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Cherry Whole Orchard Recycling: Investigating Fruiting Bodies Viability of Cherry Fungal Pathogens

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SUMMARY

Whole orchard recycling (WOR), is the on-site chipping or shredding of the previous orchard tree residues and incorporation of the ground or chipped biomass into the topsoil prior to replanting. This process has become more common practice in recent years, due to the air quality regulations that restrict growers' ability to manage biomass by burning, closure of many biomass power generation plants, and the ones still open are no longer paying for woodchips. The whole orchard recycling has been established as a viable best management practice strategy in almond but its effect has not yet been investigated in cherry orchard systems. In order to answer one of the main questions we have been asked by cherry growers, whether shredding and soil incorporation of whole trees can lead to an early fungal canker disease caused by the main plant-pathogenic fungi *Calosphaeria pulchella* and *Cytospora sorbicola*, that could affect newly planted orchards, we conducted an experiment to investigate the survival of Calosphaeria and Cytospora fungi in fungal fruiting bodies still present in infected wood chips incorporated during orchard recycling and in shredded pruning wood between tree rows in a mature cherry orchard. Results showed that spore viability declined significantly over sampled time. Following a second-year spore trapping study we confirmed the high aerial dissemination of spores when shredding of infected branches between tree rows. We also confirmed the correlation between applying water via the over-tree sprinklers or after a rain event at the end of monitoring period, and fungal spore release (Nouri et al. 2021 cherry report). This information is of great importance as it helps to identify production practices responsible for the spread of these fungal pathogens within cherry orchards, or provides insights for the use of different IPM management practices to manage canker diseases under such production practices.

In our last objective, we established a nitrogen trial in a first-year orchard in Linden, CA to measure nitrogen requirements of first year cherry trees following WOR. This trial is still ongoing, and first year data will be collected this coming season and results will be presented in the 2023 Cherry Research Reports.

OBJECTIVES

1. 1) Continue the investigation into the survivability of fungal pathogens in fruiting bodies still present in wood chips incorporated during orchard recycling
2. 2) Second year spore-trapping studies to confirm our first-year results: verify if chipping and incorporating the diseased branches with cankers between tree rows could increase fungal spore dispersal in the orchard
3. 3) Continue to examine soil organic matter and stored carbon on the WOR orchard by continuing soil surveys annually, and assess impacts of N treatments on first year trees growth.

1) Continue the investigation into the survivability of fungal pathogens in fruiting bodies still present in wood chips incorporated during orchard recycling

Material and methods

Knowing that fruiting bodies of the fungus *Eutypa lata* are rarely encountered in cherry orchards, we only investigated how long *Calosphaeria pulchella* and *Cytospora sorbicola* fungi can continue to produce ascospores/pycnidiospores from perithecia/pycnidia on shredded twigs/branches, which are left on the orchard floor or buried under soil surface. This experiment was initiated in June, 2021. Infected branches/shoots covered with pycnidia of Cytospora and perithecia of Calosphaeria were shredded and placed in Brite aluminum screen mesh. The shredded wood chips were either buried or placed on the soil surface in different areas of the experimental whole orchard recycling (WOR) site, and under mature cherry trees in a second irrigated orchard with a high incidence of fungal disease where the grower adopted shredding tree prunings between tree rows. We performed monthly sampling of the infected sweet cherry branches covered with fruiting bodies for both orchards until there was no recovery of these two pathogens. Samples were collected and transferred to the laboratory for processing as described by Nouri et al. (2021 cherry report).

Results and Discussion

This experiment was conducted under two different environmental conditions. For the WOR site, the viability of these fungal pathogens was assessed under dry conditions, as the orchard did not receive any irrigation/rain until planting date. For the mature cherry orchard, fungal pathogen viability was assessed under wet conditions. Percent germination of both types of spores continued to decline significantly over time on each sampling date at each site. The WOR site was replanted on March 31, 2022. Unfortunately, we lost some of the buried/surface-placed wood during the site preparation for planting, and our last data collected dates from April 07, 2022. Results showed that the viability of asco- and pycnidiospores of both pathogens declined significantly by approximately 82% and 79% respectively after about 10 months on the soil. The decline is similar to what have been observed from shredded wood pieces buried under mature cherry trees. Thus, we believe that fungal recovery may not exceed 14-16 months in the soil.

For the shredded wood pieces placed/buried under mature cherry trees, our last data was collected on August 12, 2022. The last sampling period did not result in pathogen recovery from sampled wood pieces, and the wood was totally decayed with no signs of fruiting bodies that we could sample from. The viability of asco- and pycnidiospores declined significantly over time by

approximately 96% and 94% respectively after about 13 months on the soil, and no pathogens was recovered after 14 months. Pycnidia of *Cytospora* and perithecia of *Calosphaeria* can remain viable for extended time periods, especially when left in the tree branches/scaffolds. Survival was much lower if diseased tissue was on the orchard floor and even lower when buried. Growers should remove diseased tissue from the trees (pruning) as early as possible, shred it and incorporate it in the soil.

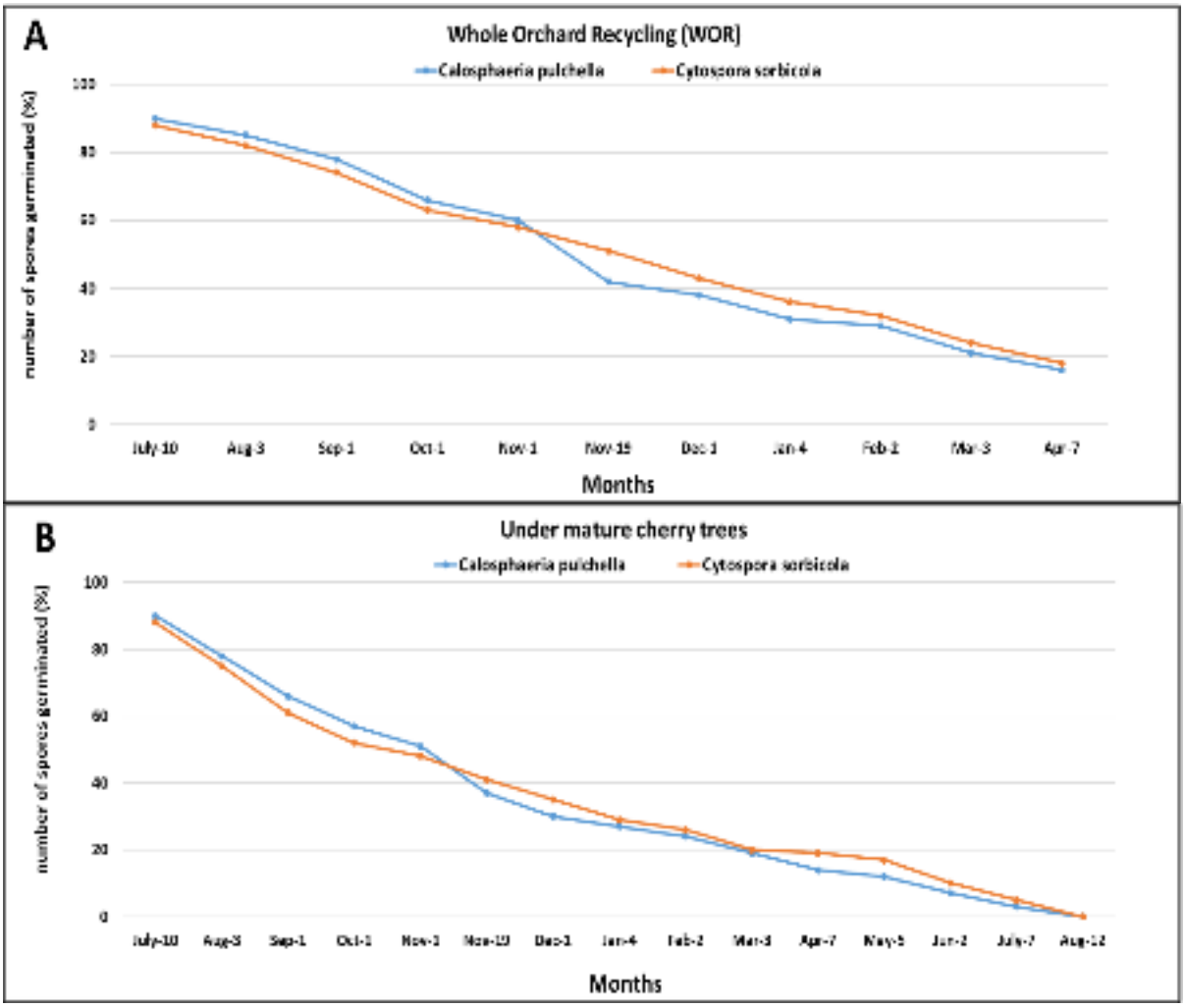

Fig. 1. Germination rates for Cytospora pycnidiospores and Calosphaeria ascospores isolated from fruiting bodies found under the periderm of shredded infected cherry branches. Shredded wood was **A.** placed/buried on the soil surface in different areas of the WOR, **B.** placed/buried on the soil surface in cherry orchard under mature cherry trees. Germination starting point at: 90% and 88% for Calosphaeria and Cytospora respectively.

2) Second year spore-trapping studies to confirm our first-year results: verify if chipping and incorporating the diseased branches with cankers between tree rows could increase fungal spore dispersal in the orchard.

Material and methods

For this year study, we repeated the experiment in: 1) one orchard in San Joaquin County from June 10, 2022 to November 03, 2022, where the grower adopted the wood shredding between tree rows, 2) the same two orchards in Kern County (used for the first-year study) from June 09, 2022 to November 07, 2022: Orchard 1 showed high fungal disease incidence where the grower adopts wood shredding between tree rows, and the second orchard developed less canker diseases compared to the previous one and the grower removes most pruned material from the orchard – in both orchards, overhead water-based cooling systems are being used as early as June through the sampling period in November. The spore trapping method described by Nouri et al. (2021 cherry report) was utilized for this second-year study. Traps were changed and analyzed weekly in the laboratory. Fungal colonies of *Cytospora* and *Calosphaeria* fungi were identified by colony morphology and growth characteristics.

Results and Discussion

Our second-year spore trap study results were consistent with those of the first-year study (see Nouri et al. 2021 cherry report). For the orchard located in San Joaquin County, we confirmed the high aerial dissemination of spores following grinding/shredding of infected branches on the orchard floor (few spores were also captured after approximately one week from the day of shredding). Here we noticed that the captured spores for both *Cytospora* and *Calosphaeria* fungi were lower compared to last year (Fig. 2). This orchard showed less incidence of dieback and fungal canker diseases compared to last year, as the grower followed excellent management practices in pruning diseased branches/scaffolds, and spraying fungicide (every other row) right before and after wood shredding between tree rows. In Kern County, we also confirmed the correlation between the overhead water-based cooling systems, which were turned-on during very hot days in both orchards, and fungal spore release. While it is hard to confirm the spore release due to shredding in the orchards in the southern valley because of the use of the overhead cooling last year as both activities took place at the same time, we have not detected spore release after shredding of pruned twigs and branches that took place in the middle of September this year (orchard 1) (Fig. 3A). A relationship between spore release and precipitation was also detected during the third week of September 2022 and early November 2022, in the three experimental sites (North and South); This was consistent with other spore trap studies in California cherry orchards; monitoring the release of spores of *Calosphaeria* (Trouillas et al. 2012).

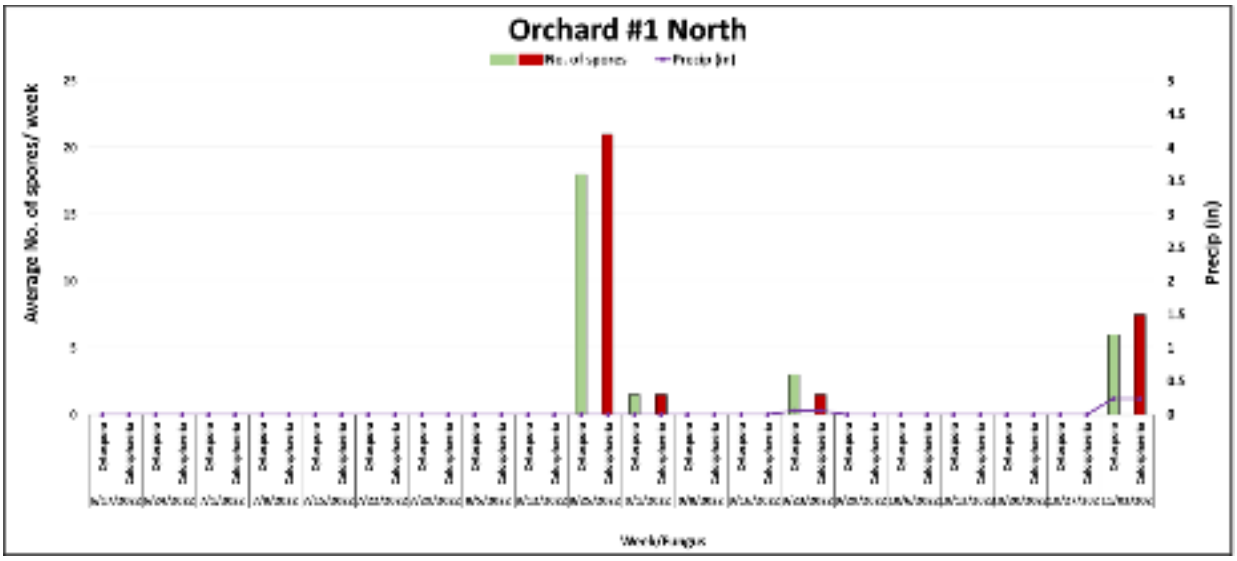

Fig. 2. Second-year spore trapping; total number of *Cytospora* and *Calosphaeria* spores trapped per week correlated with the shredding of infected branches between tree rows in one cherry orchard in San Joaquin County. Precipitation (in) is included in the graph.

Furthermore, overhead water-based cooling systems used in the southern San Joaquin valley orchards contributes to wetting tree branches/scaffolds, thus contributing significantly to the release of *Cytospora* pycnidiospores and *Calosphaeria* ascospores and increase the risk of canker disease development to susceptible twigs and branches. Spore release events associated with shredding of dead branches and/or rain or the use of overhead cooling system can coincide with pruning events increasing the probability of high infection of fresh wounds that are created by hedging or hand pruning. For example, hedging and hand pruning in orchards 1 and 2 in kern County took place during the first two weeks of September during an active spore release. This work emphasizes the importance of spore-trapping study as a valuable tool to gain knowledge of the epidemiology of *Cytospora* and *Calosphaeria* fungi in California cherry orchards under different cultural practices. Information generated from this study provides valuable information to help growers better manage canker diseases of cherry.

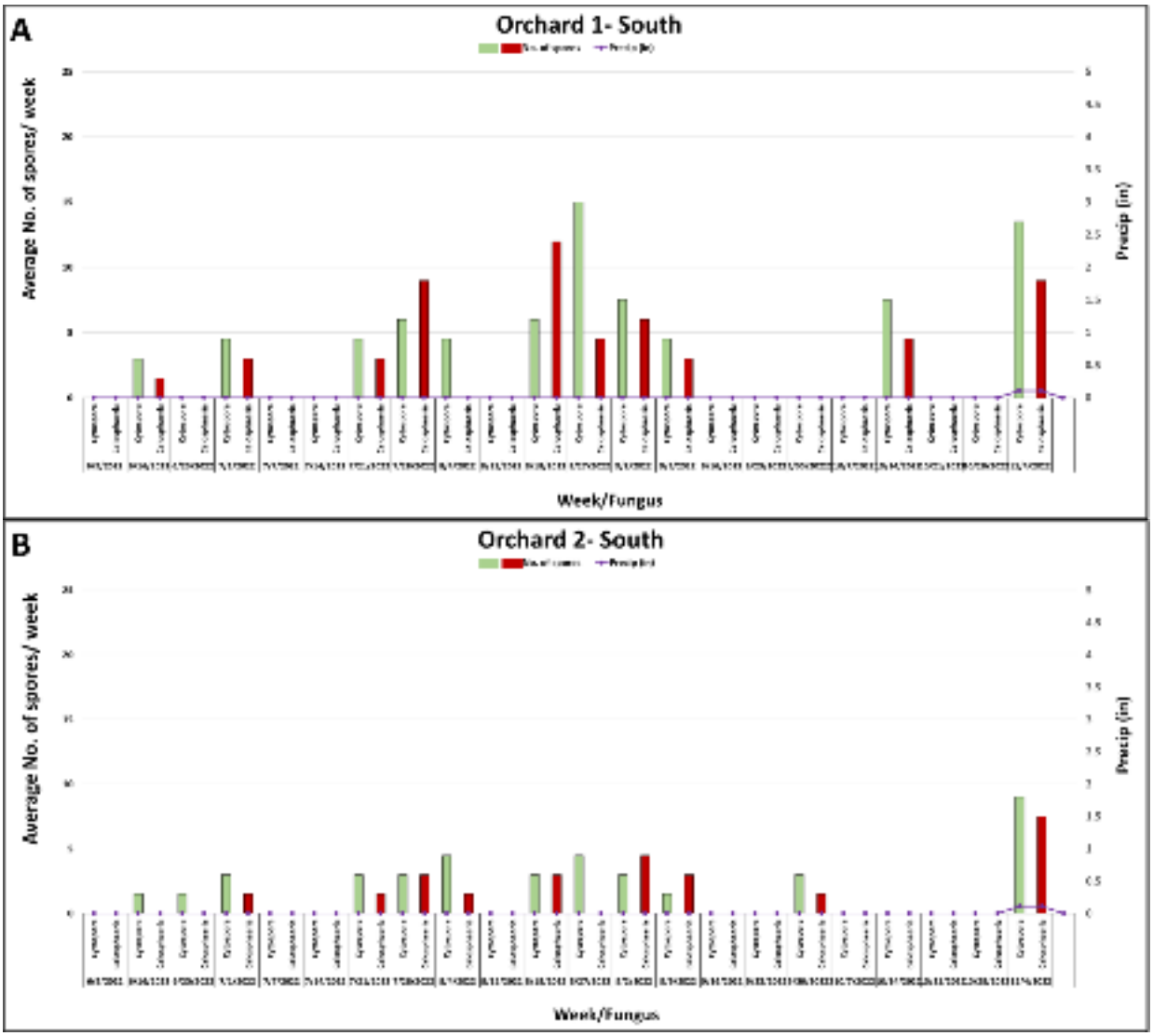

Fig. 3. Second-year spore trapping; total number of *Cytospora* and *Calosphaeria* spores trapped per week correlated with the overhead water-based cooling systems in two cherry orchards in Kern County. Precipitation (in) and shredding of infected branches between tree rows are included in the graphs.

3) Continue to examine soil organic matter and stored carbon on the WOR orchard by continuing soil surveys annually, and assess impacts of N treatments on first year trees growth.

Material and methods

Part 1- With the new orchard was replanted on March 31, 2022, annual soil sampling is still ongoing to quantify the residual soil nitrate, total nitrogen, organic carbon and total carbon in the WOR site. In March 25, 2021, right before tree planting, we collected soil samples from across the WOR field at two depths, 0-6 and 6-12-inches. Samples were analyzed by a commercial laboratory.

Part 2-

Whole orchard recycling is a practice for managing orchard biomass. By incorporating a large quantity of organic C into the soil, WOR has the potential to improve soil health properties, but a tradeoff may be that nitrogen (N) becomes limiting for subsequent crops for the first year. Our understanding of nutrient cycling and availability is most advanced in almond WOR sites replanted back to almond. As the higher carbon to nitrogen ratios of wood chips can decrease the availability of N to the trees, and based on the almond WOR research results, the UC recommendation is to double the N application in the first year after replanting by applying it gradually over the year to avoid tree stunting. Here in this objective (2022), we established a nitrogen trial in the first-year cherry orchard in Linden to see if we could determine more accurately the N requirements of first year trees after WOR. Three treatment rates were put out with three rows replicates (62 trees/row): "one ounce" (control), based on the UC 2017 samples costs to establish a sweet cherry orchard (~10 Lb. N/acre), "three ounces" and "five ounces". In order to precisely apply N, triple 15 granular fertilizer (15-15-15) was hand-applied to each tree. Nitrogen (N) was applied gradually: 1st application, May 20, 2022; 2nd application, June 23, 2022; 3rd application, July 28, 2022 and 4th application, August 25, 2022. The rest of the orchard consist of the grower's N application/fertigation through his double-line drip system at a rate of 3 ounces of N applied monthly from May to August. Here during each fertigation time, the grower turned off the valves for the double-line drip system on each row of our treatments.

Fig. 4. Experimental design in the WOR site located in Linden area, San Joaquin County.

Results and Discussion

Part 1- Our second-year soil sampling collected from across the WOR field at two depths, showed that there was approximately 5.4 ppm nitrate-N in the top 6 inches and 4.7 ppm from 6-12 inches, for a total residual nitrate level of approximately 18.5 pounds per acre in the top foot of soil. Total Organic Carbon (measured only in 2022) averaged 2.2 percent across all the field in the top foot of soil. Total carbon and total N were significantly greater in 2022 compared to 2021 (Fig. 5). Soil surveys will continue to be conducted annually.

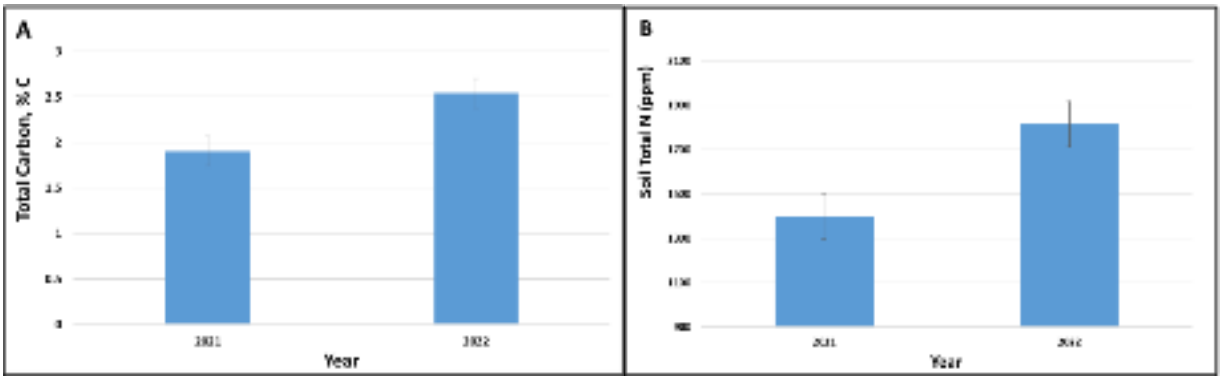

Fig. 5. Soil properties in 2021 and 2022 averaged across all the WOR site and two depth increments (0-6 and 6-12 inches). **A.** Soil Total Carbon was greater in 2022 compared to 2021. **B.** Soil total N was also greater in 2022 compared to 2021.

Part 2-

We believe that the hand-applied granular nitrogen was not immediately available as the double-line drip system could increase the time to effectively dissolve the granular nitrogen, thus no differences in shoot growth were visible between treatments in the first few months after our first application (April). Tree trunk diameter/circumference was measured right after the first N application for all trees (for the three treatments) as baseline, and will be measured again every year for the next 2-3 years. Leaf analysis will also be conducted this coming year, and results will be presented in the 2023 Cherry Research Reports.

ACKNOWLEDGEMENTS

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Testing for Potential Resistance of Spotted Wing Drosophila (SWD) to Commonly Used Insecticides in Cherry Orchards

Jhalendra Rijal

Institution/Organization: University of California Cooperative Extension-Stanislaus County
Project Year: 2022-2023
Principal Investigator(s): Jhalendra Rijal
Cooperating Personnel: Mohamed Nouri (UCCE), Frank Zalom (UC Davis), Fatemeh Ganjisaffar (UC Davis)

Executive Summary.
Spotted wing drosophila (SWD) is the major pest for cherry growers, and having multiple options, including choices of insecticide active ingredients, is critical for resistant management. During the 2022 season, we collected a wild population of SWD from a commercial cherry orchard. We tested the adults for potential resistance to commonly used insecticides – malathion, spinosad, and zeta-cypermethrin (a proxy for lambda-cyhalothrin). The study found that the corrected mortalities (the mortality after discounting the control mortality) of the flies exposed to the discriminating doses of malathion, spinosad, and zeta-cypermethrin were 89.2%, 92.0%, and 97.5%, respectively, indicating the resistance. Incorporating the newer and additional active ingredients into the SWD management program helps to slow down the potential resistance.

Background
Spotted Wing Drosophila (SWD), *Drosophila suzukii*, is one of the major pests of sweet cherry in California. The most common insecticides for SWD management in cherry are pyrethroid and spinosyn products. These insecticides are sprayed several times within a short period when fruits are most susceptible, from the color-break stage to the harvest. However, repeated use of these insecticides can have several negative consequences, such as pest resistance, impact on natural enemy populations, and potential outbreaks of secondary pests such as scale insects (Rijal et al., 2016).

Nearly 35% of the California cherries are for the export market, with the major market in several countries in Asia-Pacific, Europe, North America, and Latin America. Due to differences in maximum residual limits (MRL) set by importing countries, satisfying those limits for multiple pesticides is one of the significant challenges for cherry growers. A repeated spray of the same insecticides close to harvest for SWD management may result in higher crop pesticide residue and interfere with the cherry export (Haviland and Beers 2012). The residual limits set by the US EPA for US domestic use are not universally accepted. Therefore, the selection of insecticide depends on the efficacy and the MRL concerns for the export market. Because of the MRL risk, using a few insecticide active ingredients is common for SWD control in cherry. There have been indications of SWD flies developing resistance to spinosad (Success) insecticide in caneberries in coastal California (Gress and Zalom 2018). The study reported that the LC50 of

spinosad on SWD collected from the treated field was up to 7.7 folds higher than the SWD collected from unsprayed fields. In San Joaquin Valley, in the last 3-4 years, some cherry growers have anecdotally reported a weaker efficacy of major insecticides against SWD in cherry orchards. Therefore, we began to explore the potential resistance of SWD populations.

Objective

1. To conduct preliminary testing of wild spotted wing drosophila flies to detect potential insecticide resistance.

Materials and Methods

Collection of live SWD flies from the cherry orchard

Collecting live flies from the field is always a challenge. In the 2022 season, we designed a trap (see figure below) to collect SWD flies from the cherry orchards. Briefly, we used a plastic container with two openings on two opposite sides. We hung the SWD commercial lure (Trece, Inc) on the lid. We placed a small window screen piece inside the trap to minimize the flies exiting from the container. Using this trap, we collected SWD males and females from a commercial cherry orchard in Stockton.

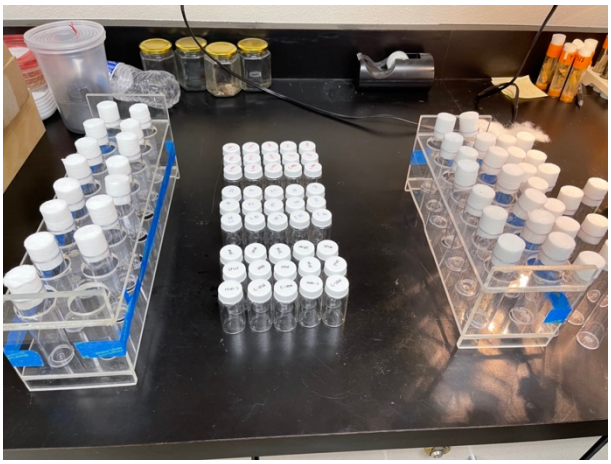
Rearing of the field-collected SWD populations

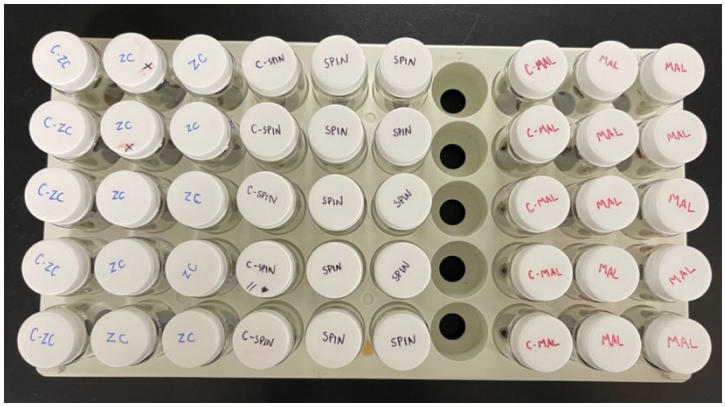
SWD adults collected from the orchard were reared in drosophila rearing vials filled with standard SWD Jazz-Mix diet at UC Cooperative Extension, Stanislaus, following a standard rearing protocol Rijal's lab has followed in the last five years. It is a crucial step as hundreds of flies of the same age are needed for insecticide susceptibility testing for each insecticide for each replicate. We used F3 generation of the field-collected SWD population in the trial.

Testing of SWD populations for pesticide resistance

In 2022, we performed a study using SWD populations collected from a cherry orchard and tested against three insecticide active ingredients — spinosad, malathion, and zeta-cypermethrin (zeta-cypermethrin as a proxy for type II pyrethroids, e.g., lambda-cyhalothrin, i.e., Warrior II). The lab assay was done following a rapid laboratory resistance test protocol developed and used

in multiple studies (e.g., Ganjisaffar et al. 2022a) to identify the potential resistance developed by these flies. For this, flies were exposed to vials containing indiscriminating doses of insecticides (i.e., $LC_{90} \times 8$ for malathion and zeta-cypermethrin, and $LC_{99} \times 2$ for spinosad) and also vials without insecticide (Control vials). The discriminating dose vials were prepared at Zalom lab, UCD, following the published protocol (Ganjisaffar et al. 2022a). Briefly, 1-ml solution of desired insecticide concentration was pipetted into test vials (20-ml capacity, Fisher Scientific, Pittsburgh, PA), capped, and turned gently for uniform distribution of the solution on the interior surface of the vial. The excess liquid of the vial was emptied and dried overnight. The zeta-cypermethrin solution was prepared using acetone as a solvent, and the acetone-treated vials were used as a Control. For each insecticide tested, we released 10 female flies (4-7 days old) in each vial (experimental unit) and replicated 10 times for the insecticide-treated and 7-8 times for the Control (7 times for zeta-cypermethrin; 8 times for spinosad and malathion). The mortality of the flies for treated and Control vials was evaluated after 6 hours for zeta-cypermethrin and malathion and after 8 hours for spinosad. The corrected mortality was calculated using the formula, Corrected mortality = (% Test mortality - % Control mortality)/(100-% Control mortality).





Results and Discussion

The progenies of the field-collected SWD flies were tested against the discriminating dose of selected insecticides. In our study, the corrected mortalities (the mortality after discounting the control mortality) of the flies exposed to the discriminating doses of malathion, spinosad, and zeta-cypermethrin were 89.2%, 92.0%, and 97.5%, respectively (see Table). The discriminating dose used in these experiments was 8 times the LC_{90} values of malathion and zeta-cypermethrin and 2 times the LC_{99} for the spinosad. These mortalities seem to be not bad if we consider the products' overall field efficacy. However, the concentrations we used for the assays were much higher than the field application rates. Also, the flies in assays were directly exposed to the insecticide concentration without the effects of any other external factors such as coverage, environmental conditions like rain, sunlight exposure, etc. So, not having 100% mortalities in these assays is the early indication of SWD flies tolerant to these insecticides.

Table: Insecticide susceptibility of cherry orchard collected SWD populations to three commonly used insecticide active ingredients

	Malathion	Zeta-Cypermethrin	Spinosad
Avg. % test mortality (MT)	89.2	97.5	92.5
Avg. % control (MC)	0.0	1.4	6.3
MT-MC	89.2	96.1	86.3
100-MC	100.0	98.6	93.8
% Corrected mortality [(MT-MC)/(100-MC) x 100]	**89.2**	**97.5**	**92.0**

SWD is also a key pest in caneberries (raspberry, blackberry) and strawberries in California's Central Coast, where SWD has already built resistance to spinosad and some pyrethroids (Gress and Zalom 2018, Ganjisaffar et al. 2022a, Ganjisaffar et al., 2022b). One of the studies reported that the LC_{50} of spinosad on SWD collected from the treated field was up to 7.7 folds higher than the SWD collected from unsprayed fields. Another study (Ganjisaffar et al. 2022b) reported that field-collected SWD populations are resistant to a Type I pyrethroid, bifenthrin (Brigade 2 EC), and a Type II pyrethroid, zeta-cypermethrin (Mustang Maxx 0.8 EC). One difference is that cherries are grown in the orchard system and have a shorter susceptibility period (4-6 weeks) compared to the season-long for caneberries. However, the SWD feeding behavior and the putative resistance mechanisms are likely to be the same regardless, and our 1-yr study results of testing the field-sourced SWD flies from a cherry orchard have shown a concerning trend. Therefore, future research is warranted to continue this work by incorporating flies representing several orchards and regions in the San Joaquin Valley. In addition to three insecticides – spinosad, zeta-cypermethrin, and malathion, it would be good to include a few more active ingredients, such as cyantraniliprole (e.g., Exirel) for potential tolerance development by SWD flies.

As SWD flies sourced from cherry orchards showed some tolerance to frequently used insecticides – spinosad, zeta-cypermethrin, and malathion, growers need to consider incorporating additional active ingredients and other alternative tools into the SWD management program. Our previous research (Rijal and Gyawaly, 2021) demonstrated good efficacy of diamide products, Verdepryn (cyclaniliprole), and Exirel (cyantraniliprole). These products can be a good fit for SWD resistance management (https://ipm.ucanr.edu/agriculture/cherry/spotted-wing-drosophila). Similarly, some neem-based products might be a good alternative for organic orchards because of their efficacy and oviposition deterrent activities against SWD (Gyawaly and Rijal, 2022).

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Developing a Low-Cost, Low-Tech Assay for Identification of Commonly Grown Sweet Cherry Varieties in California

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Problem, significance, and objectives

Sweet cherry (*Prunus avium* L.) is one of the most popular stone fruit crops cultivated in the temperate region. Sweet cherries are delicious and rich in vitamins and mineral nutrients that benefit human health. In California, sweet cherries are grown not only for local consumption, but also for shipping to other states in the U.S. and internationally. The CCB research program identified sweet cherry varietal identification as a research priority for the 2021-2022 fiscal year: *there is a need to develop a cost-effective assay that would permit the identification of sweet cherry varieties using fruit tissue samples.* Specifically, the assay should be able to identify nine sweet cherry varieties that are considered as important by CCB. Such an effort is to ensure that consumers are informed of the sweet cherry varieties that they purchase from stores. These assays could also be used towards varietal identification of scions received at the orchard.

Our goal is to develop a molecular diagnostic assay that could be useful to the California sweet cherry industry. The following objectives will facilitate the achievement of this goal.

Objective 1. To identify molecular markers that differ among commonly grown sweet cherry varieties in California.
Objective 2. To establish and validate a low-cost, low-tech assay for identification of commonly grown sweet cherry varieties in California.

Research update

In 2021-2022, we collected leaf and fruit tissues from nine sweet cherry varieties and carried out high-molecular-weight (HMW) genomic DNA extraction. Of the nine sweet cherry DNA samples, seven passed the stringent quality test for integrity and purity, while Royal Hazel and Brooks did not meet the standard for library construction. We then proceeded to whole genome library construction and sequencing with the seven sweet cherry samples with success, including Coral Champagne, Lapin, Tulare, Black Pearl, Royal Tioga, Sweet Heart, and Bing. Bioinformatics analysis of the whole genome sequence data identified a large number of DNA sequence variations for these commercial sweet cherry varieties relative to the published cherry reference genome. We were also able to use this information to identify DNA sequence variations that are unique to each of the seven sweet cherry varieties that had gone through whole genome sequencing.

The HMW genomic DNA from the two cherry varieties Brooks and Royal Hazel not passing quality control could be attributed to the less than optimal quality of the leaf tissue being used. We then collected new leaf samples for Brooks and Royal Hazel in May 2022. These leaves were kept on ice after being collected at the orchard and were flash frozen in liquid nitrogen in our lab in Davis. We extracted HMW genomic DNA from these new leaf samples using the same protocol that worked successfully for the other seven

sweet cherry varieties. However, the HMW genomic DNA did not meet the standard for sequencing. It is possible that certain components present in Brooks and Royal Hazel leaves, but absent in leaves of the other seven sweet cherry varieties, interfere with the HMW genomic DNA extraction. We are currently testing the use of a commercial DNA extraction kit manufactured by Bionano Genomics to obtain high-quality HMW genomic DNA from Brooks and Royal Hazel.

Our next step

The genomic DNA extracted from Brooks and Royal Hazel will be submitted to Novogene for quality testing, whole genome library construction and sequencing. The bioinformatics pipeline for data analysis has already been established; sequence mapping as well as structural variants, SNPs, and InDels analyses will be conducted for Brooks and Royal Hazel. We will also perform bioinformatics analysis on the genome sequence data of all nine sweet cherry varieties (the seven varieties that were analyzed previously plus Brooks and Royal Hazel) to identify DNA sequence variations that are unique for each variety. SNP and SSR markers will be designed based on these DNA sequence variations, and primers will be custom ordered and used for PCR amplification of the sweet cherry DNA. PCR products will be analyzed by gel electrophoresis and also sequenced to verify whether the primers for the SNP and SSR markers can differentiate the nine sweet cherry varieties.

Plant-Based Irrigation Management in Sweet Cherry to Reduce Water Needs While Maintaining Yield and Quality

Kosana Suvocarev

Principal Investigators: Kosana Suvočarev, Kenneth Shackel, Giulia Marino

Collaborators: Kari Arnold, Mohamed Nouri

Graduate student: Jarin Tasnim Anika

Objectives

While using previously set continuous measurements of ET, install applied irrigation flowmeters, measure soil moisture and midday-stem water potential (SWP) to collect data to guide full irrigation vs. periodic mild stress pre-harvest and post-harvest irrigation, allowing mild/moderate stress levels. Use deep soil moisture measurements to track soil water depletion patterns and inform water management strategies.

Methods

Within the experimental design, we are using previously installed eddy covariance towers in three different orchards, i.e., Go, Kahn and Dasso with different planting density and orientation to quantify evapotranspiration of fully irrigated sweet cherry orchards in San Joaquin County. To ensure full irrigation, we assessed weekly/biweekly stem water potential in the trees that have been monitored over the past three years from 2019-2021. At the project onset, we met with our grower collaborator and irrigation managers to collect the information and compile different datasets that can help us decide how to design our experiment. We used growers' suggestions on which rows are optimal to apply deficit irrigation and monitor plant water status through adding trees from those rows in our weekly and continuous SWP measurements. Our grower-collaborator helped us select with careful consideration single rows within each of three orchards to be under regulated deficit irrigation in the post-harvest period during summer 2022.

The main experiment started at the end of July, where the selected rows were either the control (water application following the growers' irrigation practice) or deficit treatments (50% reduction of the growers' irrigation practice). Two rows of trees in each orchard having one control row and a deficit row were selected (**Fig 1**). These rows included the trees (T_1 to T_4) that we have been observing weekly/biweekly for stem water potential (SWP) from 2019. We added T_5 and T_6 in the deficit and control rows, respectively as replicates for better understanding of the SWP values. In the selected control and deficit rows we selected one tree in each row from the previously observed trees and installed a microtensiometer (FloraPulse) that measures continuously real-time SWP from the water-carrying tissues (**Fig 2**). This continuous dataset will help us monitor the daily basis cycle of stress that the trees experience. Additionally, we have been observing and comparing the SWP values both from the weekly manual measurement with pressure chamber and the FloraPulse to understand the compatibility of both. The combination of continuous Eddy Covariance data from the previously established ET station and FloraPulse data will help us determine the actual and optimum level of water that the orchards need.

Figure 1: Map of the experimental areas with the rows and trees under observation, the location of the ET station and flowmeters.

Figure 2: FloraPulse sensor with protective layer.

At the beginning of August, before implementing the deficit treatments, we installed flowmeters (2/row) and valves (**Fig 3**) in the selected experimental rows to monitor water application in the

control and deficit treatments and manipulated water application. After continuous observation of evapotranspiration, SWP, soil moisture, etc. for the past three years, 50% deficit irrigation as post-harvest irrigation treatment was started in the selected deficit rows in August. Our goal is to observe how this level of stress application affects next year's fruit set, yield and fruit quality of the trees under deficit treatment with respect to the other trees getting full irrigation in the orchards having different planting density and orientation. (**Table 1**). Based on the observations including fruit analysis, e.g., yield, fruit weight, unitary mass, texture, soluble solid concentration, etc. at harvest in 2023, we will decide how much pre-harvest deficit irrigation should be applied in the following year.

Figure 3: Two types of installed flowmeters, WNT series (left) and T10 series (right).

Table 1: Details of the areas under observation with control and deficit treatment

Site	Irrigation	Planting density	Control trees under observation	Deficit trees under observation
Go	Drip	20x22 ft	24	25
Kahn	Drip	16x16 ft	31	31
Dasso	Drip	20x20 ft	10	12

Initial and anticipated results

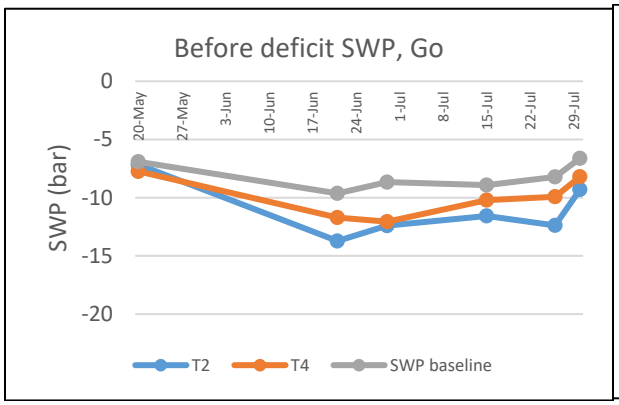
We continued to measure evapotranspiration through use of eddy covariance stations, installed in 2019, and funded partly by California Cherry Board and partly by the lab resources of PI Suvočarev. Since this method measures ET on landscape level, we cannot capture the signal of single rows of trees that are under regulated deficit irrigation. It is rather serving us to compare what is the full irrigation requirement of each orchard.

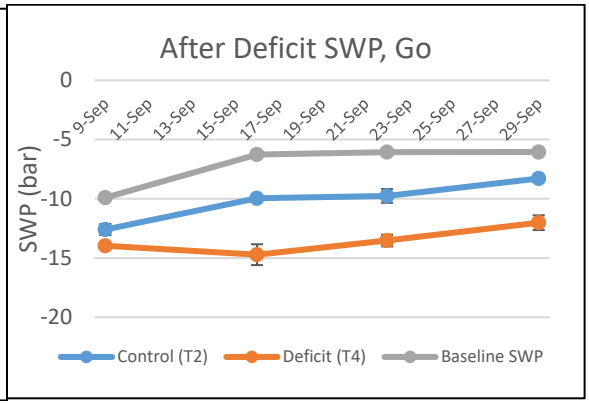
Our weekly/biweekly SWP measurements were analyzed together with water use, irrigation and soil moisture (neutron probe soil moisture collected by the farm irrigation managers) for easier interpretation between applied water and plant water status. Continuous measurements of stem water potential in the rows where the regulated deficit irrigation was applied starting in August 2022 were monitored for better insights into stress initiation during daytime and potential stress recovery during nighttime and after irrigation applications. In addition to the two planned orchards

in the project proposal, we decided to include a third orchard where frost damage had significant impact on the fruit loss in the year 2022 in order to monitor effects on evapotranspiration and plant water status under the conditions of low bearing. In addition to flowmeters installed in the fully irrigated orchards, we were monitoring the RDI water applications to quantify potential for water conservations.

Stem Water Potential with Pressure Chamber before and after deficit application

Before applying deficit, we analyzed the SWP of 4 trees (T_1 to T_4) in each orchard from measurements taken from the beginning of the previous experiment (since 2019). From these trees, we selected T_2 and T_4 in 'Go' and 'Kahn', and T_1 and T_4 in 'Dasso' for our observation for control and deficit, respectively (**Fig 1**). After deficit application, we have been monitoring 6 trees in each orchard for SWP. As a guide, we used the UC Davis Fruit and Nut Research Information on targeted SWP values for prunes. We considered these values to be the closest baseline to cherry research where baseline has not been developed yet, (*http://informatics.plantsciences.ucdavis.edu/Brooke_Jacobs/datainterpretation.html*), -14 to -18 bar is considered acceptable in the month of September. Our weekly SWP measurements with the pressure chamber in September after 50% deficit application in August show that, in the station 'Go', the range of SWP in deficit irrigated trees was within -13.8 to -10.5 bar, in the station 'Kahn' it was within -20.4 to -12.4 bar, and in the 'Dasso' station the range was -11.3 to -7.2 bar (**Fig 4.1, Fig 4.2, Fig 4.3**). In 'Kahn' the tree T_4 was already showing signs of stress before the water was cut off (**Fig 4.2**), T_4 in 'Dasso' became more stressed than the control (T_1) after some time of deficit application (**Fig 4.3**), whereas, in 'Go', T_4 was less stressed than the control (T_2) before but more stressed after the deficit not exceeding the mentioned acceptable range (**Fig 4.1**). Overall, in all three orchards and according to the weekly pressure chamber measurements, the deficit irrigation can be observed as added stress level when compared to the targeted range for prunes and the control treatment.





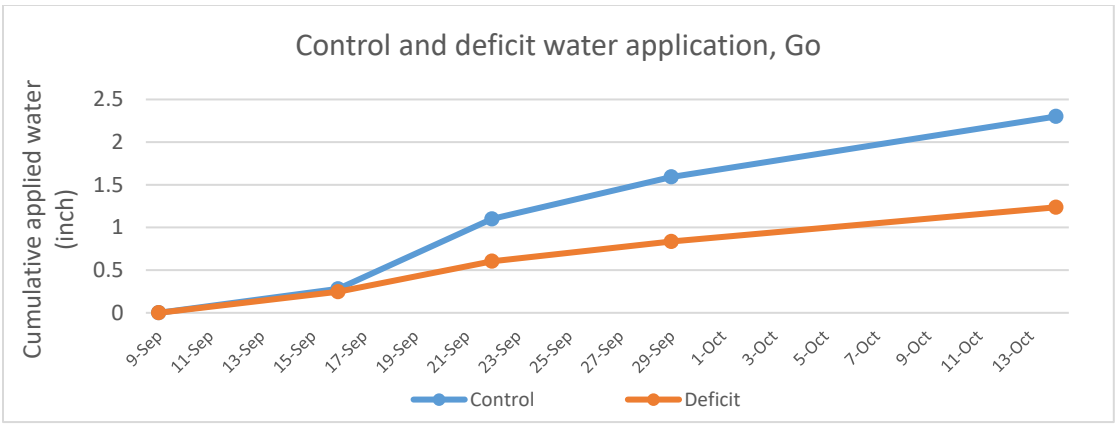


Figure 4.1: SWP with Pressure Chamber of the selected trees before (top left) and after (top right) water cut off, and cumulative water applications (bottom) in Go orchard.

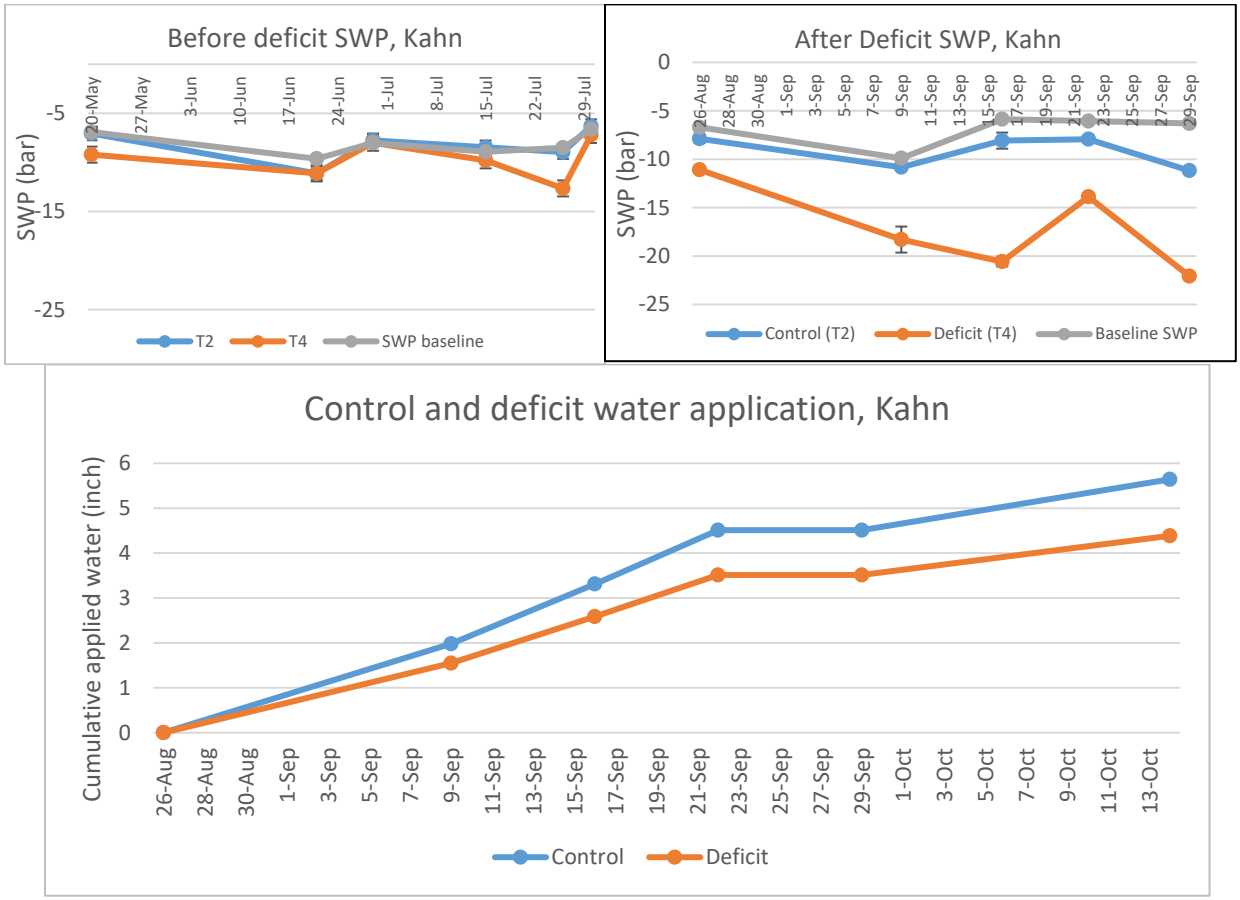


Figure 4.2: SWP with Pressure Chamber of the selected trees before (top left) and after (top right) water cut off, and cumulative water applications (bottom) in Kahn orchard.

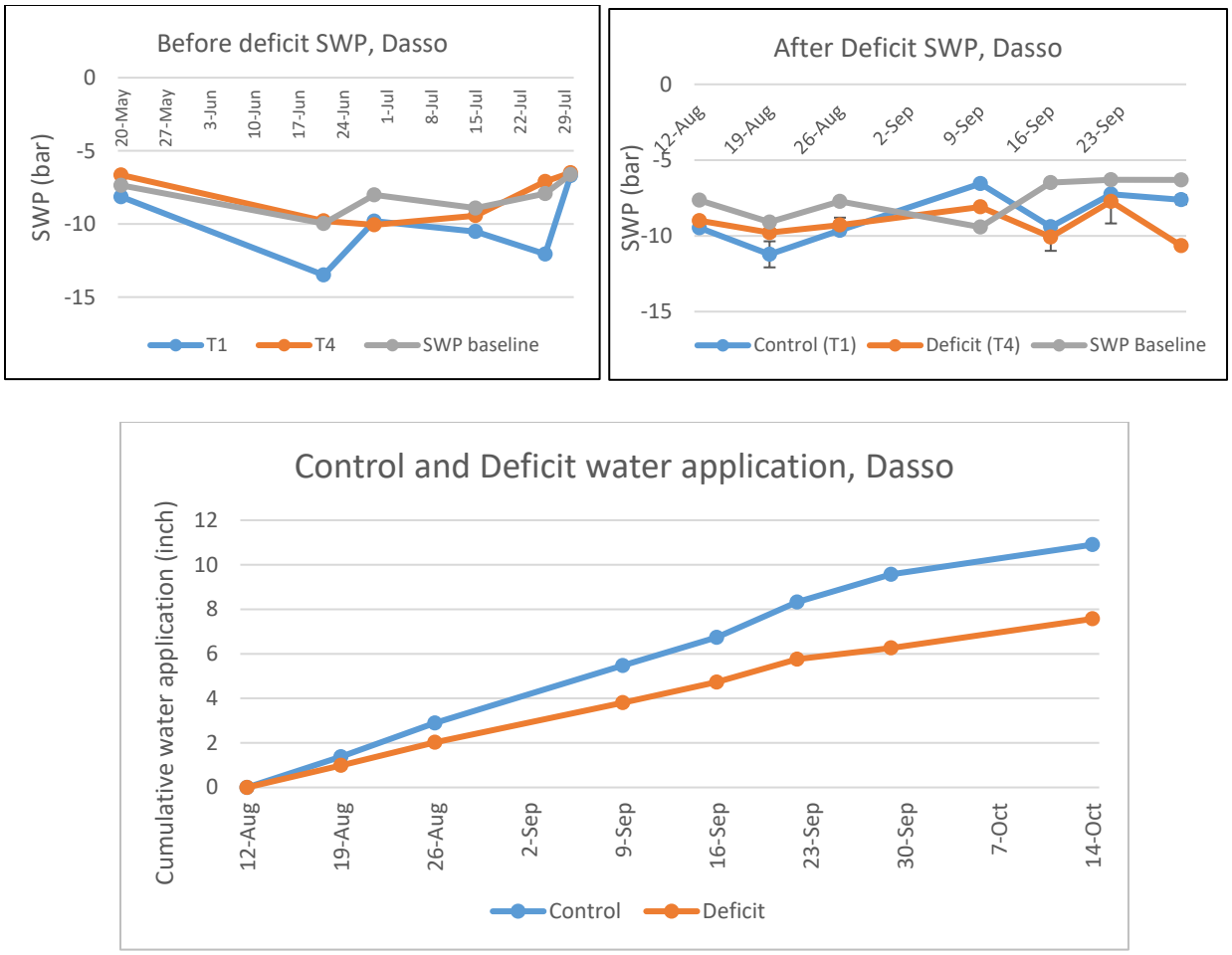


Figure 4.3: SWP with Pressure Chamber of the selected trees before (top left) and after (top right) water cut off, and cumulative water applications (bottom) in Dasso orchard.

Fig. 4.4, 4.5 and 4.6 show the daily continuous SWP by FloraPulse of the stations before and after deficit application. In 'Kahn' and 'Dasso' it shows a similar situation like that of the pressure chamber, i.e., T_4 in Kahn was more stressed than the control (T_2) both before and after deficit, in 'Dasso' T_4 became more stressed than the control one (T_1) after deficit application although remained within the acceptable range. But in 'Go', pressure chamber and FloraPulse showed somewhat different situation before deficit water application. The pressure chamber data before deficit application showed that the selected tree for deficit treatment was less stressed than the control tree whereas the FloraPulse data showed that it was already stressed than the control one before deficit application. However, we will be continuing our observation to calibrate the FloraPulse data based on the chamber measurements which are the direct measurement and the gold standard.

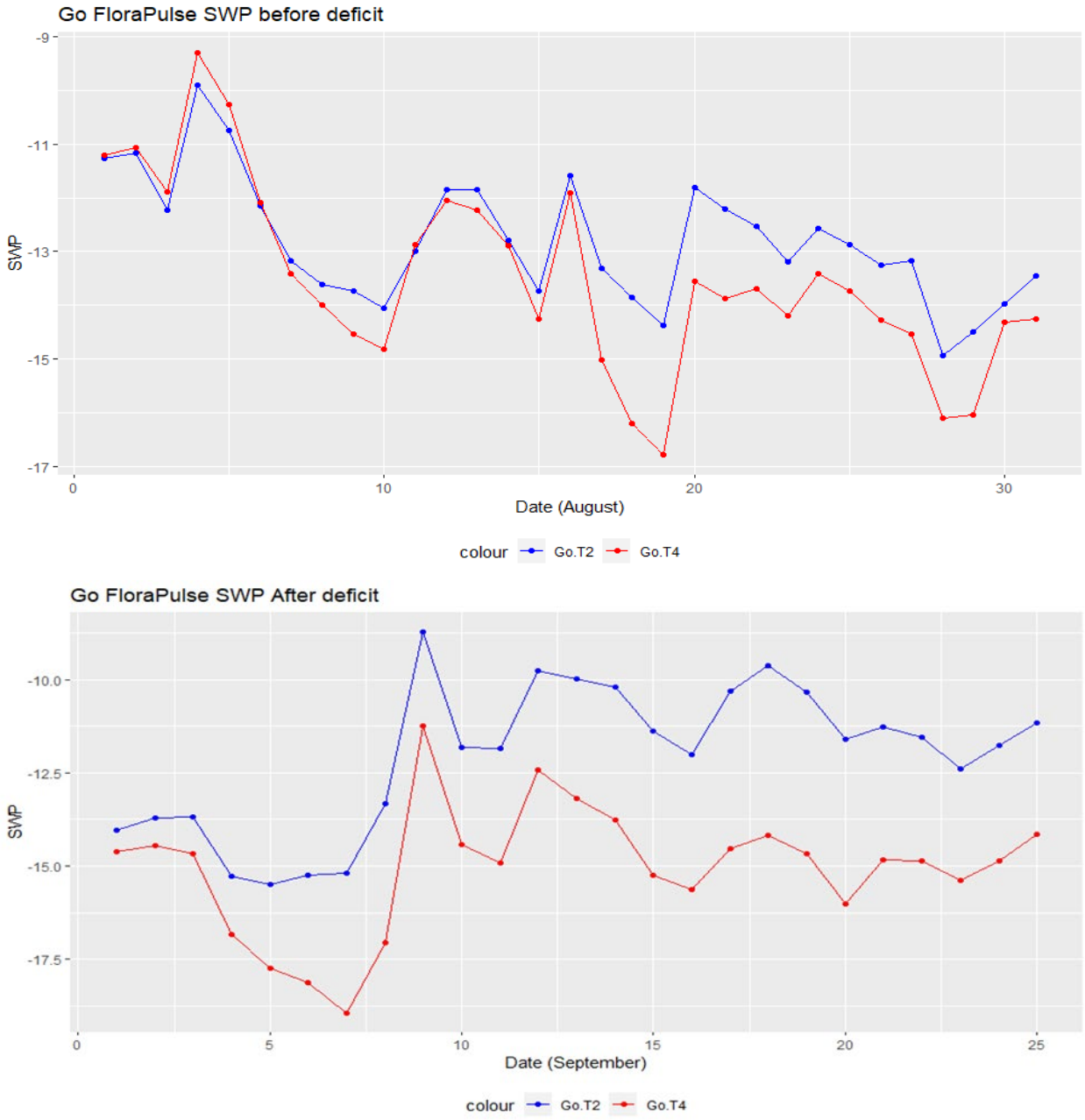


Figure 4.4: SWP by FloraPulse of the selected trees before and after water cut off in Go orchard.

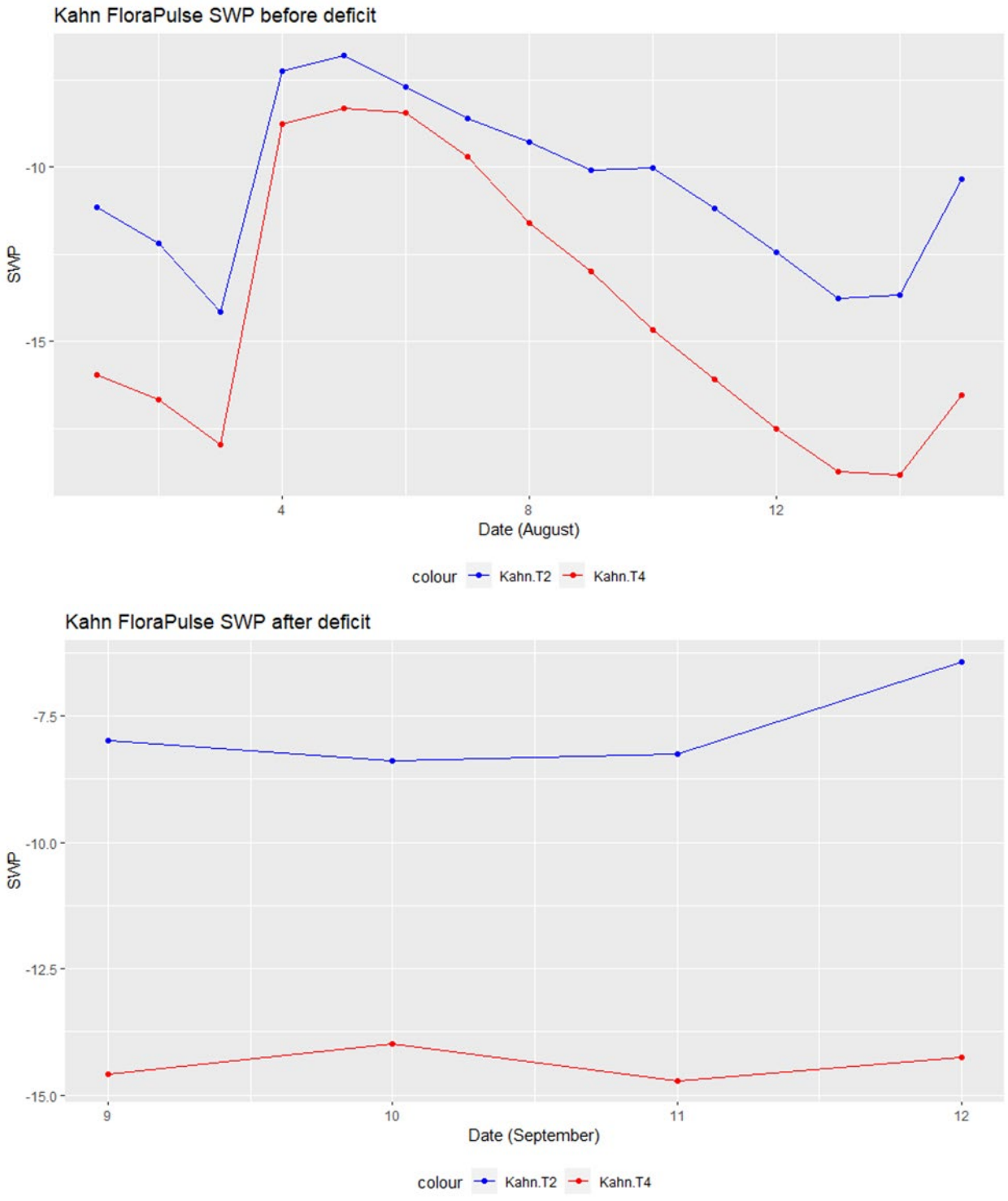


Figure 4.5: SWP by FloraPulse of the selected trees before and after water cut off in Kahn orchard.

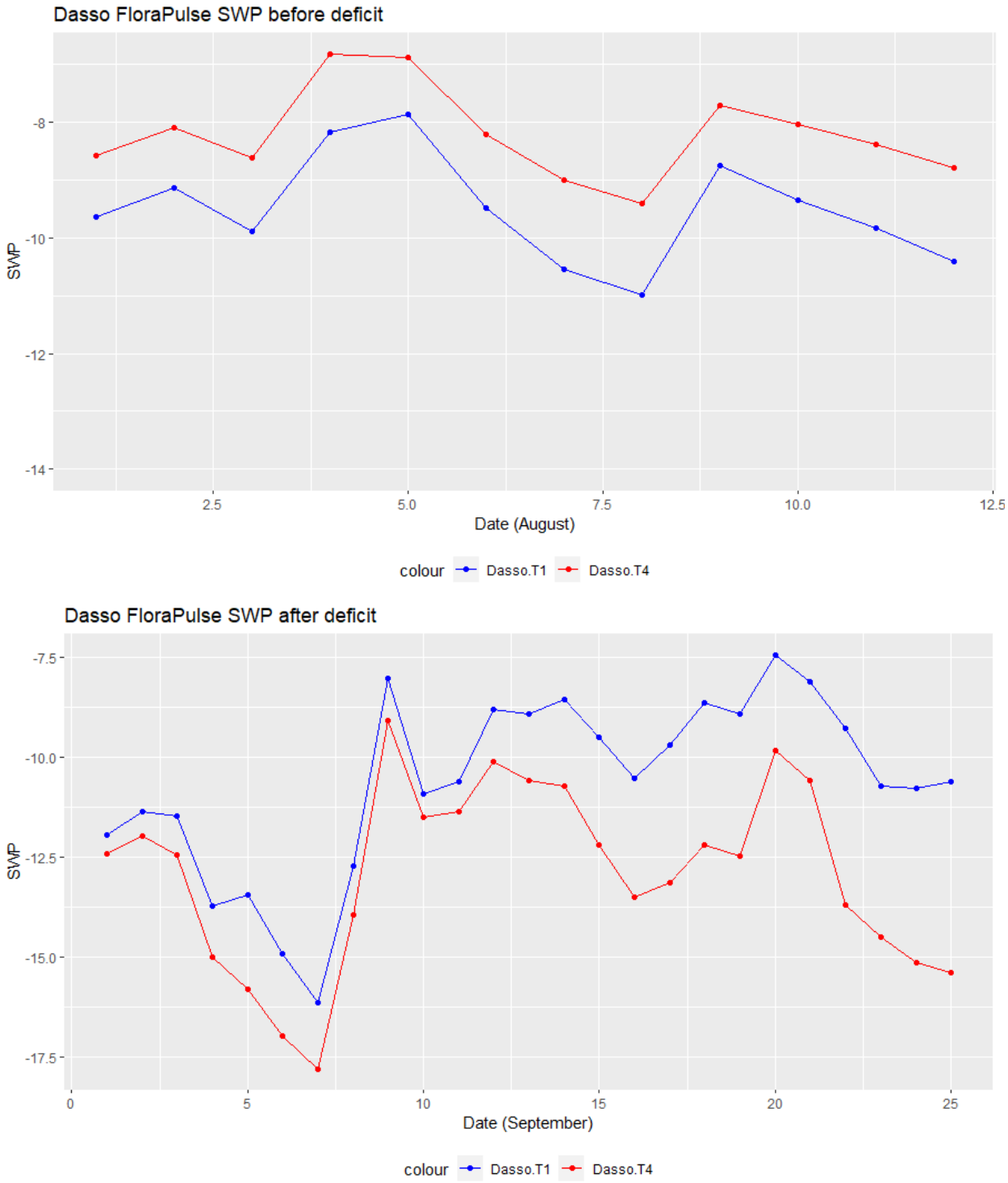


Figure 4.6: SWP by FloraPulse of the selected trees before and after water cut off in Dasso orchard.

Conclusions and future plans

So far, we had an opportunity to analyze data collected on irrigation applications, evapotranspiration and soil moisture relationships using our previous study results on evapotranspiration, SWP and neutron probe soil moisture collected by irrigation managers in the past three years. These relationships are helping us guide our decisions on water applications in RDI to start a new measurement season in March 2023. Some differences that have been observed between the control and RDI water applications, indicate the increased stress in the trees that were irrigated at 50 %, however, the fruit sampling for its quality and quantity at the harvest time in 2023 will be the best indicator of the potential effects of stress on the sweet cherry production. If no effect is observed on the fruit with the level of stress applied in postharvest season of 2022, we might consider with grower collaboration to increase the deficit to larger area. FloraPulse sensors are still under evaluation and calibration and more data points in the next season will help us develop better values.

Irradiation as a Phytosanitary Treatment

Peter Follett

"Australia is exporting sweet cherries to Vietnam and Indonesia using irradiation. If the industry ever seriously considers the use of irradiation for phytosanitary purposes, I would be happy to advocate, either at a CCB board meeting or the annual review meeting."
– Peter Follett

The Investigation into Dormancy Breaking Agents and the Dynamic Chill Portions Model in CA Cherries Via Carbohydrates and Solar Radiation

Giulia Marino

Principal Investigators: Giulia Marino, Kari Arnold, Mohamed Nouri
Collaborators: Mohammad Yaghmour, Maciej Zwieniecki, Paula Guzmán-Delgado, Kosana Suvočarev,

Katherine Jarvis-Shean, Louise Ferguson, Emily Santos, Katelyn Cooper

1) Rationale

Main objectives of this project are to improve identification of cherry winter dormancy status and the efficacy of dormancy breaking agents' application, developing information on 1) tree carbohydrate dynamics during winter and 2) the relative contribution of solar radiation on tree bark temperature.

2) Methods

2.1 Study orchard characterization and experimental design

Four experimental locations were selected at the beginning of August 2020, located within two main cherry production regions, Kern County and San Joaquin (SJ) county, characterized by different climatic conditions (Fig. 1). The SJ county orchards were close to Linden, which reaches on the average 80 chill portions, as observed from the analysis of the data of the closest CIMIS station (#70, Manteca). In the Kern County location, close to Bakersfield, the chill portion accumulation calculated for the closest CIMIS station (#125, Arvin-Edison) is 13% lower (about 70 chill portion).

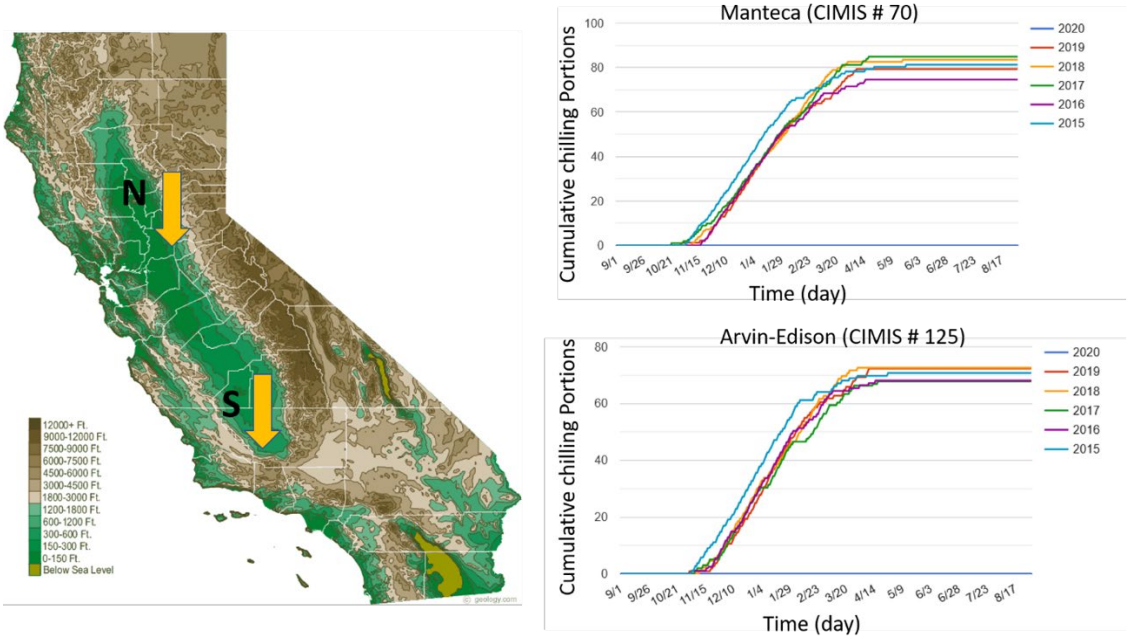


Fig. 1 – Localization of the Northern and Southern experimental sites and chill portion accumulation calculated from Fruit & Nut Research & Information Center for the CIMIS stations of Manteca and Arvin- Edison.

In the SJ county location we selected 3 plots-orchards: the first one is planted with Bing on Mazzard treated with CAN 17 (Fig. 2 a); the second one is planted with Coral on Mazzard and treated with Dormex (Fig. 2 b); the third one is planted with Bing on Mazzard and treated with Dormex (Fig. 2 c). At the end of September 2020, we selected 4 blocks within each plot-orchard, located in 4 cardinal points: North-East (NE), North-West (NW), South-East (SE) and South-West (SW). Within each block, 4 trees were selected, of which two are our "Control" treatment, that are acquired by bagging them just before spray to avoid contact with dormancy breaking agents, while the other two trees are our "Treated" treatment, that receive normal orchard practices (sprayed with dormancy breaking agents).

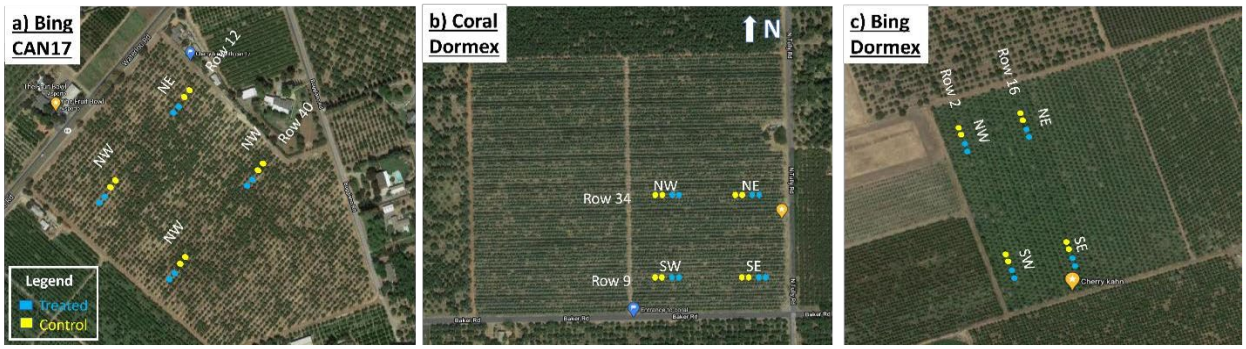


Fig. 2 – Experimental blocks and design in the 3 orchards in the Northern location (SJ county, Linden).

In Kern County one orchard was selected planted with the cultivars Brooks and Coral on Mahaleb and treated with Dormex (Fig. 3). One portion of the orchard (the first 10 rows from the South) has been treated only with CAN 17 for several years, because of its closeness to a lemon block that would defoliate completely in response to the drift from the chemical treatment. Within the orchard, 3 plots were selected at the end of September 2020. Two of them (B1 and B3), located in the south area of the orchard are treated only with CAN17. The third plot, located in the North part of the orchard, is treated only with Dormex. Within each plot, the cultivar and the "Control" and "Treated" treatments were distributed randomly.

Fig. 3 – Experimental blocks and design for the orchard in the Southern location (Kern county, Bakersfield). 2.2 Orchard instrumentation

Weather stations have been installed in the different experimental sites at the end of October 2020 (Fig. 4) to monitor continuously air temperature and humidity within canopy, incoming solar radiation, soil moisture, tree wood temperature, tree phenology and trunk shrinkage.

Fig. 4 - Bio-meteorological station installed in the orchards (a), details of the temperature and humidity sensor (b), phenocamera (c), soil moisture sensors (d), dendrometer (e), and bark temperature sensor (f).

2.3 Tree bagging

Control trees were bagged with plastic tissues just before the dormancy breaking agent spray, to avoid the contact of the chemical (Fig 5). Bagging happened on only in the Kern County orchard and in the Coral orchard in the SJ County. The others were not bagged for technical problems.

Fig 5 – Picture of the bagging process and example of bagged trees in one block 2.4 Sampling for non structural carbohydrate (NSC) analysis

Twigs were collected bi-weekly for NSC analysis starting at the beginning of November and were delivered to Dr. Zwieniecki's laboratory at UC Davis, for characterizing NSC dynamics through the season as affected by the interaction of cultivar, environmental conditions and rest breaking agent applications.

2.3 New activities 2022-2023

We started in November 2022 the third and last year of data collection, for the 2022-2023 season. We installed the meteorological stations back in the fields at the and added leaf wetness sensors (Fig. 6), to catch the effect of surface water on tree temperature. In the Kern County location, where in winter above canopy irrigation is applied to maintain tree temperature lower, we added a new monitoring site where we took out the above canopy sprinkler. In this way, we are measuring bark temperature and leaf wetness in trees with and without above canopy irrigation. Carbohydrates samples are collected biweekly from early November 2022. We are also sampling extra shoots every three weeks and placing them in a growth chamber to force bloom and monitor the dormancy status of the trees (Fig 7). Specifically, we sampled three branches per block, 12 per orchard, around 40 cm long from the S exposed side of the trees. In the lab, we make a fresh cut on the base of the branches and placed them in a 5% sucrose solution. The branches are maintained at 25 °C during a photoperiod of 16 h and at 18 °C during a dark period of 8 h, with a constant relative humidity of 65%. After 5 days, the sucrose solution is changed, and the basal branch cuts refreshed. The branches were maintained in the growth chamber for 10 days to accumulate sufficient heat. Flower buds are counted at time 0, and flower number at 5 days and at 10 days after the beginning of the forcing treatment.

Fig. 6 - Leaf wetness sensor installed close to the thermocouples measuring tree temperature. We aim to observe the impact of trunk wetness on tree temperature

Fig. 7 – Shoots forced in growth chamber to push budbreak and identify trees dormancy stage.

3. Results

3.1 Relation between tree bark temperature and air temperature

Second year meteorological data collection confirmed and strengthened first-year data. Tree temperature (T_{tree}) was higher than air temperature (T_{air}). The average T_{tree} was up to 10 oF higher than T_{air}, and this difference increased to 20 oF when considering southern exposed limbs (T_{tree_South}). The correlation between T_{air} and T_{tree} was highly significant ($P < 0.001$). Using all the data collected every 30 minutes over 2 years in one orchard (Fig. 8) we were able to predict T_{tree} with an R^2 of 0.9 using only T_{air} as a

predictor. The slope of the relation was 1.32, which means that for every degree increase in T_{air}, T_{tree} will increase 0.33 degrees more. For example, when T_{air} is 70 oF we can predict T_{tree} to be 80 oF (orange dashed lines in Figure 8) with a prediction error of 8 oF. We are now incorporating incoming radiation and relative humidity in the prediction model to further increase the prediction efficiency.

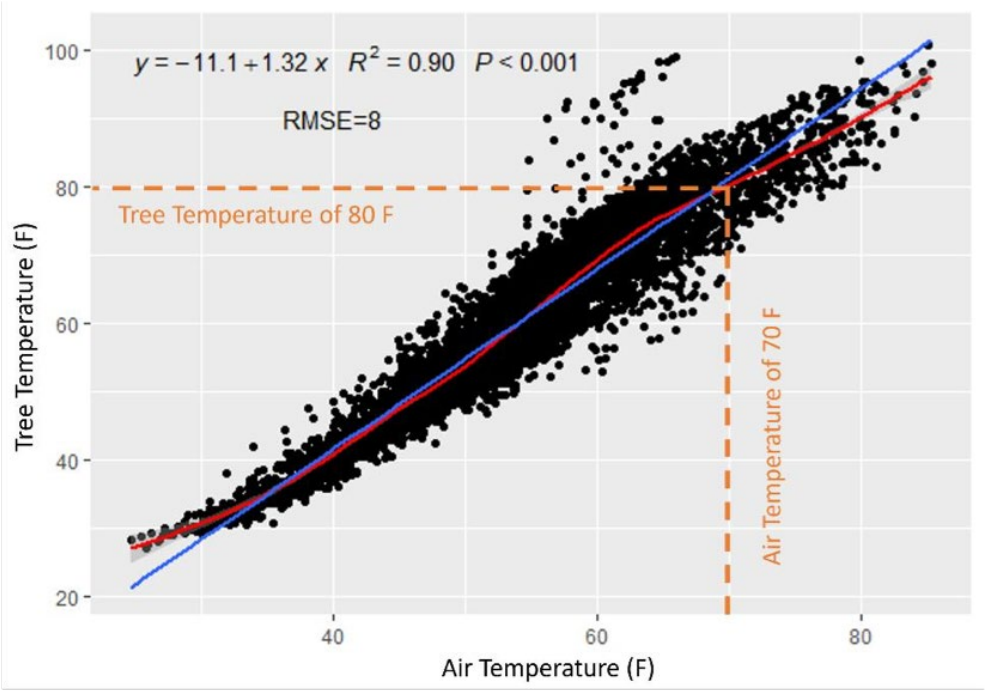


Fig. 8 – Relation between air temperature and tree temperature developed using two seasons of data collected in the San Joaquin County Coral orchard. The orange line shows that when air temperature was 70oF the tree temperature was 80oF.

In Fig. 9 and 10 we report the chill accumulation calculated using the dynamic model and both tree and air measured temperatures, for the San Joaquin Valley orchard (here used as an example representative of other locations). At the end of January, chill accumulation calculated using the temperature of the air was around 60 chill portions (CP) in SJ County in both 2021 and 2022 seasons (blue line in Fig. 9 and 10). When the tree temperature was used instead of air temperature to calculate chill accumulation, we had only 40 CP in the same location and time. The chill accumulation was continuous through the entire winter when air temperature was used as input in the dynamic model; as opposite, when tree temperature was used as input in the dynamic model, chill accumulation was mainly happening between the first week (middle) of December and the middle (end) of January, depending on the year.

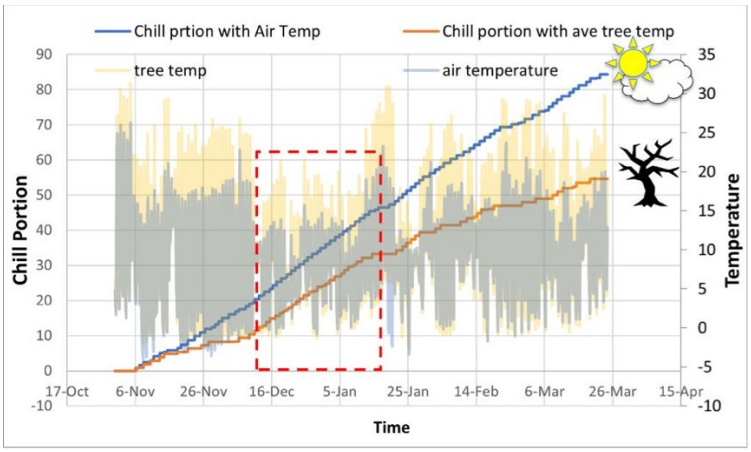


Fig. 9 Chill Portion accumulation (dynamic model) calculated using the temperature of the air (blue line) and the temperature of the tree (orange line) in 2021 in the SJ county orchard. The red dashed box shows the period of maximum chill accumulation based on tree temperature.

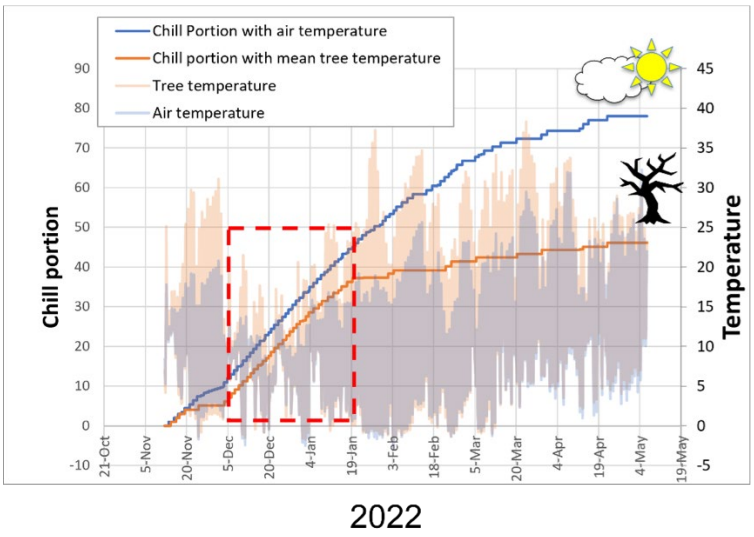


Fig. 10 Chill Portion accumulation (dynamic model) calculated using the temperature of the air (blue line) and the temperature of the tree (orange line) in 2022 in the SJ county orchard. The red dashed box shows the period of maximum chill accumulation based on tree temperature.

Figure 11 shows how incoming radiation was the main driver of tree-based chill accumulation rates. The period of main chill accumulation in 2022, between December 4 and January 15, corresponds exactly to the period when the lowest incoming solar radiation is recorded (Fig. 11). Similar pattern is observed for other years and locations.

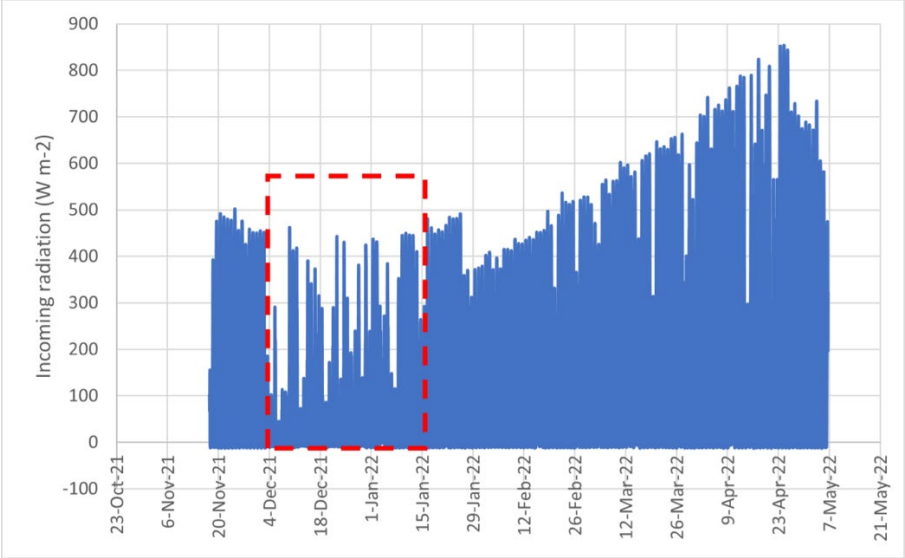


Fig. 11 – Seasonal trend of incoming solar radiation (W m^{-2}) in San Joaquin County orchard. The red box corresponds to the period when chill accumulation calculated using tree temperature is the highest based on Fig 10.

3.2 Tree carbohydrate dynamics

Nonstructural carbohydrate (NSC) dynamics for all the monitored orchards in 2021 and 2022 seasons are reported in Fig. 12 and 13. NSC varied strongly through the winter, highlighting tree physiological changes. The spray treatment did not significantly affect NSC values. However, the seasonal trend of NSC was highly significant and with common patterns well related to temperature changes across orchards. We identified two periods of relevance for the chill accumulation based on NSC changes: the first occurred between mid-December and mid-January and consisted of a strong and fast decline of starch that reached values of 40 mg g^{-1} and 15 mg g^{-1} in the wood and the bark, respectively. In this period, we also observed an increase of sugars levels up to 45 mg g^{-1} and 75 mg g^{-1} in the wood and the bark, respectively. This corresponded to the period of maximum chill accumulation calculated using tree temperature as input of the dynamic model (Fig 9 and 10) and is highlighted by yellow arrows in the graphs. We hypothesize that this sugar accumulation is what triggers chill accumulation and it is needed to break dormancy. Around mid-January, this trend was totally flipped, and we observed increasing or constant starch levels and a continuous decline of sugars levels in bark and wood. This period is highlighted by green arrows in the graphs. Sugars reached minimum values of about 20 mg/g in the wood and 40 mg/g in the bark around early to mid-February which we hypothesize triggered bloom. Considering that sugars and starch tend to have opposite trends, we develop an index, the starch to sugar ratio. Based on our hypothesis, a change in this index from a decreasing to an increasing trend highlights when chill is satisfied.

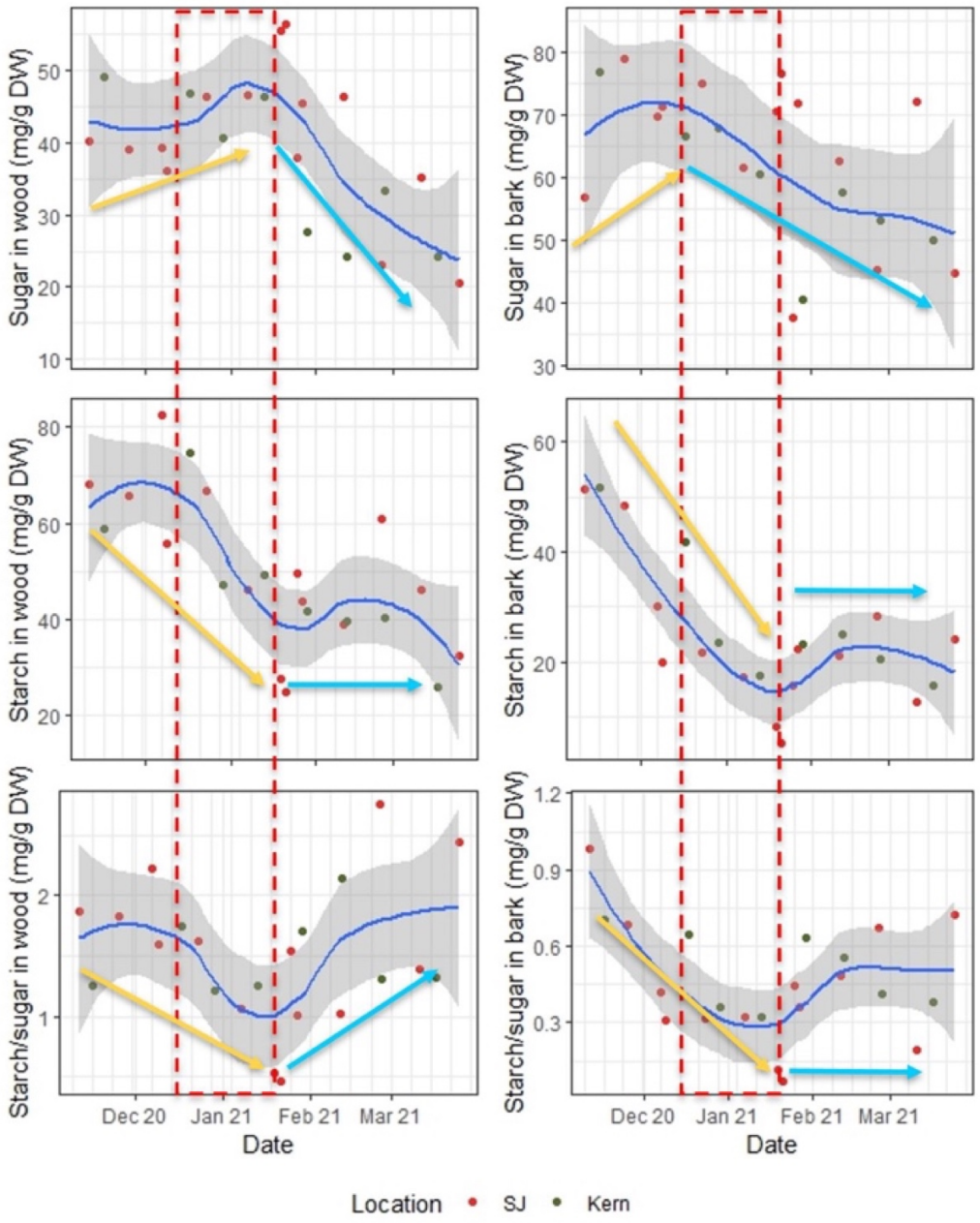


Fig. 12 - Seasonal trend of sugars, starch and their ratio (starch:sugar) in bark and wood of twigs of cherry trees located in SJ County and Kern County in 2021. Arrows of different colors indicate the two periods of relevance for the chill accumulation based on NSC changes (see text for details). The red box shows the period when maximum chill accumulation is recorded using the dynamics model with tree temperature as input (see figure 9 and 10).

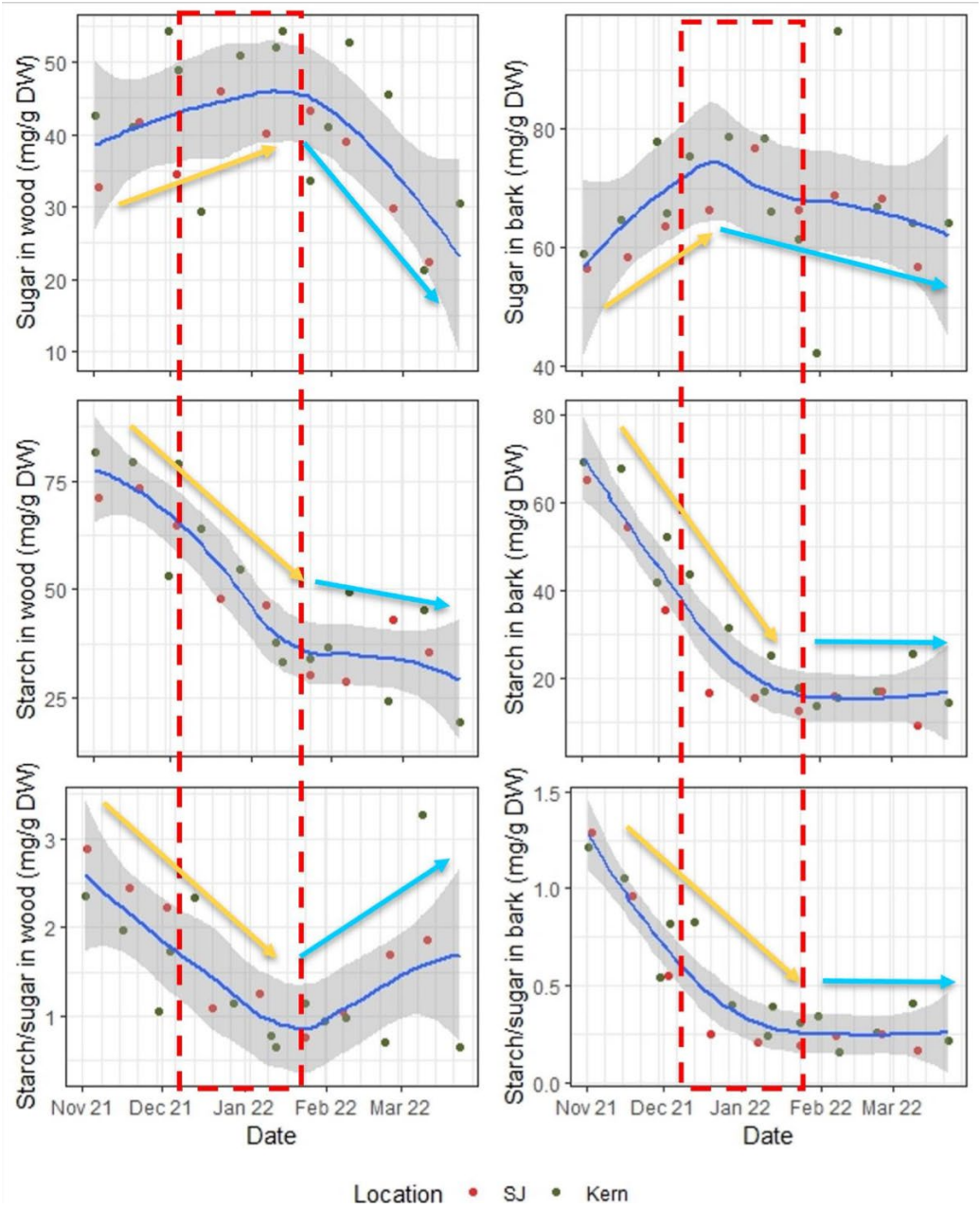


Fig. 13 - Seasonal trend of sugars, starch and their ratio (starch:sugar) in bark and wood of twigs of cherry trees located in SJ County and Kern County in 2022. Arrows of different colors indicate the two periods of relevance for the chill accumulation based on NSC changes (see text for details). The red box shows the period when maximum chill accumulation is recorded using the dynamics model with tree temperature as input (see figure 9 and 10).

Discussion: The results of this experiment are developing new information and tools for understanding and managing winter chill in cherry in a more precise way. We were able to get a good prediction of tree temperature using only air temperature as an input. We want now to expand the model adding more environmental factors to improve the tree chill accumulation estimation. We also want to use our data set to test the possibility of using environmental data from close CIMIS stations to estimate tree temperature during winter.

The carbohydrate winter dynamics highlighted very clear changes that corresponded with chill accumulation milestones. This suggest that NSC could be used as a management tool to better predict chill accumulation and improve spray efficiency. Hence, our next objectives are to 1) use existing dataset to predict tree temperature at any location based on meteorological data from CIMIS stations, 2) use tree temperature to predict changes in NSC, and 3) test the possibility to time spray application using NSC change predictions.

Developing Nutrient Budget and Early Spring Nutrient Prediction Model for Nutrient Management in Citrus

Patrick Brown

Project Leaders

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INTRODUCTION

Increasing awareness of the environmental impact of excess nitrogen (N) and new N management regulations demand user-friendly tools to help growers make fertilization decisions. Currently, nutrient management decisions in cherries are based on leaf analysis and critical value interpretation which only indicates a deficiency or sufficiency and is performed too late to respond to deficiencies or plan N applications. In other high value crops such as Almond, Pistachio and Walnut, nutrient management is increasingly based on yield and vegetative growth estimated crop demand coupled with an understanding of seasonal nutrient demand dynamics. This approach has not been developed for cherry cultivars in California and hence cherry growers do not have improved fertilizer management decision tools to apply the right rate of fertilizer at right time, to optimize productivity and avoid environmental losses. Current approaches to nutrient management in cherries rely heavily on leaf sampling collected during late summer which is too late to respond to deficiencies or adjust fertilizer regimes. The concept of demand driven nitrogen management is not widely practiced but is essential to meet ILRP guidelines and achieve a high efficiency of N use. Critical data on N export rates, seasonality of N demand and differences between cultivars and practices in N dynamics, is not currently available from California cherry production.

OBJECTIVES

Our goal is to develop knowledge of the pattern of nutrient uptake and allocation of nutrients in cherry and to provide insight into nutrient allocation patterns, the storage of nutrients in perennial tissue and the role of nutrient remobilization in supplying early season nutrient demand and direct application for the management of nutrients in commercial orchards.

DESCRIPTION

The study is being conducted in three high yielding commercial cherry cultivars “Bing”, “Coral”, and “Rainier” orchards in the California Central Valley. All varieties were grafted on Mazzard rootstock with an approximate planting density of 202 trees per acre.

We are currently monitoring three replicated blocks of trees (3 trees per block, totaling 9 trees per orchard) for each cherry cultivar (“Bing”, “Coral”, and “Rainier”) for changes in nutrient concentrations in annual (leaves and fruits) and perennial organs (roots, trunk, scaffold, canopy branches and small branches) six times during the season at different phenological stages.

A new nutrient BMP will be developed by integrating the findings from whole tree nutrient curves and early season tissue analysis. The combination of nutrient budget, seasonal changes in tree N content and in-season prediction of tissue nutrient status will help is developing a robust new fertilizer management tools for cherry growers of California.

RESULTS AND DISCUSSION

Tree biomass and nutrient content

Total nutrient amounts per tree was obtained by summing the nutrient content of tree organs calculated by multiplying the dry weight of each tree organ by its nutrient concentration. Data refer to the average of six trees excavated in 2020-2021 for each cultivar. Canopy branches and large roots accounted for the majority of the biomass (~40-60%) in all orchards. Canopy branches and large roots also included a notable fraction of nutrients present in below- and aboveground tissues as shown in Figure 1.

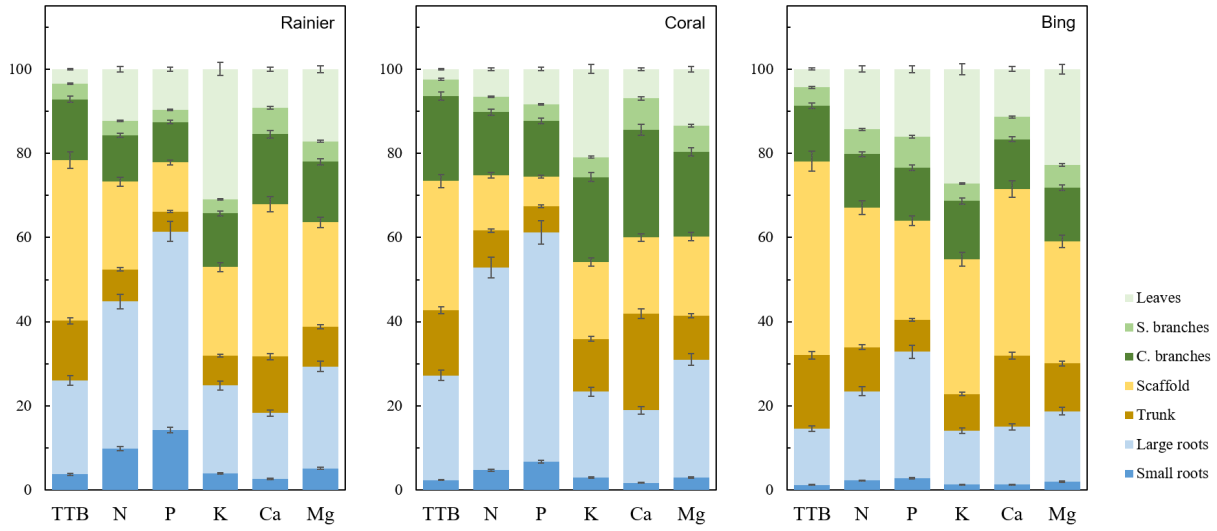

Figure 1. Tree partitioning (% of total) of total tree biomass (TTB) and macronutrients (N, P, K, Ca, and Mg) content. Data refer to cherry cultivars “Rainier”, “Coral”, and “Bing”. Bars represent standard errors.

Dynamics of Nitrogen uptake during the season

Seasonal N content in perennial organs (trunk, scaffold, canopy branches and roots), and leaves of cherry trees are shown in Figure 2. Data refer to the average of 9 trees per orchard for each species.

The seasonal demand of N in cherry is high early in the season from March through

September. Knowing the dynamics of nutrient uptake during the season is a requirement to allow the management of the timing of nutrient supply with nutrient needs. Preliminary data suggest that nutrients should be available in the soil for root to uptake by cherry trees from March to October. In contrast, from November to February, no net increase in nutrient was observed during this period.

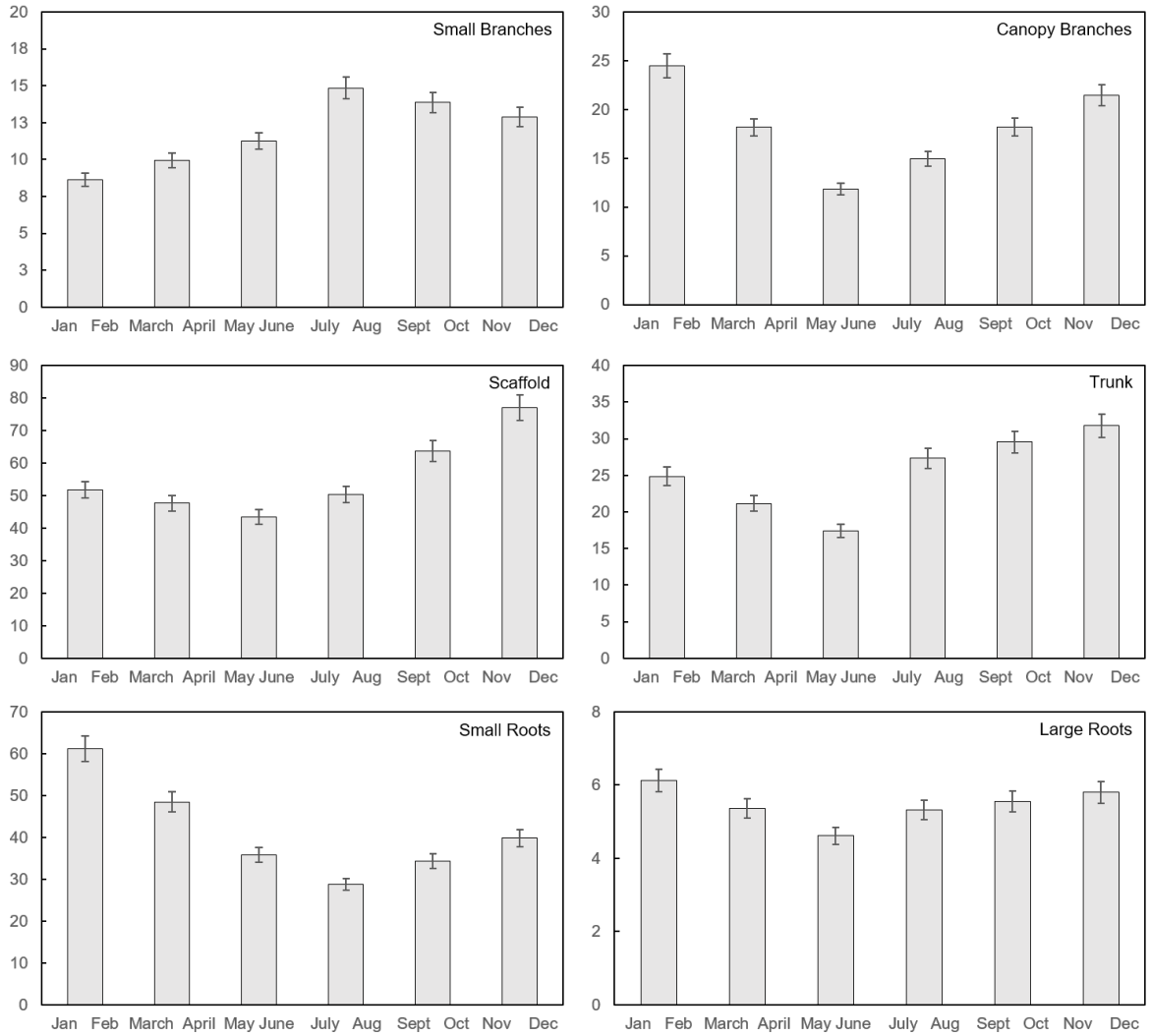

Figure 2. Seasonal trends in Nitrogen partitioning in fruits, leaves, and perennial organs (trunk, scaffold, canopy branches and roots) of mature cherry trees. The overall average is weighted for the number of observations in all trials (n = 27). Bars represent standard errors.

Nitrogen removal during the season

On average, preliminary data suggests that cherry offtake of N was estimated to be 2.52 lb. per 1000 lbs. of fresh fruit. In addition, N requirement for tree development (biomass accumulation) was estimated to be 28.3 lbs. acre (Table 1). Nitrogen use efficiency can be optimized by adjusting fertilization rate based on realistic, orchard specific yield, accounting for all N inputs and adjusting fertilization in response to spring nutrient status and yield estimates.

Table 1. Nitrogen removal in cherry cultivars. The overall average is weighted for the number of observations in each trial (*n = 9*).

Variety	***Removal at harvest (lbs N/1000 lbs of fruits)***
Rainier	2.74
Coral	2.73
Bing	2.32
Weighted Average	**2.59**

	Tree development (lbs N/acre*)
Rainier	28.99
Coral	28.41
Bing	27.51
Weighted Average	**28.30**

*Planting density of 202 trees per acre.

It is important to note that the data shown in this report is a **preliminary data** from year 1 and 2 of a 3-year project, then no conclusive data are shown. Our goal is to develop knowledge of the pattern of nutrient uptake and allocation during three seasons (2020-2023) in cherry trees to develop a nutrient prediction model for cherry cultivars “Rainier”, “Coral”, and “Bing” to guide fertilizer application based on crop phenology for the State of California.

TAKE-HOME MESSAGE

As a best management practice, fertilizer application in a cherry orchard should be based on expected yield estimated at flowering and fruit set followed by analysis of leaves to diagnose any deficiency. The combination of nutrient budget determination, nutrient response information, improved sampling and monitoring strategies, and yield determination provide a theoretically sound and flexible approach to ensure high productivity and good environmental stewardship.

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ACKNOWLEDGEMENTS

We would like to thank the California Cherry Board (CCB), the California Department of Food and Agriculture(CDFA) and the Fertilizer Research and Education Program (FREP)

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IMPROVING THE SANITARY STATUS OF SWEET CHERRY PLANTING MATERIAL

Florent Trouillas

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Commodity: Sweet Cherry

Objectives:
Objective 1: Determine the critical stages of fungal pathogen infection and contamination sources during tree production at the nursery **(year 1)**

Objective 2: Determine the efficacy of various compounds for the protection of tree wounds following budding/grafting of cherry planting material **(year 1 and year 2)**

Objective 3: Investigate the occurrence of the X-Disease Phytoplasma and Little cherry viruses in cherry propagation materials and orchards **(year 1 and year 2)**

Objective 4: Promote outreach activities and education **(year 1 and year 2)**

Summary of major outcomes:

Previous research by our laboratory revealed a common occurrence of fungal infection by canker pathogens in cherry planting stocks that occurred during the tree production process at the nursery. We identified pruning wounds on rootstocks made following budding and growth of the scion as the main infection court for canker/wood decay pathogens. This year we tested various fungicidal treatments including a biocontrol agent, a sealant and chemical products in field trials to prevent infection of such pruning wounds by fungal pathogens *Diaporthe ambigua*, *Trametes versicolor* and *Schyzophyllum commune*. Two trials were established at Kearney and UC Davis in the spring 2022. Results indicated a best efficacy for the Doc Farewell's grafting seal and the biocontrol product Vintec to protect pruning wound, reducing infection by up to about 80% when compared to non-treated trees. These results

confirmed last year results obtained in nursery trials showing Doc Farewell's and Vintec as best products to protect pruning wounds in nursery stocks. Furthermore, we continued to assess the occurrence in symptomatic orchards of the X-Disease Phytoplasma, Little cherry viruses and other common cherry viruses. Testing for the pathogens was conducted in collaboration with the diagnostic lab at Foundation Plant Services (FPS) in Davis using real-time qPCR assays and selected markers currently available for the targeted pathogens. This year, one additional cherry orchard with trees grafted onto Mahaleb rootstock in San Joaquin County was identified expressing symptoms of a sudden decline similar to Cherry X-disease. Symptoms included zippering under the bark at the bud union and a sudden collapse with leaves turning bronze. However, testing by FPS using real-time qPCR and selected markers for common Phytoplasma as well as High Throughput Sequencing technologies did not reveal the presence of phytoplasma or viruses from samples in this orchard. While this issue appears to be severe in orchards and a few orchards were identified so far expressing this syndrome, the cause of this problem remained unknown. Accordingly, further research is needed to investigate the cause of this sudden decline.

Objective 1: Determine the critical stages of fungal pathogen infection and contamination sources during tree production at the nursery:
Completed, refer to 2021 annual report

Objective 2: Determine the efficacy of various compounds for the protection of tree wounds following budding/grafting of cherry planting material
We evaluated conventional fungicides, paste and biocontrol agents for wound protection against main canker and wood decay fungi affecting cherry planting material. For this objective, two field trials were established on 4/19/22 (Kearney Agricultural Research and Extension Center) and 5/27/21 (UC Davis), respectively. The goal of these trials was to identify products to protect pruning wounds occurring during the tree budding and tree production process at the nursery, which, as illustrated in our previous research, are sites of contamination for wood decay fungi (*Schizophyllum, Trametes*) and canker pathogens (*Diaporthe/Phomopsis*)

Materials and methods:

In the Kearney trials, pruning wounds were made in 2-year-old branches in cherry trees (Benton cv) planted in the field. Fresh pruning wounds were then then treated with either sterilized water (negative control), Vintec (Trichoderma-based biological control product), Topsin M + Rally, Quilt Xcel, Luna Sensation and the Doc Farwell's grafting seal (Table 1). Applications were made with hand- held spray bottles at the label rate, and wounds were sprayed until runoff. The grafting sealant was applied using a paint brush. Eight tree replicates per treatment were included in a randomized complete block design. Twenty-four hours after fungicide treatments, trees were inoculated with grinded mycelium suspensions of Schizophyllum, Trametes and Diaporthe (Phomopsis) commonly associated wood decay and canker in rootstocks.

In the UC Davis Trial, we evaluated the efficacy of five wound protectants (Table 1) against the most common canker pathogens isolated from sweet cherry planting material (*Diaporthe ambigua*, *Trametes versicolor* and *Schyzophyllum commune*). Lignified branches (2nd to 3rd year wood) of 4- year-old cherry trees (cultivar Lapins grafted onto rootstock Krymsk 6) were pruned to make a flat wound. The experimental design included six treatments: wounds were treated with either sterilized water (negative control) or one of the five protectants listed in Table 1 (Vintec, Topsin M + Rally, Quilt Xcel, Luna Sensation and the Doc Farwell's grafting seal). Sterilized water was used as a negative control. Twelve trees per pathogen were used, with six wounds per tree, with one wound per treatment. Treatment applications were made with hand-held spray bottles and wounds were sprayed until runoff. For the protectant Doc Farewell's, which comes as a paste formulation, application was done with a paint brush.

All treatments were applied 24h before pathogen inoculations. The experiment was set up in May 2022 in an experimental orchard at the Plant Pathology field station in Davis, CA. At the time of pathogen inoculations, wounds were misted with sterilized water to provide high relative humidity and these wounds were inoculated with 100 µL of a mycelium suspension of the canker pathogens *Diaporthe ambigua* and *Schyzophyllum commune*.

Treated branches were collected approximately 4 months after inoculation in September 2022 and brought to the laboratory for fungal isolations. Presence (1) or absence (0) of the inoculated fungal species was recorded for each treatment. Low rates of fungal recovery were correlated with high product efficacy. Infection rate was calculated as the percentage of pruning wounds from which the pathogen was recovered, out of the total number of inoculated pruning wounds.

Table 1. Products tested for preventing cherry pruning wound infections by *Diaporthe ambigua* and *Schyzophyllum commune*.

Products	Active ingredient(s)	Application rate
Vintec	*Trichoderma atroviride* SC1	1g/L
Quilt Xcel	azoxistrobin + difenoconazole	2.03 mL/L (26 oz/ac)
Topsin M + Rally	thiophanate-methyl + myclobutanil	1.8 g/L (1.5 lbs/ac) and 0.63 g/L (8 oz/ac)
Luna Sensation	fluopyram + tebuconazole	0.6 mL/L (7.6 fl oz/ac)
Doc Farewell's	*proprietary*	brushed as formulated

Results and discussion:

In the Kearney trial, *Diaporthe ambigua* and *Schyzophyllum commune* were highly recovered from the water-treated control with infection rates of 62.5% and 100%, respectively. *Trametes versicolor* was not recovered from this experiment, suggesting the mycelium grindate suspension was inappropriate for this pathogen to infect pruning wounds. All product tested reduced infection by the fungal pathogens. The Doc Farwell's grafting seal performed best in this experiment, with infection rates of 12.5% and 25%, respectively for *Diaporthe ambigua* and *Schyzophyllum commune* (**Figure 1**). The biocontrol agent product was the second most effective product with infection rates at pruning wounds of 37.5% and 12.5% respectively for these pathogens. There was a 37.5% infection rate for wounds treated with Quilt Xcel. While Topsin M performed well against *Diaporthe ambigua* (25% infection rate), it did not perform well against *Schyzophyllum commune* (87.5% infection rate). Luna sensation showed moderate activity against the fungal pathogens with 50% and 37.5% infection rates, respectively (**Figure 1**).

In the UC Davis trial, pathogen infection rates in wounds treated with water ranged from 67% for *Diaporthe ambigua* to 92% for *Schyzophyllum commune*. Inoculations with fragments of mycelium in suspensions were thus successful at establishing infections of pruning wounds with these two pathogens that do not produce spores readily in cultures. However, as with the Kearney trial, *Trametes versicolor* was not recovered from the water-only control, and so no results were obtained for this pathogen. The five products tested all reduced the infection rate of *Diaporthe ambigua*. However, Quilt Xcel and Luna Sensation still had relatively high infection rates (both 50%) and thus were moderately effective at protecting pruning wounds from this pathogen. In contrast, there was no detectable infection in pruning wounds treated with Doc Farewell and the mixture Topsin M + Rally; these two products were the best performing against this pathogen (**Figure 2**). There was a 25% infection rate in wounds treated with the biological control product Vintec. Results regarding the pathogen *Schyzophyllum commune* were not as promising as only the product Vintec offered a large reduction in infection rate (17%) by this pathogen, while infection rates in wounds treated with the other four products were ≥ 50% (**Figure 2**).

Objective 3: Investigate the occurrence of the X-Disease Phytoplasma and Little cherry viruses in cherry propagation materials and orchards

Materials and methods:

With the collaboration of Mohamed Nouri (farm advisor San Joaquin County) and Dr. Maher Al Rwahnih (Director of Foundation Plant Services), we continued to assess the occurrence in symptomatic orchards of the X-Disease Phytoplasma, Little cherry viruses and other common cherry viruses. Testing for the pathogens was conducted at FPS using real-time qPCR assays and selected markers currently available for the targeted pathogens. Detection of the various pathogen-specific nucleic acids was performed on DNA samples extracted directly from leaf petiole composite samples

from cherry mother blocks used for budwood. High Throughput Sequencing was used also to detect additional viral pathogens from field samples in orchards with unidentified decline.

Results and discussion:

In 2022, one additional orchard in San Joaquin County with trees expressing symptoms of sudden decline (sudden tree collapse) was brought to the attention of Mohamed Nouri. Trees were Black Tartarian (pollinator) and Bing varieties grafted onto Mahaleb rootstock. Trees exhibited symptoms similar to the Cherry X-disease, including zippering under the bark at the bud union and a sudden collapse with leaves turning bronze (**Figure 3**). Using real-time qPCR and selected markers for common Phytoplasma and viruses, no phytoplasma or virus was detected from this orchard. A follow- up detection using High Throughput Sequencing technologies also was utilized to detect putative virus causal agents and determine the cause of this sudden collapse. To date, no pathogen was detected from trees sampled from this orchard, and the cause of this problem remained unknown. Further research is needed to investigate the cause of this sudden decline. The possibility of a genetic rootstock-scion incompatibility may be considered, although it is unlikely due to the diversity of rootstock/scion combinations associated with this problem.

Objective 4: Promote outreach activities and education

Outreach and extension activities on fungal canker and wood decay diseases of cherry was provided to nurserymen through in-person meetings. Observations, findings, and testing protocols resulting from this work and previous research were presented to nursery staff and leaders, and training on disease management was provided. In addition, extension meetings were organized by farm advisors to raise awareness among CA cherry growers and nurserymen about the epidemiology, symptomology, management and introduction risks of Little Cherry disease and cherry X-disease in California.

Figures:

Figure 1. Kearney Trial. Infection rates of cherry pruning wounds following artificial inoculations with *Diaporthe ambigua* and *Schyzophyllum commune*. Protectants were sprayed or paint brushed (Doc Farewell's) onto pruning wound 24h before pathogen inoculations.

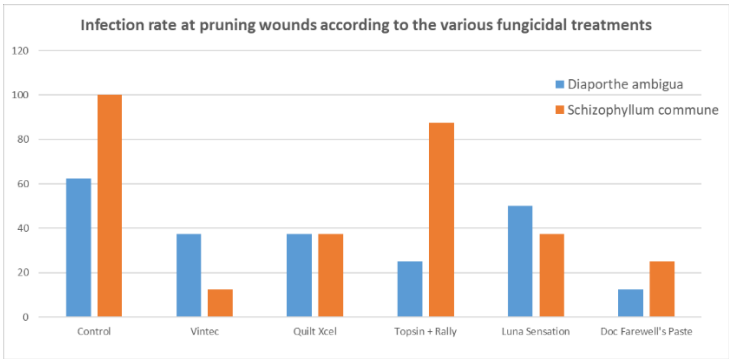

Figure 2. UC Davis trial. Infection rates of cherry pruning wounds following artificial inoculations with *Diaporthe ambigua* and *Schyzophyllum commune*. Protectants were sprayed or paint brushed (Doc Farewell's) onto pruning wound 24h before pathogen inoculations.

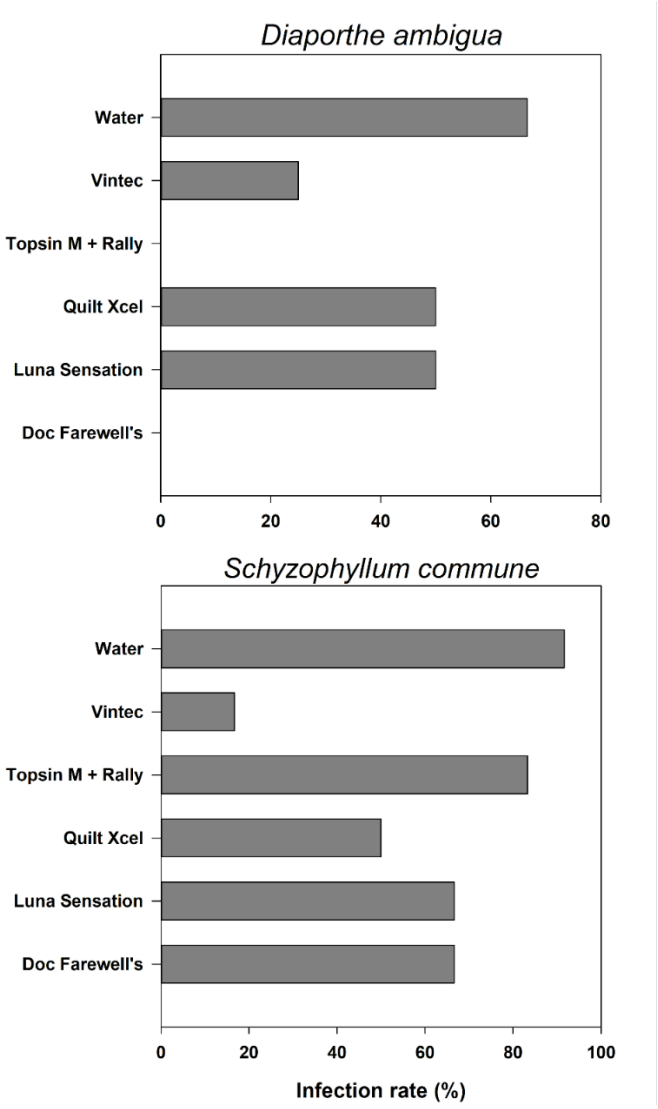

Figure 3: New orchard identified in San Joaquin County with symptoms of sudden tree collapse, zippering under the bark at the bud union and bronzing of leaves. No pathogen was yet detected from these trees.

Epidemiology of Fungal Canker Disease of Sweet Cherry

Florent Trouillas

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Cooperating personnel:

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Kari Arnold, Farm advisor, UCCE Stanislaus County
Mohammad Yaghmour, Farm advisor, UCCE Kern County

Commodity: Sweet Cherry

Objectives:

Objective 1: Determine the seasonal susceptibility of pruning wounds (winter vs summer pruning) to fungal canker pathogens

Objective 2: Investigate new infection pathways of fungal canker pathogens

Objective 3 (new): Determine the efficacy of peracetic acid for pruning wound protection against fungal canker pathogens.

Summary of major outcomes:

Our laboratory recently conducted various field studies in order to expand our understanding of the biology and epidemiology on fungal canker diseases affecting sweet cherry. During 2022, we investigated the susceptibility of pruning wounds in relation to winter vs. summer pruning. We also investigated the main infection courts, other than pruning wounds, that can lead to substantial infection of cherry trees. This work aims to improve control methods and develop IPM strategies for the management of canker diseases.

Last year, five field experiments were set in multiple sweet cherry orchards in Davis to assess the seasonal susceptibility (winter vs. summer) of pruning wounds to fungal canker pathogens including *Calosphaeria pulchella*, *Cytospora sorbicola* and *Eutypa lata*. Results from this study indicated, that pruning wounds made during winter (December-January) were generally not or only poorly susceptible to infection by *Cal. pulchella* compared to pruning wounds made during summer (June). These findings agree with our previous in vitro temperature studies indicating that *Cal. pulchella* requires temperatures above 15° C for ascospores germination and optimal mycelial growth. Therefore, pruning of sweet cherry trees under cold winter temperatures can significantly reduce the risks of infection by *Cal. pulchella*. These findings are particularly relevant in cherry orchards or regions where Calosphaeria canker is the most prevalent canker disease. However, winter pruning may not prevent infection by *E. lata* or *Cyt.*

sorbicola. Nevertheless, winter pruning may be considered as part of an integrated management strategy for Calosphaeria canker, especially in cherry orchards with abundant inoculum sources of *Cal. pulchella*. Similarly, winter pruning may be considered as a management strategy in the southern sweet cherry producing counties of California (Fresno, Tulare and Kings), where the inoculum sources of *E. lata* are absent. While few fungicides are effective at protecting pruning wounds against Calosphaeria canker, our previous work demonstrated that products such as Topsin M, Quilt Xcel, and Vintec are highly efficient at protecting pruning wounds against *Cyt. Sorbicola* and *E. lata*.

Furthermore, we investigated the main infection pathways associated with Calosphaeria, Eutypa and Cytospora canker diseases and examined the role of leaf, fruit and bud scars, in addition to pruning wounds, as possible infection courts for canker pathogens. Field experiments were set in multiple cherry orchards in Davis. Wounds occurring through natural processes due to leaf senescence (leaf scars) and budbreak (bud scars) were inoculated at the time when these processes occurred naturally in the field. Manual harvest of mature fruits was done in June to mimic wounds occurring during harvest, including superficial wounds on fruit spurs caused by pulling fruit peduncles and wounds from broken fruit spurs commonly occurring during commercial harvest. Results from this study revealed that wounds caused by the harvesting of fruit (detachment of fruit peduncle or breakage of fruit spurs) can serve as important infection sites for fungal canker pathogens. Infection rates from wounded or broken spurs were 42.9%, 65.9% and 30%, respectively following artificial inoculations with spore suspensions of *Cal. pulchella*, *Cyt. sorbicola* and *E. lata*. We demonstrated also that bud scars following budbreak can serve as additional infection sites for *Cyt. sorbicola*. Overall, our results suggest that a fungicide application immediately after harvest should be considered to limit further infection of sweet cherry trees by canker pathogens.

Finally, following recommendation from the California Cherry Board research committee, we investigated the efficacy, both preventively (applied 24-hour prior to infection) and curatively (applied 24-hour post infection), of peracetic acid and hydrogen peroxide (OxiDate® 5.0) for the protection of pruning wounds against *Cal. pulchella*, *Cyt. sorbicola* and *E. lata.* Results from this study showed that there were no to only limited benefits in spraying OxiDate® 5.0 in a preventive manner against canker pathogens. When OxiDate® 5.0 was sprayed in a curative manner i.e. 24h after inoculations of pruning wounds with canker pathogens, the largest reduction in infection relative to water treated wounds was for the pathogen *Cal. pulchella*, dropping from 78% to 44%. However, the benefit of OxiDate® 5.0 applied curatively was only moderate against the pathogens *Cyt. sorbicola* and *E. lata*. OxiDate® 5.0 also was tested to reduce infections from wounded or broken fruit spurs following harvest. Our results show no benefits of using OxiDate® 5.0 for the protection of harvest wounds from fungal canker pathogens.

Objective 1: Determine the seasonal susceptibility of pruning wounds (winter vs summer pruning) to fungal canker pathogens

We compared susceptibility of pruning wounds following winter and summer pruning to Calosphaeria, Cytospora and Eutypa cankers. Field experiments were set in multiple cherry orchards in Davis.

Materials and methods:

A total of five trials were established in Davis in 2022 to compare the susceptibility of pruning wounds to *Calosphaeria pulchella, Cytospora sorbicola*, and *Eutypa lata* between winter and summer. Trials took place in three different orchards (Orchard-1, -2 and -3). Cherry trees were pruned either in January (winter pruning) or June (summer pruning) 2022. *Cal. pulchella* ascospores and conidia, *Cyt. sorbicola* conidia, and *E. lata* ascospores were used as inoculum. For each pruning time (summer and winter), a flat cut was made on each selected branch with pruning shears, and wounds were sprayed with DI water to

mimic rain before inoculating with 100 µL spore suspensions in water at concentrations of 1×10^4 spores/mL (i.e., 1,000 spores). Two different concentrations of *Cal. pulchella* ascospores and conidia at 1×10^4 and 1×10^6 spore/mL (i.e., 100,000 spores) were tested in each trial. For each treatment, both old branches (3- to 5-year-old) and young branches (1- to 2-year-old) were inoculated. For each treatment, infection rates (= recovery rate from wounds) between summer and winter pruning trials were compared. Each trial was conducted using 1 branch per treatment per tree with a total of 12 tree replicates and included all three pathogens. Sterile deionized (DI) water was used as negative control to test for presence of local inoculum. Summer trials were collected after three months post-inoculation, whereas winter trials were collected after 4-5 months post-inoculation. A flame-sterilized knife was used to remove the exposed bark layer and approximately 0.5 cm of dried wood below the cut surface was removed. The remaining branch segment was then surface sterilized by brief flaming. Ten wood fragments of 3 mm × 3 mm × 3 mm were cut from the margin between discolored and clean tissues for each branch sample and plated onto PDA-Tet. Isolations were incubated at the laboratory ambient temperature (22-24°C) and natural photoperiod. Plates were examined every 2 days for prospective colonies of *Cal. pulchella, Cyt. sorbicola, and E. lata* from which we transferred hyphal tips to fresh PDA-Tet and allowed to grow for 12 days as pure culture. Pathogen identity was determined based on colony morphology. The infection rate was assessed as the % recovery of the pathogen re-isolated from each branch samples out of the total number of branches inoculated per treatment. We defined a positive pathogen recovery from a treatment when the pathogen was isolated from at least one of the ten diseased wood pieces in a plate. To determine statistical significance of incidence between summer and winter pruning susceptibility, confidence intervals (CI) for recovery differences in proportions were calculated as:

$CI = p_1 - p_2 \pm 2.58 \times \sqrt{[(p_1 \times q_1/(n_1 - 1)) + (p_2 \times q_2/(n_2 - 1))]}$ where p_1 and p_2 were proportions of recovery from each pruning time, q_1 and $q_2 = 1 - p$, n_1 and n_2 were the sample sizes of each treatment, 2.58 corresponded to the z-score of the 99% confidence level, and degrees of freedom (df) = $(n_1 - 1) + (n_2 - 1)$.

Results and discussion:

Results from this study showed significant differences in seasonal susceptibility of pruning wounds to *Cal. pulchella* (CI = [0.709, 0.865], P-value = 0.01, df = 409) and *E. lata* (CI = [0.053, 0.337], P-value = 0.01, df = 11) infection. No significant differences were found in seasonal susceptibility of pruning wounds to *Cyt. sorbicola* (CI = [-0.198, 0.262], P-value = 0.01, df = 112). Pruning wounds inoculated with water were not infected by any known canker-causing pathogens, indicating that pathogens recovered in these trials originated from inoculations during this study. The infection rate in winter (January 2022) and summer-inoculated (June 2022) branches (all branch age, spore types and concentrations combined) were 87.3% and 8.5%, respectively, for *Cal. pulchella*; 66.2% and 69.4%, respectively, for *Cyt. sorbicola*; and 78.5% and 98.0%, respectively, for *E. lata* (**Figure 1**). Our results on seasonal pruning wound susceptibility to *Cal. pulchella* was consistent with findings reported in the CCB 2020 report (**Figure 2**), which also demonstrated that recovery of *Cal. pulchella* from winter-inoculated branches was significantly lower than that of summer inoculations. No significant differences in infection rates were found between wood age for all pathogens (**Figure 3**). Additionally, no significant differences were found between the infection rates of *Cal. pulchella ascospores* and conidia (**Figure 4**). No significant difference was found in the infection rates between the two concentrations (1×10^4 and 1×10^6 sp. /mL) of ascospore and conidia of *Cal. pulchella* during summer (**Figure 4**). However, the recovery of *Cal. pulchella* from pruning wounds inoculated with 10^6 ascospores/mL was significantly greater than that of 10^4 ascospores/mL inoculations during winter, with 14% and 0% recovery, respectively (CI = [0.012, 0.268], P-value = 0.01, df = 98). Similarly, recovery from 10^6 conidia/mL inoculations was significantly greater than that of 10^4 conidia/mL inoculations during winter, with 20.4% and 0% recovery, respectively (CI = [0.090, 0.318], P-value = 0.05, df = 97).

Overall, our study indicates that pruning wounds made during summer were significantly more susceptible to *Cal. pulchella*. In other words, summer pruning of sweet cherry trees in California poses greater risks of infection by *Cal. pulchella,* while winter pruning can avoid the disease. In contrast, *E. lata* was significantly more infectious on pruning wounds made during winter than those of summer. Pruning of cherry trees in summer and in winter may pose equal risks to *Cyt. sorbicola* infection if inoculum is present, although the seasonal abundance of *Cyt. sorbicola* conidia in sweet cherry orchards has not been studied. A shift from winter to summer pruning likely has favored the emergence of Calosphaeria canker as a major canker disease in California cherry orchards during the past two decades. A previous survey of cherry orchards in California also found *E. lata* to be of low incidence relative to *Cal. pulchella*, particularly in regions of the southern San Joaquin Valley where rainfalls are low (Trouillas et al. 2012). This work indicates that pruning of sweet cherry trees under cold winter temperatures can significantly reduce the risks of infection by *Cal. pulchella*. However, winter pruning will not prevent infection by *E. lata* or *Cyt. sorbicola*. Nevertheless, winter pruning may be considered as part of an integrated management strategy for Calosphaeria canker, especially in cherry orchards with abundant inoculum sources of *Cal. pulchella*. Similarly, winter pruning may be considered as a management strategy in the southern part of the state (another important sweet cherry production for California), where the inoculum sources of *E. lata* are absent. A survey of vineyards across 21 California counties by Úrbez-Torres et al. (2006) reported little to no detection of *E. lata* from grapevine cankers in southern counties including Santa Barbara, Fresno, Kern, Tulare, and Riverside County. Similarly, Trouillas and Gubler (2010) reported no occurrence of perithecia of *E. lata* from a great diversity of plant hosts surveyed in southern counties such as Madera, Fresno, Kings, and Tulare Counties. *Cytospora* spp. release spores year-round with the fewest amounts during mid- to late-winter (Bertrand and English 1976). Thus, pruning during mid- to late-winter in CA Southern counties poses lesser risks to infection by *Cal. pulchella* and *E. lata*, especially during dry and cold weather conditions. Nevertheless, wound protectants should be applied immediately after pruning. Our previous work demonstrated products such as Topsin M, Quilt Xcel, and Vintec to have high efficacy in reducing pruning wound infections by *Cyt. Sorbicola* and *E. lata*.

Objective 2: Investigate new infection pathways of fungal canker pathogens

We investigated the main infection pathways associated with Calosphaeria, Eutypa and Cytospora cankers and examined the role of leaf, fruit and bud scars, in addition to pruning wounds, as possible infection courts. Field experiments were set in multiple cherry orchards in Davis.

Materials and methods:

Field experiments were conducted to determine the role of leaf scars, bud scars and wounds on spurs resulting from fruit harvesting as infection pathways for fungal canker pathogens. Experiments were conducted in a 30-year-old orchard at the Plant Pathology Research Field of the University of California, Davis. Wounds occurring through natural processes due to leaf senescence (leaf scars) and bud break (bud scars) were inoculated at the time when these processes occurred naturally in the field. Manual harvest of mature fruits was done in June to mimic superficial spur scars (harvest by pulling fruit peduncle) and broken spurs scars (harvest by breaking the whole fruit spurs) occurring during commercial harvest. All wound types were inoculated to determine their susceptibility to infection by fungal canker pathogens. A total of 4 treatments including *Cal. pulchella* ascospores, *Cyt. sorbicola* conidia, *E. lata* ascospores, and sterile DI water as control were spray-inoculated directly onto each potential infection sites on Bing cultivar cherry trees. All spores were collected from fruiting bodies found naturally on diseased wood and spore suspension were prepared and their concentration were adjusted to 1×10^6 spores/mL using a hemocytometer. Spray bottles were used to apply inoculum onto the various plant wounds by spraying until runoff (~2 to 4 mist sprays).

For leaf scars, each treatment was inoculated onto all leaf scars on each of 10 bud clusters or spurs during December 2022 when leaves senesced. Bud scars were inoculated in March 2022 after bud scales had fell off following bud break and blooming of flowers. For each treatment, 10 flower bud clusters were inoculated and repeated over 10 trees. Spur scars resulting from the pulling of fruit at harvest were inoculated in June 2022. Two types of wounds were inflicted on fruiting spurs during harvest: (1) superficial spur scars (SSS) created from harvesting fruit clusters by the peduncle, which caused superficial scars where the peduncles were attached; and (2) broken spur scars (BSS) resulted from harvest by the fruiting spur, which caused partial or complete breakage of the spur. Each treatment was applied on 5 wounds per wound-type and repeated over 4 trees.

After 3 months following inoculations of each potential infection site, the various samples were collected for re-isolation and to determine the rate of infection of each pathogen at each site. Tissues were collected for each treated bud and spur scar. The bark of samples was then removed and cross- sections of wood tissue above, below, and at the inoculated wound site were taken. Ten 3 mm × 3 mm × 3 mm wood fragments of each section were surface sterilized with 0.875% sodium hypochlorite solution. This was followed by two rounds of 60 seconds rinsing in sterile deionized (DI) water and allowed to dry over clean paper towel before plating onto PDA-Tet. All isolation plates were incubated at the laboratory ambient temperature (22-24°C) and natural photoperiod. Cultures were checked every two days for growth of prospective canker-pathogen colonies, which were then transferred to fresh PDA-Tet petri dishes and allowed for growth as pure cultures. The identity of isolates was determined by colony morphology. The infection rate or disease incidence was assessed as the % recovery of the pathogen re-isolated from samples out of the total number of samples of that infection court inoculated per treatment. We defined a positive pathogen recovery from a treatment when the pathogen was isolated from at least one of the ten diseased wood pieces in a plate.

Results and discussion:

Cytospora sorbicola was recovered from 20% of inoculated bud scars, while *Cal. pulchella* and *E. lata* were not recovered. The recovery of *Cal. pulchella*, *Cyt. sorbicola* and *E. lata* from spur scars (wound-types SSS and BSS combined) were 42.9%, 65.9% and 30%, respectively (**Figure 6**). For the different spur wound-types, recovery of each canker pathogen from SSS were 35%, 42.6% and 25%, respectively, while recovery from BSS were 50%, 90% and 35%, respectively (**Figure 7**). One leaf scar trial was inoculated in December 2022. Data for this remaining trial are not yet available.

Overall, our results from infection court inoculations in 2022 demonstrated that wounds on fruiting spur (spur scars) created during harvest are susceptible to infection by *Cal. pulchella*, *Cyt. sorbicola*, and *E. lata*. Spur scars of the SSS-type, which were superficial scars created from peduncle detachment, resulted in lower infection rates when compared with wounds of the BSS-type, which were scars created from the detachment or breakage of spurs. Peduncle detachment mainly exposes the inner bark tissues, while a broken or detached spur usually exposes the sapwood and pith. This difference could explain the higher infection rates of broken spurs as fungal canker pathogens require woody tissue for infection. We also demonstrated that bud scars are not important infection sites for *Cal. pulchella* and *E. lata*; however, *Cyt. sorbicola* was shown to infect via bud scars. The high incidence of fungal canker pathogens below pruning wounds from Objective 1 supports that pruning wounds also act as major entry point for canker infection.

In conclusion, this is the first study to demonstrate that wounds on spurs caused by manual fruit harvesting can serve as an additional and substantial infection court for major fungal canker pathogens of sweet cherry. The high incidence of fungal canker pathogens below pruning wounds confirm that pruning

wounds also act as the primary entry point for canker infection. Although less susceptible, bud scars could lead to significant infections in orchards due to the large amount of such wounds produced each year during bud break in the spring. The infection of such wounds also could explain the decline of shoot tips with no apparent pruning or other mechanical injury. Results overall suggest that a fungicide application immediately after harvest should be considered to reduce further infection of cherry trees by canker pathogens.

Objective 3 (new): Determine the efficacy of peracetic acid and hydrogen peroxide for pruning and harvest wound protection against fungal canker pathogens

Following recommendation from the California Cherry Board research committee, we investigated the efficacy of peracetic acid and hydrogen peroxide for the protection, both preventively and curatively, of pruning wounds against fungal canker pathogens.

Materials and methods:

We evaluated the efficacy of peracetic acid combined with hydrogen peroxide (OxiDate® 5.0) as a wound protectant against the three main fungal canker pathogens of sweet cherry trees (*Calosphaeria pulchella, Cytospora sorbicola,* and *Eutypa lata*). Lignified branches (2nd to 3rd year wood) of 4-year-old cherry trees (cultivar Lapins grafted onto rootstock Krymsk 6) were pruned to make a flat wound. The experimental design included three treatments: (1) wounds were treated with either sterilized water (negative control) or (2) OxiDate® 5.0 (1:256 dilution rate) 24h before pathogen inoculations (preventive) or (3) 24h after pathogen inoculations (curative). A total of nine trees was used, with three trees per treatment and nine wounds per tree, with three wounds per pathogen per tree. Treatment applications were made with hand-held spray bottles and wounds were sprayed until runoff. The experiment was set up in May 2022 in an experimental orchard at the Plant Pathology field station in Davis, CA. At the time of pathogen inoculations, wounds were misted with sterilized water to provide high relative humidity and these wounds were inoculated with 100 µL of a spore suspension of the fungal canker pathogens *Cal. pulchella, Cyt. sorbicola,* and *E. lata* at a concentration of 1,000 spores per wound. Treated branches were collected approximately 4 months after inoculation in September 2022 and brought to the laboratory for fungal isolations. Presence (1) or absence (0) of the inoculated fungal species was recorded for each treatment. Low rates of fungal recovery were correlated with high product efficacy. Infection rate was calculated as the percentage of pruning wounds from which the pathogen was recovered, out of the total number of inoculated pruning wounds. Finally, we attempted to protect wounded spurs resulting from the pulling of fruit or breakage of fruit spurs at harvest (see Objective 2). Wounded spurs (15 replicates per treatment) were inoculated in June 2022 with spore suspensions (1 x 10^6 spores/mL) of *Cal. pulchella, Cyt. sorbicola.* After 24h, OxiDate® 5.0 (1:256 dilution rate) was applied in an attempt to cure the freshly inoculated spurs wounds. After 3 months following inoculations of wounded spurs and treatment with OxiDate® 5.0, the various spur samples were collected for re-isolation and to compare the rate of infection of each pathogen between the OxiDate® 5.0 treatment and a water control.

Results and discussion:

In the wounds treated with water, pathogen infection rates varied from 78% for *Calosphaeria pulchella* to 89% for both *Cytospora sorbicola* and *Eutypa lata*, illustrating the success of the artificial inoculations in the field. When OxiDate® 5.0 was sprayed in a preventive manner, 24h before pathogen inoculations, infection rates for the pathogens *Calosphaeria pulchella* and *Cytospora sorbicola* were 100% and thus higher than in the water treated wounds. There were no benefits in spraying OxiDate® 5.0 in a preventive

manner for these two pathogens, and the benefit was only marginal for wounds inoculated with *Eutypa lata* (78% infection rate, compared to 89% in water treated wounds).

When OxiDate® 5.0 was sprayed in a curative manner, 24h after pathogen inoculations, the largest reduction in infection relative to water treated wounds was for the pathogen *Calosphaeria pulchella*, dropping from 78% to 44%. The benefit of OxiDate® 5.0 applied curatively was moderate against the pathogens *Cytospora sorbicola* and *Eutypa lata* (**Figure 8**). Our results also show no effect of OxiDate® 5.0 at reducing infection of harvest wounds by fungal canker pathogens (**Figure 9**).

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Trouillas, F. P., & Gubler, W. D. 2010. Host range, biological variation, and phylogenetic diversity of *Eutypa lata* in California. Phytopathology 100(10), 1048-1056.

Trouillas F.P., Peduto F., Lorber J.D., Sosnowski M.R., Grant J., Coates W.W., Anderson K.K., Caprile J. and Gubler W.D. 2012. Calosphaeria canker of sweet cherry caused by *Calosphaeria pulchella* in California and Australia. Plant dis 96:648-658.

Úrbez-Torres, J. R., Leavitt, G. M., Voegel, T. M., and Gubler, W. D. 2006. Identification and Distribution of *Botryosphaeria* spp. Associated with Grapevine Cankers in California. Plant Dis 90:1490-1503.

Figures:

Figure 1. Seasonal pruning wound susceptibility to *Cal. pulchella*, *Cyt. sorbicola* and *E. lata*. Sterile H2O was inoculated as negative control to test for local inoculum in the orchard. Percentage recovery was calculated using combined average values of all 3 orchards. Average recovery of each pathogen was compared between summer and winter-inoculated pruning wounds on sweet cherry. (*P < 0.05)

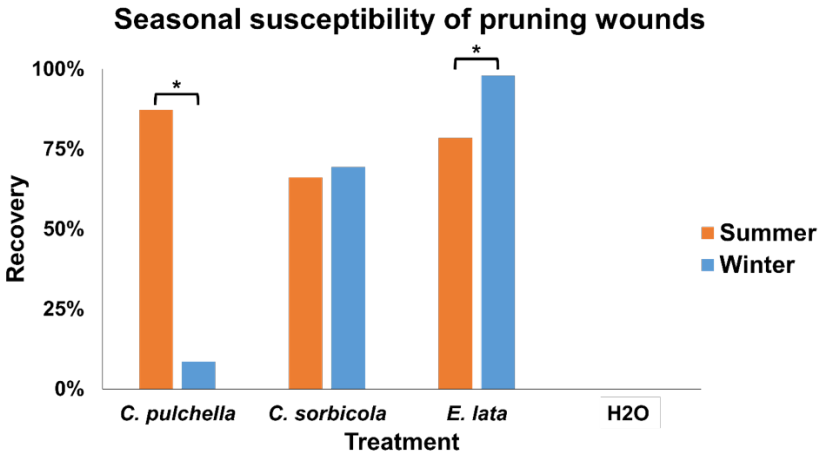


Figure 2. Seasonal pruning wound susceptibility to *Cal. Pulchella* asocospores and conidia. Sterile H2O was inoculated as negative control to test for local inoculum in the orchard. Average recoveries were compared between summer and winter-inoculated pruning wounds on sweet cherry.

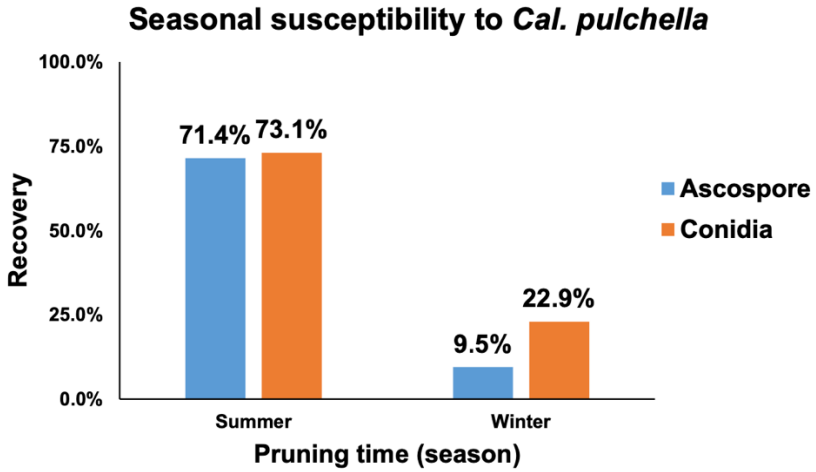


Figure 3. Pruning wound susceptibility of wood age to *Cal. pulchella*, *Cyt. sorbicola*, and *E. lata* during (a) summer and in (b) winter. Average recoveries were compared between inoculated old and young branches, where wood age is 3-to-5-years-old and 1-to-2-years-old, respectively

Fig. 3a

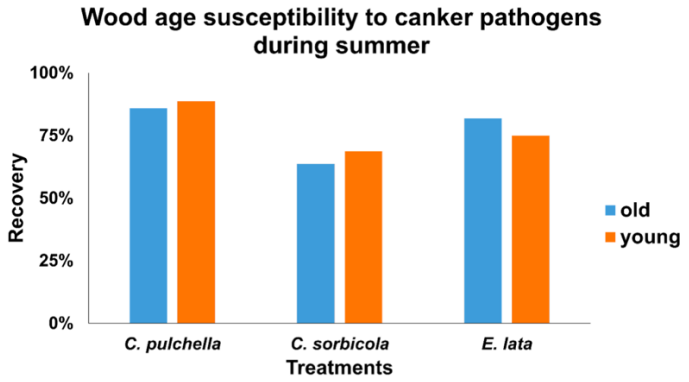


Fig. 3b.

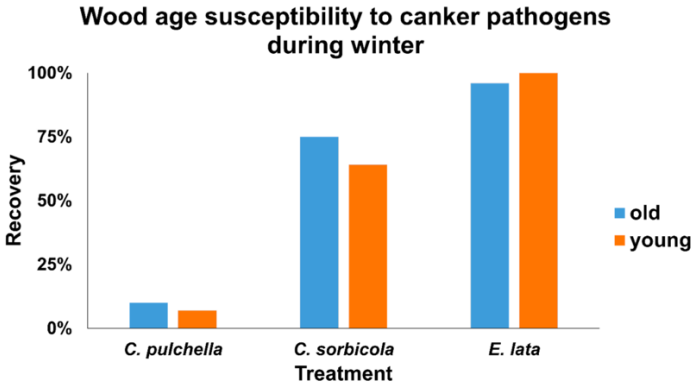


Figure 4. Pruning wound susceptibility between ascospores and conidia of *Cal. pulchella* were compared. These trials were performed both in summer and in winter.

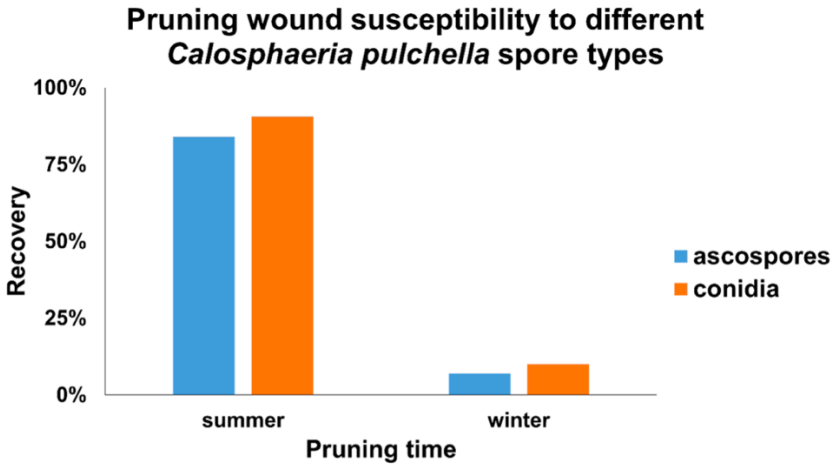


Figure 5. Pruning wound susceptibility to *Cal. pulchella* in two different spore inoculum concentrations, 1 x 10^4 and 1 x 10^6 spores per ml, done in (a) summer and in (b) winter. Two different spore types, ascospores and conidia, were used in comparison between the two inoculum concentrations. Average recoveries from each type of spore inoculations were used to assess their pathogenicity. (*P < 0.01)

Fig. 5a

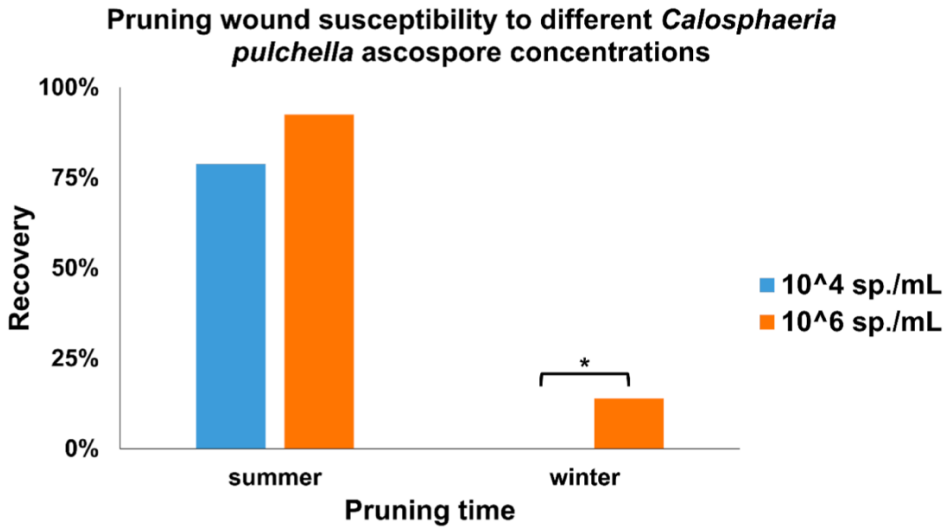


Fig. 5b.

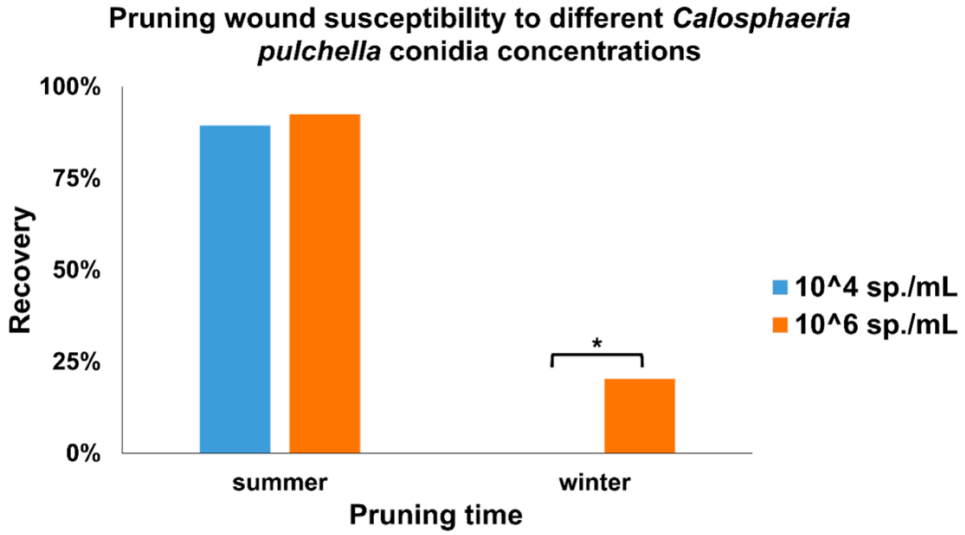


Figure 6. Average recovery rates of *C. pulchella*, *C. sorbicola*, and *E. lata* from inoculated wounds due to bud break and mechanically induced wounds of fruiting spurs of sweet cherry. Sterile DI H2O was used as control to test for presence of local inoculum.

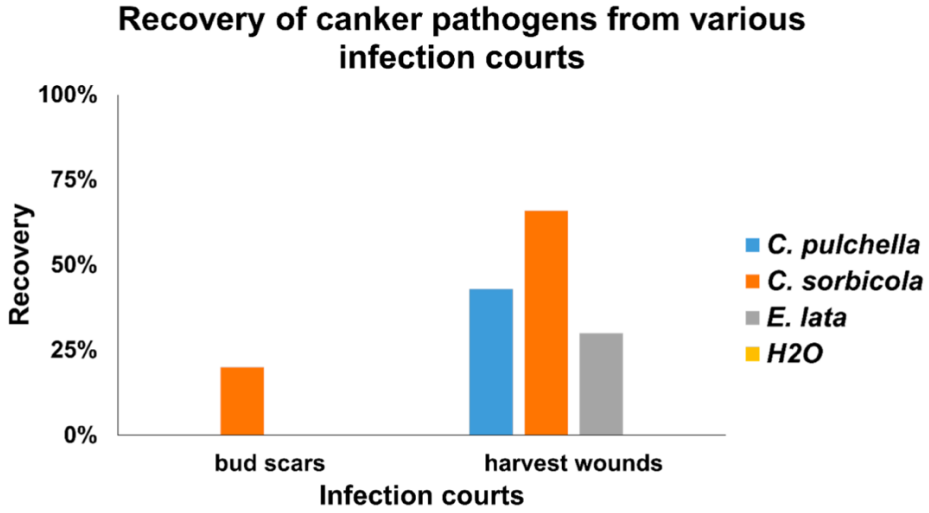


Figure 7. Comparison of recovery from two distinct types of spur wounds due to harvest. Superficial wounds are caused by harvesting fruit clusters by the peduncle. Breakage wounds are caused by harvesting fruit clusters by the spur.

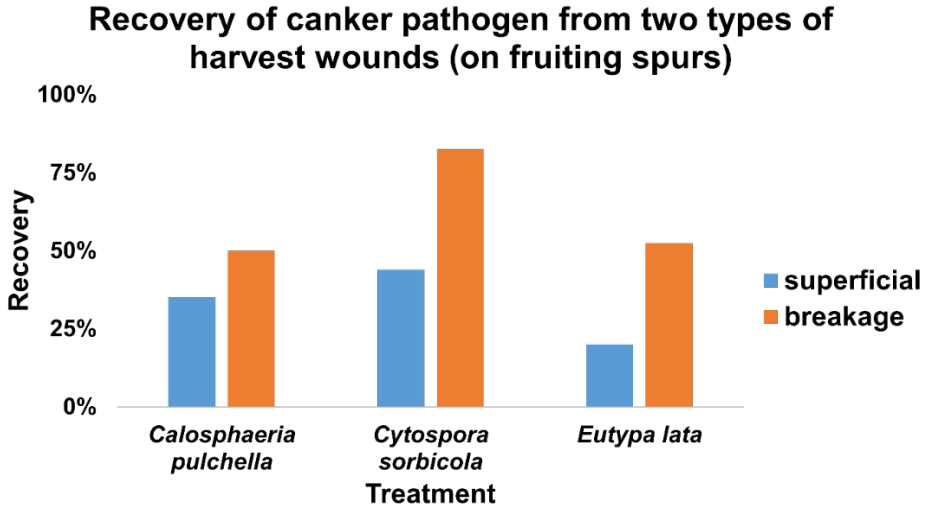


Figure 8. Pruning wound infection rates following artificial inoculations with *Cal. pulchella*, *Cyt. sorbicola*, and *E. lata*. Pruning wound were sprayed either with water 24h before inoculations, or OxiDate® 5.0 (peracetic acid + hydrogen peroxide) 24h before inoculations (preventive), or OxiDate® 5.0 24h after inoculations (curative).

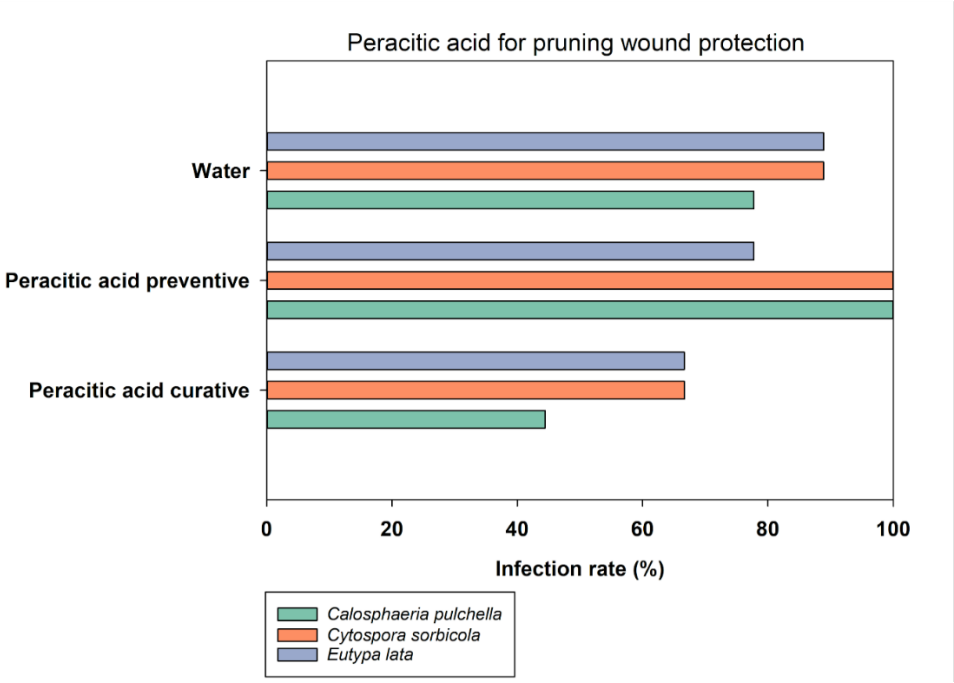
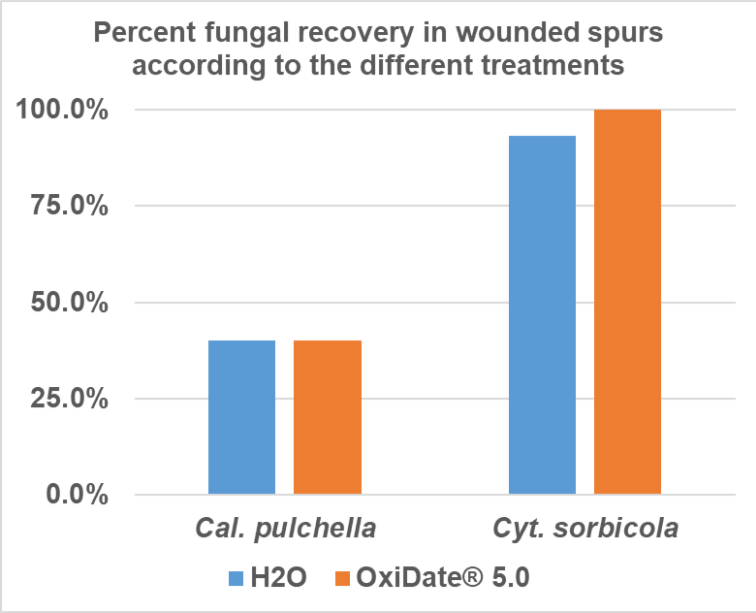


Figure 9. Infection rates on wounded spurs following artificial inoculations with *Cal. pulchella* and *Cyt. sorbicola* and treatment with water or OxiDate® 5.0 (peracetic acid + hydrogen peroxide) 24h after inoculations (curative).



Management and Epidemiology of Pre- and Postharvest Diseases of Sweet Cherry

Jim Adaskaveg

Annual Report - 2022

Prepared for the California Cherry Advisory Board

Project Title:	Management and Epidemiology of Pre- and Postharvest Foliar and Fruit Diseases of Sweet Cherry
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SUMMARY

In 2022, we continued our efforts in developing new management strategies for major foliar and fruit diseases of sweet cherry in California including bacterial blast and canker caused by *Pseudomonas syringae* pv. *syringae*, powdery mildew caused by *Podosphaera clandestina*, blossom blights and fruit rots caused by *Monilinia* and *Botrytis* spp., postharvest decays, and Phytophthora root and crown rots.

1) **Bacterial blast and canker**:
   a. Bacterial blast: We cooperated in a study evaluating 13 strains of *Pseudomonas* spp. for their pathogenicity and virulence on Coral cherry. Wide differences in emerging leaf and flower infection were observed.
   b. Bacterial canker: In a study on cv. Coral Champagne, canker size and the amount of gumming was lowest following treatment with Kasumin or a mixture of Kasumin and Mycoshield. The sanitizers Oxidate and Perasan mostly resulted in intermediate efficacy, however, a mixture of Kasumin with Perasan was less effective than Kasumin by itself.
   c. Kasumin is fully registered on cherry in California and the United States since 2018. A registration of oxytetracycline on sweet cherry is with support of the registrant through the IR-4 program has been pending at EPA for several years. Based on our latest communications, the new PRIA date is March 2023 (Note: Unlike kasugamycin, oxytetracycline is used in human medicine and as such, its registration is controversial).

2) In a **powdery mildew** study in San Joaquin Co., Sercadis, Fontelis, Luna Experience, Luna Sensation, Miravis Duo, Miravis Prime, and GF5003 had the lowest levels of disease, and these fungicides were also among the most effective in previous years. They contain active ingredients of DMI, SDHI, and/or QoI compounds which are known to have high activity against powdery mildews. The biologicals ProBlad and Serifel significantly reduced the disease only on outside shoots, and Gargoil did not show any efficacy. Proquinazid, a powdery mildew-specific fungicide, was accepted into the IR-4 program.

3) A field study was conducted on the efficacy of **fungicide treatments applied 6 days preharvest**.
   a. **Brown rot**: All preharvest-applied conventional fungicides significantly reduced the incidence of brown rot on non-washed and washed fruit after non-would-inoculation with *M. fructicola* to very low levels. The biological treatments Problad, Gargoil, and Serifel showed very little or no activity in protecting fruit. In wound-inoculations, only treatments containing a FC 3 compound significantly reduced decay, and the most effective treatments included Cevya, Regev, Mibelya, and Miravis NXT.

b. **Gray mold** again was much more difficult to manage. On non-washed fruit, only Merivon, Miravis NXT, and GF5003 effectively reduced the incidence of decay on wound- and non-wound inoculated fruit. On washed fruit, Miravis Prime was the most effective treatment, but only after non-wound inoculation.

5) **Postharvest studies** focused on the evaluation of new biologicals in laboratory studies and on the efficacy of ozone in a commercial packinghouse study.
   a. Biofungicides (i.e., natamycin, polyoxin-D) and conventional fungicides (e.g., Chairman, Mibelya, Miravis NXT, and GF5003) were evaluated. Spray applications with natamycin were equivalent in efficacy or slightly less effective than conventional fungicides against brown rot, gray mold, and Rhizopus rot.
   b. The natamycin formulations BioSpectra and Cerafruta were certified by OMRI for organic postharvest use in the United States.
   c. Ozonated water used in a hydrocooler or in a T-jet was ineffective against brown rot and gray mold when wound-inoculated fruit were treated. For non-wound-inoculated fruit, a significant reduction in brown rot was shown for both application methods. The T-Jet application, however, was more effective than the hydrocooler application of ozone. In comparison, T-Jet applications with tebuconazole provided nearly 100% control of both decays.

6) Field and greenhouse studies were conducted on the evaluation of new fungicides for management of **Phytophthora root and crown rot**.
   a. In surveys in two cherry orchards with tree decline, only *Phytopythium vexans* was recovered from roots, soil, and cankers. In trunk inoculation studies, this species was found to cause cankers that were approximately half the size as those caused by *Phytophthora citricola.* Several species of *Phytophthora* were recovered in previous years' surveys. *Ph. vexans* has been reported as a root rot pathogen of several fruit and ornamental tree species, and therefore, this species may also be a pathogen of sweet cherry trees causing tree decline and sometimes death.
   b. In field efficacy studies with inoculated trees, treatments with Orondis, Elumin, Presidio, or Revus significantly improved tree health as compared to the controls, and they were numerically more effective than Ridomil Gold or ProPhyt. Improved tree health ratings in the UC Davis plot were reflected by reduced stem water potential readings indicating that trees were under no or much reduced water stress.
   c. IR-4 residue studies with Orondis are currently ongoing to obtain registration of this fungicide on sweet cherry in the United States. Ethaboxam (Elumin) was accepted into the IR-4 program in 2022.

INTRODUCTION

Management of bacterial blast and canker. Bacterial canker and blast are important diseases of sweet cherry that can impact production in seasons with favorable environmental conditions and can also have long-term effects on tree health. *Pseudomonas syringae* pv. *syringae* (*Pss*) is the main pathogen causing these diseases of sweet cherry and other stone fruit crops in California. Cold, wet conditions favor both phases of the disease. After infection of twig and branch wounds, cankers with gumming around the infected, sunken bark tissue develop after several weeks to months. In contrast, blossom blast develops rapidly after infection, and flowers become dark to black, wilt, and die. Bacterial blast may be confused with brown rot blossom blight and is more commonly found on early-blooming varieties and on trees where rest-breaking treatments are applied and that subsequently bloom earlier and may experience cooler, wet spring environments. Bud death and spots on leaves and developing fruit are additional symptoms of the blast phase of the disease.

Because copper resistance in the pathogen populations is widespread in California, we are looking for potential alternatives. In our previous studies, kasugamycin (Kasumin), an antibiotic that is not used in

animal or human medicine, significantly reduced bacterial blast of sweet cherry and was the only compound that consistently reduced the severity of bacterial canker of inoculated branches. Based on our efforts, Kasumin was registered for management of these diseases of sweet cherry in early 2018. It is important to continue to evaluate its efficacy under different environmental conditions to optimize its use and to identify other copper alternatives that could be used in rotations and mixtures. We are pursuing oxytetracycline (Mycoshield) for registration because is showed good efficacy in our studies. Registration of this antibiotic on sweet cherry has been postponed by EPA until March 2023. Other alternatives we evaluated include the biocontrols Actinovate (fermentation product of *Streptomyces lydicus*) and Blossom Protect/Botector (*Aureobasidium pullulans*), copper-enhancing compounds, inhibitors of the type III bacterial secretion system that has a major role in plant infection, a nano-particle zinc compound, Cinetis, a nutritional stress reducer, and oxidizing sanitizers (e.g., Oxidate). Two GRAS antibacterial food additives (i.e., nisin and ε-poly-L-lysine) showed promising results in some studies. In collaboration with a chemical company, agrochemical formulations have been designed for nisin and ε-poly-L-lysine, and these need to be tested. Additional natural products, such as cinnamon-derived compounds, and biocontrols are becoming increasingly available in recent years for evaluation against bacterial diseases of plants.

Management of powdery mildew, blossom blight, and fruit rot. Powdery mildew of sweet cherry is an ongoing problem for growers in California because warm temperatures with low rainfall and high humidity from dews or irrigation are highly favorable for its development. Flower sepals, leaves, and fruit may be infected. Symptomatic fruit need to be removed during sorting, or the lot will be downgraded. Powdery mildew can also provide entry points for infection of fruit decay organisms. Additionally, powdery mildew is a quarantine disease in some export markets, and fruit for shipment may have to be certified as disease-free. With decreased powdery mildew sensitivity to Quintec, new, highly effective materials such as proquinazid, as well as new combinations and rotations of registered fungicides are being evaluated. Alternative fungicides that we evaluated in previous field trials include the FRAC Code (FC) 3 (DMI) Procure (triflumizole), the FC 7 (SDHI) fluopyram, fluxapyroxad, penthiopyrad, and pyraziflumid, the FC 19 polyoxin-D, as well as pre-mixtures of FC 3/7 (Luna Experience - tebuconazole/fluopyram, Miravis Duo - difenoconazole/ pydiflumetofen, Mibelya, formerly UC-2 - mefentrifluconazole/fluxapyroxad), FC 3/11 (Quadris Top - azoxystrobin/ difenoconazole), FC 7/11 (Luna Sensation - fluopyram/trifloxystrobin, Merivon - fluxapyroxad/ pyraclostrobin), and of FC 7/12 (Miravis Prime - pydiflumetofen/fludioxonil). In 2022, we evaluated some these and biologicals based on plant extracts (FC BM-01; i.e., ProBlad, Gargoil) or microorganisms (FC BM-02, i.e., Serifel) as well as several numbered experimental compounds. New effective compounds need to be identified to obtain new rotation alternatives not only for powdery mildew, but also for other bloom, petal fall, and preharvest diseases. Fungicides and bactericides evaluated in 2022 for management diseases of sweet cherry are listed in Table 1.

For management of brown rot and Botrytis blossom blight and fruit rot of sweet cherry caused by *Monilinia fructicola* and *M. laxa* as well as *Botrytis cinerea,* respectively, in the past, we found selected fungicides belonging to FCs 3, 7, 9, 11, 12, 17, and 19 to be effective. The pre-mixtures Quadris Top, Luna Experience, Pristine, Luna Sensation, and Merivon represent some of the best treatments along with tank mixtures of FC 3 and 7 fungicides. Still, more new fungicides are being developed. They generally belong to the same FCs as previously registered compounds, but their activity against fungal pathogens is often different due to their different affinity to fungal target sites. Thus, the newer FC 7 Miravis (pydiflumetofen) and the FC 3 Cevya (mefentrifluconazole) have extremely high in vitro activities. The new pre-mixture Regev (FC BM-01/3 – tea tree oil/difenoconazole) and several experimentals were included in our evaluations in 2022. Thus, we continued to evaluate the efficacy, spectrum of activity, and persistence of residues of new fungicides and pre-mixtures, as well as the integration of these materials into a comprehensive management program. The preventative and post-infection activity of many fungicides for control of blossom blight was evaluated extensively in previous years, and this has helped to develop our delayed bloom fungicide application model for improved timing of fungicide treatments. Although DMI

fungicides are highly effective against brown rot, they generally have to be complemented with other materials to obtain high efficacy against gray mold.

Management of postharvest fruit decay with postharvest treatments. We are also continuing our efforts to provide effective and economical treatments for management of postharvest fruit decays such as brown rot, gray mold, and Rhizopus rot. Currently, seven postharvest fungicides, Tebucon (tebuconazole, FC 3), Mentor (propiconazole, FC 3), Scholar (fludioxonil, FC 12), Chairman (fludioxonil/propiconazole, FC 3/12), Penbotec (pyrimethanil, FC 9), and the biofungicide natamycin (BioSpectra, Cerafruta, FC 48) are registered on sweet cherry. Judge (fenhexamid) was withdrawn from postharvest use. Natamycin is the first postharvest biofungicide and is exempt from tolerance in the United States. Another biofungicide, an organic formulation of polyoxin-D (i.e., Oso) is planned for registration. These fungicides are effective against brown rot and gray mold, but Penbotec and Oso are not active against Rhizopus rot. The DMI propiconazole (Mentor) is also effective against sour rot, a less common decay on sweet cherry. Chairman has the broadest spectrum of activity with controlling all four decays. Of the compounds registered, Tebucon and Mentor are not 'reduced-risk' fungicides. Scholar, Penbotec, and Mentor received Food Additive Tolerances (FAT) in Japan, and the registrant of BioSpectra has submitted for a FAT. Thus, continued studies on how to use these fungicides most efficiently for the Japanese export market are critical to the industry. Laboratory studies were conducted on the efficacy of new treatments, and in a commercial

packinghouse study the efficacy of ozone as a hydrocooler or T-Jet treatment was compared to a T-Jet treatment with Tebucon.

Table 1: Fungicides and bactericides used in 2022 studies*.

FRAC Code	Trade name	Active ingredient
Oxidizer		
	Perasan	peroxyacetic acid
Single active ingredients		
3	Cevya	mefentrifluconazole
4	Ridomil Gold	mefenoxam
7	Fontelis	penthiopyrad
7	Sercadis	fluxapyroxad
19	Ph-D, Oso	polyoxin-D
22	Elumin	ethaboxam
24	Kasumin	kasugamycin
40	Revus	mandipropamid
41	Mycoshield	oxytetracycline
43	Presidio	fluopicolide
48	BioSpectra	natamycin
49	Orondis	oxathiapiprolin
P07 (33)	ProPhyt	potassium phosphite
Experimentals	G296	not disclosed
	GF4536	not disclosed
	GF 4536 + Fontelis	not disclosed + penthiopyrad
	GF 5003	not disclosed
Biologicals		
BM-01	Gargoil	cinnamon oil and garlic
BM-01	Guarda	thyme oil
BM-01	ProBlad	extract of *Lupinus albus*
BM-01	Timorex ACT (TACT)	tea tree oil
BM-02	Serifel	*Bacillus amyloliquefaciens* strain MBI600
Premixtures		
3 + BM-01	Regev	difenoconazole + tea tree oil
3 + 7	Mibelya	mefentrifluconazole + fluxapyroxad
3 + 7 + 11	Miravis NXT	difenoconazole + pydiflumetofen + azoxystrobin
3 + 12	Chairman	propiconazole + fludioxonil
7 + 3	Miravis Duo	pydiflumetofen + difenoconazole
7 + 3	Luna Experience	fluopyram + tebuconazole
7 + 11	Luna Sensation	fluopyram + trifloxystrobin
7 + 11	Merivon	fluxapyroxad + pyraclostrobin
7 + 12	Miravis Prime	pydiflumetofen + fludioxonil
49 + 40	Orondis Ultra	oxathiapiprolin + mandipropamid

* - Only those chemicals are included in the table where efficacy data were obtained, and they are sorted by Fungicide Resistance Action Committee (FRAC) code or mode of action. Some treatments were used with adjuvants such as DyneAmic.

Etiology and Management of Phytophthora root and crown rot. P. cambivora is considered a major pathogen of Phytophthora root and crown diseases of sweet cherry in California, however, no extensive surveys on the causal pathogens have been conducted since the 1980s. With current increasing reports of

cherry trees declining from apparent *Phytophthora* spp. infection, research is warranted to identify the species. Surveys in several orchards in the main cherry growing regions in California were initiated in 2021 and continued in 2022 where isolations were conducted from roots, crowns, and rhizosphere soil.

We have identified several new fungicides with different modes of action for managing Phytophthora root and crown rot diseases of tree fruit crops. Oxathiapiprolin (FC 49), mandipropamid (FC 40), and fluopicolide (FC 43) are now registered on citrus, and we are seeking registration on cherry and almond with the registrants (i.e., Syngenta and Valent). Other compounds such as ethaboxam (FC 22) and picarbutrazox (FC U17) can also be evaluated. We are determining the in vitro toxicity of these new fungicides to isolates of *Phytophthora* spp. from cherry. We established two field trials each at UC Davis and UC Riverside (4 total) where trees were inoculated, and preliminary data were obtained on the efficacy of the new treatments. In collaboration with growers, field plots were also initiated in naturally infested orchards. Our goal is to develop efficacy data for the new compounds so they can be made available to the sweet cherry industry and to identify best treatment strategies. This will allow for the development of resistance management programs with rotation and mixtures of different fungicides. Additionally, in greenhouse studies with Mahaleb, Mazzard, and Krymsk rootstocks, we demonstrated systemic properties of Presidio and Orondis as well as ProPhyt and Ridomil Gold after soil treatment. This will benefit treatment efficacy because roots and crowns are protected from infection for extended periods.

Objectives:

1. Evaluate new products against bacterial blast in flower inoculation studies and against canker in twig inoculation studies.
   a. Biologicals/natural products (e.g., Blossom Protect, new formulations of nisin and ε-poly-L-lysine, as well as essential oils without and with selected adjuvants): small-scale field trials.
   b. Antibiotics – kasugamycin, oxytetracycline: small- and large-scale trials under favorable environments and trials to improve penetration into plant tissue.
2. Evaluate under field conditions bloom and preharvest applications of new compounds: Cevya, pyraziflumid, new premixtures (Miravis Top, Miravis Prime, UC-2-Mibelya), and biologicals for control of brown rot and Botrytis blossom blight, powdery mildew, and preharvest brown rot and gray mold fruit decay.
3. Evaluate new fungicides as postharvest treatments:
   a. Continue to evaluate Chairman and support Scholar-natamycin mixtures for approved or pending food additive tolerance (FAT) in Japan, respectively.
   b. Continue to monitor for postharvest fungicide resistance in target pathogen populations.
   c. Continue to evaluate new (e.g., Yarden) and 'exempt from tolerance' biofungicides (natamycin and polyoxin-D), organic (e.g., polyoxin-D, Oso) or nominated for organic compounds (e.g., natamycin), and new biological treatments including biocontrols and natural products.
4. Evaluate new fungicides for managing Phytophthora root and crown rot of cherry
   a. Conduct surveys on causal agents of cherry trees declining from apparent *Phytophthora* spp. infection
      ii. Collect root, crown, and rhizosphere soil from declining cherry trees and isolate the causal pathogens by direct plating or pear baiting and identify pathogens based on morphology and DNA sequencing
   b. Establish in vitro baseline sensitivities to oxathiapiprolin, mandipropamid, fluopicolide, and ethaboxam (and possibly others like picarbutrazox) for collected isolates of *Phytophthora* spp. This data will be used for future reference in detecting potential resistance in the pathogen.
   c. Conduct studies in experimental orchards at UC Davis and UC Riverside, in commercial orchards in collaboration with growers, and in the greenhouse to evaluate the new fungicides for the management of Phytophthora root and crown rot.

i. Inoculate soil in experimental orchards and use commercial orchards with natural pathogen populations and apply fungicides to the soil and trunk. Soil applications will be done to dry and pre-wetted soil.
ii. Conduct greenhouse studies with selected cherry rootstocks to characterize fungicide mobility inside plants and compare virulence of *Phytophthora* spp.
iii. Support registration of mandipropamid for use in container greenhouse trees during propagation.

MATERIALS AND METHODS

Evaluation of new products against bacterial canker in twig inoculation studies and comparative evaluation of pathogenicity of* Pseudomonas *sp. strains on leaves and flowers. In a study to evaluate antibiotics and oxidizing sanitizers against bacterial canker, 1- to 2-year-old branches of cv. Coral cherry trees were puncture-wounded laterally in Dec. 2021 using a nail to expose the cambium and wood (3 wounds/branch). Wounds were spray-treated and inoculated with *Pss* (approximately 5 x 10^7 cfu/ml) after air-drying. Branches were evaluated for gumming and canker formation in late-April 2022. Thirteen strains of *Pseudomonas* spp. obtained from cankers of *Prunus* spp. were compared for their pathogenicity on sweet cherry in cooperation with F. Trouillas and M. Nouri. Partially emasculated flowers (pistils, stamens, and part of the petals were cut off using scissors) and newly emerging injured leaves (the tips were cut off with scissors) of cv. Coral were spray-inoculated with bacterial suspensions on 3-9-22, bagged overnight, and evaluated for disease on 3-23-22.

Evaluation of new fungicides for control of powdery mildew. In a field trial in San Joaquin Co., treatments were done on 3-15 (50% bloom), 4-6, and 5-11-22. These applications were targeted to provide protection from primary ascospore inoculum from overwintering chasmothecia and from infection by secondary conidial inoculum. Biological treatments, single fungicides, pre-mixtures, and three numbered experimentals were evaluated (see Fig. 3). The incidence of powdery mildew was evaluated on 5-22-22 and leaves from 4 random shoots each from inside and outside of the tree were rated using the following scale: 0=healthy, 1 = 1-3 lesions, 2 = <25%, 3 = up to 50%, and 4 = >50% of leaf area diseased. Data were analyzed using analysis of variance and mean separation procedures of SAS 9.4.

Evaluation of new fungicides for control of brown rot and Botrytis fruit decay. Preharvest fungicide applications for control of fruit decay were evaluated in a commercial orchard in San Joaquin Co. Treatments were applied 6 days PHI using a back-pack sprayer calibrated to deliver 100 gal/A. Fruit (8 fruit from each of three or four single-tree replication) were harvested, wounded with a glass rod (1 x 1 x 0.5 mm), and inoculated with 20 µl of a conidial suspension of *M. fructicola* (50,000 spores/ml) or *B. cinerea* (30,000 spores/ml) or 16 fruit from each replication each were non-wound drop-inoculated with *M. fructicola* (50,000 spores/ml) or *B. cinerea* (300,000 spores/ml in 50% cherry juice). Some fruit were postharvest washed before inoculation, and this was done by gently agitating fruit in running water for 2 min. Fruit were incubated for 5-10 days at 20-24 C, >95% RH. Percent incidence of infection was determined as the number of fruit infected of the total number of fruit evaluated. Data were analyzed as described above.

Efficacy of new and registered postharvest treatments for managing brown rot, gray mold, and Rhizopus rot of sweet cherry. One focus of our postharvest studies was the evaluation of ozone treatments that are sometimes being advertised as effective alternatives to postharvest fungicide treatments. Fruit were wound- or non-wound inoculated as described above and treated in a commercial packinghouse with 5.1 ppm ozone by T-Jet or in a hydrocooler for 3 min. For comparison, some fruit were treated with a T-Jet application of Tebucon. Fruit were then incubated for 7 days at 20C and evaluated for decay development.

In laboratory studies, Chairman, BioSpectra, and Oso were compared to several new fungicides that potentially could be registered as postharvest treatment (*see* Fig. 7). Fruit were wound-inoculated with *M. fructicola, B. cinerea,* or *R. stolonifer* as described above and treated after 12-13 h by spraying using an air-nozzle sprayer. After treatment, fruit were incubated for 4-7 days at 20 C, >95% RH. Incidence of decay in

these studies was determined as the number of infected fruit of the total number of fruit evaluated. Data were analyzed using analysis of variance procedures of SAS 9.4.

Determine* Phytophthora *spp. currently affecting tree health in California cherry orchards. Two orchards with declining cherry trees suspect of Phytophthora infection were brought to our attention by growers and we collected roots, crown cankers, and rhizosphere soil from 15 trees from each location. Symptomatic root pieces were plated onto selective medium (PARHFB; V8C agar amended with antibiotics, pimaricin, hymexazol, fludioxonil, and benomyl; agar medium without the addition of hymexazol was also used because some species of *Phytophthora* are inhibited by this compound). For pathogen detection in soil, pear baiting was done. For this, each sample was immersed in 400 to 500 ml deionized water in 1-liter plastic bags, and one mature 'D'Anjou' pear was placed into each bag. The bags were incubated at 20°C for up to 4 weeks. Internal tissue from the margin of brown, firm pear decay lesions was plated onto PARHFB-V8C. Representative colonies from the different isolations were sub-cultured and verified for species identity using morphological characteristics and species-specific TaqMan qPCR (Hao et al. 2018). ITS and Cox sequences were obtained from isolates that could not be identified this way, and sequences were submitted to a BLAST search.

The pathogenicity of *Phytopythium vexans* in comparison to *P. citricola* was evaluated on potted Mahaleb plants in the greenhouse. Trunks were wounded using a cork borer, the bark inside the wound was removed, a colonized agar plug was placed on the wound, and the inoculation site was wrapped with parafilm. Parafilm was removed after one week, and canker lengths under the bark were measured after two additional weeks. Data were analyzed using analysis of variance procedures of SAS 9.4.

Evaluate new fungicides for managing Phytophthora root rot and crown rot. Field studies on the evaluation of new fungicides were done in three orchards with Coral scions on Mahaleb rootstock, including the second orchard at UC Davis (planted in Jan. 2021), and two orchards at UC Riverside (planted in Jan. 2021 and May 2022), as well as in two commercial orchards with young trees. Treatments (shown in Fig. 9) at the first UC Riverside plot were done to wet soil in May and Oct. 2021 followed by soil inoculation at the base of the trunks with a mixture of *P. cactorum, P. cambivora,* and *P. citricola*. Tree health was rated in Aug. 2022 using a scale from 0-4 with 0 = full canopy, no gumming, 2 = reduced canopy and/or partial gumming, and 4 = tree dead. Phytotoxic was rated using a scale from 0 (= healthy leaves) to 4 (= severe marginal necrosis on the majority of leaves). The causal agent of two dying trees was determined by isolation on agar media. Treatments and inoculation of the second UC Riverside plot were done in fall 2022.

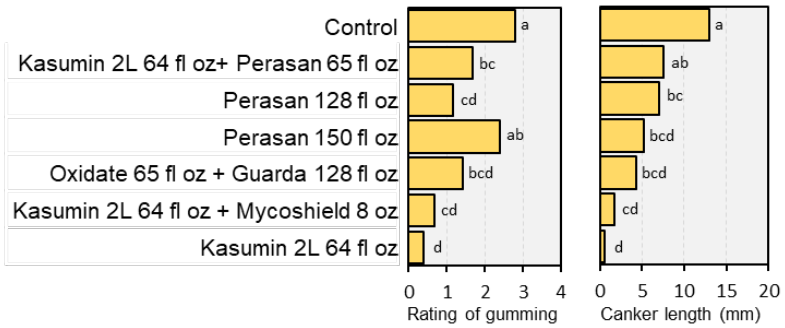
At UC Davis, treatments (shown in Fig. 10) were applied to wet soil Sept 2021 and April 2022 around the tree trunks and were watered in. Trees were inoculated one week after the first treatment and in Oct. 2022. The inoculum of a mixture of *P. citricola, P. cactorum*, and *P. cambivora* was buried next to the injured tree crown. Trees were evaluated for health in Sept. 2022 using a scale from 0 = no canker and no gumming to 3 = extensive canker development and gumming; two control trees were dead and were given a rating of 4. Leaf stem water potential measurements were done in July one day after trees were irrigated. Midday stem water potentials for fully irrigated trees based on current temperature and relative humidity were subtracted from the measurements. Higher resulting values indicate that the trees were water-stressed, whereas low values indicate that trees were able to obtain their full water needs. Data were analyzed using analysis of variance procedures of SAS 9.4.

RESULTS AND DISCUSSION

Evaluation of treatments for control of bacterial blast and canker. A trial on bacterial canker focused on the use of antibiotics and oxidizing sanitizers. Among treatments evaluated, canker size and the amount of gumming was lowest following treatment with Kasumin (Fig. 1). A mixture of Kasumin and Mycoshield was numerically the second most effective treatment. The sanitizers Oxidate and Perasan mostly resulted

in intermediate efficacy, however, a mixture of Kasumin with Perasan was less effective than Kasumin by itself. The high efficacy of Kasumin confirms our previous years' data where the antibiotic was also very effective in reducing the incidence of bacterial blast. Mycoshield also often showed good results but sometimes was inconsistent. Registration of Mycoshield is currently pursued with support of the registrant through the IR-4 program. The PRIA date was postponed several times by EPA, and our latest communications indicate that EPA changed the PRIA date to March 2023. Because oxytetracycline is used in human medicine, new registrations on plants such as cherry are controversial and difficult. Oxidizing sanitizers showed some efficacy in our study in 2022, and these treatments could be helpful in reducing epiphytic inoculum on cherry trees before infection events. They have, however, no residual activity. Because inoculum populations will rapidly build up again, treatments will need to be applied before each predicted infection period.

Fig. 1. Evaluation of antibacterial treatments for protection of cv. Coral branches from bacterial canker - 2022



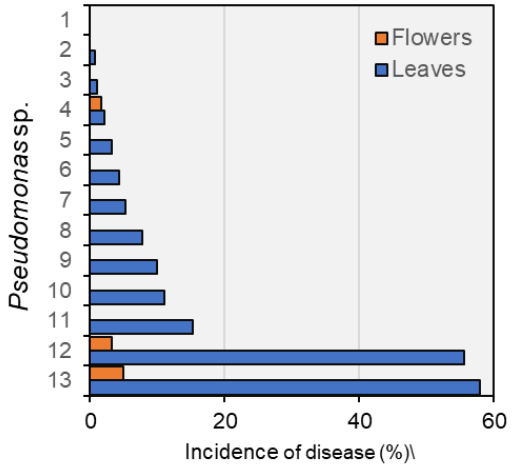
On 12-15-21, 1- to 2-year-old branches were wounded (3 wounds/branch; 2 mm deep, 2 mm in diameter) on the tree, sprayed with selected treatments using a hand sprayer, and spray-inoculated with *Pseudomonas syringae* (4 x 10^7 cfu/ml) after air-drying (TI). For the treatment using Perasan at 128 ppm, wounds were first inoculated and then treated (IT) after air-drying. Disease was evaluated on 4-26-22. Gumming of wounds was rated using a scale from 0 = no gum to 4 = extensive gumming. Canker length was measured after removing the bark.

In a collaborative study with F. Trouillas and M. Nouri comparing pathogenicity and virulence of 13 strains of *Pseudomonas* spp. from blasted flowers or cankers of *Prunus* spp. in California, disease incidence on newly emerging leaves varied widely. Some strains caused no or little disease, whereas for two strains an incidence of nearly 60% developed. Only low levels of disease developed on flowers, however, the two strains with the highest leaf blast incidence also caused the most disease on flowers (Fig. 2). The wide range of virulence among strains of *Pseudomonas* indicates that the risk of blast cannot be predicted based on inoculum population sizes. Future work by Trouillas and Nouri that we will

cooperate with will focus on determining the genetic relatedness of these strains and if they possibly belong to different species.

Evaluation of new fungicides for control of powdery mildew of sweet cherry. Our epidemiological studies have shown that mildew sequentially develops on 1) leaves of inside shoots (water sprouts); 2) leaves of outer shoots; 3) green stems of fruit; and 4) on ripening fruit (fruit with color). The disease has not been found on epi- or mesocarp tissues of green fruit, and young leaves are more susceptible than old leaves. The efficacy of registered and new fungicides (i.e., Cevya, Sercadis, Miravis Duo, Miravis Prime, Mibelya, and several numbered compounds (i.e., GF4536, GF5003, G296), as well as of several biological compounds based on plant extracts (i.e., Gargoil, ProBlad) or bacteria (i.e., Serifel) was evaluated in a trial in San Joaquin Co. in a three-application program starting at 50% bloom. Environmental conditions were favorable for powdery mildew development at our trial site in the spring

Fig. 2. Comparative evaluation of pathogenicity of 13 strains of *Pseudomonas* sp. on leaves and flowers of cv. Coral cherry 2022

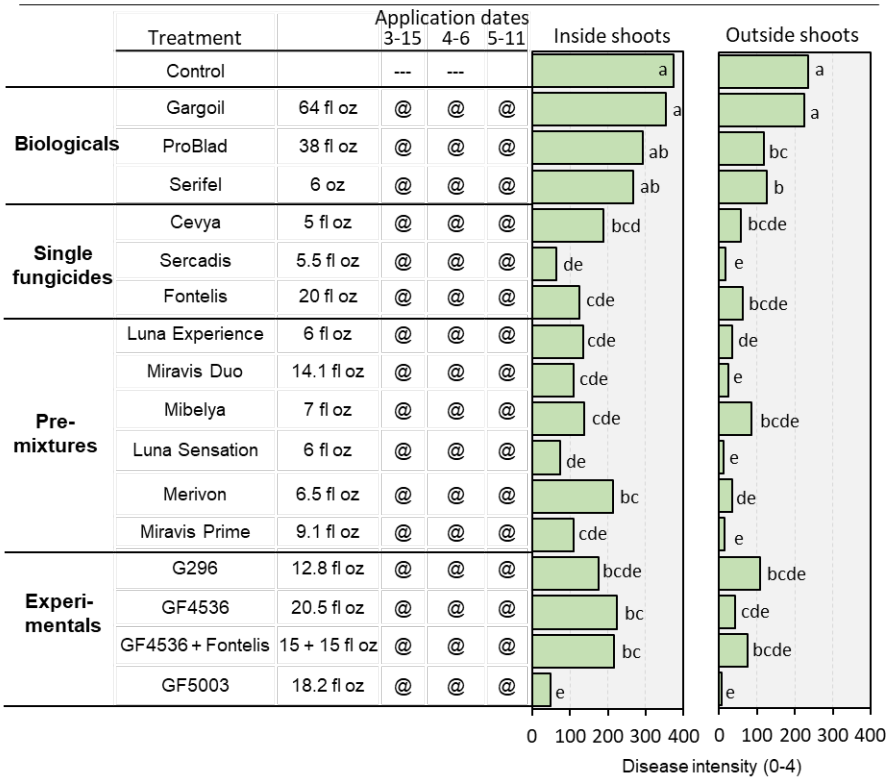


Done in cooperation with F. Trouillas and M. Nouri

Partially emasculated flowers and newly emerging injured leaves were spray-inoculated with *Pseudomonas* sp. suspensions on 3-9-22, bagged overnight, and evaluated for disease on 3-23-22.

of 2022. At evaluation in early June, all evaluated leaves on control trees were diseased, but disease severity on shoots inside the canopy was higher than on outside shoots.

Fig. 3. Evaluation of preharvest fungicide treatments for management of powdery mildew of Bing cherries in San Joaquin Co. 2022



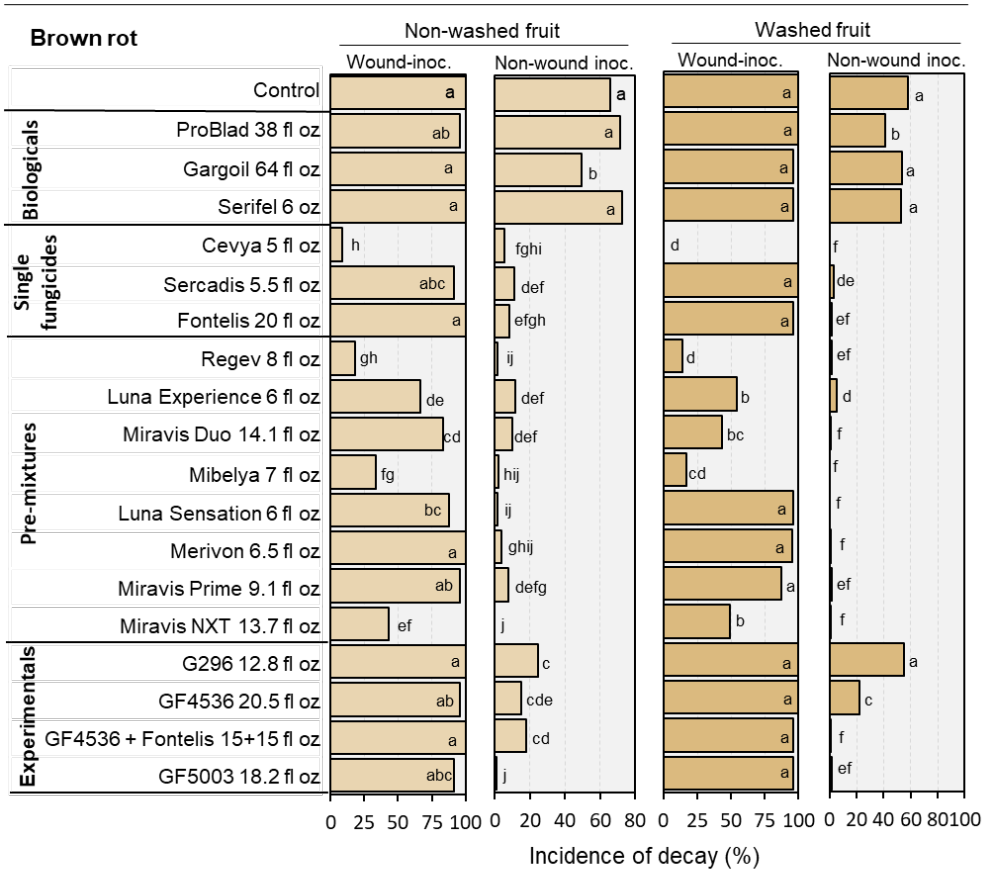
Applications were done using an airblast sprayer at 100 gal/A starting at 50% bloom. DyneAmic (6 fl oz /A) was added to treatments in the second and third applications. For evaluation on 5-22-22, leaves from 4 random shoots each from inside or outside of the tree were sampled and rated using a scale from 0=healthy to 4 = >50% of leaf area diseased. Incidence was calculated based on the number of infected leaves of the total number of leaves evaluated. Disease intensity is the multiplication product of disease incidence and severity.

All fungicides significantly reduced the disease as compared with the control on outside and inside shoots (Fig. 3). Numerically, Sercadis, Fontelis, Luna Experience, Luna Sensation, Miravis Duo, Miravis Prime, and GF5003 had the lowest levels of disease on both types of shoots, and these fungicides were also among the most effective in previous years. They contain active ingredients of DMI, SDHI, and/or QoI compounds which are known to have high activity against powdery mildews. Thus, several FCs can be used in rotations (as we demonstrated in previous years) as an anti-resistance strategy. The biological treatments, ProBlad and Serifel only significantly reduced the disease on outside shoots, and Gargoil was not effective on both types of shoots. Additionally, the new powdery mildew-specific fungicide proquinazid was nominated and accepted into the IR-4 program in 2022.

Our research demonstrates excellent activity of several registered and experimental compounds against powdery mildew. We show that the disease can be reduced to acceptable levels by properly timed applications. Because of the potential of resistance to single-site mode of action fungicides, pre-mixtures or tank mixtures of FC 3, 7, 11, and 19 fungicides will be most sustainable. This limits the use of any single-site mode of action fungicide (i.e., single FCs) and reduces the pressure for selecting for fungicide resistance. Limiting the number of applications of any one mode of action (i.e., FC) will also reduce the residue and ensure that MRLs are not exceeded with any of the trade partners of the cherry industry. Under conditions where fungicides need to be used as post-infection treatments when visible symptoms

are already present on fruit, we showed previously that Ph-D can be used with a multi-site fungicide like Kaligreen or with FC 3 fungicides like Procure for effective suppression of the disease.

Fig. 4. Efficacy of 6-day preharvest fungicide treatments for management of postharvest brown rot of Bing cherries - San Joaquin Co. 2022



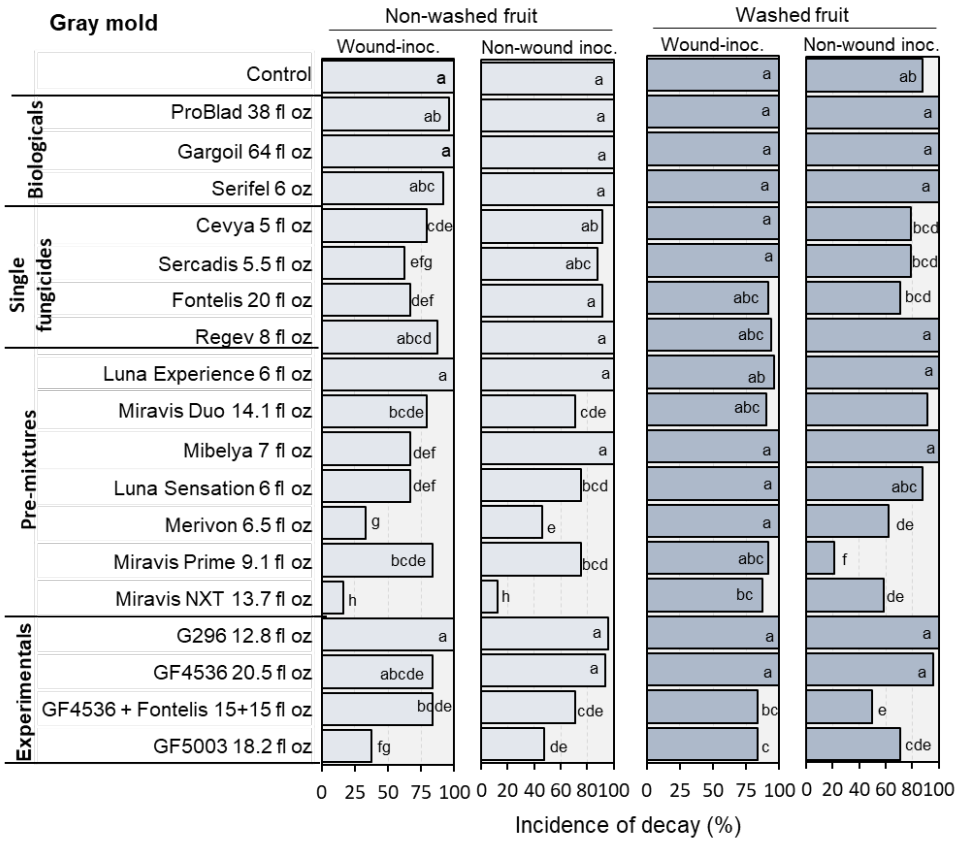
Treatments were applied on 5-11-22 using an air-blast sprayer at a rate of 100 gal/A, and all except Regev were done in combination with DynAmic at 8 fl oz/A. Treatments were also applied on 3-15 and 4-6-22 as part of a powdery mildew program, except for Regev and Miravis NXT that were only applied on 5-11-22. Harvested fruit were washed by gently agitating in water for 2 min. Fruit were wound-inoculated with *M. fructicola* (50,000 spores/ml) or non-wound drop-inoculated (50,000 spores/ml). Fruit were then incubated for 5-10 days at 22C.

Evaluation of preharvest treatments for management of fruit decays. Preharvest treatments were applied 6 days PHI in a commercial cv. Bing orchard. Fruit were not washed or were washed before inoculation. This was done to evaluate the fungicides' persistence after a postharvest hydrocooler treatment. Persistence is important in an additive integrated pre- and postharvest program for decay control or if no postharvest fungicide treatment can be done. Harvested fruit were wound- or non-wound inoculated with *M. fructicola* or *B. cinerea*.

All preharvest-applied conventional fungicides (except the experimental G296) significantly reduced the incidence of brown rot fruit decay on non-washed and washed fruit after non-would-inoculation with *M. fructicola* to very low levels, but the biological treatments Problad, Gargoil, and Serifel showed very little or no activity (Fig. 4). In wound-inoculations, only treatments containing a FC 3 compound significantly reduced decay as compared to the control on non-washed and washed fruit, and the most effective treatments included Cevya, Regev, Mibelya, and Miravis NXT. Thus, as established previously, fungicides that contain a FC 3 compound can effectively prevent brown rot decay that is initiated in wounds occurring after treatment, and they retain their high efficacy after postharvest washing that simulates a hydrocooler treatment. Gray mold again was much more difficult to manage. On non-washed

fruit, only Merivon, Miravis NXT, and GF5003 effectively reduced the incidence of decay on wound- and non-wound inoculated fruit (Fig. 5). On washed fruit, Miravis Prime was the most effective treatment, but only after non-wound inoculation.

Fig. 5 Efficacy of 6-day preharvest fungicide treatments for management of postharvest gray mold of Bing cherries - San Joaquin Co. 2022

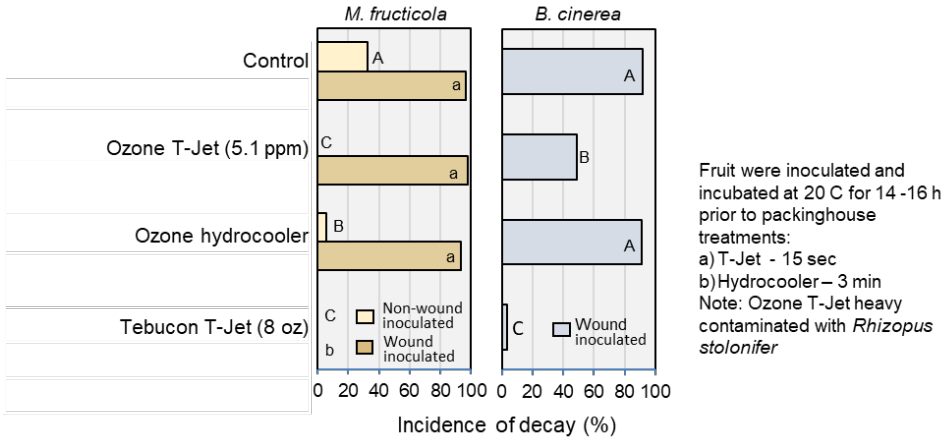


Treatments were applied on 5-11-22 using an air-blast sprayer at a rate of 100 gal/A, and all except Regev were done in combination with DynAmic at 8 fl oz/A. Treatments were also applied on 3-15 and 4-6-22 as part of a powdery mildew program, except for Regev and Miravis NXT that were only applied on 5-11-22. Harvested fruit were washed by gently agitating in water for 2 min. Fruit were wound-inoculated with *B. cinerea* (30,000 spores/ml) or non-wound drop-inoculated (300,000 spores/ml 50% cherry juice). Fruit were then incubated for 5-10 days at 22C.

Our studies demonstrate that preharvest treatments with a range of conventional fungicides can effectively protect fruit from infections before and during harvest when inoculum of *Monilinia* and *Botrytis* spp. is dispersed to the non-wounded fruit surface or when pre-existing wounds are treated. When wounds occur after treatments and are then contaminated with inoculum, the new Cevya was found to be highly effective in preventing brown rot decay, but several other treatments containing a DMI fungicide such as Miravis Duo, Quadris Top, Regev, and Mibelya were also very effective and apparently penetrate into the fruit where they are present at high enough amounts to stop fungal development. Postharvest decays, however, can still develop due to injuries occurring during bulk handling of fruit if the fungicides lack local systemic action. Additionally, hydrocooling will remove residues of many fungicides from fruit although in this year's and previous studies that we conducted, the efficacy of Cevya, Quash, Ph-D+Procure, Quadris Top, Miravis Duo, and Mibelya after 2-min washes of fruit was similar to no washing. Our studies also indicate that more effective treatments are becoming available against gray mold that has been always more difficult to manage than brown rot. Our results suggest that Miravis Prime has high potential as an effective brown rot and gray mold preharvest treatment. This fungicide was accepted in 2021 into the IR-4 residue program for registration on sweet cherry and

proquinazid for specific use against powdery mildew was accepted in 2022. Postharvest fungicides are still the most effective strategy to reduce decay to the lowest levels possible for shipping and marketing fruit to distant locations and to minimize claims.

Fig. 6. Evaluation of ozonated water treatments for managing postharvest decays of Bing cherry in a commercial packinghouse study in 2022

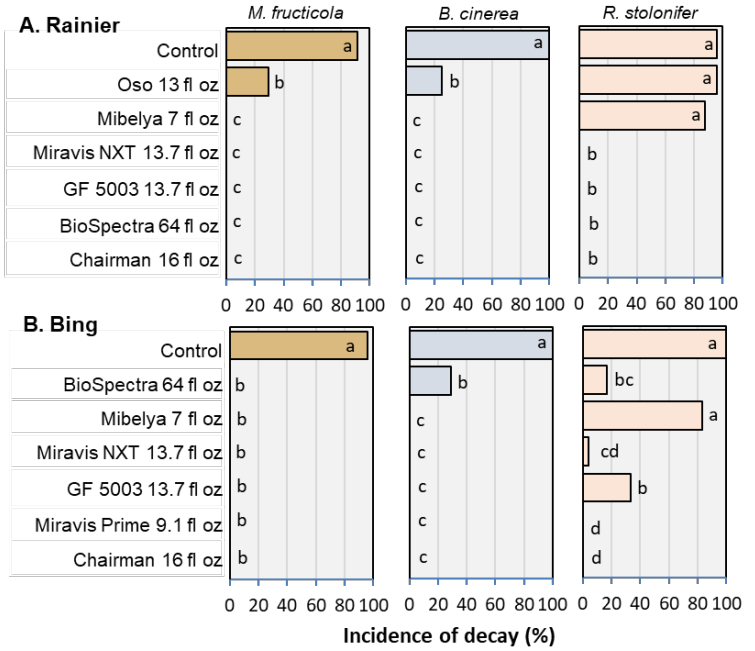


Efficacy of ozonated water for managing brown rot, gray mold, and Rhizopus rot of sweet cherry. In a commercial packinghouse study, Bing cherry fruit were either wound-inoculated or non-wound inoculated with conidia of *M. fructicola* or wound-inoculated with conidia of *B. cinerea.* Fruit were incubated for 14 h prior to treatment with ozonated water in a hydrocooler or as a T-Jet application on a netted belt in a commercial packinghouse. Ozonated water used in a hydrocooler or in a T-jet (measured as 5.1 ppm) was ineffective against both decays when wound-inoculated fruit were treated (Fig. 6). For fruit non-wound-inoculated with *M. fructicola*, a significant reduction in decay was shown for both application methods (T-jet or hydrocooler) of ozonated water as compared to the untreated control. The T-Jet application, however, was more effective than the hydrocooler application of ozone. T-Jet applications with tebuconazole (e.g., Tebucon) that were used for standard comparison provided nearly 100% control of both decays. These studies indicate that ozone is similar to other oxidizers that are ineffective in preventing decay of wounded fruit regardless of application method. The reason for this is that reducing sugars in fruit wounds block the oxidative activity, and the spores in the wounds can survive and cause decay even when treated with ozone. Additionally in these studies, viable *Rhizopus* spores were detected on the netted belt used for T-Jet applications even with continuous exposure to ozone. The packinghouse where the study was done wanted to be entirely organic, however, decay pathogens shortened the storage-ability and marketing of the fruit. A suggestion for organic packinghouses would be to use the OMRI-approved natamycin as a T-Jet application of cherry fruit on the netted belts.

Efficacy of new postharvest treatments for managing brown rot, gray mold, and Rhizopus rot of sweet cherry. Postharvest laboratory studies were performed where treatments were applied to fruit approximately 12-13 h after inoculation. Two studies focused on comparative evaluations of new biofungicide treatments and conventional fungicides that could be developed for postharvest management of cherry decays. Biofungicides included natamycin and polyoxin-D, and conventional fungicides evaluated included Chairman, Mibelya, Miravis NXT, and GF5003 (Fig. 7). Among biofungicides, spray applications of natamycin were equivalent in efficacy to conventional fungicides against brown rot, gray mold, and Rhizopus rot on Rainier and slightly less effective against gray mold and Rhizopus rot on Bing cherry but still significantly reduced decay as compared with the control (Fig. 7). Natamycin (formulated as the commercial products BioSpectra or Cerafruta) was approved for organic use by OMRI in 2022. Polyoxin-D formulated as Oso is organically approved as a preharvest treatment and showed significant reduction of brown rot and gray mold but not Rhizopus rot in our postharvest studies (Fig. 7). We are currently working with the registrant (i.e., Certis) to expand the registration of Oso to include postharvest use so that the cherry industry can have

two active ingredients that are organically approved. Yarden, a mixture of fludioxonil and tea tree oil (evaluated in 2021), and Miravis NXT were highly effective, similar to Scholar and Chairman treatments against all three decays. Mibelya was highly effective against brown rot and gray mold but not Rhizopus rot; whereas GF 5003 was effective against all three decays on Rainier and moderately effective against Rhizopus rot on Bing cherry (Fig. 7).

Fig. 7. Evaluation of new and registered fungicides as postharvest treatments to manage major decays of cherry in laboratory studies in 2022



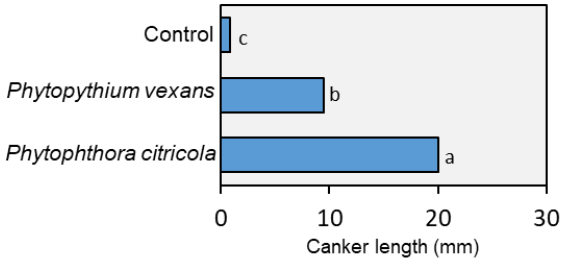
Fruit were wound-inoculated with spores of *M. fructicola* (40,000 spores/ml), *B. cinerea* (30,000 spores/ml), or *R. stolonifer* (30,000 spores/ml) and incubated for 12-13 h at 20C. Aqueous treatments were applied using an air-nozzle sprayer. Fruit were incubated at 20C for 4-7 days.

Our studies indicate that postharvest decays of sweet cherry can be effectively and economically managed using currently registered fungicides that became available through our research (Table 2). MRLs have been established and FATs were approved for most of the compounds, including propiconazole and fludioxonil, the active ingredients of Chairman. Other conventional products with promise include Miravis NXT, GF 5003, and possibly Mibelya. MRLs have not yet been established for natamycin in many international markets. Natamycin is a biofungicide that is 'exempt from residue tolerance' in the United States and in 2022 was approved by OMRI as an organic postharvest treatment. Although limited to domestic markets (including Canada), the registered organic certification is a major step forward for the cherry industry. Additionally, natamycin is an exciting compound because resistance has never been reported in filamentous fungi. Therefore, it can have an important role in reducing the risk of selecting resistant sub-populations of the decay pathogens to other registered postharvest fungicides when mixed with these fungicides. An organic formulation of polyoxin-D is now planned for postharvest registration, and the two fungicides will provide a treatment option for organically grown fruit. Although polyoxin-D is not effective against Rhizopus rot, sanitizing fruit, removal of injured and over-ripe fruit, and cold-temperature management can prevent Rhizopus rot from causing postharvest crop losses. With increasing emphasis on food safety and consumer concerns, natamycin and polyoxin-D with 'exempt from tolerance'

status and OMRI certification may become important components of postharvest decay management in the future once CODEX accepts this US biopesticide classification and MRLs are established worldwide.

***Determine* Phytophthora *spp. currently affecting tree health in California cherry orchards*.** Two newly planted orchards with declining cherry trees were surveyed for the presence of the pathogen in the spring of 2022. Trees in one orchard had a high incidence of trunk cankers, whereas in the other orchard, trees showed a general decline, and some trees were dead. The identity of isolated fungi was determined by morphology and DNA sequencing of rDNA and cytochrome c oxidase (cox) genomic regions. No *Phytophthora* spp. were detected in soil, roots, and trunk cankers in both orchards, but *Ph. vexans* (previously *Pythium vexans*) was recovered from cankers or roots from 8 or 9 of the 15 trees sampled at each location, respectively. This species was recovered at high frequency previously in our surveys, and we found it to be pathogenic to cherry in greenhouse studies when trunks of potted trees were wound-inoculated. As shown in Fig. 8, trunk canker length after *Ph. vexans* inoculation was approximately half as compared to inoculation with *P. citricola*. There have been numerous reports in recent years of *Ph. vexans* being a root rot pathogen of several fruit and ornamental tree species, and therefore, this species may also be a pathogen of sweet cherry trees causing tree decline and sometimes death. We will continue our surveys on the causal pathogens of cherry tree decline in 2023.

Fig. 8 Comparative virulence of *Phytopythium vexans* and *Phytophthora citricola* in causing stem cankers of Mahaleb plants in a greenhouse study at UCR 2022



Trunks of potted Mahaleb trees were wound-inoculated with isolates of *P. vexans* or *P. citricola*. Canker lengths were measured after 3 weeks after removing the bark.

Table 2. Postharvest fungicides registered or pending registration on sweet cherry

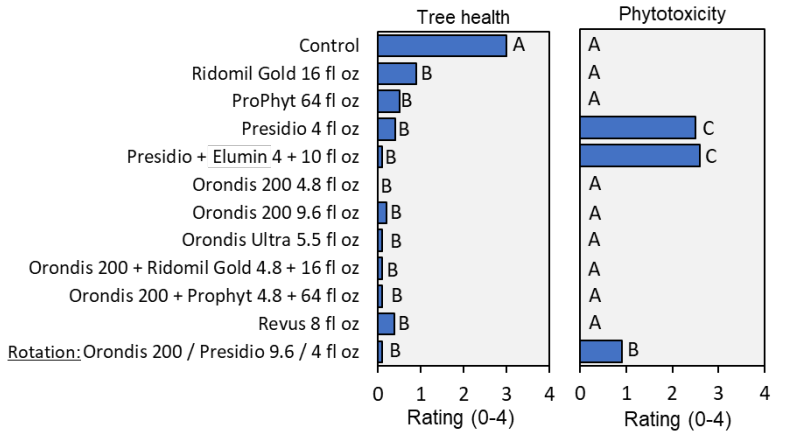
Trade names	Active ingredients	FRAC Code	FAT	Activity against			
				Brown rot	Gray mold	Rhizopus rot	Sour rot
Tebucon, Teb	tebuconazole	3	no	+++	++	++	-
Mentor	propiconazole	3	yes	+++	++	++	+++
Scholar	fludioxonil	12	yes	+++	+++	+++	-
Chairman	fludioxonil/propiconazole	3/12	yes	+++	+++	+++	+++
Penbotec	pyrimethanil	9	yes	+++	+++	-	-
BioSpectra, Cerafruta	natamycin	48	no	++	++	++	+
Oso (pending)	polyoxin-D	19	no	+++	++	-	-

* - Efficacy is rated from +++ (= excellent) to 0 (= no efficacy)

Evaluate new fungicides for managing Phytophthora root rot and crown rot. We previously reported on the in vitro toxicity of four new Oomycota fungicides against four species of *Phytophthora* recovered from sweet cherry as well as *Ph. vexans.* For *Ph. vexans*, we found ranges of sensitivities similar to those for the *Phytophthora* species, and EC_{50} values were very low. *Ph. vexans*, however, was not inhibited by mandipropamid at 40 mg/ml. Therefore, if this species is confirmed as a major cherry root and trunk pathogen, it likely can be managed with selected fungicides. Applications with Orondis were done in two commercial orchards where *Ph. vexans* was detected, and tree health will be evaluated in 2023.

In field studies in experimental orchards on the efficacy of Orondis, Elumin, Presidio, and Revus, trees were inoculated at the wounded crown with a mixture of three *Phytophthora* spp. after the soil around the trunk was treated. At the UC Riverside orchard, tree health that was rated in Aug. 2022 was significantly improved by all treatments applied as compared with the untreated control (Fig. 9). Three of the control trees had died, and *P. citricola* (roots), *P. cactorum* from soil, and *P. cambivora* from trunk cankers were recovered There were no statistical differences among treatments, but numerically, those that included Orondis resulted in the best tree health. Treatments containing Presidio caused some phytotoxicity that was visible as marginal leaf burn. This has never been observed in all our studies using this fungicide in other locations on cherry or almond and possibly is related to the type of soil (i.e., a monserate sandy loam) at UC

Fig. 9. Efficacy of new fungicides for management of Phytophthora root and crown rot of cv. Coral cherry on Mahaleb rootstock in a field trial at UCR 2022



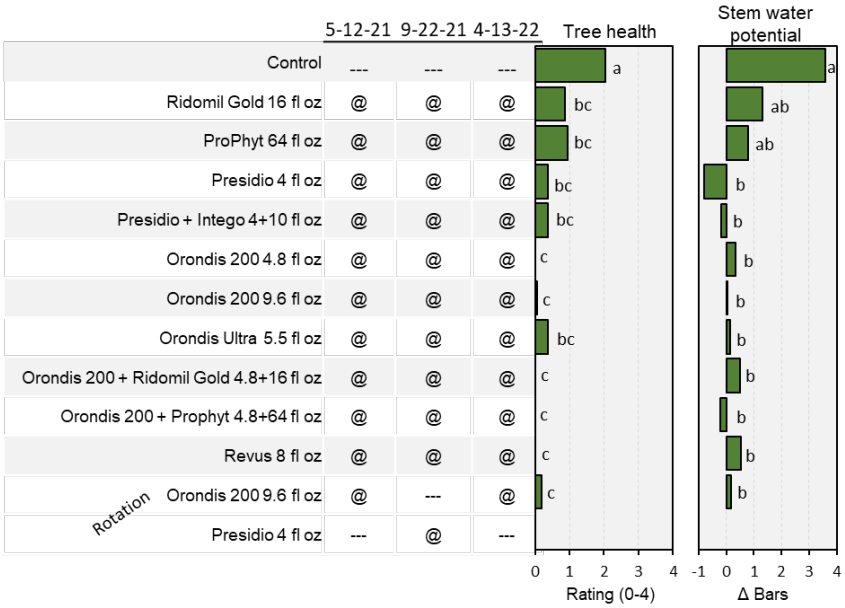
Trees were planted in Jan 2021. Treatments were done to wet soil in May and Oct. 2021 followed by soil inoculation at the base of the trunks with a mixture of *P. cactorum*, *P. cambivora*, and *P. citricola*. Gumming and cankers were first observed in late May 2022 on control trees. Tree health was rated in Aug. 2022 using a scale from 0-4 with 0 = full canopy, no gumming 2 = reduced canopy and/or partial gumming, and 4 = tree dead. Phytotoxicity was rated using a scale from 0 (= healthy leaves) to 4 (= severe marginal necrosis on the majority of leaves).

Leaf marginal chlorosis in Presidio treatment

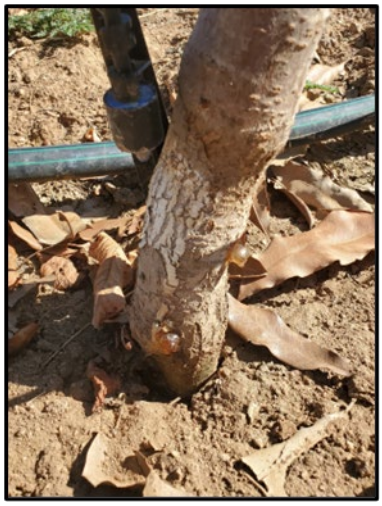
Riverside as opposed to the clay-loam soils at UC Davis and other locations where we saw no phytotoxicity.

In the second UC Davis plot (data for the first plot were reported on previously), trunk cankers from the Oct. 2021 inoculation were first observed in late May 2022. At evaluation time in Sept., two of twelve control trees were dead and *P. cambivora and P. cactorum* were recovered from trunk cankers. *Phytophthora* species were not isolated from roots. Similar results for fungicide efficacy were obtained as in the UC Riverside trial, and any treatment containing the new fungicides resulted in numerically better tree health than treatments with Ridomil Gold or ProPhyt (Fig. 10). Additionally, improved tree health ratings were reflected by stem water potential readings in this plot. Thus, readings were highest for control trees and lowest for trees treated with any of the new fungicides (Fig. 10). This indicates that the latter trees were under no or much reduced water stress. Treatments and evaluation of these orchards is ongoing. Additionally, treatments and inoculations in a second orchard at UC Riverside will be initiated in spring 2023. IR-4 residue studies with Orondis are currently ongoing to obtain registration of this fungicide on sweet cherry in the United States. Ethaboxam (Elumin) was accepted into the IR-4 program in 2022.

Fig. 10. Efficacy of soil-applied fungicide treatments for management of Phytophthora crown and trunk cankers in a field study at UC Davis 2022



Trees were planted in Jan 2021. Treatments were applied as aqueous solutions to wet soil around the tree trunks and were watered in. Trees were inoculated. The inoculum of a mixture of *P. citricola*, *P. cactorum*, and *P. cambivora* was buried next to the injured tree crown. Trunk cankers were first observed in late May 2022. Trees were evaluated for health in Sept. 2022 using a scale from 0 = no canker and no gumming to 3 = extensive canker development and gumming; two control trees were dead and were given a rating of 4. Leaf stem water potential measurements were done in July one day after trees were irrigated. Midday stem water potentials for fully irrigated trees based on current temperature and relative humidity were subtracted from the measurements. Higher resulting values indicate that the trees were water-stressed, whereas low values indicate that trees were able to obtain their full water needs.



Canker gumming of lower Mahaleb roostock on cv. Bing cherry trunk