*, and one *P. atlantica* × *P. integerrima* cross. In addition, four Prunus rootstocks with known nematode responses and one walnut were included as checks in this test. The plants were available at varying sizes at the time of planting; only newly from seed started genotypes were smaller, all other plants established well. On May 17, plants were planted in two-plant plots in a randomized complete block design with four replications. On July 10, each plant was inoculated with 1,012 vermiform RLN and 12 infective stages of RKN using infested soil. On November 7, plant height and diameter were measured, soil samples 0-18 inch deep were collected from the root zones of the plants and extracted for nematode detection. Different nematode species were found. In examining RLN, there were minor trends among the genotypes, but overall numbers were much lower than under walnut, and somewhat lower than in the susceptible Prunus.

The second experiment was planted on August 11 in tanks (11)12 × 8 ft area and ca. 4 ft deep filled with sand. Vertical plastic divisions were installed 18 inches deep to allow every plant a growth area of 2.5 × 2.5 ft. Each of these tanks was planted to fifteen plants in randomized complete block design. Each of the five tanks was one replication. On September 1, every plant was inoculated with 1,500 ring nematodes. Here, eight clonal UCB1, one seed UCB1, one *P. atlantica*, three prunus checks, one *P. integerrima*, and one *P. atlantica* × *P. integerrima* cross were planted. The plants established vigorously despite the challenging growing conditions in sand. A data collection was not scheduled yet.

Conclusion

Two field experiments for evaluating responses of various plant-parasitic nematodes on UCB1 clones and controls of previously used rootstock genetics have been established. Plants grow appropriately vigorously but limited nematode numbers have been detected in the root zones of these pistachio plants. The observation of few nematodes present in the root zones or roots of nut crop rootstocks is common, and a much better assessment is expected at completion of the second vegetation period.

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Improving the online pistachio educational program to train pistachio new growers and handlers

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Introduction

For the last 5 years, we have been funded partly from the California Department of Food and Agriculture (CDFA) Specialty Crop Block Grant Program (SCBGP). The first grant allowed us to establish peer-reviewed and updated online fruit and nut resources on our website.
In 2015, we detected the need and high demands for online extension resources for beginner farmers. Classroom and online educational materials are important to support current and future performance of beginner growers in competitive markets. Thus the FNRIC proposed the creation of a fruit and nut production online educational training program to complement our annual classroom extension course. The project *Online Nuts and Tree Fruits Education Program to Train California Specialty Crops Growers* is funded by the CDFA SCBGP (2015-2018) and the California Pistachio Research Board (2017-2018). The objective is to train California's beginning and transitioning farmers in improved orchard management practices and sustainable fruit production, with topics such as principles of tree biology, commodity production, food safety, technology tools, environmental conservation, fruit maturity and harvesting techniques. The project consists in developing interactive courses for each crop, including a series of online videos as well as quizzes, engagement activities and supplemental information. Courses are developed for four different crops: Pistachio, Stone Fruits, Walnut and Almond. Additionally a Postharvest course has also been developed. Each of these course will be offered in English and Spanish. The inexpensive and time-flexible FNRIC online fruit and nut production program will provide beginner farmers and others with the knowledge and tools to become sustainable and productive growers.

Results and Discussion

The English and Spanish Pistachio online courses (*Advances in Pistachio Production* and *Avances en la Producción de Pistachio*) are finished since August 2017. We have prepared scripts and illustration material based on the Pistachio production manual and additional materials. These scripts have been reviewed by experts (UC Davis Faculty, UCCE Specialists and Farm advisors) in each field, while pictures were collected and/or taken for video production. The audio track was recorded by a professional voice company. The videos were created using video editor software *Camtasia*, taking in account the feedback received from a small group of experts. Supplemental information (reading material, videos or any other useful tool), engagement activities (quiz or specific exercise) and entrance and exit tests were also prepared. All this material and tools were uploaded to our UC Davis online teaching platform *Canvas*, along with the entrance/demographic survey previously prepared and the scripts provided in pdf format for each video. The Pistachio course is divided into five modules and 16 sections.

The students' progress and knowledge gain in the FNRIC online program is being evaluated comparing results of the registration-entry questionnaire and of the tests at the end of each module. In order to measure expected outcomes and collect feedback from students, we will gather baseline data using an entrance demographic survey and a finale evaluation.

The two Pistachio courses, along with other completed courses (Stone Fruits and Postharvest) have been offered for free to 200 Californian growers among people who either took or were interested

in our “hand on” classroom extension course “Principles of Fruit and Nut Tree Growth, Cropping and Management” in the past. Many of them are beginner growers or people working in the Californian fruit and nut industry. The course was open on October 30th to all people who answered us with an interest in taking one or several of our six ready courses, establishing a first large test group. The course was also offered to a few extra test groups in our network. We expect to have first data collected in the next three months, and be able to collect the data from a first set of Californian growers by April 2018 like planned in the initial proposal. As of November 22, we have already 50 positive answers, with 20 for the English Pistachio course. This will allow us to collect the first outcome data in the next few months.
After the CDFA SCBGP grant period, there will be a registration fee charged to participants, kept at a minimum to cover the continued management and operational costs of the online program.

Conclusion

The Pistachio course will educate beginner Pistachio growers on how to improve orchard management practices, food safety, crop losses & causes, agricultural accounting, and respond to changing environmental conditions.

Produce Safety Alliance (PSA) curriculum training for pistachio growers

Authors:

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Introduction

The Food Safety Modernization Act (FSMA) Produce Safety Rule requires that at least one supervisor or responsible part from a farm subject to the rule must have successfully completed food safety training that is at least equivalent to that received under standardized curriculum recognized as adequate (§ 112.22(c)). At this time, the Produce Safety Alliance (PSA) Grower Training Course is the only course that is recognized as adequate. The course materials can be delivered in an 8-h period but it is generally accepted that attempting to offer the course in a single day does not provide enough opportunity for participants to understand the materials and to have adequate opportunity to respond to questions. Recognized Trainers receive a complete slide set that must be used in the delivery of the course. While those slides must be presented, trainers have flexibility to exchange photos or examples in the slides and may add additional, crop-specific slides to enhance the materials.

The objectives of this project were to:

1. Modify the PSA Grower Training slide set to incorporate photographs and examples relevant to California pistachio production practices.
2. Deliver three 2-day PSA Grower Training courses to pistachio growers or representatives from affiliated industries.
3. Conduct a needs-analysis for the development of targeted pistachio grower supplemental materials including a review of the current (2009) pistachio grower's good agricultural practices document and grower self-assessment questionnaire.
4. Develop, as appropriate key supplemental materials identified in the needs assessment (Objective 3).

Results and Discussion

Objective 1. The PSA Grower Training slide set was modified to incorporate a significant number of photographs of California Pistachio Production practices. Additional slides were developed that expanded upon the concepts illustrated in each section of the training program and that focused on pistachio-specific practices or data. These slides were used at a July 2017 workshop.

Objective 2. One 2-day PSA Grower Training course was offered July 25-26, 2017 at the Fresno County Farm Bureau. A total of 48 people registered for the course; 46 received certificates of attendance for the course. Thirty-eight different pistachio growers or harvesting companies were represented along with one farm advisor from Fresno County Cooperative Extension. The training was well received although it was very clear that pistachio farming, harvesting, and postharvest handling do not fall neatly into the examples provided in the standardized curriculum. The modified slides were useful but the addition of supplementary slides was not as well received. We have addressed this issue by modifying our presentation approach for the second and third workshops. At this time there are a number of guidance documents that have yet to be published by the Food and Drug Administration (FDA). At the time of the training there was significant uncertainty regarding the production agricultural water testing requirements. Since that time the FDA has published documents that provide some clarification and they have extended the deadline for compliance with this part of the rule. The attendees of the workshop were alerted to these changes and links to the supporting documents posted on the website (see below).

A second 2-day PA Grower Training course was scheduled for November 28-29, 2017 in Bakersfield. However, this course was cancelled due to low enrollment (16). The next two workshops will be scheduled in the spring where we believe attendance will be higher.

Objective 3. An evaluation was provided at the July training that included questions on needed support materials. A more formal needs assessment will be administered at the second and third training sessions planned for early 2018 (dates not yet confirmed).

Objective 4. We have established a website (http://ucfoodsafety.ucdavis.edu/Pre-_-_Post-harvest_Produce/Pistachio_Grower_Resources/) where we have begun to gather resources that have been identified as potentially useful to pistachio growers as they establish a farm food safety plan and work to meet the Produce Safety Rule. Larger growers will need to comply with parts of the rule beginning January 2018. The website has links to important documents on Compliance Dates, Agricultural Water, Farm Food Safety Plan Resources and links from the training manual. Under development is a factsheet on commercial harvesters and their roles and responsibilities in the Produce Safety Rule. We have begun a review of the pistachio grower's good agricultural practices document and grower self-assessment questionnaire.

Conclusion

This project is roughly 30% complete. We expect to complete the objectives by June 2018.