California Cherry Research Review

Thursday, February 3, 2022

CALIFORNIA CHERRY RESEARCH REVIEW

February 3, 2022

Sponsored by the California Cherry Board and University of California

8:00 **Registration – Only those who RSVP will be able to attend**

8:50 **Welcome**

PRESENTATIONS ON ANNUAL AND FINAL REPORTS

9:00 **The investigation into dormancy breaking agents and the dynamic chill portions model in CA cherries via carbohydrates and solar radiation**
Giulia Marino, Department of Plant Sciences, UC Davis

9:20 **Development of nutrient budget and nutrient demand model for nitrogen management in cherry** | Patrick Brown, Dept. of Plant Sciences, UC Davis

9:40 **Developing a low-cost, low-tech assay for identification of commonly grown sweet cherry varieties in California** | Li Tian, Department of Plant Sciences, UC Davis

10:00 **Cherry whole orchard recycling: investigation into fruiting body viability of cherry fungal pathogens** | Jim Adaskaveg, Professor, Dept. of Plant Pathology, UC Riverside

10:25 **Break**

10:40 **Exploring new and alternative insecticides for resistance management of spotted wing drosophila** | Jhalendra Rijal, UCCE IPM Advisor, Statewide

11:05 **Improving the sanitary status of sweet cherry planting material**
Florent Trouillas, Associate CE Specialists

11:30 **Management and epidemiology of pre- and postharvest diseases of sweet cherry**
Jim Adaskaveg, Professor, Dept. of Plant Pathology, UC Riverside

11:30 **Questions**

12:00 **Lunch**

CDPR and Private-Applicator CE Credits Available

CALIFORNIA CHERRY BOARD (CCB) - RESEARCH BUDGET

April 1, 2021 - March 31, 2022

	Project Leader	Project Title	Status	Project Objectives	2021 Approved Funding
1	Rijal	Exploring new and alternative insecticides for resistance management of spotted wing drosophila	On-going (yrs. 2 of 2)	Year 1: $19,490; Year 2: $30,083; **Obj. 1:** To evaluate insecticide active ingredients against spotted wing drosophila in cherry orchards; **Obj. 2:** To test the impact of Erythritol in SWD behavior and egg-laying activities; **Obj. 3:** To conduct preliminary testing of wild spotted wing drosophila flies to detect potential insecticide resistance	$ 30,083
2	Marino	The investigation into dormancy breaking agents and the dynamic chill portions model in CA cherries via carbohydrates and solar radiation	On-going (yrs. 2 of 3)	Year 1: $48,686.20; Year 2: $19,000; Year 3: $19,000; **Obj. 1:** Understand the physiological impact of traditional rest breaking agent applications (hydrogen cyanamide and CAN 17) on NSC seasonal dynamics; **Obj 2:** Develop methods to improve precision and effectiveness of rest breaking agent applications	$ 19,000
3	Brown	Development of Nutrient Budget and Nutrient Demand Model for Nitrogen Management in Cherry	On-going (yrs. 2 of 3)	Year 1: $50,000; Year 2: $50,000 = $100,000; **Obj. 1:** Develop nutrient demand curves to guide the quantity and time of fertilizer application in cherry: repeat for most representative cultivars and production systems; **Obj. 2:** Develop and extend nutrient Best Management Practices (BMP) for cherry cultivars.	$ 50,000
4	Adaskaveg	Management and epidemiology of pre- and postharvest diseases of sweet cherry	On-going (yrs. 2 of 4)	Year 1: $57,000; Year 2: $57,000; Year 3: 59,346; Year 4: $61,049; **Obj. 1:** Evaluate new products against bacterial blast in flower inoculation studies and against canker in twig inoculation studies; **Obj. 2:** Evaluate under field conditions bloom and preharvest applications of new compounds; **Obj. 3:** Evaluate new fungicides as postharvest treatments; **Obj. 4:** Evaluate new fungicides for managing Phytophthora root and crown rot of cherry.	$ 57,000
5	Trouillas	Improving the sanitary status of sweet cherry planting material	New (yrs. 1 of 2)	Year 1: $59,816; Year 2: $56,160; **Obj. 1:** Determine the critical stages of fungal pathogen infection and contamination sources during tree production at the nursery; **Obj. 2:** Determine the efficacy of various compounds for the protection of tree wounds (and eventually evaluate the use of root/rootstock/scion soaking in chemical and biocontrol agent solutions before budding/grafting and any production steps involving mechanical wounding of the planting material); **Obj. 3:** Investigat the occurence of X-Disease Phytoplasma and Little Cherry Viruses in cherry propagation materials; **Obj. 4:** Promote outreach activities and education	$59,816
6	Tian Sudarshana Jiang	Developing a low-cost, low-tech assay for identification of commonly grown sweet cherry varieties in California	New (yrs. 1 of 1)	**Obj. 1:** To identify molecular markers that differ among commonly grown sweet cherry varieties in California; **Obj. 2:** To establish and validate a low-cost, low-tech assay for identification of commonly grown sweet cherry varieties in California	$ 51,200
7	Nouri	Cherry whole orchard recycling: investigating fruiting bodies viability of cherry fungal pathogens	New (yrs. 1 of 1)	**Obj. 1:** Whole cherry orchard recycling survey; **Obj. 2:** To investigate the viability of fungal pathogen survival in fruiting bodies still present in wood chips incorporated during orchard recycling; **Obj. 3:** To verify if grinding or chipping and incorporating the diseased branches with cankers between tree rows could increase fungal spore dispersal in the orchard; **Obj. 4:** To examine soil organic matter (SOM) and stored carbon (C) after whole orchard recycling, by conducting soil surveys annually	$ 9,000
8	Follett	Phytosanitary Irradiation using an In-House Cabinet X-ray System for Sweet Cherries	New (yrs. 1 of 1)	Year 1: $15,000; Year 2: $15,000; **Obj. 1:** Evaluate two different prototype x-ray systems in terms of cost, throughput, logistics, and other parameters: a. BUGS II (Applied Energy Systems, Albuquerque, NM); b. LEHP Processing (Rayfesh Foods, Ann Arbor, MI); **Obj. 2:** Demonstrate irradiation treatment of California sweet cherries using both types of equipment by preparing videos and providing samples of irradiated fruit	$ 15,000
	Total Research Funding				**$ 291,099.00**

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Project Title: The investigation into dormancy breaking agents and the dynamic chill portions model in CA cherries via carbohydrates and solar radiation

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, Harleen Dhillon, Katelyn Cooper, Esme Hassell-Thean, Suzuka Kawaguchi, Natalie Espanol

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).

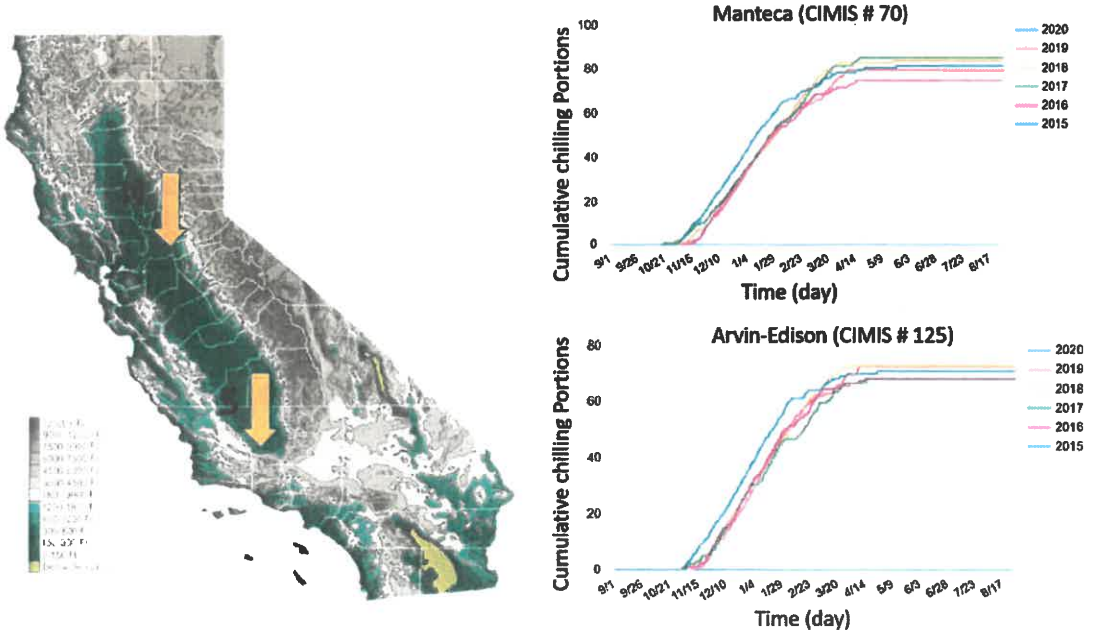

Fig. 1 – Localization of the Northern and Southern experimental sites and chill portion accumulation calculated from Fruit & Nut Research & Information Center for the CIMIS stations of Manteca and Arvin-Edison.

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).

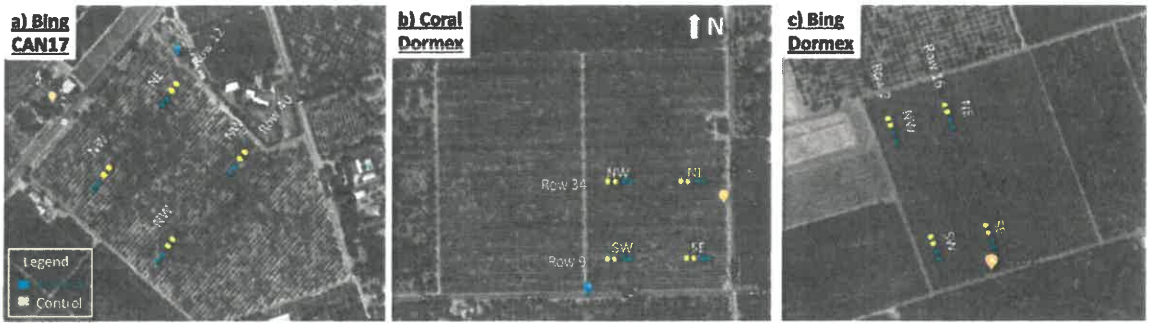

Fig. 2 – Experimental blocks and design in the 3 orchards in the Northern location (SJ county, Linden).

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.

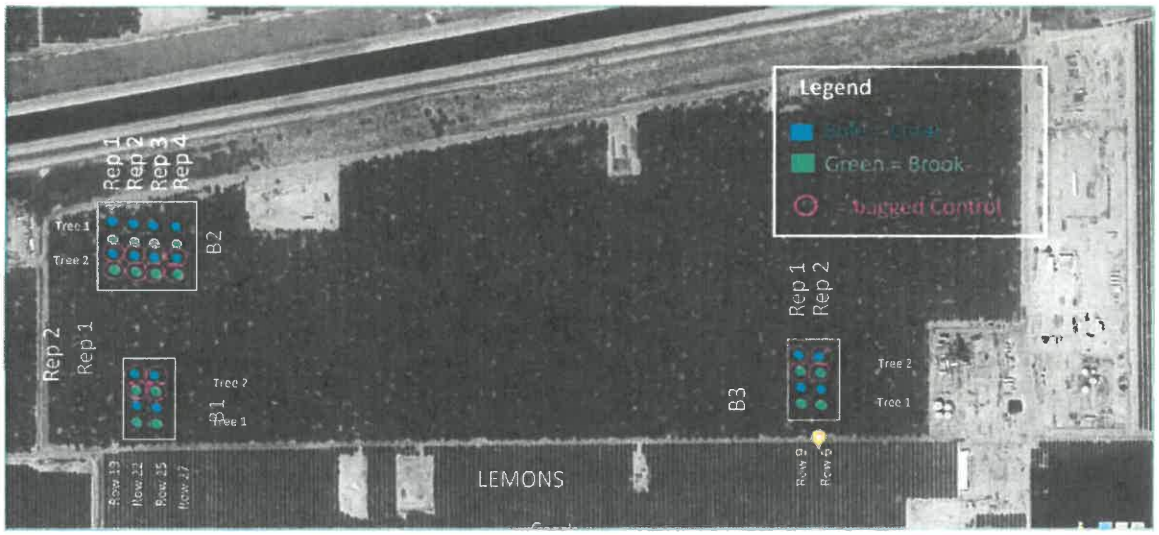

Fig. 3 – Experimental blocks and design for the orchard in the Southern location (Kern county, Bakersfield).

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.

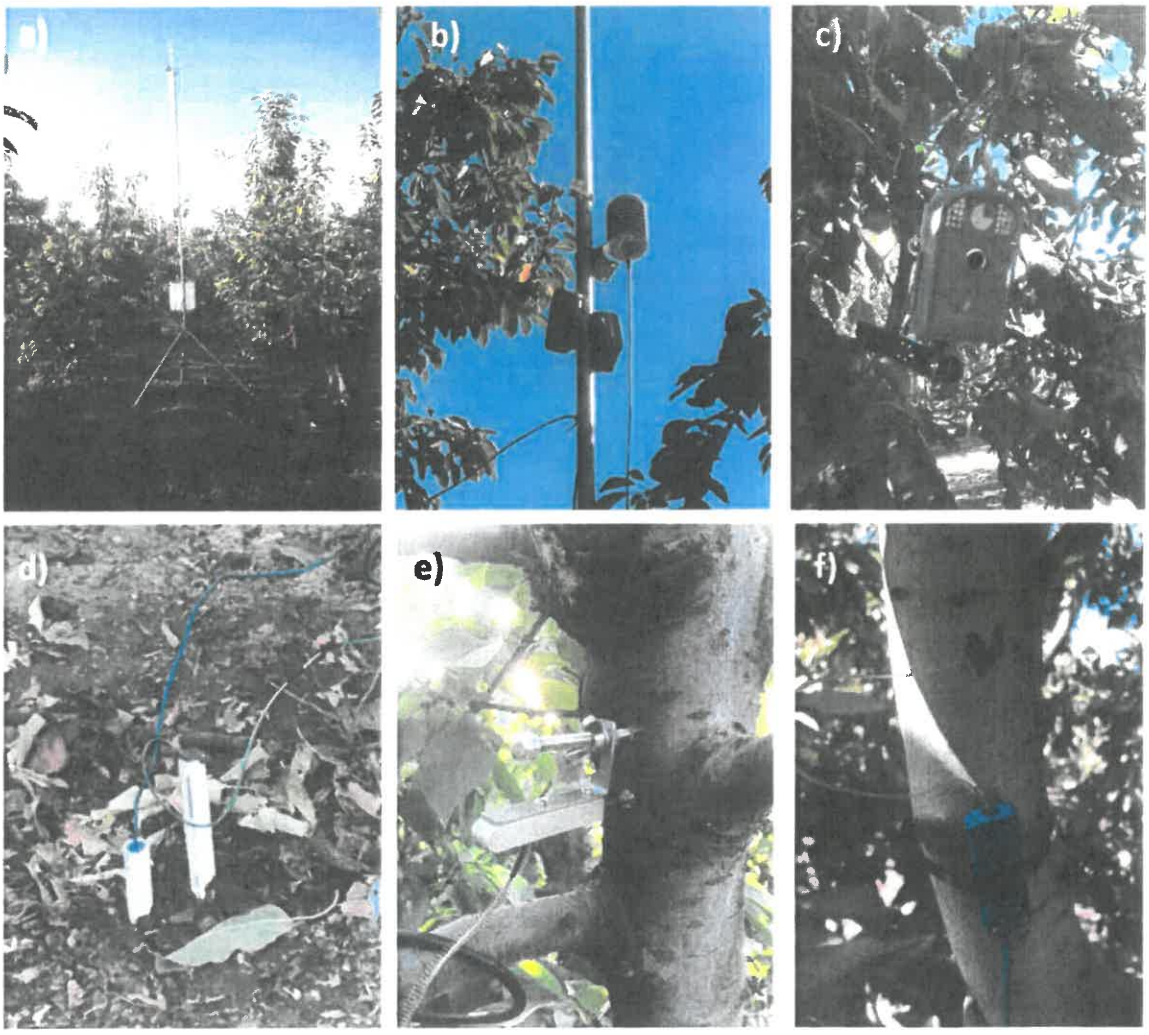

Fig. 4 Bio-meteorological station installed in the orchards (a), details of the temperature and humidity sensor (b), phenocamera (c), soil moisture sensors (d), dendrometer (e), and bark temperature sensor (f).

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 the Feb 3rd in the CAN17 orchard in the SJ County, Jan 19th in the Coral orchard in the SJ County and Jan 30th in the orchard in Kern County. The Bing Dormex was not bagged for technical problem.

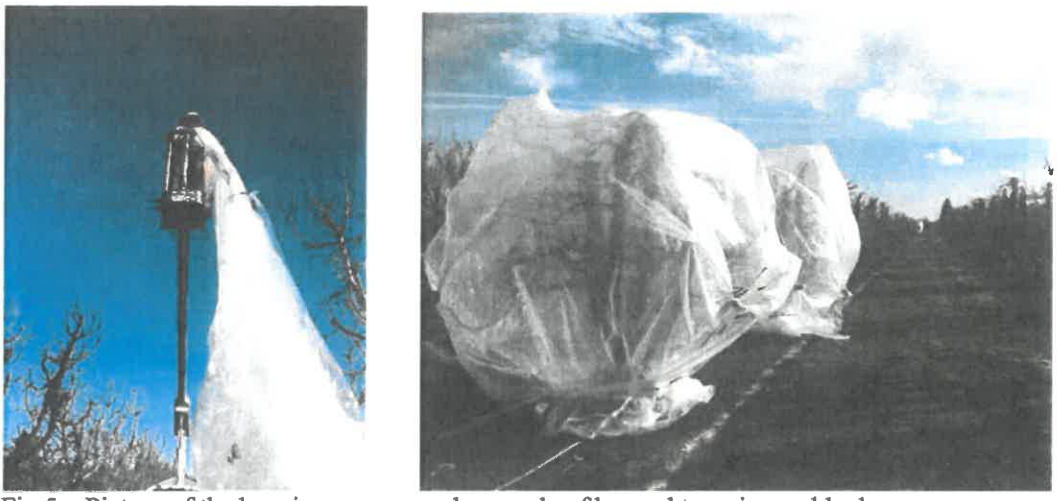

Fig 5 – Picture of the bagging process and example of bagged trees in one block

2.4 Sampling for carbohydrate analysis

Twigs were collected bi-weekly for carbohydrate (CHO) analysis starting October 1 and were delivered to Dr. Zwieniecki's laboratory at UC Davis, for characterizing CHO dynamics through the season as affected by the interaction of cultivar, environmental conditions and rest breaking agent applications.

3. Results

3.1 Relative contribution of solar radiation on tree bark temperature

Air temperature was lower than tree bark temperature during daytime, but similar during nighttime. The largest temperature difference was observed in the South and West side of the branches that were directly exposed to the solar radiation due to the winter absence of leaves. In particular, in the Kern County orchard, the bark temperature of branches exposed to South was on the average 30°F higher than the air temperature, with peaks of 45°F. In the San Joaquin County orchard, bark temperature of branches exposed to South was about 20°F higher than air temperature, with peaks of 35°F (Fig. 6).

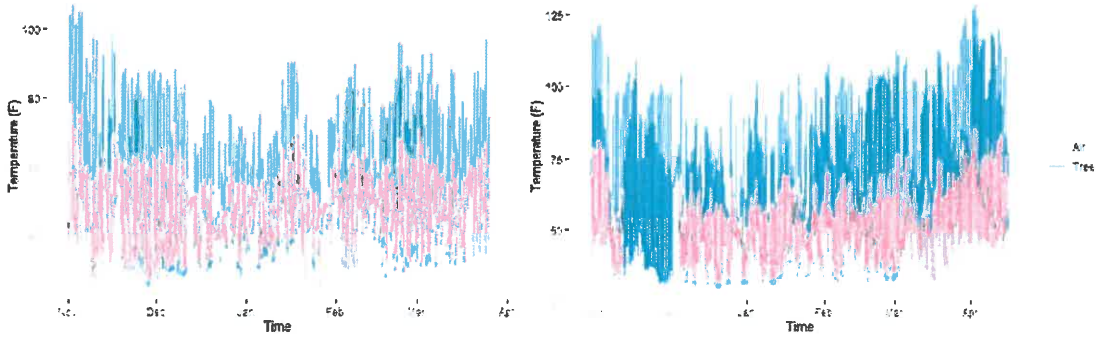

Fig. 6 - Seasonal trend (hourly values) of air temperature (red) and tree temperature (blue) from the San Joaquin County (Left) and Kern County (Right) measured with a thermocouple installed below bark in the South side exposed to solar radiation of one main branch.

Incoming solar radiation was the main driver of the difference between air and bark temperature (Fig. 7). During foggy days with low radiation (200 Wm^{-2}), daily maximum air and bark temperature were the same (60°F) and much lower than those observed on clear days with high solar radiation (900 Wm^{-2}), when the air temperature was 65/70 °F and the bark temperature 90 °F (in San Joaquin County) and 110 °F (in Kern County).

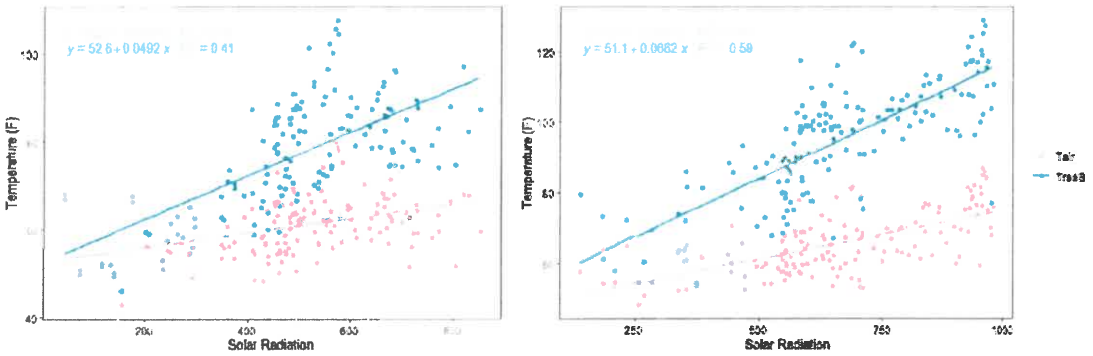

Fig. 7 - Relationship between daily maximum solar radiation (W m^{-2}) and maximum air temperature (red) or bark temperature (blue) in San Joaquin County (Left) and Kern County (Right). The thermocouple was installed in the south exposed side of one main branch.

Through a deeper analysis of the relationship between net radiation and temperature (fig 7), we observed a group of datapoint (highlighted in black in the left graph of fig. 8) that showed higher tree temperature for medium radiation levels with respect to the average. We noticed that the points corresponded to measurements made before Dec 11th, when a big rain event restored soil moisture (red circle in the right graph of fig. 8) and probably wetted the bark, decreasing its temperature by evaporative cooling.
This suggest that integrating other environmental factors in addition to net radiation and air temperature could increase our capability to predict winter tree temperature.

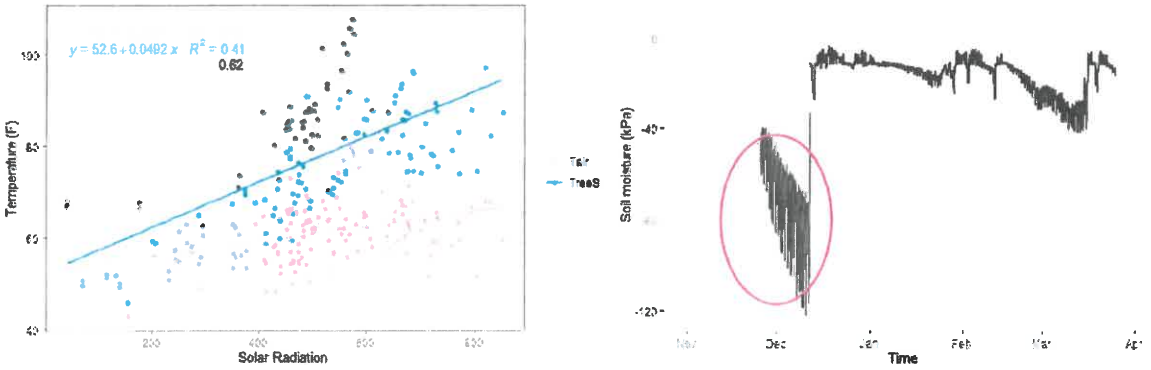

Fig. 8 - Relationship between daily maximum solar radiation (W m^{-2}) and maximum air temperature (red) or bark temperature (blue) in SJ County (left) and soil moisture (in kPa) measured with a watermark 5 inch below ground (right). Black points and red circle refer to period before big rain event in Dec 11th.

Chill accumulation, calculated using the temperature of the air, was 85 chill portions (CP) in SJ County and 72 CP in Kern County (Fig. 9). When the temperature of the bark was used to calculate chill accumulation, we had only 40 CP in SJ County, and 20 CP in Kern County.

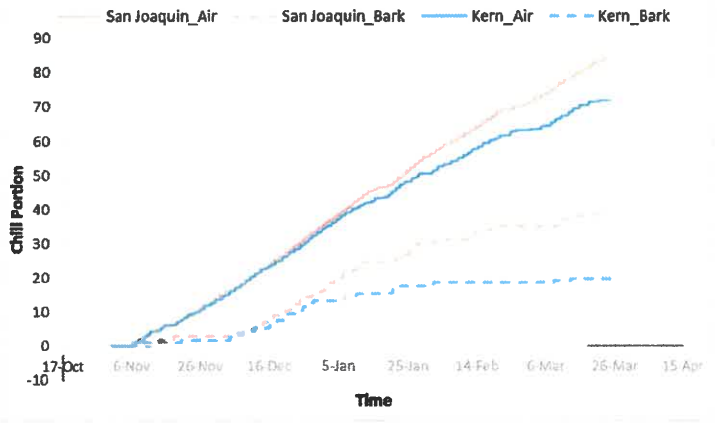

Fig. 9 Chill Portion (dynamic model) calculated using the temperature of the air (solid line) and the temperature of the bark (dotted line) in the Kern (blue) and SJ (orange) county orchards.

3.2 Tree carbohydrate dynamics during winter

The cameras installed in the field allowed us to characterize main visible phenological changes happening during the dormancy period and compare them with observed changes in the physiological parameters measured in the field.

Nonstructural carbohydrate (NSC) dynamics for the Coral orchard located in the SJ County are reported in Fig. 10 as an example. NSC varied strongly through the winter, highlighting tree physiological changes. In particular, we identified the following four stages:

Stage 1 - paradormancy - between senescence (a, Nov 5th) and defoliation (b, Dec 5th) - characterized by an opposite trend in both bark and wood NSC, with starch decreasing in bark and increasing in wood, and sugars decreasing in wood and increasing in bark. This is associated with remobilization of reserves, hardening for winter and decrease of temperature that promotes starch degradation.

Stage 2 – dormancy – from beginning of Dec 5th to mid-Jan - no visible phenological indicators - characterized by a steady increase sugar in the wood, a constant (high) level of sugar in the bark, and a decrease in starch in bark and wood. Starch is used for basal respiration and transformed into sugars under low temperatures. This period is when 'chill' is mainly accumulated.

Stage 3 - ecodormancy – from Mid-Jan to end of Feb- no visible phenological indicators- characterized by a clear increase in starch and a decrease in sugar – changes in temperature are promoting starch synthesis.

Stage 4 – 'internal bloom' - no visible phenological indicators – characterized by an increase in starch and a decrease in sugar that happens 10 days before evident visible bloom. When flowers start opening (March 11st), this trend was reversed, with sugars decreasing, and starch decreasing in wood and increasing in bark. The chemical applications did not significantly impact NSC in this orchard ($P>0.05$). However, this could be masked by changes in temperature during the treatment. In fact, a strong decrease in temperature was observed the week after the chemical was applied.

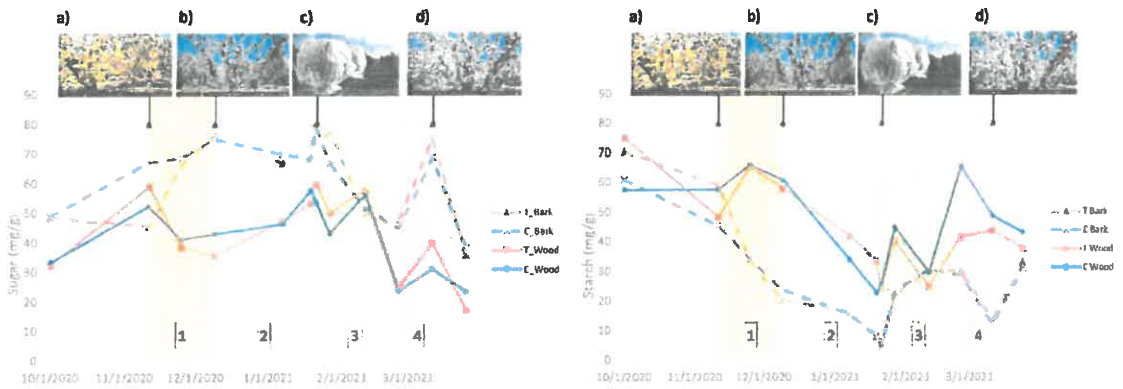

Fig. 10 - Seasonal trend of sugar (left) and starch (right) in bark (dotted line) and wood (solid line) of twigs of cherry trees (cultivar Coral) located in SJ County, treated (orange) or non-treated (blue) with Dormex. Picture on the top of the graph indicate the occurrence of leaf senescence (a), leaf drop (b), spray/bagging (c) and full bloom (d). Dormancy stages are highlighted with colors: yellow=para-dormancy, blue = endodormancy, green = pre bloom or internal bloom and pink = bloom.

The dendrometers showed some neat changes of trunk diameter happening through the winter period that could be potentially correlated with environmental, phenological and physiological factors (Fig. 11). In particular, dendrometers showed three distinct patterns of trunk diameter:

Pattern 1: wide daily shrinkage and swelling, recorded during paradormancy, pre-bloom and bloom. Indication of exchange of water with the environment.

Pattern 2: sharp continuous increase recorded at the beginning of endodormancy and before and during bloom

Pattern 3: sharp and instantaneous decrease of trunk diameter, associated with very low temperatures and freezing events.

Pattern 4: not detectable growth and shrinkage, in the second part of endodormancy, indicating trees are isolated from environment.

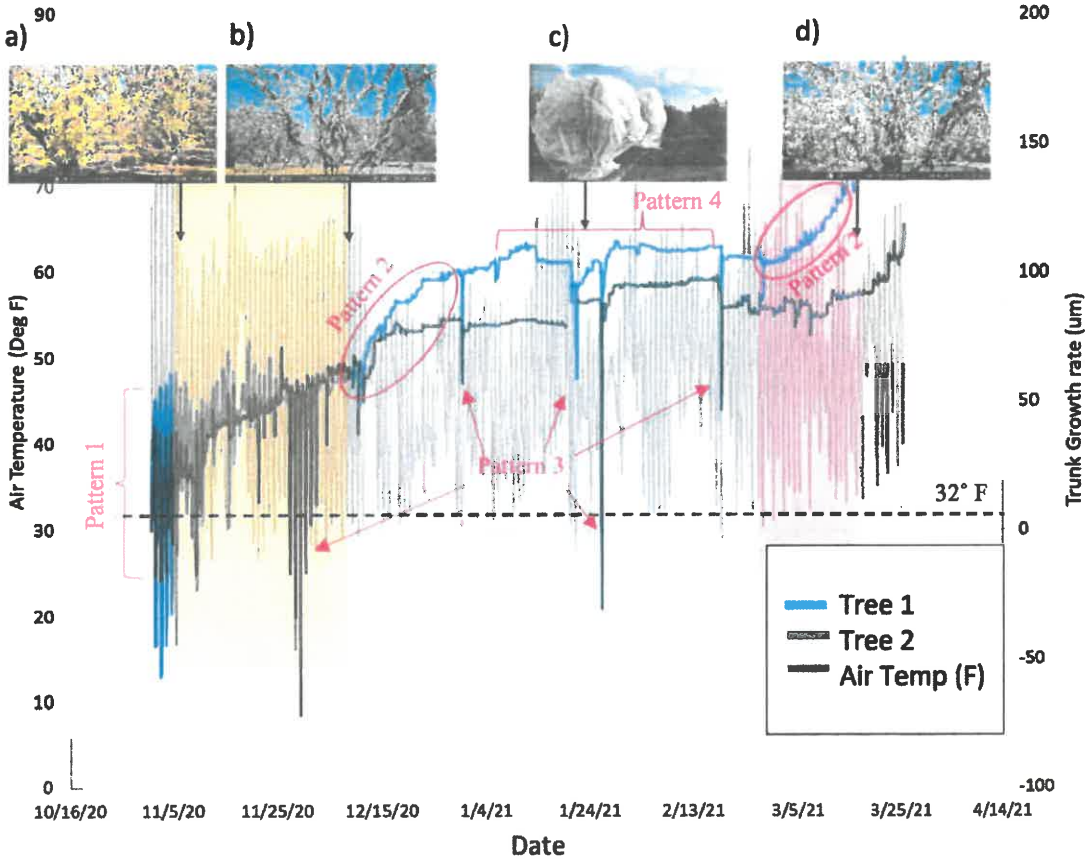

Fig. 11 – Example of the seasonal trend of cherry trunk shrinkage measured with trunk dendrometers in two trees (tree1 and tree2) in comparison with air temperature (grey line). Picture on the top of the graph indicate the occurrence of leaf senescence (a), leaf drop (b), spray/bagging (c) and full bloom (d). Dormancy stages are highlighted with colors: yellow=para-dormancy, blue = endodormancy, pink= pre bloom and full bloom. The black dashed horizontal line indicates the threshold of 32 °F corresponding to pattern 3 in the dendrometers. Example of recurrent pattern of dendrometers are shown with red arrows and brackets and circles.

Discussion: Despite still very preliminary stages of this research project, the data analysis of this experiment are extremely encouraging. We were able to model with good precision the impact of radiation on tree temperature. We believe that adding more environmental factors into the model, such as rain and associated trunk wetness, can improve the model estimation of the chilling accumulation. The carbohydrate and trunk diameters dynamics highlighted changes not visible with phenological observations that could be used to increase our understanding of chill accumulation and improve spray efficiency. However, data from the other orchards/locations and from multiple years are needed to have replicable results and reliable conclusions by smoothing out the effect of site- and year- specific conditions

Project Title
Development of Nutrient Budget and Nutrient Demand Model for Nitrogen Management in Cherry

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.

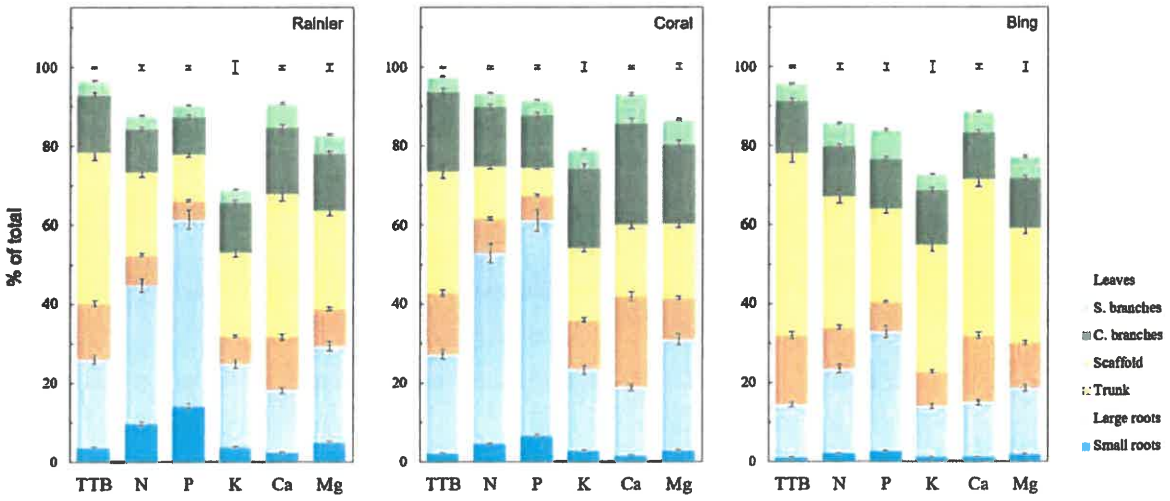

Figure 1. Tree partitioning (% of total) of total tree biomass (TTB) and macronutrients (N, P, K, Ca, and Mg) content. Data refer to cherry cultivars "Rainier", "Coral", and "Bing". Bars represent standard errors.

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.

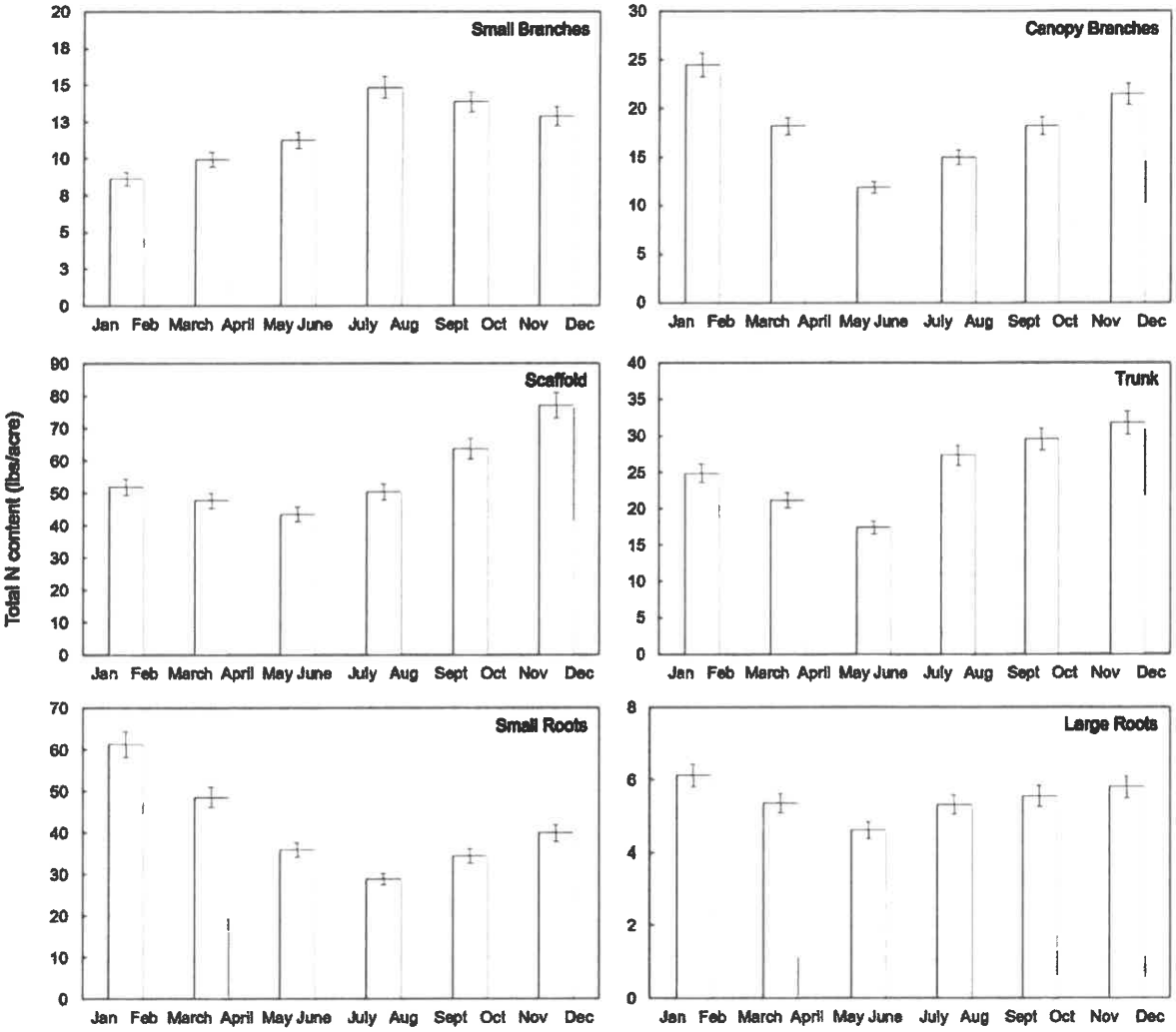

Figure 2. Seasonal trends in Nitrogen partitioning in perennial organs (trunk, scaffold, canopy branches and roots) of mature cherry trees. The overall average is weighted for the number of observations in all trials (n = 27). Bars represent standard errors.

Nitrogen removal during the season

On average, preliminary data suggests that cherry offtake of N was estimated to be 13.4 lb. per 1000 lbs. of fruit. In addition, N requirement for tree development (biomass accumulation) was estimated to be 28.3 lbs. per 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 DW)*
Rainier	13.99
Coral	13.96
Bing	12.13
Weighted Average	**13.36**

	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**, then no conclusive data are shown. Our goal is to develop knowledge of the pattern of nutrient uptake and allocation during three seasons (2020-2022) 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.

LITERATURE CITED

Benbi and Biswas. 1999. Nutrient budgeting for phosphorus and potassium in a long-term fertilizer trial. Nutr. Cycling Agroecosyst. 54 (2), 125–132.

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ACKNOWLEDGEMENTS

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California Cherry Research Board Project Annual Report

Project Title: Developing a low-cost, low-tech assay for identification of commonly grown sweet cherry varieties in California

Project duration: April 1,2021 - March 31, 2022
Reporting period: April 1, 2021 - January 15, 2022

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Executive Summary

Sweet cherry (*Prunus avium* L.) is an economically important crop in California. Its fruit is favored by consumers for the sensory properties and nutritional values. To ensure that consumers are informed of the sweet cherry varieties that they purchase from stores, the California Cherry Board (CCB) set sweet cherry variety identification as a research priority in 2021. However, the commercial sweet cherry varieties cannot be reliably distinguished by the morphological traits of fruit. Therefore, a molecular diagnostic assay is needed to rapidly and effectively detect differences in various sweet cherry varieties. In this reporting period (April 2021 - Jan 2022), we conducted high-molecular-weight DNA extraction, whole genome library construction and sequencing, and bioinformatics analysis, and identified a large number of DNA sequence variations for the commercial sweet cherry varieties relative to the reference genome. Currently, we are using this information to determine the sequence variations that are distinct among all 7 sweet cherry varieties that went through whole genome sequencing. Molecular markers will be subsequently designed based off these sequence variations and tested using fruit tissues of the sweet cherry varieties.

Problem and its significance

Sweet cherry 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 eight sweet cherry varieties that are considered as important by CBB. 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.

Objectives

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.

Experimental procedures and results

Tissue collection and processing

In preparation for genomic DNA extraction, library construction and sequencing, and molecular marker identification (Objective 1), leaves of 9 sweet cherry varieties were collected from Dave Wilson Nursery in Hickman, CA on April 5, 2021. These include Bing, Coral Champagne, Tulare, Brookes, Royal Tioga, Lapin, Sweet Heart, Royal Hazel, and Black Pearl. Leaves were flash frozen in liquid nitrogen immediately after collection at the nursery and brought to the lab to store at -80°C until used (Figure 1).

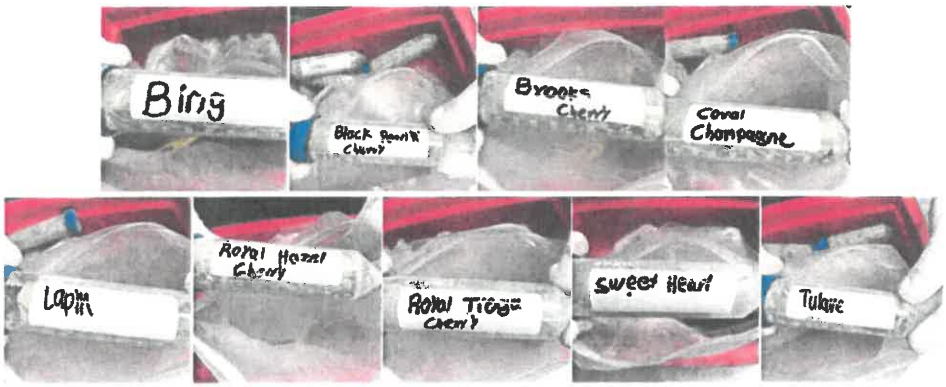

Figure 1. Leaves of 9 sweet cherry varieties were collected from Dave Wilson Nursery and frozen in liquid nitrogen to preserve the tissue until analysis.

In preparation for developing a low-cost, low-tech assay for sweet cherry variety identification (Objective 2), sweet cherry fruits were obtained from Morada Produce, prepared for purees, and stored at -80°C until analysis (Figure 2). Genomic DNA will be extracted from these fruits and used for testing molecular markers developed in Objective 1.

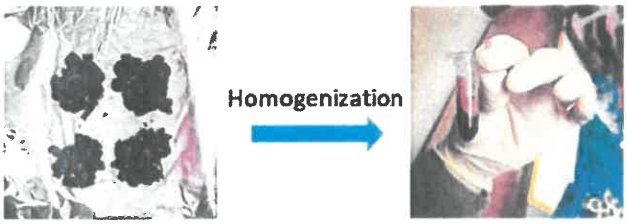

Figure 2. Fruits of 9 sweet cherry varieties were obtained from Morada Produce, cut into pieces, homogenized, frozen in liquid nitrogen, and stored at -80°C to preserve the tissue until analysis.

High-molecular-weight genomic DNA was extracted from whole genome library construction and sequencing

Following leaf tissue collection (Figure 1), we extracted high-molecular-weight genomic DNA from sweet cherry leaves using an established protocol. The method worked well and the genomic DNA fragments were larger than 25,000 bp, which is desirable for whole genome library sequencing (Figure 3).

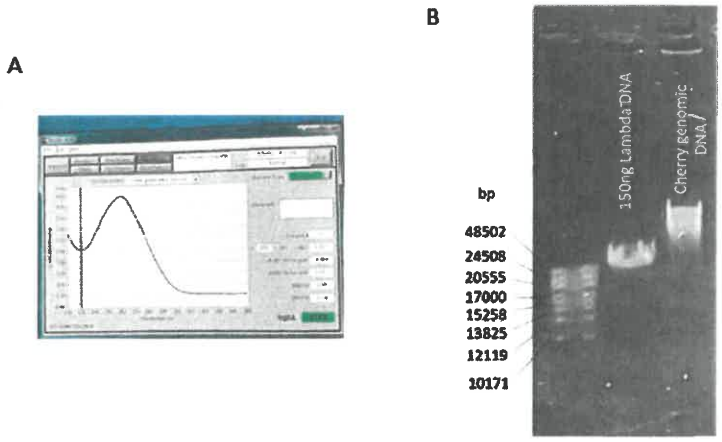

Figure 3. High-molecular-weight genomic DNA was extracted from sweet cherry leaves. A. The genomic DNA was of high quality as shown by the Nanodrop reading. B. The genomic DNA was separated on a 1% agarose gel (4.5 hours at 60 V) and demonstrated a size range of greater than 25,000 bp.

Of the 9 cherry DNA sample, 7 passed the stringent quality test of integrity and purity while Royal Hazel and Brookes did not meet the standard for library construction (Figure 4). We therefore proceeded to whole genome library construction with the 7 sweet cherry samples, including Coral Champagne, Lapin, Tulare, Black Pearl, Royal Tioga, Sweet Heart, and Bing.

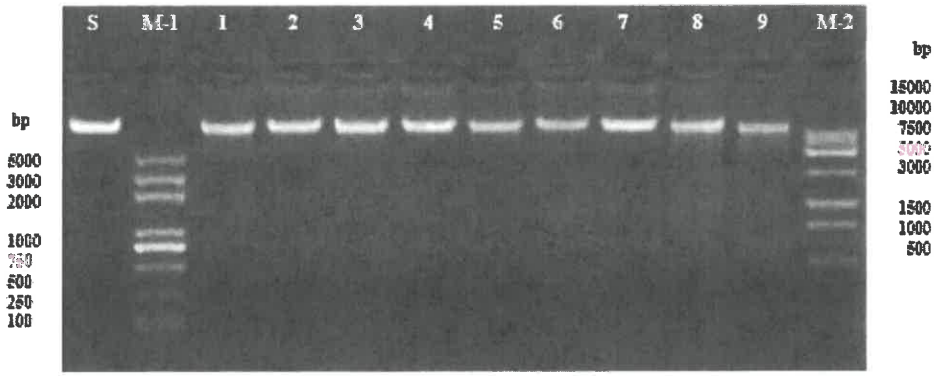

Figure 4. Electrophoresis results of 9 cherry high-molecular-weight genomic DNA samples. S, standard sample (50 ng); M-1, Trans 15k plus DNA ladder; 1, Coral Champagne; 2, Lapin; 3, Tulare; 4, Black Pearl; 5, Royal Hazel; 6, Royal Tioga; 7, Sweet Heart; 8, Brookes; 9, Bing.

Whole genome library construction and sequencing

The high-molecular-weight genomic DNA was used for whole genome library construction and sequencing with the PacBio Long-Read Technology, which is a third-generation sequencing technology using single molecule real-time sequencing (SMRT). For the SMRT library construction, the genomic DNA was fragmented to appropriate sizes, which were damage repaired, end repaired, and A tailed. The double stranded DNA templates were ligated to adapters and purified. The sequencing primers were then annealed to the DNA templates, followed by binding of the polymerase to the annealed templates (Figure 5).

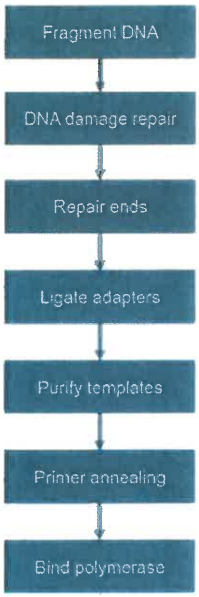

Figure 5. Workflow of library construction.

The SMRT whole genome libraries were subjected to PacBio sequencing. The statistics of the high-fidelity (HiFi) reads obtained are as follows (Table 1). The HiFi reads length distributions are shown in Figure 6. The highest amounts of HiFi reads for the sweet cherry varieties had the size of around 5,000 bp, except for Sweet Heart that peaked between 10,000 bp and 15,000 bp (Figure 6).

Sample	HiFi reads bases (bp)	Total bases (G)	HiFi reads number	Average HiFi reads length	N50
Coral Champagne	2574409991	2.57	353471	7283	9621
Royal Tioga	2107358498	2.11	289022	7291	10731
Tulare	1965286170	1.97	236747	8301	11831
Lapin	1351775238	1.35	149658	9032	13261
Black Pearl	1792832010	1.79	201767	8885	13056
Sweet Heart	2050660989	2.05	192601	10647	13301
Bing	2400656345	2.40	482616	4974	6141

Table 1. Statistics of HiFi reads.

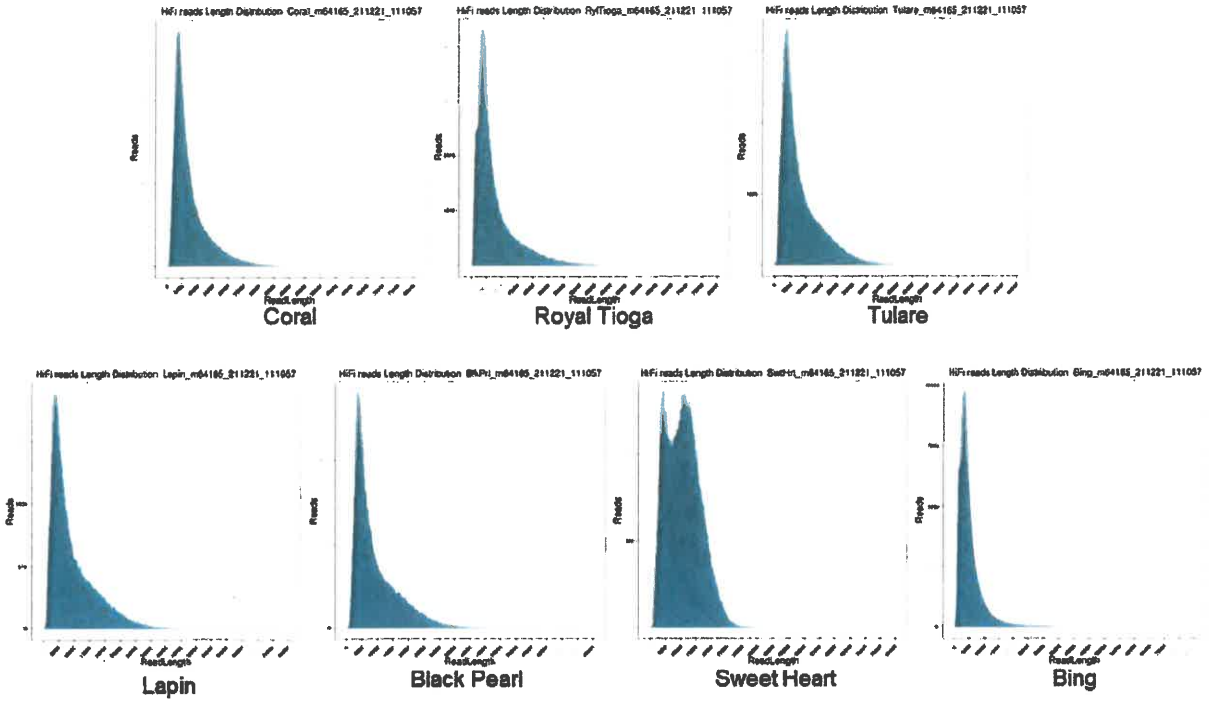

Figure 6. HiFi reads length distributions.

The HiFi sequence reads were mapped to the reference cherry genome downloaded from the Ensembl Genome Brower website (https://ftp.ebi.ac.uk/ensemblgenomes/pub/release-52/plants/fasta/prunus_avium/dna/Prunus_avium.PAV_r1.0.dna.toplevel.fa.gz) (Table 2).

Seq number	Total length	GC content (%)	Gap rate (%)	N50 length	N90 length
10,148	**272,361,615**	37.72	9.39	219,566	19,672

Table 2. Statistics of the reference genome. Seq number, the total number of the assembled genomic sequences. Total length, the total length of the assembled genomic sequence. GC content, the GC content of the reference genome. Gap rate, the proportion of unknown sequence (N) in the reference genome assembly. N50 length, the length of scaffold N50, of which 50% of the sequence is higher than this level. N90 length, the length of scaffold N90, of which 90% of sequence is higher than this level.

All 7 sweet cherry samples had 100% mapping rate, suggesting that they are very similar to the reference genome. We have also achieved ~90% coverage of the cherry genome for all samples, with an average depth between 4.91- to 9.36-fold.

Sample_Name	Total	Mapping Rate	Average Depth	Average Coverage	Coverage_10X
Coral Champagne	349849 (100%)	349849 (100.00%)	9.36	92.62%	22.19%
Royal Tioga	284236 (100%)	284236 (100.00%)	7.14	89.85%	7.64%
Tulare	233706 (100%)	233706 (100.00%)	6.92	90.51%	7.33%
Lapin	147888 (100%)	147888 (100.00%)	4.91	86.84%	2.42%
Black Pearl	199582 (100%)	199582 (100.00%)	6.51	90.29%	6.50%
Sweet Heart	190509 (100%)	190509 (100.00%)	7.42	90.84%	8.60%
Bing	474927 (100%)	474927 (100.00%)	8.66	92.59%	14.48%

Table 3. Mapping statistics.

Detection of sequence variants

Our goal is to differentiate the sweet cherry varieties using the DNA sequence information. We analyzed the genome sequences of the 7 cherry varieties to detect different types of sequence variations, including single nucleotide polymorphisms (SNPs), insertions and deletions (InDels), and structural variants (SVs) (Tables 4-6). Single nucleotide polymorphism (SNP) refers to a variation in a single nucleotide which may occur at some specific position in the genome, including transition and transversion of a single nucleotide. InDel refers to the insertion or deletion with length less than 50 bp. Structural variants (SVs) are genomic variation with mutations of relatively larger size (>50 bp), including deletions, duplications, insertions, inversions, and translocations.

Sample Name	Upstream	Stop gain	Stop loss	Synonymous SNV	Nonsynonymous SNV	Intronic	Splicing	Downstream	Upstream; Downstream	Intergenic
Coral Champagne	132886	2290	668	46037	76017	162545	712	122632	25140	599750
Royal Tioga	110879	1778	503	37682	61571	140659	563	101873	20295	443468
Tulare	104351	1702	489	34930	57486	129295	537	94692	19940	437983
Lapin	79130	1232	366	26127	41928	97778	413	70443	15338	313491
Black Pearl	102610	1680	483	34915	56636	129242	521	92611	18688	423380
Sweet Heart	107684	1766	502	35869	58338	128013	549	96191	20128	478731
Bing	130323	2338	712	47805	78282	158101	722	122679	24113	605938

Table 4. Summary of SNPs detected in the genomes of 7 sweet cherry varieties. Upstream, SNPs located within 1 kb upstream (away from transcription start site) of the gene. Stop gain/loss, a nonsynonymous SNP that leads to the introduction/removal of stop codon at the variant site. Synonymous SNV, single nucleotide mutation without changing amino acid sequence. Nonsynonymous SNV, single nucleotide mutation changing amino acid sequence. Intronic, SNPs located in intronic region. Splicing, SNPs located in the splicing site (2 bp range of the intron/exon boundary). Downstream, SNPs located within 1 kb downstream (away from transcription termination site) of the gene region. Upstream;Downstream, SNPs located within the < 2 kb intergenic region, which is in 1 kb downstream or upstream of the genes. Intergenic, SNPs located within the > 2 kb intergenic region.

Sample Name	Upstream	Stop gain	Stop loss	Intronic	Splicing	Downstream	Upstream; Downstream	Intergenic
Coral Champagne	37774	585	53	48652	277	31720	7107	105951
Royal Tioga	30165	396	37	39319	207	25273	5655	80356
Tulare	28643	392	39	36817	205	23915	5538	77902
Lapin	20705	290	26	25941	149	17077	4135	54103
Black Pearl	28130	399	33	36488	207	23425	5291	75734
Sweet Heart	30048	448	40	36815	238	24736	5635	82262
Bing	33785	542	53	43560	256	29317	6279	98724

Table 5. Summary of InDels detected in the genomes of 7 sweet cherry varieties. Upstream, InDels located within 1 kb upstream (away from transcription start site) of the gene. Stop gain/loss, a nonsynonymous InDel that leads to the introduction/removal of stop codon at the variant site. Intronic, InDels located in intronic region. Splicing, InDels located in the splicing site (2 bp range of the intron/exon boundary). Downstream, InDels located within 1 kb downstream (away from transcription termination site) of the gene region. Upstream;Downstream, InDels located within the < 2 kb intergenic region, which is in 1 kb downstream or upstream of the genes. Intergenic, InDels located within the > 2 kb intergenic region.

Sample Name	Upstream	Exonic	Downstream	Intronic	Upstream/ Downstream	Intergenic	Splicing	Others	BND	CNV	DEL	DUP	INS	INV	Total
Coral Champagne	5100	4501	4603	5089	1032	15229	48	622	8684	513	15533	945	10488	60	36225
Royal Tioga	4878	4169	4254	4761	956	13654	40	608	6290	418	15292	829	10442	49	33321
Tulare	4646	3977	4185	4505	924	12979	50	618	5838	390	14814	831	9968	43	31885
Lapin	4023	3475	3555	3999	808	11019	42	504	3744	288	13613	623	9113	34	27426
Black Pearl	4456	3976	3947	4469	881	12840	43	582	5756	384	14445	795	9791	33	31205
Sweet Heart	4633	4034	4035	4464	901	13388	44	584	6268	420	14720	833	9776	46	32054
Bing	4902	4241	4389	4875	977	14058	48	615	8064	465	15058	809	9861	46	34104

Table 6. Summary of SVs detected in the genomes of 7 sweet cherry varieties. Upstream, SVs located within 1 kb upstream (away from transcription start site) of the gene. Exonic, SVs located in exonic region. Intronic, SVs located in intronic region. Downstream, SVs located within 1 kb downstream (away from transcription termination site) of the gene region. Upstream/Downstream, SVs located within the < 2 kb intergenic region, which is in 1 kb downstream or upstream of the genes. Intergenic, SVs located within the > 2 kb intergenic region. Splicing, SVs located in the splicing site (2 bp range of the intron/exon boundary). Others, SVs located in other region. BND, numbers of translocation; translocations include interchromosomal and intrachromosomal translocations. CNV, numbers of copy number variation. Copy number variation also defined as unbalanced structural variants; variants that change the number of base pairs in the genome. DEL, numbers of deletion. DUP, numbers of duplation. INS, numbers of insertion. INV, numbers of inversion.

Our next step

We are currently analyzing the sequence data for SNP and simple sequence repeat (SSR) markers that can distinguish the 7 commonly grown sweet cherry varieties. These molecular markers will then be validated using genomic DNA extracted from the fruit tissue of the sweet cherry varieties.

Cherry whole orchard recycling: Investigating fruiting bodies viability of cherry fungal pathogens.

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SUMMARY

During our recent surveys, we documented the adoption of Whole Orchard Recycling (WOR) in few cherry orchards in Linden area. WOR is the on-site grinding or shredding of whole trees during orchard removal and incorporation of the ground or chipped biomass into the topsoil prior to replanting. WOR 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. Due to promising results from almond orchard recycling, and the addition of WOR to CDFA's Healthy Soils Initiative, cherry growers may qualify for funding opportunities, and this practice could provide a sustainable method of tree removal that could enhance both air and soil quality. Although the effect of WOR has yet to be investigated in cherry orchard systems, in-person survey results showed that more growers are interested in adopting this practice. Results indicated also that shredding diseased wood and incorporating them into the soil is a major concern to the growers, raising the question whether shredding and soil incorporation of whole trees can lead to an early fungal canker diseases – caused by the plant-pathogenic fungi *Calosphaeria pulchella*, *Eutypa lata* and *Cytospora sorbicola* – that could affect newly planted orchards. We initiated 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. Spore viability declined significantly over sampled time, and we will continue monthly sampling until there is no further recovery of the pathogens.

A four-month spore trapping study was conducted to determine the abundance and spore discharge of the mentioned above fungi. Here we analyzed the correlation between shredding of infected branches between tree rows (following maintenance/cleaning pruning) in both northern and southern regions, the overhead water-based orchard cooling systems – used on very hot days in the southern regions – and Cytospora/Colosphaeria spore release. Overall, based on colony counts, we detected high aerial dissemination of spores when shredding of infected branches between tree rows in the northern region. In the Southern region, recovery of spores occurred during or soon after applying water via the over-tree sprinklers or after a rain event at the end of monitoring period, and here we could not conclude that there was a clear relationship between shredding and spore release in the south despite presence of fungal fruiting bodies on wood chips on soil surface.

This information is of great importance as it helps to identify production practices responsible for the spread of these fungal pathogens within cherry orchards.

Soil samples collected from across the WOR field at two depths, showed that there was approximately 2.4 ppm nitrate-N in the top 6 inches and 2.3 ppm from 6-12 inches, for a total residual nitrate level of approximately 12 pounds per acre in the top foot of soil. Soil surveys will continue to be conducted annually, and we will assess the impacts of different Nitrogen treatments on first year trees growth (cherry trees planting will be in the spring of 2022).

OBJECTIVES

1) Whole cherry orchard recycling survey.
2) To investigate the viability of fungal pathogen survival in fruiting bodies still present in wood chips incorporated during orchard recycling.
3) To verify if grinding or chipping and incorporating the diseased branches with cankers between tree rows could increase fungal spore dispersal in the orchard.
4) To examine soil organic matter (SOM) and stored carbon (C) after whole orchard recycling, by conducting soil surveys annually.

1) Whole cherry orchard recycling survey

Last year, we documented the adoption of Whole Orchard Recycling (WOR) in few cherry orchards in Linden area. With promising results from almond orchard recycling, and the addition of Whole Orchard Recycling to CDFA's Healthy Soils Initiative, cherry growers may qualify for funding opportunities. In-person survey results showed that more growers are interested in adopting this practice. We also found that one of the main concerns regarding WOR is whether shredding and soil incorporation of whole trees can lead to an early fungal canker diseases. Our investigation will continue this year to see if more cherry growers would be willing to adopt WOR as an alternative, while trying to better understand what growers foresee as limitation to such practice in cherry orchard systems.

2) Investigate the viability of fungal pathogen survival in fruiting bodies still present in wood chips incorporated during orchard recycling.

Material and methods

Fungal pathogens reported to occur in cankers in sweet cherry in California have included *Calosphaeria pulchella*, *Eutypa lata* and *Cytospora sorbicola*. Knowing that Eutypa fruiting bodies are rarely encountered in cherry orchards, questions have been raised about how long Calosphaeria and Cytospora fungi can continue to produce ascospores/pycnidiospores from perithecia/pycnidia on shredded twigs/branches, which are left on the orchard floor or even buried.

Two trials were established in Linden area in two different locations; the first, on an approximately 15-acre site, following cherry orchard recycling. The second in a 30-year-old cherry orchard. Infected branches/shoots covered with pycnidia of Cytospora and perithecia of Calosphaeria have been collected and shredded to the average size of commercial shredding (Fig. 1A and B). In July

of 2021, these shredded pieces were placed in Brite Aluminum Screen Mesh, which were either buried or placed on the soil surface in different areas of the cherry WOR (Fig. 1C). The WOR orchard used for this study consist of 35-40-year-old Bing trees, which were shredded and incorporated into the topsoil in August of 2020. The grower is planning to plant the new cherry orchard early spring of 2022 – this period of time will allow us to assess the viability of these fungal pathogens under dry conditions, as the orchard is already prepared and no irrigation until planting.

Shredded wood pieces (as described above) were also placed/buried under mature cherry trees at the second site in an orchard with a high incidence of fungal diseases – where the grower adopts shredding of prunings between tree rows. This experiment (as part of objective 3) will allow us to assess the viability of these fungal pathogens under wet conditions (Fig. 1D).

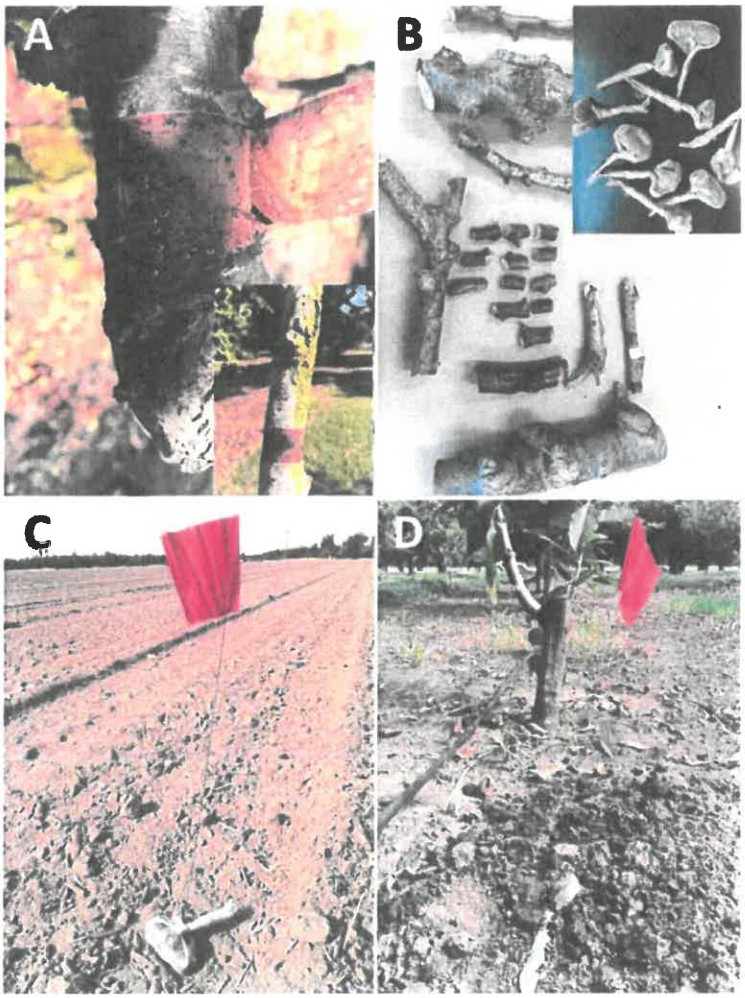

Fig. 1. A and B. Shredded cherry wood pieces covered with pycnidia of Cytospora and Perithecia of Callosphaeria placed in Brite Aluminum Screen Mesh: **C.** buried and/or placed on the soil surface in different areas of WOR orchard and **D.** buried and/or placed on the soil surface under mature cherry trees.

Initial sampling of the infected sweet cherry branches covered with fruiting bodies took place immediately after placing them into the two orchards. In the laboratory, ascospores of Calosphaeria were collected from fruiting bodies found under the periderm of infected branches: Perithecia were first collected with sterile tweezers using a dissecting microscope and affixed to a glass-microscope slides covered with Vaseline. The slides were then placed in sterile Petri dishes filled with sterile deionized water (SDW) and incubated at room temperature for approximately 30 min. After drying the slides containing perithecia on sterile paper towels, perithecia were returned to sterile Petri dishes and incubated for approximately one hour for spore discharge. Ascospore masses were eventually collected by adding 3ml SDW in each Petri dish, and mixed into a suspension adjusted to 10^3 ascospores per ml using a hemacytometer. From the suspension, two 100µl aliquots were spread on two petri plates containing 2% water agar. After spreading the spore suspension, plates were dried for 10 min inside a laminar flow hood, and then incubated at 30°C. After 37 hours, the germination of the ascospores was evaluated under a 40x magnification light microscope by counting the number of germinating spores in each Petri plate.

Conidia of Cytospora fungi were also collected from the fruiting bodies found when peeling the outer layer of the bark. Fruiting structures containing spore masses were collected with a razor blade and placed into 1.5-mL microcentrifuge tubes filled with SDW. Tubes were then spined using a vortex for approximately 10 seconds to discharge spores. Spore suspensions were adjusted, and then plated. Plates were then incubated at 25°C and analyzed as described above.

Results and Discussion

When infected sweet cherry branches/shoots – covered with pycnidia of Cytospora and perithecia of Calosphaeria – were collected in June 2021, percent of spore germination was determined at approximately 90% and 88% for Calosphaeria and Cytospora, respectively. Pycnidia and perithecia were still viable in infected branches after four and a half months on the soil surface or buried in both orchards. Percent germination of both types of spores declined significantly over time on each sampling date. While shredded wood pieces placed/buried on the soil surface of the WOR orchard had viable asco- and pycnidiospores, ascospores of Calosphaeria and Cytospora pycnidiospores viability declined significantly by approximately 53% and 42% respectively after four and a half months on the soil (42% of the Calosphaeria ascospores germinated and 51% of the Cytospora pycnidiospores germinated) (Fig. 2A).

Similarly, percentage of spore germination declined significantly from both fungi collected from shredded wood pieces placed/buried under mature cherry trees. The overall spore germination had declined by approximately 54% for Cytospora and 58% for Calosphaeria after four and a half months (41% of pycnidiospores and 37% of ascospores germinated). This decline is less than the decline observed in the orchard where WOR was performed (Fig. 2B). Here we believe that the dry conidia/ascospores had greater longevity than wet conidia/ascospores: storage of these fruiting bodies at high relative humidity (RH)/moisture values – created mainly by sprinkler irrigation –

reduces viability of spores. Monthly sampling is still ongoing until there is no further recovery of these two pathogens.

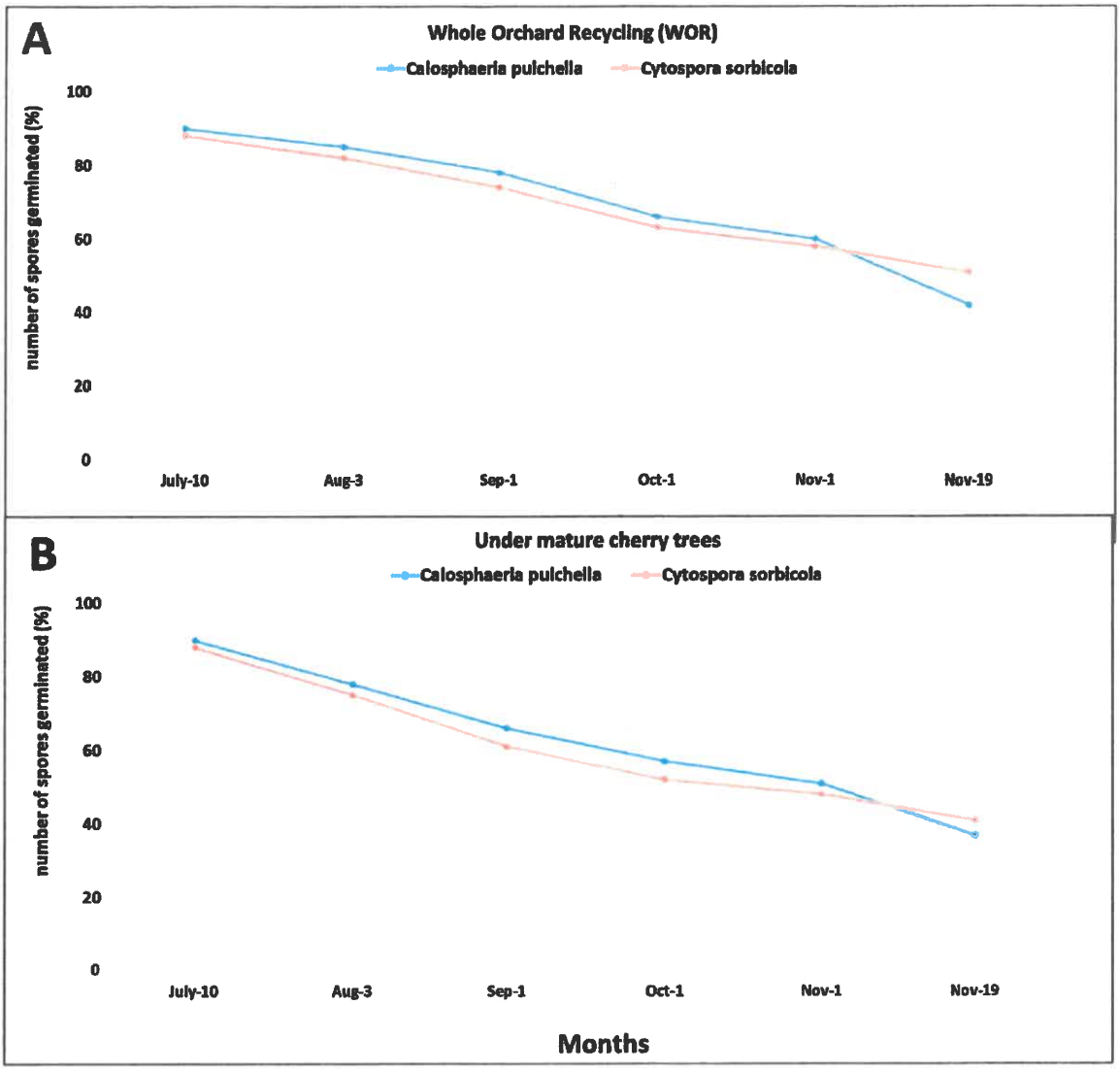

Fig. 2. Germination rates for Cytospora pycnidiospores and Calosphaeria ascospores isolated from fruiting bodies found under the periderm of shredded infected cherry branches. Shredded wood was **A.** placed/buried on the soil surface in different areas of the WOR, **B.** placed/buried on the soil surface in cherry orchard under mature cherry trees. Germination starting point at: 90% and 88% for Calosphaeria and Cytospora respectively.

3) **Objective 3: To verify if shredding or grinding and incorporating the diseased branches with cankers between tree rows could increase fungal spore dispersal in the orchard.**

Material and methods

Four cherry orchards were selected for this study. Two orchards showing high incidence of dieback and fungal canker diseases where growers adopt the wood shredding between tree rows in San Joaquin County. Two other orchards in Kern County – one showing 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 on very hot days.

Spores were trapped on glass microscope slides (25 by 76 mm) coated on both sides with a thin layer of white petroleum jelly and placed on cherry branches. Ten spore traps were placed randomly on the lower canopy of separate trees in the middle of the four orchards, in several cases positioned in areas where fruiting bodies occurred. Spore traps were changed weekly and individually collected in sterile 50-ml conical tubes, then transferred to the laboratory for processing.

In the laboratory, spores were removed by adding 3 ml DI water into each tube, and then gently shaking by hand for 30-40 second. Two 100µl-aliquots were spread on two 85-mm-diameter Acidified Potato Dextrose Agar (APDA) Petri plates. After spreading the solution, plates were dried for 10 min inside a laminar flow hood, and then incubated in the laboratory at room temperature (~ 25°C) with approximately 12 h of daylight and 12 h of darkness. Fungal colonies were counted after 4-7 days. Fungal colonies of Cytospora and Calosphaeria fungi were identified by colony morphology and growth characteristics. Spores counting in each trap was performed using the following formula: number of spores = number of colonies x dilution factor. In our case the dilution factor = 3ml/0.2ml = 15. (where 0.2ml corresponds to the total final volume placed on the two replicate petri plates).

Results and Discussion

This study was conducted from mid-June to the end of October 2021, in Kern County, and from the end of June to mid-October 2021 in San Joaquin County (approximately a month and half before and after wood shredding in San Joaquin County).

In San Joaquin County, overall, recovery of Cytospora and Calosphaeria spores occurred mainly during or soon after wood shredding events. We determined high aerial dissemination of spores following grinding/shredding of infected branches on the orchard floor mainly during the last week of August 2021 for Orchard 1, and during the second week of August 2021 for orchard 2. For both orchards, few spores were captured after approximately 10 days from the day of shredding (Fig. 3A & B).

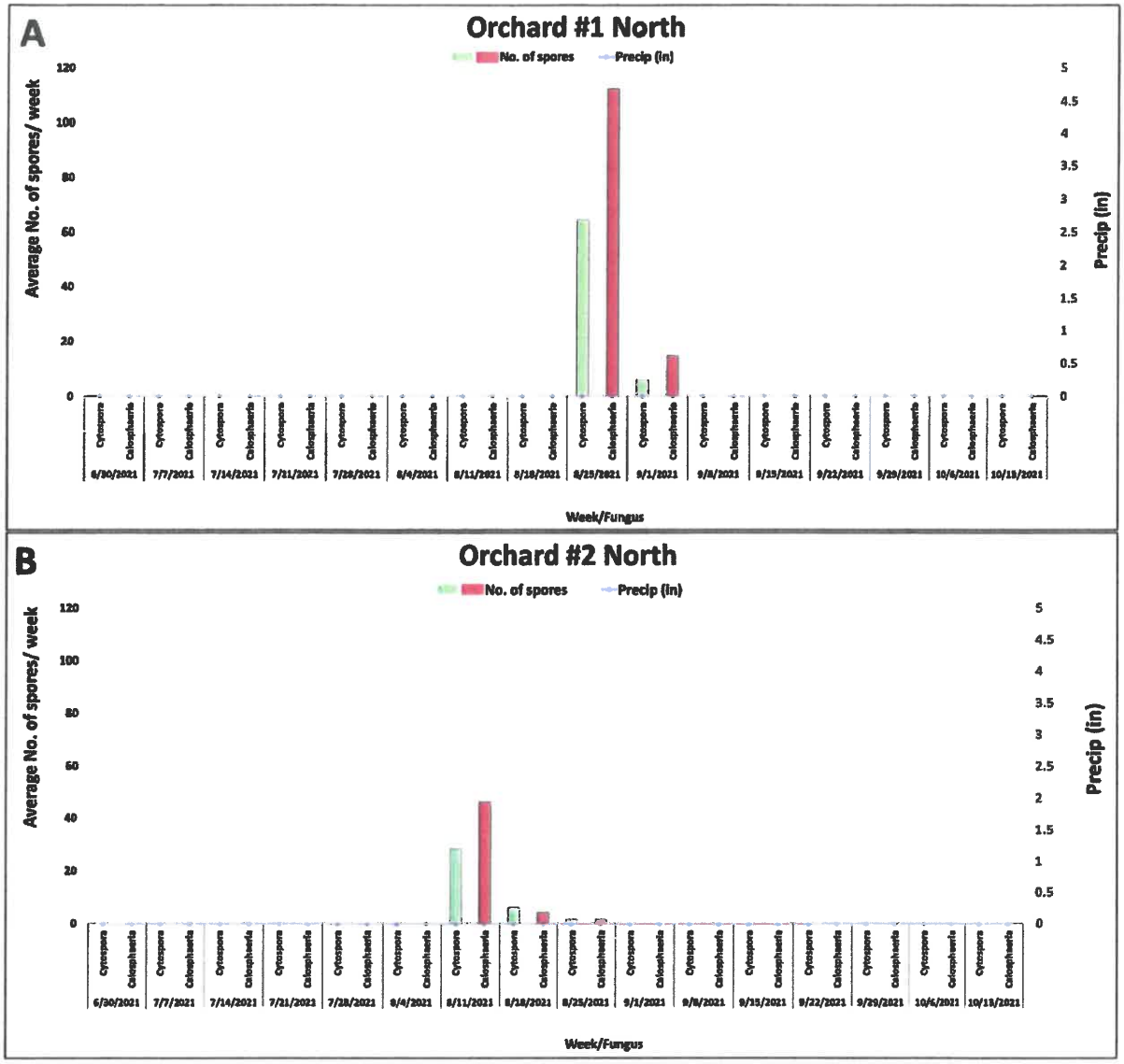

Fig. 3. Total number of Cytospora and Calosphaeria spores trapped per week correlated with the shredding of infected branches between tree rows in two cherry orchards in San Joaquin County. Precipitation is included in the graphs (which was 0 mm during the spore trapping period)

In Kern County, our spore trap study indicated that spore release of Cytospora and Calosphaeria fungi occurred mainly when the overhead water-based cooling systems were turned-on during very hot days in both orchards. In addition, spores were also captured during the rainy event that occurred at the end of October 2021. While we can not conclude for sure that the increase of spore release by the end of October was due to shredding and not due to the rain event on the 25th of

October, it is worth noting that many wood chips and wood pieces shows visible signs of fungal fruiting bodies on the soil surface between tree rows.

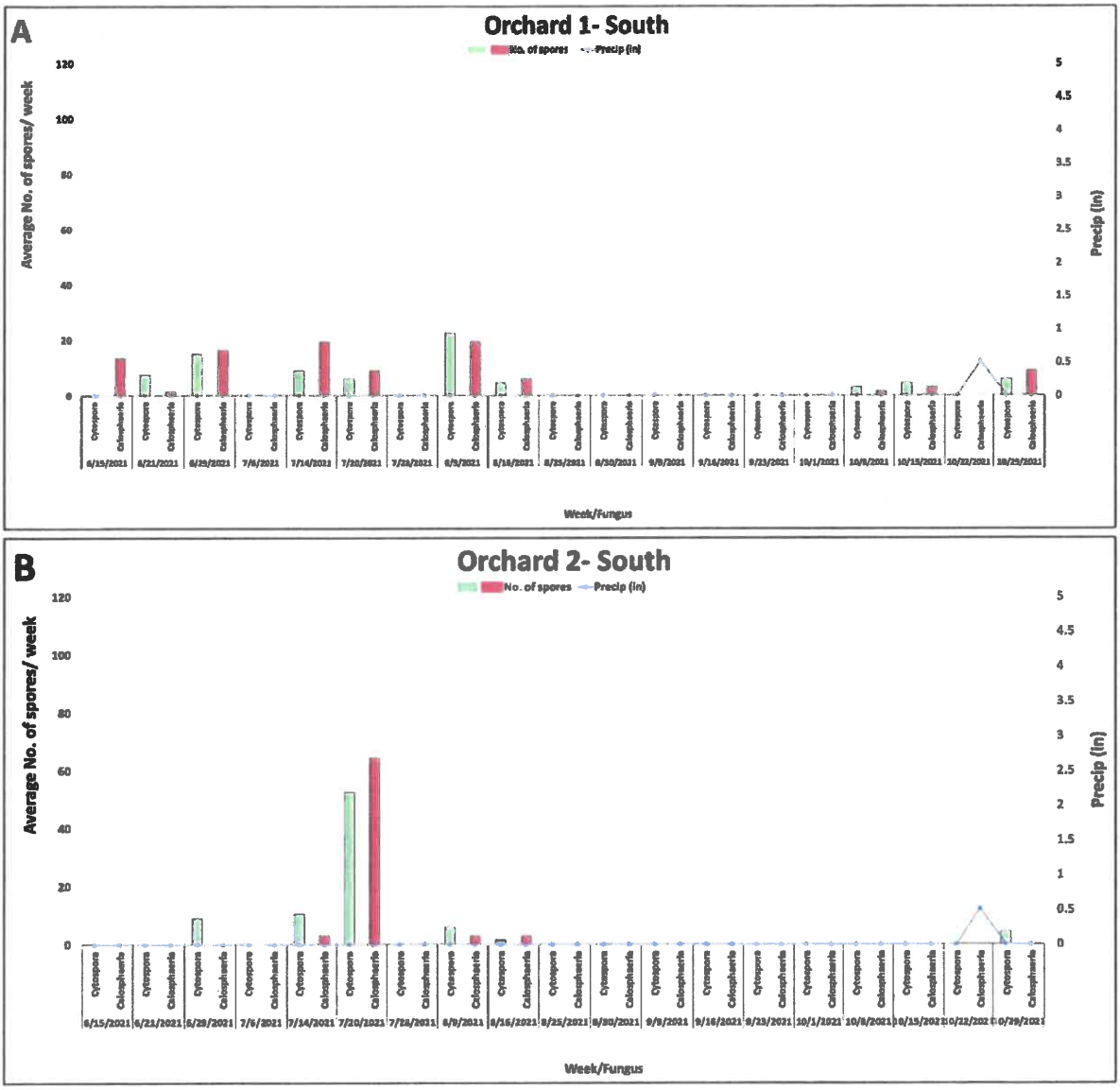

Fig. 3. Total number of Cytospora and Calosphaeria spores trapped per week correlated with the overhead water-based cooling systems in two cherry orchards in Kern County. Precipitation and shredding of infected branches between tree rows (orchard 1) are included in the graphs

This unique spore release event associated with shredding of dead branches also occurred shortly after maintenance pruning thus creating fresh pruning wounds, which could lead to high infection

rates of pruning wounds in these orchards. 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. 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.

4) **Objective 4: To examine soil organic matter (SOM) and stored carbon (C) after whole orchard recycling, by conducting soil surveys annually.**

In June of 2021, we collected soil samples from across the WOR field at two depths, 0-6 and 6-12-inches. Samples were analyzed by a commercial lab to quantify the residual soil nitrate, total nitrogen, organic carbon, which will be used as a baseline for future studies. There was approximately 2.4 ppm nitrate-N in the top 6 inches and 2.3 ppm from 6-12 inches, for a total residual nitrate level of approximately 12 pounds per acre in the top foot of soil. We also collected wood chips to be analyzed.

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. Previous research conducted by Dr. Brent Holtz and his team at WOR sites that were replanted back to almond found that extra N was needed in the first year to avoid stunting. The UC recommendation is to double the N application in the first year after replanting, applying it gradually over the year. Here in this objective, we are planning to assess the impacts of different N treatments on first year cherry trees growth in the WOR plot in Linden.

CALIFORNIA
CHERRIES

CALIFORNIA CHERRY BOARD
ANNUAL RESEARCH REPORT

Institution/Organization: University of California Cooperative Extension-Stanislaus County
Project Year: 2021-2022
Principal Investigator(s): Jhalendra Rijal
Cooperating Personnel: Sudan Gyawaly, Mohammad Nouri, Kari Arnold, Frank Zalom, Joanna Chiu

Project Title: Exploring new and alternative insecticides for resistance management of spotted wing drosophila

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 2020 season, we tested several new insecticides against SWD adults in laboratory settings. These studies showed that lambda-cyhalothrin (Worrier II) as an industry-standard performed the highest mortality (avg. of the flies mortality 98%), followed by other insecticides-cyantraniliprole (Exirel) with 93% mortality, cyclaniliprole (Verdepryn) with 88% mortality, Minecto Pro with 84% mortality, and pyrethrin (Pyganic) with 84% at 48 hours after exposure. In continuation of these efforts, we tested these insecticides in a replicated trial setup in a 5-acre cherry orchard in Stockton in the 2021 season. At 1 and 7 days after the insecticide application, cherry fruits were collected from, brought to the lab, and tested for: a) adult mortality and b) adult emergence. These studies showed that, in addition to industry-standard Warrior II, Exirel insecticide is an effective rotation product against SWD. In addition, we explored the ovipositional and larvicidal effects of these insecticides in the lab. We showed that Verdepryn, Warrior II, and Exirel all showed promising results as a presumed ovicide and/or larvicide against SWD. We continue these ovi- and larvicidal aspects of the study in 2022 for conclusive results.

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 natural enemy populations, and potential outbreaks of secondary pests such as scale insects.

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. The repeated spray of the same insecticides close to harvest for SWD management may potentially result in higher pesticide

residue in the crop and interfere with the cherry export (Haviland and Beers 2012. J. Integ. Pest Mngmt. DOI: http://dx.doi.org/10.1603/IPM11034). 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; Pest Manag Sci.75: 1270–1276). 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. There have been anecdotal reports of the ineffectiveness of recommended insecticide programs in the northern San Joaquin Valley to control the SWD population, creating concern among cherry producers. In this context, exploring various insecticide active ingredients with potentially shorter residues in that fruit is desirable so that they can be used in rotation to minimize the resistance build-up is necessary. Therefore, we conducted studies to evaluate additional insecticide active ingredients for managing SWD and explore potential insecticide resistance issues in major producing growing areas in California.

Objectives.

1. To evaluate insecticide active ingredients against spotted wing drosophila in cherry orchards.
2. To conduct preliminary testing of wild spotted wing drosophila flies to detect potential insecticide resistance.

Objective 1. Insecticide efficacy in the lab and field.

In 2020, we screened several conventional and organic insecticides approved by the CA Cherry Board Research Committee. The study was conducted in the lab by exposing SWD flies to insecticide-treated fresh cherries and assessing fly mortality. Insecticide active ingredients used were, 1) cyantraniliprole (Exirel @16 fl. oz/ac); 2) cyclaniliprole (Verdepryn 11 fl. oz/ac); 3) spirotetramat (Movento @ 9 fl. oz/ac); 4) cyantraniliprole+abamectin (Mintecto Pro 12 fl. oz/ac); 5) Erythritol 0.1 M, Erythritol 1.5 M + Sucrose 0.5 M; 6) pyrethrin (Pyganic 1.4 EC 2 qt/ac), *Chromobacterium subtsugae* (Grandevo 3 lb/ac); 7) *Burkholderia* spp. strain A396 (Venerate 4 qt/ac); 8) lambda-cyhalothrin (Warrior II 2.56 fl. oz/ac) and 9) water control. This study showed that, in addition to industry-standard (Warrior II, avg. mortality 98%), Exirel (93%), Verdepryn (88%), Minecto Pro (84%), and Pyganic (84%) were highly effective against SWD adults at 48 hours after exposure (see Table 1). The rest of the insecticides were at par with the control mortality.

Table 1. Effect of insecticide treatments on SWD adult mortality. Means within the column with the same letters are statistically not different (ANOVA, $p > 0.05$).

SN	Treatments	Adult SWD mortality (%) (Mean ± SE) for various insecticide treated cherry fruits after exposure in the lab.		
		6 h	24 h	48 h
1	Exirel	10 ± 4.4 c	71 ± 4.8 b	93 ± 3.0 a
2	Minecto Pro	10 ± 3.3 c	64 ± 3.7 b	84 ± 4.2 a
3	Pyganic	42 ± 7.1 b	74 ± 5.6 ab	85 ± 6.7 a
4	Venerate	0 ± 0 c	5 ± 5.0 c	17 ± 7.7 bc
5	Grandevo	0 ± 0 c	6 ± 2.2 c	21 ± 4.3 b

6	Erythritol	0 ± 0 c	4 ± 2.6 c	14 ± 4.0 bc
7	Sucrose + Erythritol	0 ± 0 c	0 ± 0 c	0 ± 0 d
8	Warrior II	63 ± 5.7 a	90 ± 4.2 a	98 ± 2.0 a
9	Control	0 ± 0 c	2 ± 1.3 c	13 ± 4.4 bc
	Statistics	$P<0.05$	$P<0.05$	$P<0.05$

In the 2021 season, we tested the efficacy of the selected insecticides from the 2020 season against the SWD population in the field. Treatments were Exirel, Verdepryn, Minecto Pro, and Pyganic, Warrior II, and negative control (untreated control). The study was conducted in a portion of the 5-acre orchard in Stockton, CA, using one tree as an experimental unit and replicated five times. We kept one tree between two treated trees as a border and only used fruits from the central portion of the treated trees for evaluation to minimize the contamination. A power backpack sprayer (Stihl SR 200) was used to spray the insecticides after the cherry fruit developed its color. The insecticides were applied at a rate of 100 gallons/acre. At 1 and 7 days after the insecticide application, cherry fruits were collected, brought to the lab, and tested for: a) adult mortality and b) adult emergence.

a) Adult mortality

In the study with fruits exposed to field environmental factors for 1 d after spray, the insecticide treatments differed significantly with regard to fly mortality ($P < 0.05$). At the end of the 72 h, only Exirel and Warrior II caused significantly higher mortality of adult SWD than untreated control ($F = 26.64$, $df = 5, 24$, $P < 0.001$). In the study with fruits picked 7 d after spray; however, only the Warrior insecticide caused significantly higher mortality than that of untreated control ($F = 4.09$, $df = 5, 24$, $P < 0.008$) (See Table 2). Overall, the field residue study showed that, in addition to industry-standard Warrior, Exirel insecticide is an effective rotation product against SWD.

Table 2. Adult SWD mortality in sweet cherry fruits exposed to 1 d or 7 d field-weathered insecticide residues in various periods. Means within the column with the same letters are statistically not different (ANOVA, $P > 0.05$).

SN	Treatments	Adult SWD mortality (%) (Mean±SE) in 1-day field-weathered insecticide residue on cherry fruits after exposure in the lab for			Adult SWD mortality (%) (Mean±SE) in 1-week field-weathered insecticide residue on cherry fruits after exposure in the lab for	
		24 h	48 h	72 h	24 h	48 h
1	Exirel	8 ± 3.3 a	40 ± 10.1 bc	88 ± 5.2 b	14 ± 4.5	28 ± 5.9 ab
2	Movento	0 ± 0 a	2 ± 1.7 a	4 ± 3.5 a	2 ± 1.7	10 ± 0 a
3	Pyganic	4 ± 2.1 a	8 ± 3.3 a	16 ± 6.0 a	6 ± 2.1	12 ± 1.7 a
4	Verdepryn	8 ± 3.3 a	22 ± 7.6 ab	34 ± 9.2 a	8 ± 5.2	20 ± 7.4 ab
5	Warrior	24 ± 3.5 b	54 ± 7.2 c	76 ± 8.7 b	16 ± 5.3	40 ± 7.4 b
6	Control	0 ± 0 a	0 ± 0 a	4 ± 2.1 a	4 ± 2.1	14 ± 2.1 a
Statistics		$P<0.05$	$P<0.05$	$P<0.05$	$P<0.05$	$P<0.05$

b) Adult emergence.

We assessed the intensity of fruit damage in various treatments by collecting treated fruits from the trees at 1 or 7 days after the treatment, putting them in ventilated containers (12 oz.), and letting the adults emerge from these treatments. However, no flies emerged from any treatments, including untreated Control. This was not completely surprising given 2021 being the very dry year, and natural infestation of SWD in the field this year appeared to be very low compared to other years; certainly, it was the case in the orchard where did our trial.

c) Efficacies of insecticides against SWD eggs and larvae.

We also examined the ovicidal and larvicidal effects of these five insecticides in the lab. For this study, we released ~50 SWD flies into a 1 ft. x. 1 ft fabric cage with over 100 cherry fruits inside. After 24 hours, we counted oviposition stings (marks) in these fruits and grouped them into 3 fruits with a total of 5 oviposition marks as one replicate. For the ovicidal effect study, cherry fruits were treated with insecticides immediately, and we ran 6 replications for each treatment, including control. For the larvicidal effect study, after removing the SWDs from the containers in 24 h, we left cherry fruits in labs for two more days. Then, we treated them with the insecticide 74 h after oviposition. There were only 3 replications for larvicidal study. This study is still preliminary, and we can not make any conclusions. However, we found that Verdepryn may be very effective against SWD eggs (Table 3).

Table 3. SWD eggs and larva mortality after exposure to insecticides in sweet cherry fruits over 4 weeks.

SN	Treatments	SWD mortality (%) (mean ± SE) after exposure to insecticides in the egg stages in sweet cherry fruits over 4 weeks (n=10)	SWD mortality (%) (mean ± SE) after exposure to insecticides in the larval stages in sweet cherry fruits over 4 weeks (n=3)
1	Exirel	78.0±10.0	66.6±17.6
2	Movento	64.0±11.0	40.0±11.5
3	Pyganic	58.0±12.0	NA
4	Verdepryn	98.0±2.0	66.6±24.0
5	Warrior	72.0±12.7	86.6±6.6
6	Control	64.0±10.6	40.0±30.5

Objective 2. Testing for insecticide resistance.

Because of the low SWD populations and issues throughout the valley this year, and the difficulty in finding cherry orchards with SWD insecticide issues, we could not conduct this objective this year. I coordinated with Dr. Frank Zalom at UCD about this study, but the lack of flies in the field to test made it difficult to accomplish this objective. The COVID19 and the University's guidelines and restrictions make it challenging, especially for early-season fly collection and coordination. We continue to look for the opportunity to accomplish this goal in the 2021 season. Since I have not utilized the budget for this objective in the current funding cycle, we will finish this work in 2022 season without requesting additional funding from the Cherry Board.

2021 ANNUAL REPORT

Project Title: IMPROVING THE SANITARY STATUS OF SWEET CHERRY PLANTING MATERIAL

Principal investigator: Florent Trouillas, Associate C.E. Specialist, flotrouillas@ucanr.edu

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Mohammad Yaghmour, Farm advisor, UCCE Kern County
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Rosa Jaime Frias, Laboratory Assistant, KARE

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 **(year 1 and year 2)**

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

Summary of major outcomes:

This work confirmed critical stages of fungal pathogens infection during the tree production process at the nursery and identified pruning wounds on rootstocks made following budding as the main infection court for canker/wood decay diseases of cherry planting stocks. Fungicidal treatments including biocontrol, a sealant and chemical products were tested in the field at a nursery to prevent infection of such pruning wounds by fungal pathogens. Two trials were established at a nursery during the spring 2021. Results indicated the efficacy of the biocontrol product Vintec and the sealant Doc Farewell's to protect pruning wound infection of the rootstock reducing infection by up to 62.5% when compared to non-treated trees. In nursery trials however, the overall infection of trees, which relied on natural inoculum, was low and additional trials using artificial inoculation under controlled conditions will be necessary to confirm results. These trials will be established at KARE in the spring 2022. In addition, important sources of inoculum for pathogenic Basidiomycota fungi (Trametes, Schizophyllum) were identified at a nursery and recommendations were made to destroy sources to improve sanitation at the nursery. Finally, molecular detection tools were used to sample two nursery mother blocks for the detection of several viruses including LChV-1 and LChV-2 and the X-disease phytoplasma responsible for little cherry disease. Although these pathogens were not detected at the nurseries, the molecular detection tools proved to be effective to detect Little Cherry virus 1, the X-disease phytoplasma as well

as other common cherry viruses in commercial cherry orchards. Extension and outreach activities about improving the sanitary status of nursery planting material at the nursery were provided to nurserymen. Similarly, information about the status of Cherry X disease and Little Cherry viruses in California was presented to cherry growers at farm advisors' meetings and field days.

Materials and methods:

Objective 1: Determine the critical stages of fungal pathogen infection and contamination sources during tree production at the nursery

For this objective, three main nurseries supplying sweet cherry trees in California were visited to monitor the various steps of the tree production process and identify steps as well as specific conditions likely to induce infection and contamination of trees. This work included the sampling of young rootstocks in the field, budded trees prior to shipping, bud wood as well as mother blocks in the field. Multiple rootstock varieties and scion cultivars were sampled using standard culture-dependent (isolation in culture medium) methodologies for the detection of fungal pathogens. Approximately 10 wood pieces of the sampled tissues (rootstock wood) was surface-sterilized by immersion for 2 mins in a 1.5 % sodium hypochlorite solution and washed twice with sterile distilled water. Pieces were then placed onto petri dishes filled with potato dextrose agar (PDA) amended with 100 ppm tetracycline (PDA-tet) for isolation of fungi. Fungal identification of growing colonies was conducted morphologically. Finally, putative sources of contamination or sources of inoculum at the nurseries were investigated. Various host plants, dead plants, and plant debris at the nursery and in the nursery close vicinity were inspected for fruiting structures of fungal pathogens responsible for canker and wood rot diseases.

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 4/29/21 and 5/12/21, respectively, on cherry trees planted in the field at a nursery. Planting, budding, pruning, irrigating and the overall nursing of trees were done as usually performed by the nursery. Following budding, pruning of rootstock was made to head back (topping) rootstocks following the standard budding/pruning operations of the nursery. Pruning wounds were 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. 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. Fifty tree replicates per treatment were included in a randomized complete block design. Infection of wounded trees at the nursery relied on natural airborne inoculum. Treated trees were collected in October 2021 and brought to the laboratory for fungal isolations. Presence (1) or absence (0) of fungal pathogens were recorded and averaged for all treatments. Lowest rates of fungal recovery (# of pathogens) was correlated with highest product efficacy. Additional rootstocks (Colt Seedlings) were ordered from a nursery and planted in the field at the Kearney Agricultural Research and Extension Center (KARE) in June 2021. The fungicide experiment at KARE will be conducted using artificial inoculation of fungal pathogens during the spring 2022.

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

Little cherry disease is an important disease of sweet cherry with currently epidemic levels across Washington State and northern Oregon. This disease is caused by one or more of three pathogens: Little Cherry Virus 1 (LChV-1), Little Cherry Virus 2 (LChV-2), and the X-disease phytoplasma (Candidatus Phytoplasma pruni). With the collaboration of CA nurseries, farm advisors and Dr. Maher Al Rwahnih,

Director of Foundation Plant Services (FPS) we assessed the occurrence, in propagation materials (i.e. budwood, mother blocks) of the X-Disease Phytoplasma, Little cherry viruses and other common cherry viruses. Testing 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.

Objective 4: Promote outreach activities and education
Results and outcomes from this research will be disseminated to nurseries through in-person meetings. Observations, findings and protocols resulting from this work will be shared with nurseries and training will be provided so that disease management and detection strategies can be implemented routinely in nurseries willing to participate in a training program. 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.

Results and discussion:

Objective 1: Determine the critical stages of fungal pathogen infection and contamination sources during tree production at the nursery:
Three main nurseries propagating cherry trees in California were visited to determine the sanitary status of cherry planting materials and identify putative infections routes in the tree production pipeline and potential contamination sources at the nursery. For each nursery, we examined the various steps of tree production to determine practices and conditions likely to induce infection and contamination of trees with fungal pathogens. Trees and various plant parts were sampled at different stages of tree propagation and included rootstock cuttings and budded trees in the field, budwood sticks in storage and mother trees in the field as well as trees stored in sawdust bins prior to shipping. Multiple rootstock varieties and scion cultivars were sampled at these different stages from three nurseries. One out of three nursery sampled had trees infected with wood decay or canker pathogens near the bud union. Sampling of the various plant materials confirmed previous observations indicating that mainly wounds made to head back rootstocks following budding served as entry sites for fungal canker and wood decay pathogens. Main pathogens encountered in trees sampled in the spring of 2021 included *Eutypa lata* (20% to 30% of trees infected), *Phomopsis* sp. (20% of trees infected) and a Basidiomycete (10% of trees infected). No additional infection routes for fungal pathogens or contamination events were detected at the nursery.

In addition, we investigated putative sources of inoculum at the nurseries for fungal canker and wood decay pathogens. This last objective aimed to improve nursery cleanliness by identifying and removing contamination sources including dead plants and plant debris with fungal fruiting structures occurring on-site at the nursery. At one location, we detected fruiting structures of common fungal pathogens responsible for wood rot and wood decay diseases. Fungal fruiting bodies occurred on dead cherry stumps left in the orchards (Figs. 1-2-3). Fungi identified included *Trametes versicolor* and *Schyzophyllum commune* also previously identified by our laboratory as common contaminants of cherry planting materials from the same nursery. The occurrence of mature walnut orchards in nursery fields also indicated these may serve as putative sources of inoculum for *Phomopsis* sp, a common pathogen of walnut recently reported as a canker pathogen of cherry planting stocks. Recommendations were made to nurserymen to remove all dead plant material including tree stumps from nursery fields. Additional sampling of cherry planting materials at another nursery indicated that trees growing in open fields or new planting grounds with no mature orchards in the vicinity showed no infection at pruning wounds resulting from budding.

Objective 2: Determine the efficacy of various compounds for the protection of tree wounds following budding/grafting of cherry planting material:
Two field trials were established at a nursery on 4/29/21 and 5/12/21, respectively. In this experiment we relied on natural inoculum for the infection of treated and control trees. Percent infection of pruning wounds or trees treated with water-only was 12% and 16% for Trial 1 and Trial 2, respectively, thus infection rates appeared low to moderate in our control treatments. Main pathogens isolated from both trials included, *Eutypa lata*, *Phomopsis* sp., *Botryosphaeria* sp. and a Basidiomycetes sp. All pathogens isolated in our trials corresponded to fungal pathogens previously identified in association with contaminated nursery stocks. Of the 5 compounds tested against, Vintec and the Doc Farewell's sealant performed best, each providing 50% and 62.5% disease reduction, in Trial 1 and Trial 2, respectively (Figs. 10 and 11). In addition, Topsin M + Rally, Quilt Xcel, and Luna Experience provided moderate disease control varying between 16.5% to 32.5% disease reduction. Experiments confirmed the superior efficacy of Trichoderma product Vintec in protecting pruning wounds, as shown in previous experiments. Also, experiments indicated that isolate *Trichoderma atroviride* SC1 from Vintec was recovered from 80% to 86% of the treated trees, six months after treatment was applied. This suggests that such product may provide long term protection of pruning wounds and trees against fungal pathogens. This information together with protection results has been of interest to nurseries and the application of Trichoderma products as a standard practice to durably protect nursery stocks from fungal infection is currently being discussed with nurserymen. The overall low disease incidence in control treatments and the likelihood that some trees may have been already infected prior to the start of the experiment (at or shortly after planting due to wounding during the handling of trees) suggest that controlled experiments that include artificial inoculation of fungal pathogens on treated trees is needed to determine product efficacy.

For the Kearney experiment, because of the small size of rootstocks at planting (July 2021), fungicide treatments and fungal inoculations will be applied during the spring of 2022. Trees will be treated with the same products as described above and inoculated with 100 µL of a spore/mycelial suspension of the fungal canker pathogens *Calosphaeria pulchella*, *Eutypa lata*, *Cytospora sorbicola*, *Phomopsis/Diaporthe* spp., *Trametes versicolor* and *Schizophyllum commune* at a concentration of 1,000 spores per wound.

Objective 3: Investigate the occurrence of the X-Disease Phytoplasma and Little cherry viruses in cherry propagation materials:
Thus far, two mother blocks at two different nurseries were sampled in July 2021. In each mother block, 5 to 6 cherry cultivars were selected to be sampled and trees were inspected for symptoms suspicious of cherry X-disease and viral diseases (Figs. 4-5-6). Thirty leaves per cultivar were selected and a total of 350 leaves were submitted for molecular analyses, which included testing for Phytoplasmas (P-Phyto), Little Cherry Virus 1 (LChV1), Little Cherry Virus 2 (LChV2), Prune Dwarf Virus (PDV), Prunus Necrotic Ringspot Virus (PNRSV). All sampling of nursery materials was negative for the targeted pathogens (Table 1).

The same molecular detection tools were used to test trees in grower orchards expressing symptoms suspicious of cherry X-disease as well as from orchards occurring in the vicinity of orchards formerly diagnosed with X-disease. The assay confirmed the occurrence in CA cherry orchards of the X-disease phytoplasma, LChV1, PNRSV and PDV (Table 1 & 2). The sampling and testing also confirmed the spread of the X-disease phytoplasma from diseased blocks to neighboring orchards (Table 1 & 2).

Additional orchard visits included one orchard in San Joaquin County with trees expressing symptoms of a sudden decline or sudden tree collapse. Trees were grafted on Mahaleb rootstock and exhibited symptoms characteristic of Cherry X-disease, including zippering under the bark at the bud union and leaves turning bronze (Figs. 7-8-9). Symptoms also resembled to some extent a form of rootstock/scion

incompatibility. Using real-time qPCR and selected markers for common Phytoplasma, no phytoplasma was detected from this orchard (Table 1). A detection follow-up 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, suggesting genetic rootstock-scion incompatibility could be the issue.

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.

Presentations included:

- Two Continuing Education Meetings San Joaquin Ag County (X-disease/fungal pathogens)
- One IPM Breakfast meeting Stanislaus County (X-disease)
- A dozen of in-person field education meeting (farm calls) (X-disease/fungal pathogens)

Newsletter articles:

- August 2020 San Joaquin County Newsletter: X-Disease
- UCCE San Joaquin Trees and Vines website. September 2020. X-Disease article

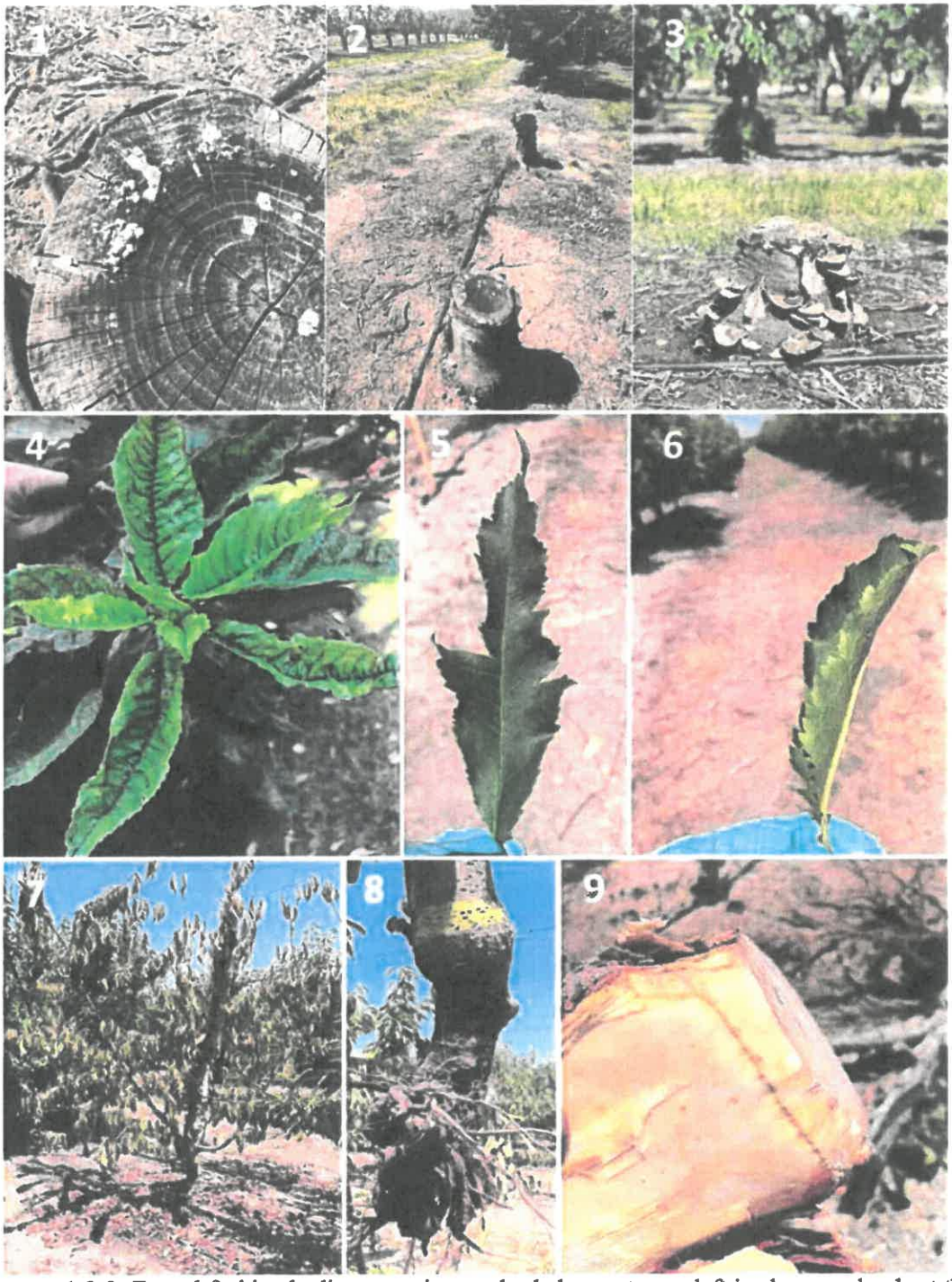
Tables & Figures

Sample #/Description	P-LChV1	P-LChV2	P-PDV	P-Phyto	P-PNRSV
#1: Kern Co, orchard previously tested positive for X Phytoplasma	POSITIVE	negative	POSITIVE	negative	POSITIVE
#2: Kern Co, orchard just South of #1	negative	negative	POSITIVE	negative	POSITIVE
#3: Nursery 1	negative	negative	negative	negative	negative
#4: Nursery 1	negative	negative	negative	negative	negative
#5: Nursery 1	negative	negative	negative	negative	negative
#6: Nursery 1	negative	negative	negative	negative	negative
#7: Nursery 1	negative	negative	negative	negative	negative
#8: Trees with Sudden Collapse, zippering of the bud union	negative	negative	negative	negative	negative
#9: Trees with Sudden Collapse, zippering of the bud union	negative	negative	negative	negative	negative
#10: Nursery 2	negative	negative	negative	negative	negative
#11: Nursery 2	negative	negative	negative	negative	negative
#12: Nursery 2	negative	negative	negative	negative	negative
#13: Nursery 2	negative	negative	negative	negative	negative
#14: Nursery 2	negative	negative	negative	negative	negative
#15: Nursery 2	negative	negative	negative	negative	negative

Table 1. Results of molecular testing in commercial cherry orchards and nursery mother blocks for Phytoplasmas (P-Phyto), Little Cherry Virus 1 (LChV1), Little Cherry Virus 2 (LChV2), Prune Dwarf Virus (PDV), Prunus Necrotic Ringspot Virus (PNRSV).

Description	P-LChV1	P-LChV2	P-PDV	P-Phyto	P-PNRSV
Tree 1	negative	negative	negative	POSITIVE	negative
Tree 2	negative	negative	negative	negative	negative
Tree 3	negative	negative	negative	POSITIVE	negative
Tree 4	negative	negative	POSITIVE	negative	negative

Table 2. Results of molecular testing in one commercial cherry orchard adjacent to an orchard formerly diagnosed with X-disease.



Figures 1-2-3: Fungal fruiting bodies occurring on dead cherry stumps left in cherry orchards at the nursery. Fungi identified included *Schyzophyllum commune* (1, 2) and *Trametes versicolor* (3); **4-5-6:** Suspicious symptoms on cherry leaves collected at nurseries (mother blocks) and submitted for testing in the FPS laboratory. All sampling from tissues collected at the nurseries was negative for phytoplasma and viral pathogens; **7-8-9:** Symptoms of an unidentified sudden tree collapse (under investigation) including zippering under the bark at the bud union and bronzing of leaves.

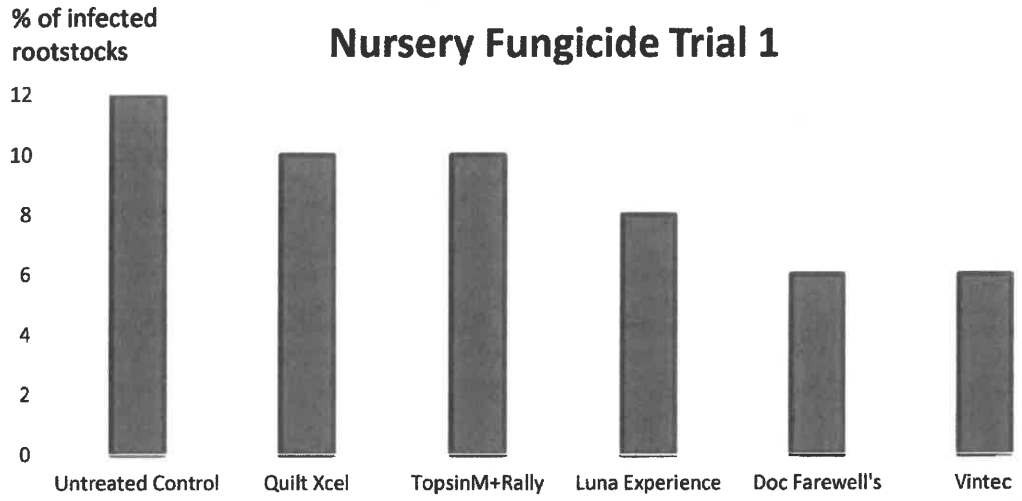


Fig. 10: Efficacy of treatments applied by handheld spray bottles or a paintbrush (Doc Farewell's) to pruning wounds resulting from the topping of cherry rootstocks in the field at a nursery. Pruning wound infection relied on natural inoculum present at the nursery. Trial 1 was established on 4/29/21.

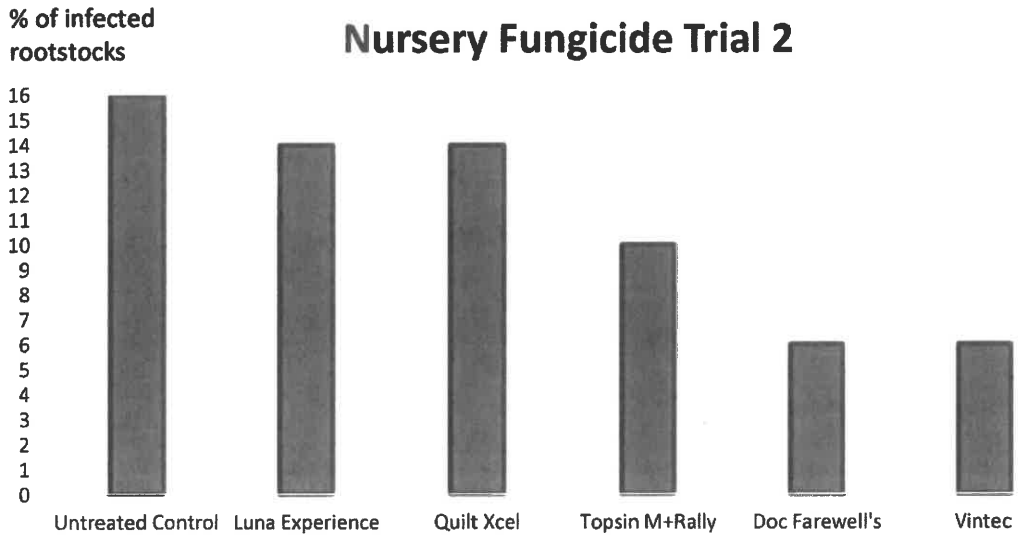


Fig. 11: Efficacy of treatments applied by handheld spray bottles or a paintbrush (Doc Farewell's) to pruning wounds resulting from the topping of cherry rootstocks in the field at a nursery. Pruning wound infection relied on natural inoculum present at the nursery. Trial 1 was established on 5/12/21.

Annual Report - 2021

Prepared for the California Cherry Advisory Board

Project Title:	Management and Epidemiology of Pre- and Postharvest Foliar and Fruit Diseases of Sweet Cherry
Project Leader:	Dr. James E. Adaskaveg, Department of Plant Pathology, University of California, Riverside, CA 92521 (951) 827-7577
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SUMMARY

In 2021, 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. New bactericides were available for evaluation, and several studies on cv. Coral Champagne were conducted on their efficacy. Due to insufficient low-temperature periods at the trial sites, however, no disease developed.
   b. 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 is pending at EPA for fall 2021. New formulations of the antimicrobial food additives nisin and ε-poly-L-lysine are being developed in collaboration with a potential registrant.

2) In a **powdery mildew** study in San Joaquin Co., the experimentals Miravis Duo and Miravis Prime, as well as the registered Cevya, Luna Sensation, and a rotation of Fontelis, Rally+Quintec, and Merivon were highly effective in reducing the disease on inside and outside shoots. The plant extracts BacStop+EF400 and ProBlad and the fungicides Quadris Top, Merivon, Ph-D+Procure, and GWN-10570 only showed good to very good efficacy on outside shoots where disease pressure was lower.

3) A field study was conducted on the efficacy of **fungicide treatments applied 7 days preharvest**.
   a. **Brown rot:** All preharvest treatments significantly reduced the incidence of brown rot on non-washed fruit after wound- and non-wound-inoculation with *M. fructicola*, but the plant extracts EcoSwing and ProBlad were only effective in non-wound inoculations. On washed fruit, the synthetic fungicides were highly effective in reducing decay when fruit were non-wound inoculated, but the two plant extracts also significantly reduced the incidence. In wound inoculations, many of the fungicide treatments showed reduced efficacy as compared with non-washed fruit. The efficacy of Cevya, Quash, Quadris Top, Miravis Duo, Ph-D + Procure, and UC-2, however, remained very high.
   b. For **gray mold**, using non-washed fruit, high efficacy was obtained on non-wound- and wound-inoculated fruit using Luna Sensation, Miravis Prime, Merivon, Fontelis+Teb, Miravis Duo, pyraziflumid, GF4536, and UC-2. On washed fruit, Luna Sensation, Miravis Prime, and the experimental GF4536 demonstrated good efficacy.

5) **Postharvest studies** focused on the evaluation of new biologicals in laboratory studies and on the comparative efficacy of registered fungicides in commercial packinghouse studies.
   a. Among biological treatments, spray treatments with a high rate of Doxall (capric acid) showed the most consistent efficacy against the three major decays. The efficacy of Howler was inconsistent, ranging from high (Rhizopus rot) or very high (brown rot, gray mold) to not effective (brown rot, Rhizopus rot). Theia, Aviv, and Timorex Act had no or very little efficacy. Yarden, a mixture of fludioxonil and tea tree oil, was similarly highly effective as Scholar or Chairman.
   b. In commercial packinghouse studies, T-Jet, drench, or dip applications with Chairman were more effective than a drench application with Scholar or a T-Jet application with Tebucon. Our data indicate that Chairman, a pre-mixture of fludioxonil and propiconazole with a broad spectrum of

activity against brown rot, gray mold, Rhizopus rot, and sour rot, sets a new standard to be the most effective postharvest treatment available for sweet cherry.

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 11 cherry orchards with tree decline, *P. cactorum, P. cambivora, P. cryptogea, P. syringae*, a currently unidentified *Phytophthora* spp., and *Phytopythium vexans* were recovered from four orchards, mostly by soil pear baiting. Inoculation studies are ongoing to determine the virulence of these species on cherry.
   b. Three field efficacy studies were established at UC Davis and UC Riverside where the soil adjacent to tree trunks was inoculated with *Phytophthora* spp. Orondis and Revus treatments resulted in improved tree health in two of the orchards where data have been obtained to date, but Presidio+Elumin was only highly effective in the UC Davis study. Two of these trials are ongoing, and additional treatments and evaluations will be done.
   c. In greenhouse studies, systemic movement of Presidio, Orondis, ProPhyt, and Ridomil Gold in Mahaleb, Mazzard, and Krymsk rootstocks was demonstrated after soil application. Canker size in comparison to the untreated control was reduced when stems of soil-treated plants were inoculated with *P. citricola* two weeks after treatment. Uptake into plant roots will benefit treatment efficacy because roots and crowns will be protected from infection for extended periods.
   d. IR-4 residue studies with Orondis are currently ongoing to obtain registration of this fungicide on sweet cherry in the United States.

INTRODUCTION

Management of bacterial blast and canker. Pseudomonas syringae pv. *syringae* (*Pss*) is the main pathogen causing bacterial blossom blast and canker of sweet cherry and other stone fruit crops in California. Cold, wet conditions are associated with both phases of the disease. Cankers with gumming around the infected, sunken bark tissue develop after several weeks to months following infection of twig and branch wounds. 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.

We evaluated numerous other compounds that could be used in rotations and mixtures. These include oxytetracycline (Fireline, Mycoshield) that we are also pursuing for registration, 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, and Cinetis, a nutritional stress reducer. Two GRAS antibacterial food additives (i.e., nisin and ε-poly-L-lysine) showed promising results. Trials were initiated in 2021 to further evaluate their effectiveness. In collaboration with a chemical company, agrochemical formulations are being designed for nisin and ε-poly-L-lysine, and these need to be tested. Additional natural products 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, as well as new combinations and rotations of registered fungicides are being evaluated. Alternative fungicides that we evaluated over several years in our field trials on sweet cherry in California include the FRAC Code (FC) 3 (DMI) Procure (triflumizole) and FC 7 (SDHI) (e.g., fluopyram, fluxapyroxad, and penthiopyrad) compounds, and the pre-mixtures Luna Sensation (fluopyram/ trifloxystrobin), Merivon (fluxapyroxad/ pyraclostrobin) (FC 7/11), and Quadris Top (azoxystrobin/ difenoconazole) (FC 3/11), as well as polyoxin-D (FC 19). In 2021, we evaluated these and other new compounds such as the experimentals Miravis Duo, Miravis Prime, pyraziflumid, and GWN-10570, as well as biologicals based on plant extracts (FC BM-01; i.e., ProBlad, BacStop, EF400). 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 2021 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 and Luna Experience (both FC 3/11) as well as Pristine, Luna Sensation, and Merivon (all FC 7/11) 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. 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 BioSpectra (natamycin, 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. All currently registered fungicides are effective against brown rot and gray mold, but Penbotec is 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 FRAC codes registered, Tebucon and Mentor are not 'reduced-risk' fungicides. Scholar, Penbotec, and recently 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. Commercial packinghouse studies were conducted with Scholar, Chairman, and Tebucon using drench, T-Jet, or dip applications in 2021 to compare efficacy against brown rot and gray mold and ultimately help identify the most efficacious and cost-effective treatment. Laboratory studies were conducted on the efficacy of new treatments: three biocontrols (Howler – *Pseudomonas chlororaphis*, and Theia and Aviv (both *Bacillus subtilis*), an essential oil plant extract (Timorex Act), and capric acid (Doxall), and Yarden, a mixture of fludioxonil and Timorex Act.

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 *Phytophthora* spp. infection, research is warranted to identify the species

Table 1: Fungicides and bactericides used in 2021 studies*.

FRAC Code	Trade name	Active ingredient
Single active ingredients		
3	Cevya	mefentrifluconazole
3	Procure	triflumizole
3	Quash	metconazole
3	Rally	myclobutanil
3	Tebucon, Teb	tebuconazole
4	Ridomil Gold	mefenoxam
7	Fontelis	penthiopyrad
7	Pyraziflumid	pyraziflumid
12	Scholar	fludioxonil
13	Quintec	quinoxyfen
19	Ph-D, Oso	polyoxin-D
22	Elumin	ethaboxam
40	Revus	mandipropamid
43	Presidio	fluopicolide
48	Orondis	oxathiapiprolin
P07 (33)	ProPhyt	potassium phosphite
Experimentals	GF4536	not disclosed
	GWN 10570	not disclosed
	GS-2	not disclosed
	UC-2	not disclosed
Biologicals		
BMO1	BacStop	essential oils
BMO1	Ecoswing	extract of *Swinglea glutinosa*
BMO1	EF400	essential oils
	Doxall (GS-2)	capric acid
BMO1	ProBlad	extract of *Lupinus albus*
BMO1	Timorex ACT (TACT)	tea tree oil
BMO2	Howler	*Pseudomonas chlororaphis*
BMO2	Theia	*Bacillus subtilis*
BMO2	AVIV	*Bacillus subtilis*
Premixtures		
3 + 11	Quadris Top	difenoconazole + azoxystrobin
3 + 12	Chairman	propiconazole + fludioxonil
7 + 3	Miravis Duo	pydiflumetofen + difenoconazole
7 + 3	Luna Experience	fluopyram + tebuconazole
BMO2 + 12	Yarden	fludioxonil + tea tree oil
7 + 11	Luna Sensation	fluopyram + trifloxystrobin
7 + 11	Merivon	fluxapyroxad + pyraclostrobin
7 + 12	Miravis Prime	pydiflumetofen + fludioxonil

* - 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.

involved. For example, on almond, two new species of *Phytophthora* have been described in the last 15 years that are highly aggressive (e.g., *P. niederhauseri* and *Phytophthora* sp. ax) and are difficult to manage. Therefore, surveys were initiated in California cherry growing areas, and isolations were conducted from cherry roots, crowns, and from rhizophere 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 started to determine the in vitro toxicity of these new fungicides to isolates of *Phytophthora* spp. from cherry. We established three field trials at UC Davis and UC Riverside where trees were inoculated. In collaboration with growers, field plots were 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.

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, nisin, ε-poly-L-lysine): small-scale field trials.
   b. Antibiotics – kasugamycin, oxytetracycline: 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: EXP-19A, Cevya (mefentrifluconazole), pyraziflumid, new premixtures (Miravis Top, Miravis Prime, Fervent, and UC-2), 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 determine EC_{50} values for baseline sensitivities and monitor for resistance in target pathogen populations to new fungicides.
   c. Continue to evaluate 'exempt from tolerance' biofungicides (natamycin and polyoxin-D) and organic (e.g., polyoxin-D) or nominated for organic compounds (e.g., natamycin).
4. Evaluate new fungicides for managing Phytophthora root rot of cherry: oxathiapiprolin, mandipropamid, fluopicolide, and ethaboxam.
   a. In vitro studies on isolates of *Phytophthora* spp. from cherry with emphasis on *P. cambivora*. This data will be used for establishing baseline sensitivities for future reference in detecting potential resistance in the pathogen.
   b. Initiate field studies with growers in newly planted orchards to prevent Phytophthora root rot in the presence of natural pathogen populations and in experimental orchards to evaluate the efficacy of each fungicide at selected rates. In experimental orchards, trees will be inoculated with *P. cambivora* or other *Phytophthora* spp.

MATERIALS AND METHODS

Evaluation of new products against bacterial blast in flower inoculation studies and against canker in twig inoculation studies. Two trials on blossom blast were done on cv. Coral cherry at UC Davis and in a commercial orchard just before a predicted cold weather period. Flowers in clusters (eight single-branch replications on different trees for each treatment) were partially emasculated by cutting pistils, stamens, and part of the petals using scissors on 3-16 and 3-17-21. Bactericide applications were made using a hand sprayer. After air-drying for 2 h, flowers were inoculated with *P. syringae* (5×10^7 cfu/ml) by hand-spraying. Inoculated branches were covered with white plastic bags overnight. Disease was evaluated after 8-14 days. In a study on bacterial canker, branches of cv. Coral cherry trees were puncture-wounded

laterally in Dec. 2020 using a nail to expose the cambium and wood. Wounds were spray-treated with bactericides 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 2021. Treatments used in bacterial blast and canker studies included Kasumin, Mycoshield, nisin, Ɛ-poly-L-lysine, ningnanmycin, as well as mixtures of Mycoshield with Kasumin, nisin with Ɛ-poly-L-lysine, or Cueva with Double Nickel. Dart, Manniplex Zn, or LI700 were added to selected treatments.

Evaluation of new fungicides for control of powdery mildew. In a field trial in San Joaquin Co., treatments were done on 3-24 (50% bloom), 4-6, and 4-20-21. These applications were targeted to provide protection from primary ascospore inoculum from overwintering chasmothecia and from infection by secondary conidial inoculum. Single fungicides, mixtures, pre-mixtures, and two rotation programs were evaluated (see Fig. 1). The incidence of powdery mildew was evaluated on 6-8-21 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 7 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 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 continued comparative evaluation of several new biological treatments that included biocontrols (i.e., *Bacillus subtilis* – Theia and Aviv, *Pseudomonas chororaphis*- Howler), essential oils (i.e., Timorex Act), capric acid (Doxall, GS-2), and Yarden, a mixture of fludioxonil and Timorex Act. In these studies, Bing cherry fruit were wound-inoculated with *M. fructicola, B. cinerea,* or *R. stolonifer* as described above and treated after 12 h by spraying using an air-nozzle sprayer. After treatment, fruit were incubated for 4-7 days at 20 C, >95% RH. In commercial packingline studies, treatments with Chairman using drench, T-Jet, or dip applications were compared to a drench application with Scholar or a T-Jet application with Tebucon. Fruit were wound- or non-wound inoculated after treatment using procedures described above. 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 and evaluate new fungicides for managing Phytophthora root rot and crown rot. Surveys were done on causal agents of cherry trees declining from apparent *Phytophthora* spp. infection in 11 commercial orchards in San Joaquin Co. Root, crown, and rhizosphere soil from symptomatic cherry trees were collected. 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 rhizosphere soil isolations, 10-g aliquots were mixed with 90 ml sterile distilled water in a 250-ml flask, shaken for 40 min, and 1 ml suspension was plated onto PARHFB-V8C medium. Plates were rinsed with deionized water after 24 h at 25°C to remove excess soil, and then further incubated for 1 to 2 days. For pathogen detection in soil, pear baiting was also done. 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 12°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.

Field studies on the evaluation of new fungicides are done in two orchards at UC Davis (Mahaleb rootstock with Bing or Coral scions planted in 2020, and Mahaleb rootstock with Coral scion planted in Jan. 2021), one orchard at UC Riverside (Mahaleb rootstock with Coral scion planted in Jan. 2021), and in two commercial orchards with young trees. In subplot A of the first UC Davis trial, treatments were done in April 2020 by pouring the fungicide solution around the base of the tree and over the lower trunk. Treatments were watered in by irrigation. Trees were inoculated on the same day with a mixture of *P. citricola, P. cactorum*, and *P. cambivora* that were grown on a rice-vermiculite-V8C juice mixture. In subplot B, trees were first inoculated and treated the following day. Trees were treated again in April 2021. The second UC Davis and the UC Riverside plots were treated and inoculated in the spring of 2021 using the same procedures as indicated above. Treatments in these studies consisted of single fungicides (i.e., Orondis, Revus, Presidio, Elumin, Ridomil Gold), a premixture (i.e., Orondis Ultra = Orondis + Revus), and mixtures (i.e., Orondis + Ridomil Gold, Presidio + Elumin). There were 8-10 trees for each treatment.

Tree health was rated in the first UC Davis trial (sub-plot 2) on 9-20-20, and in the UC Riverside trial on 10-14-21 using a scale from 0 (=healthy, vigorous) to 4 (= tree dead). Trunk diameters were measured in the first UC Davis trial (sub-plot 2) 30 cm above the graft union in mid-May 2021; canopy sizes were obtained in mid-May, 2021, using the APS Assess 2.2 software (APS Press, St. Paul, MN) and compared to a standard tarp background.

Greenhouse studies with potted Bing cherry on Krymsk, Mazzard, and Mahaleb rootstocks were initiated on the efficacy of new fungicides against Phytophthora root and crown rots. The soil of each pot was inoculated with a *Phytophthora*-colonized mixture of rice, vermiculite, and V8 juice. A mixture of *P. cambivora, P. citricola*, and *P. cactorum* was used. A solution of Orondis 200 (2.4 fl oz/A), Revus (8 fl oz/A), Presidio (6 fl oz/A), or Elumin (10 fl oz/A) was applied to each pot after 7 days. Furthermore, the potential systemic movement of fungicides was evaluated after soil applications to potted Mahaleb, Krymsk, and Mazzard plants. Two weeks after treatment, stems were wound-inoculated with colonized agar plugs 10 cm and 20 cm above the soil line on opposite sides of the stem. Three weeks after inoculation, canker length under the bark was measured and was used as an indication of the amount of fungicide present in plant tissues. All data were analyzed using the Least Square Means (LSMEANS) procedure of SAS with the Tukey adjustment.

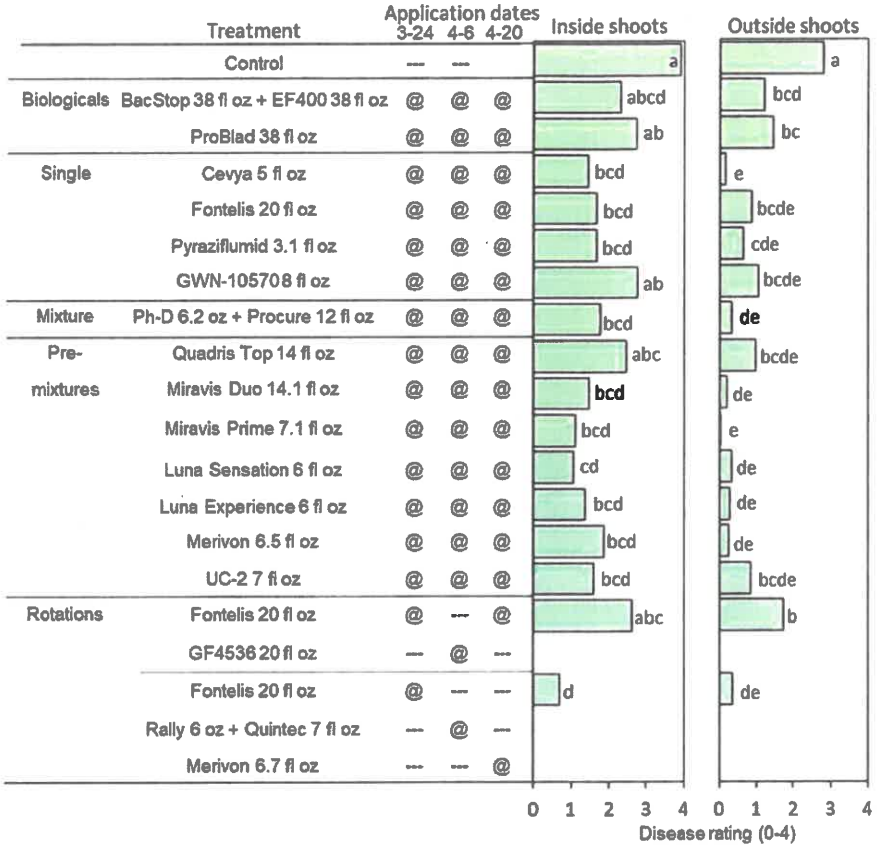
RESULTS AND DISCUSSION

Evaluation of treatments for control of bacterial blast and canker. Cold temperature periods were predicted at our trial sites, but actual temperatures were not low and long enough to cause injury that is needed for the bacterium to infect. Consequently, in the two trials conducted on bacterial blast and in the study on bacterial canker, no disease developed. Therefore, these studies will be again done in the winter of 2021/22.

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. With widespread copper resistance in the pathogen *Pss*, new effective alternative treatments are needed. In our previous years' experiments, Kasumin generally was very effective in reducing bacterial blast and canker when used at high rates, and Mycoshield/FireLine also often showed good results but sometimes was inconsistent. In 2020, the most effective treatment against blossom blast was Nisin mixed with ManniPlex Zn, whereas Mycoshield, Kasumin, BacStop, and ET91 showed intermediate efficacy. Kasumin was registered on sweet cherry in 2018. Registration of Mycoshield/FireLine is currently pursued with support of the registrant through the IR-4 program and is pending at EPA with a PRIA date of late fall of 2021.

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)

Fig. 1. Evaluation of preharvest fungicide treatments for management of powdery mildew of Bing cherries in San Joaquin Co. 2021

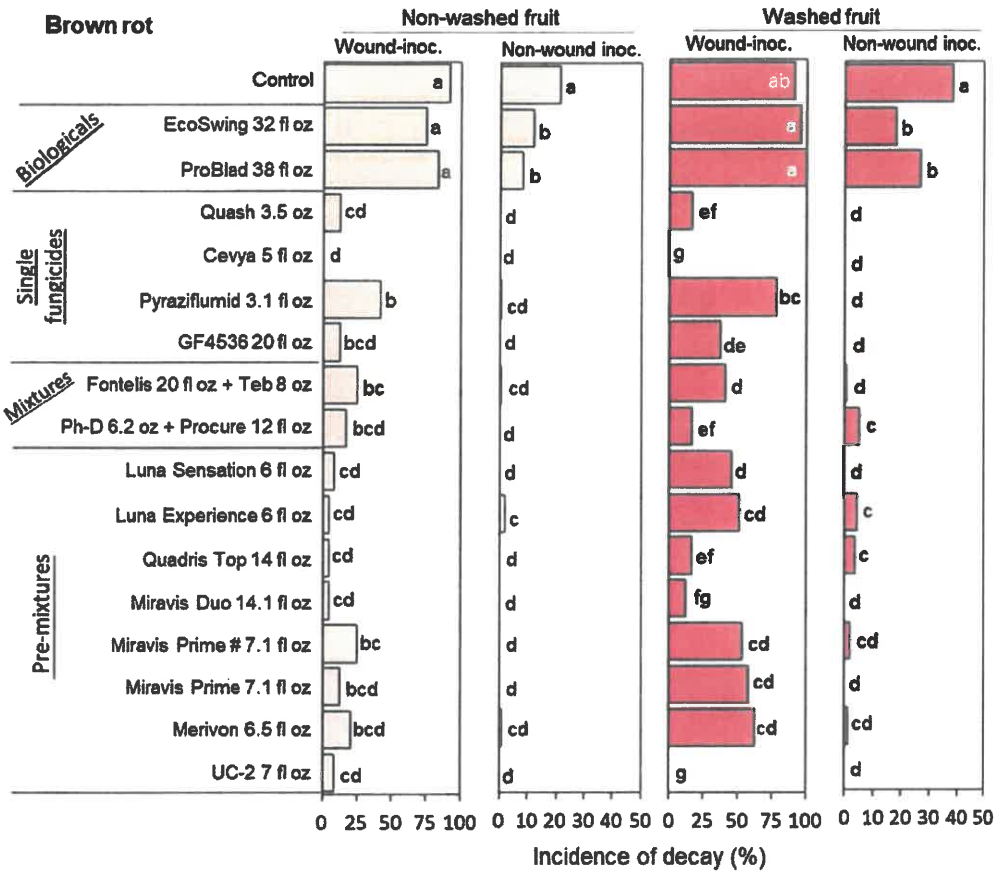


Applications were done using an airblast sprayer at 100 gal/A starting at 50% bloom, and 2 and 5 weeks after petal fall. DyneAmic (6 fl oz /A) was added to treatments in the second and third applications. For evaluation on 6-8-21, leaves from 4 random shoots each from inside or outside of the tree were sampled and rated using a scale from 0=healthy to 4 = >50% of leaf area diseased.

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, pyraziflumid, Miravis Duo, Miravis Prime, GWN-10570, UC-2, GF4536), as well as of several biological compounds based on plant extracts (i.e., BacStop, EF400, ProBlad) 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 of 2021. At evaluation in early June, disease rating on water shoots inside the canopy was 3.9 on a scale from 0 to 4, and it was 2.8 on leaves in the outer canopy.

All treatments significantly reduced the disease as compared with the control on outside shoots (Fig. 1). Numerically, Cevya, Miravis Duo, Miravis Prime, Luna Experience, Luna Sensation, Merivon, a mixture of Ph-D and Procure, as well as a rotation of Fontelis, Rally+Quintec, and Merivon had the lowest disease ratings with 0.4 or less. The latter rotation using FC 3, 7, 11, and 13 compounds was also highly effective on shoots inside the trees and resulted in the lowest disease ratings. Rotations of different FCs represent the best anti-resistance strategy, and as our data indicate, they can be very effective in managing powdery mildew. Overall, as in previous years, the experimentals Miravis Duo and Miravis Prime, as well as the registered Luna Sensation and the recently registered Cevya were highly effective on inside and outside leaves. These contain active ingredients of DMI, SDHI, and/or QoI compounds which are known to have high activity against powdery mildews. Several treatments including the plant extracts BacStop+EF400 and ProBlad, and the fungicides Quadris Top, Merivon, Ph-D+Procure, and GWN-

Fig. 2. Efficacy of 7-day preharvest fungicide treatments for management of postharvest brown rot of Bing cherries - San Joaquin Co. - 2021

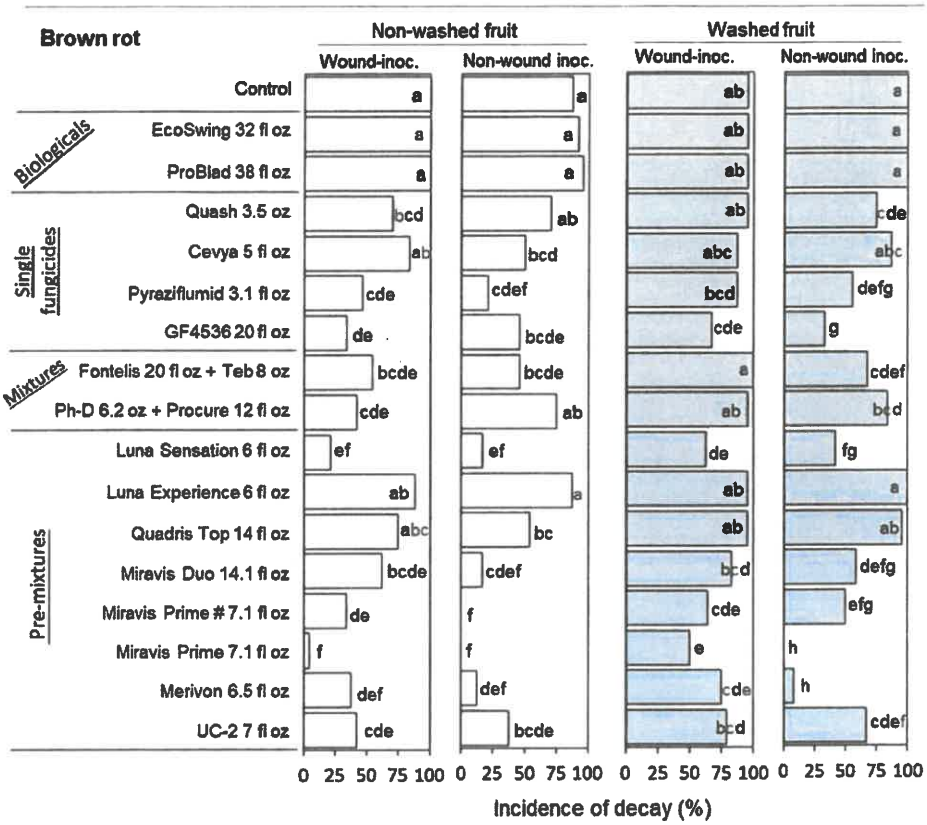


Treatments were applied on 5-25-21 using an air-blast sprayer at a rate of 100 gal/A. All treatments except EcoSwing and ProBlad were applied in combination with with DynAmic at 8 fl oz/A. Treatments were also applied on 3-24, 4-6, and 4-20-21 as part of a powdery mildew program, except for treatment No. 14 (Miravis Prime#) that was only applied on 5-25-21. Harvested fruit were washed by gently agitating in water for 2 min. Fruit were wound-inoculated with *M. fructicola* (50,000 spores/ml) *or B. cinerea* (30,000 spores/ml) or non-wound drop-inoculated with *M. fructicola* (50,000 spores/ml) or *B. cinerea* (300,000 spores/ml 50% cherry juice). Fruit were then incubated for 5-10 days at 22C.

10570 only showed good to very good efficacy on outside shoots where disease pressure was lower than on inside shoots. Treatments with intermediate activity included Fontelis, Luna Experience, pyraziflumid, and UC-2. The experimental GF4536 was not tested by itself, but in rotation with Fontelis, efficacy was reduced as compared to Fontelis by itself. Reduced sensitivity to Quintec has been reported but is still localized and can be used in rotation-mixtures as shown. Use of the fungicide in mixtures with other fungicides is highly recommended and should prolong its efficacy for the industry.

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, FC 7, FC 11, and FC 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. 3. Efficacy of 7-day preharvest fungicide treatments for management of postharvest gray mold of Bing cherries - San Joaquin Co. - 2021



Treatments were applied on 5-25-21 using an air-blast sprayer at a rate of 100 gal/A. All treatments except EcoSwing and ProBlad were applied in combination with with DynAmic at 8 fl oz/A. Treatments were also applied on 3-24, 4-6, and 4-20-21 as part of a powdery mildew program, except for treatment No. 14 (Miravis Prime#) that was only applied on 5-25-21. Harvested fruit were washed by gently agitating in water for 2 min. Fruit were wound-inoculated with *M. fructicola* (50,000 spores/ml) or *B. cinerea* (30,000 spores/ml) or non-wound drop-inoculated with *M. fructicola* (50,000 spores/ml) or *B. cinerea* (300,000 spores/ml 50% cherry juice). Fruit were then incubated for 5-10 days at 22C.

Evaluation of preharvest treatments for management of fruit decays. Preharvest treatments to Bing cherries applied 7-days PHI were evaluated in a commercial orchard. Fruit were not washed or were washed before inoculation. This was done to evaluate the fungicides' persistence after a postharvest hydrocooler passage. Persistence is important if no postharvest fungicide treatment can be done.

All preharvest treatments significantly reduced the incidence of brown rot on non-washed and washed fruit after non-would-inoculation with *M. fructicola*, including the plant extracts EcoSwing and ProBlad (Fig. 2). The conventional fungicides reduced the incidence of decay by >85%. In wound-inoculations of harvested non-washed fruit, the two plant extract products were not effective, but all fungicides significantly reduced the incidence of decay from the control where 91.7% of the fruit decayed. Treatments with Cevya, Luna Experience, Quadris Top, or Miravis Duo reduced brown rot incidence to 4.2% or less. Pyraziflumid was the least effective among the fungicides with 41.7% decay, and the remaining treatments were intermediate in efficacy. On washed fruit, many of the fungicide treatments showed reduced efficacy as compared with non-washed fruit (Fig. 2). Cevya, Quash, Quadris Top, Miravis Duo, Ph-D + Procure, and UC-2, however, remained very efficacious. Thus, as established previously, fungicides that contain a FC 3 compound can effectively prevent brown rot decay that is initiated from wounds after treatment, and they retain their high efficacy after postharvest washing that simulates a hydrocooler treatment.

For gray mold control, EcoSwing and ProBlad were not effective after treated fruit were wound- or non-wound-inoculated (Fig. 3). Several of the conventional fungicides significantly reduced the incidence

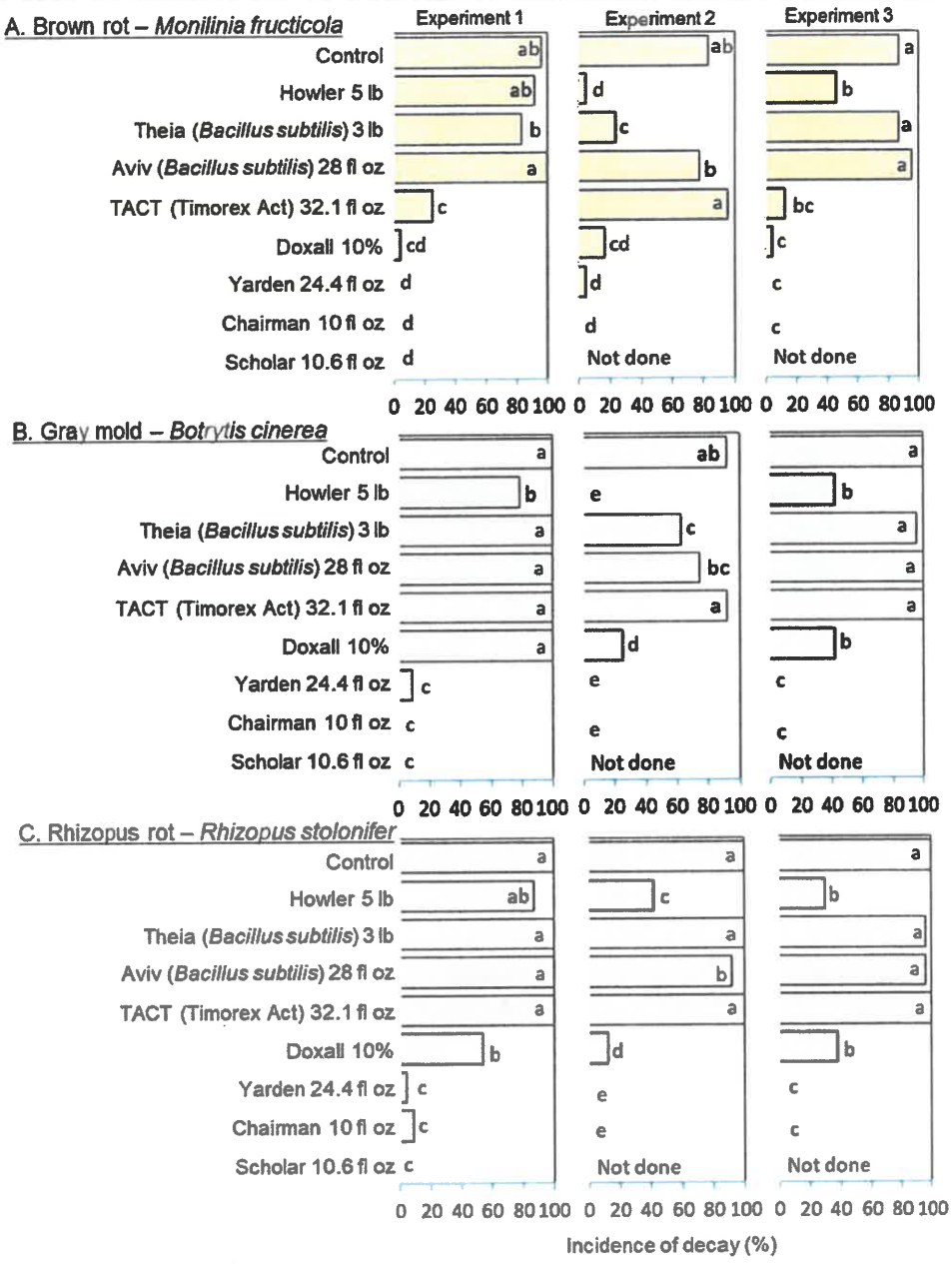
of decay on non-washed fruit using both inoculation methods, and these included Luna Sensation, Miravis Prime, Merivon, Fontelis+Teb, Miravis Duo, pyraziflumid, GF4536, and UC-2. Miravis Prime showed higher efficacy when applied in a blossom blight, mildew, and preharvest program as compared to a single preharvest spray. Efficacy of Miravis Duo, Merivon, and pyraziflumid was higher in the non-wound inoculations, and Cevya, and Quadris Top only significantly reduced decay after non-wound inoculation. This indicates that these fungicides have limited systemic action. On washed fruit, Miravis Prime and Luna Sensation had the highest efficacy after wound-inoculation. In non-wound inoculations, Merivon and one of the Miravis Prime treatments were highly effective against gray mold, but Luna Sensation and GF4536 also resulted in satisfactory control.

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, and UC-2 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 our studies, the efficacy of Cevya, Quash, Ph-D+Procure, Quadris Top, Miravis Duo, and UC-2 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. Previously, Elevate and Ph-D were top choices to manage this decay, but their performance was inconsistent in our trials. Our results suggest that Miravis Prime has high potential as an effective brown rot and gray mold preharvest treatment. This fungicide is currently in the IR-4 residue program for registration on sweet cherry. Postharvest fungicides are still warranted to reduce decay to the lowest levels possible for shipping and marketing fruit to distant locations and to minimize claims.

Efficacy of new postharvest treatments for managing brown rot, gray mold, and Rhizopus rot of sweet cherry. Several postharvest laboratory studies were performed where treatments were applied to fruit approximately 12 h after inoculation. Three studies focused on comparative evaluations of new biological treatments and conventional postharvest fungicides. Biological treatments included Howler, Theia, Aviv, Timorex Act, and Doxall; and fungicides evaluated were Yarden, Scholar, and Chairman (Table 4). Among biological treatments, spray applications with Doxall were most consistent in efficacy against the three decays (Fig. 4). At the rate evaluated (10%), it was highly active against brown rot and moderately effective against gray mold and Rhizopus rot, however, a visible residue was present on fruit after drying. The registrant recently indicated that due to possible phytotoxicity to fruit, rates will be lowered to at least 1%, and thus, this lower rate will need to be tested. Dip applications at 2.5%, however, resulted in significantly reduced efficacy against gray mold (Fig. 5). The efficacy of Howler was very inconsistent against the three decays ranging from high (Rhizopus rot) or very high (brown rot, gray mold) to not effective (brown rot, Rhizopus rot). Theia, Aviv, and Timorex Act had no or very little efficacy using an experimental setup that simulated real-life conditions (i.e., timing of application after inoculation). Yarden, a mixture of fludioxonil and tea tree oil was similarly highly effective as Scholar or Chairman (Fig. 4). A rate study with dip treatments of Chairman indicated that the 8-fl-oz rate was sufficient for highly effective brown rot control, but for gray mold and Rhizopus rot only the 12-fl-oz-rate resulted in zero levels of decay (Fig. 5).

In commercial packinghouse studies, T-Jet, drench, or dip applications with Chairman were compared to a drench application with Scholar and a T-Jet application with Tebucon to identify the most efficacious and cost-effective treatment. All treatments reduced the incidence of brown rot to zero or near zero levels after wound- and non-wound inoculation of fruit (Fig. 6). Efficacy against gray mold was not as high because fruit were inoculated *after* treatment, and high efficacy in wound-inoculations requires that fungicides have some systemic activity. Still, Chairman using either application method resulted in a significant reduction in decay as compared with the control or Scholar or Tebucon treatments. After non-wound inoculations, decay incidence using Chairman was reduced to $\leq$12.5% as compared with the control with 100% decay. Scholar

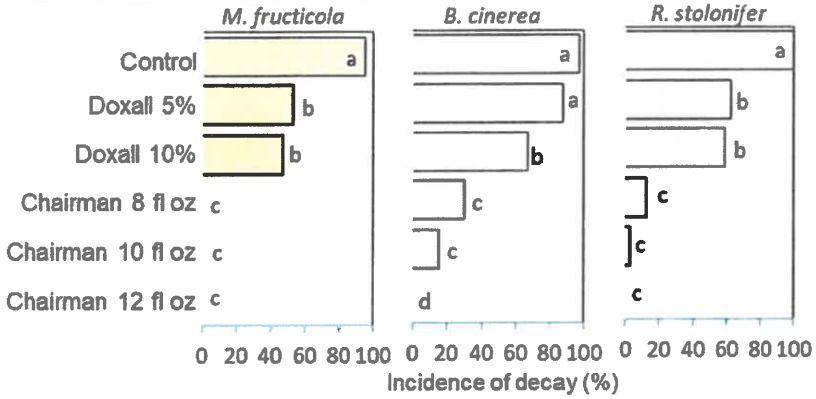
Fig. 4. Evaluation of postharvest biological and conventional treatments for managing postharvest decays of Bing cherry in laboratory studies 2021



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 h at 20C. Aqueous treatments were applied using an air-nozzle sprayer. Fruit were incubated at 20C for 4-7 days.

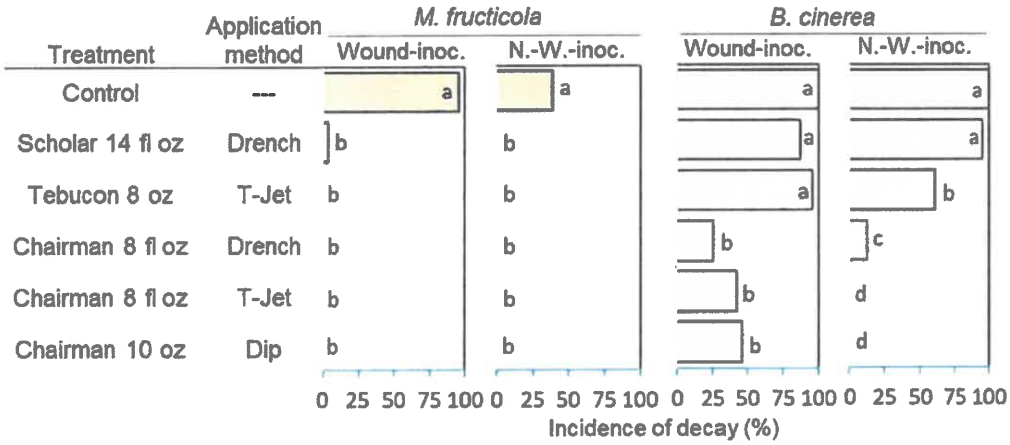
was not effective, whereas Tebucon resulted in 60% decay incidence. These data suggest that Chairman, a pre-mixture of fludioxonil and propiconazole with a broad spectrum of activity against brown rot, gray mold, Rhizopus rot, and sour rot, sets a new standard to be the most effective postharvest treatment available for sweet cherry. Still, it needs to be considered that fungicide concentrations in large-scale packinghouse studies like this are difficult to accurately adjust, and this may explain why Scholar was not

Fig. 5. Evaluation of postharvest dip treatments for managing postharvest decays of Bing cherry in laboratory studies 2021



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 h at 20C. Aqueous dip treatments were done for 10 sec. Fruit were incubated at 20C for 4-7 days.

Fig. 6. Evaluation of commercial postharvest treatments for managing postharvest decays of Bing cherry 2021



Fruit were treated in a commercial packinghouse and then wound-inoculated with *M. fructicola* (50,000 spores/ml) or *B. cinerea* (30,000 spores/ml) or non-wound (N.-W.) drop-inoculated with *M. fructicola* (100,000 spores/ml) or *B. cinerea* (300,000 spores/ml 50% cherry juice). Fruit were incubated at 20C for 7-10 days.

effective against gray mold in these studies. Additionally, different fruit lots were used for each application that may have differed in their susceptibility to decay development.

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 for the active ingredients of Chairman. Propiconazole is registered as a preharvest fungicide and thus, its postharvest use is within established MRL tolerances. MRLs have not yet been established for natamycin in many international markets. Its 'exempt from residue tolerance' status is only approved in the United States. This limits its current use to domestic markets (including Canada). Natamycin that we evaluated extensively over the last few years, however, 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. Organic formulations of polyoxin-D are planned for postharvest registration, and they provide a treatment option for organically grown fruit. This fungicide is not effective against Rhizopus rot; thus, extra care needs to be taken in removing injured and decayed fruit, sanitizing fruit and packinghouse equipment, and not marketing over-ripe fruit. No other new highly effective biological compounds were identified in 2021. With increasing emphasis on food safety and consumer concerns, natamycin and polyoxin-D with 'exempt from tolerance' status may become an important component of postharvest decay management in the future once CODEX accepts this US biopesticide classification. We will continue our evaluations of new postharvest treatments in 2022 in cooperation with commercial packinghouses and continue to work with the registrant to obtain MRLs, FAT, and organic status for natamycin.

Table 2. Postharvest fungicides currently registered on sweet cherry

				Activity against			
Trade names	Active ingredients	FRAC Code	FAT	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	natamycin	48	no	++	++	++	+

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

Determine* Phytophthora *spp. currently affecting tree health in California cherry orchards and evaluate new fungicides for managing Phytophthora root and crown rots.

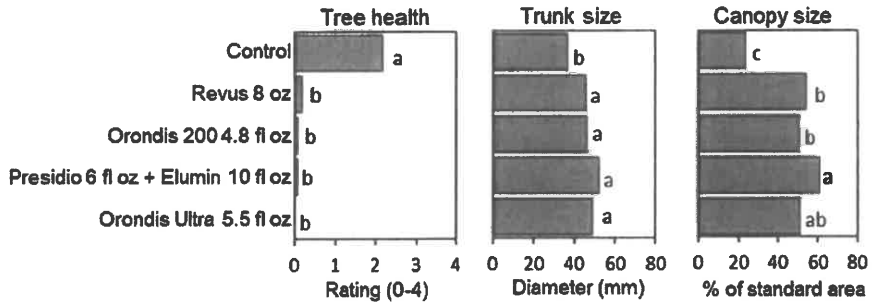
Surveys of California cherry orchards to determine the causal species of Phytophthora root and crown rot. Soil, root, and crown samples from declining trees in 11 orchards in the main California cherry production areas were collected in 2020 and 2021. The cause of tree decline in two orchards was determined as Armillaria root rot, but *Phytophthora* spp. were recovered from four orchards, mostly by soil pear baiting: *P. cactorum, P. cambivora, P. cryptogea, P. syringae*, and a currently unidentified species. Additionally, *Phytopythium vexans* (previously *Pythium vexans*) that has been reported to cause root rot on a range of host species was recovered from three of the orchards. Inoculation studies are ongoing to determine the virulence of these isolated species on cherry. Species identification was done by morphological examination and by DNA sequencing of rDNA and cytochrome c oxidase (cox) genomic regions. Thus, a diverse range of species was recovered from cherry trees including *P. cambivora* that traditionally has been regarded as the main pathogen. Because the success of *Phytophthora* spp. isolations is known to be seasonal, additional samples will be obtained in 2022. Root flushes occur in early spring and fall, and these are times when the pathogens are more likely to be recovered.

Field studies on the evaluation of new fungicides. Data for tree health of a sub-plot of the first orchard on Mahaleb rootstock at UC Davis where trees were inoculated with *Phytophthora* spp. were presented previously and are shown again in Fig. 7. For comparison, trunk diameter and canopy size measurements that were done in May 2021 are also shown in Fig. 7. Based on both measurements, tree growth was significantly increased by all fungicides as compared to the control. Canopy size after treatments with a mixture of Presidio and Elumin was significantly increased in comparison with Orondis or Revus but not Orondis Ultra.

Table 3. Species isolated in cherry orchard surveys in 2020 and 2021

Orchard	Species isolated	Number of isolates	Survey No.	Isolation Method
1	*Phytophthora cactorum*	1	2021-1	Pear baiting
2	*Phytophthora cryptogea*	2	2021-1	Pear baiting
	Phytophthora cambivora	3	2021-2	Pear baiting
	Phytophthora sp. (pending ID)	1	2021-2	Pear baiting
	Phytopythium vexans	1	2021-2	Pear baiting
3	*Phytophthora cambivora*	3	2021-2	Pear baiting
	Phytophthora sp. (pending ID)	4	2021-1	Root plating, pear baiting
	Phytopythium vexans	3	2021-1,2	Pear baiting
4	*Phytopythium vexans*	2	2021-1	Pear baiting
	Phytophthora syringae	1	2020	Pear baiting

Fig. 7. Efficacy of new fungicides for management of Phytophthora root and crown rot of cvs. Bing and Coral cherry on Mahaleb rootstock in a field trial at UC Davis 2021



Trees were planted and inoculated with *P. citricola*, *P. cactorum*, and *P. cambivora* in Jan. 2020. Soil treatments were done in April 2020 and 2021. Tree health was rated in Sept. 2020 using a scale from 0 (=healthy, vigorous) to 4 (= tree dead). Trunk diameters and canopy sizes were measured in May 2021 (see details for methods in the text).

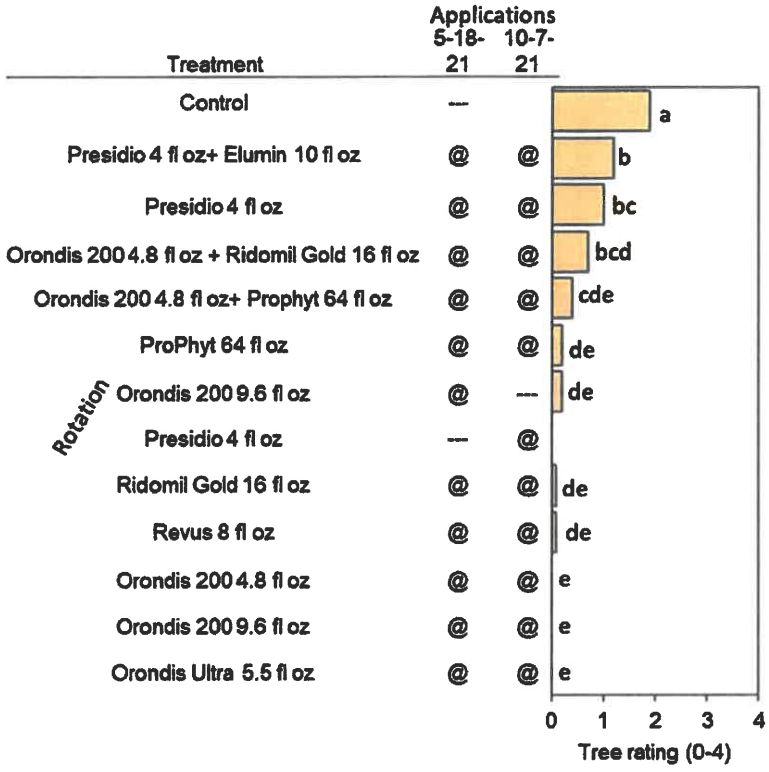
Tree health in the UC Riverside plot that was planted in Jan. 2021 and treated and inoculated in May was rated in mid-October. All treatments resulted in significantly improved tree health as compared to the control where canopy size was reduced and started yellowing and where crown cankers and gumming along the trunks were sometimes present (Fig. 8). Fungal isolations from these trees are ongoing. Trees treated with either one of three Orondis rates were rated as completely healthy. In contrast to the UC Davis plot, Presidio and Presidio+Elumin were not as effective, and this may be due to the different soil types present at the two locations. Trees treated with Orondis or Revus, however, had good ratings at both locations. These trials are ongoing, and additional treatments and evaluations will be done.

In greenhouse studies, the systemic properties of selected Oomycota fungicides were evaluated after soil treatments of Mahaleb, Mazzard, and Krymsk rootstocks. Similar results were obtained for the three rootstocks, and results for Mahaleb are presented in Fig. 9. Presidio, Orondis, ProPhyt, and Ridomil Gold all reduced canker size in comparison to the untreated control when stems of soil-treated plants were inoculated with *P. citricola* 2 weeks after treatment. This indicates that fungicide was present in the stem tissues and reduced fungal growth.

Systemic activity of ProPhyt and Ridomil Gold is known for many years, and we have recently demonstrated the systemic movement of Orondis in almond plants. Presidio, however, is not known to be taken up and transported inside plants. Although it is unlikely that these fungicides based on the rates used will be detected in foliar parts of trees in the orchard, uptake into plant roots will benefit treatment

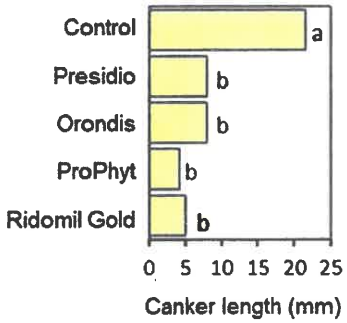
efficacy because roots and crowns will be protected from infection for extended periods. IR-4 studies with oxathiapiprolin (Orondis) are currently ongoing to obtain registration on sweet cherry in the United States.

Fig. 8. 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 2021



Trees were planted in Jan. 2021. Soil treatments were done on 5-18-21, followed by soil inoculations at the base of the trunks with a mixture of *P. cactorum*, *P. cambivora*, and *P. citricola*. Tree health was rated on Oct. 14, 2021, using a scale from 0 (=healthy, vigorous) to 4 (= tree dead). Tree yellowing and gumming at the crown were considered symptoms of Phytophthora root and crown rot.

Fig. 9. Evaluation of systemic properties of selected Oomycota fungicides in Mahaleb cherry plants after soil treatments in greenhouse studies



Fungicides were added to the soil of potted Mahaleb cherry trees based on field rates. Stems were inoculated with *Phytophthora citricola*-colonized agar plugs 10 cm and 20 cm above the soil line two weeks after treatment. Three weeks after inoculation, canker length under the bark was measured and was used as an indication for the presence of fungicide.

CBB PROJECT REPORT – December 5, 2021

Project Title: Phytosanitary Irradiation using an In-House Cabinet X-ray System for Sweet Cherries

Project Leader: Peter Follett, USDA-ARS, U.S. Pacific Basin Agricultural Research Center, Hilo, Hawaii

Objectives:

To show proof of concept for an in-line or packinghouse scale irradiation system.

1. Evaluate two different prototype x-ray systems in terms of cost, throughput, logistics, and other parameters.
    a. BUGS II (Applied Energy Systems, Albuquerque, NM)
    b. LEHP Processing (Rayfesh Foods, Ann Arbor, MI)
2. Demonstrate irradiation treatment of California sweet cherries using both types of equipment.

Background:

Irradiation is an effective alternative to methyl bromide for postharvest control of spotted wing drosophila (SWD) and other insect pests in sweet cherries. Irradiation quarantine treatment can be used to access foreign markets for California cherries such as Mexico and Australia, or to overcome internal quarantines within California or the United States during outbreaks of new invasive pests (e.g. oriental fruit fly). Sweet cherries are very tolerant of irradiation and maintain high quality at irradiation doses that control insects. Hawaii has been irradiating a variety of fruits and vegetables for export to the U.S. for more than 20 years, which demonstrates its potential as a phytosanitary treatment. Mexico is currently irradiating several fruits (mango, guava, others) for export to the U.S., and Australia is irradiating various fruits for export to the U.S. and Asian markets.

The proposed project will provide proof of concept for in-house irradiation treatment of sweet cherries. Cabinet-style x-ray irradiation systems suitable for in-line treatment are under development and have the potential to provide packinghouses with on-site quarantine treatment capability. In-line irradiation systems would have much lower throughput compared with existing methyl bromide treatment systems or high energy commercial irradiation facilities but may be practical for niche markets.

Progress:

Due to the pandemic and travel restrictions at USDA, I have not made any significant progress on my proposed objectives. As soon as possible, I plan to visit Applied Energy Systems and Rayfresh Foods to view their progress in developing cabinet x-ray machines capable of irradiating boxes of fruit for phytosanitary purposes. The objective in evaluating these systems is to irradiate boxes or sweet cherries (or a seasonal alternative if cherries are not available) to determine the ability of the machine to deliver the required dose to the product and to estimate the throughput. Australia is currently irradiating sweet cherries for export to Vietnam. This is the first commercial use of irradiation for cherries, and I communicate periodically with personnel at Steritech (Melbourne, NSW) for updates.