2020 CALIFORNIA CHERRY RESEARCH REPORT

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REPORT TO THE CALIFORNIA CHERRY BOARD

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

Project Year: 2020-2021

Anticipated Duration of Project: 3 years

Reporting period: 1st July 2020 to October 31st 2020

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Executive summary

Timing application of dormancy breaking products is essential for adequate bloom and yield in cherry. Available temperature-based chill accumulation models often fail to predict correct spray timing, particularly during years with unusual temperature patterns recently driven by climate change. Our objective is to improve identification of winter dormancy status of the trees and thus efficacy of dormancy breaking agency application, developing information on 1) tree carbohydrate and water dynamics during winter and 2) the relative contribution of solar radiation and relative humidity, in addition to air temperature, on tree bark temperature.

During the first 4 months of the project, the research team, together with grower collaborators, was able to successfully identify, scout, and instrument the experimental orchards. Two experimental locations were selected, one in Linden and one in Bakersfield, characterized by different winter chill accumulation. Six different plots were selected between the two locations, that will enable the comparison of different cultivars (Coral and Bing in Linden and Coral and Brooks in Bakersfield), treated with different chemical treatment (CAN17 and DORMEX). The experimental set up was layout to have, within each plot, four replications (or blocks) of three trees each, times two treatments: Control (no chemical will be used) and Treated (will be sprayed with either DORMEX or CAN 17).

The plots were instrumented at the end of October with bio-meteorological sensors to measure continuously: air temperature and relative humidity within the canopy, incoming solar radiation, bark temperature, soil moisture and temperature, trunk diameter fluctuation and phenological stage of development. Monthly wood sample collection for carbohydrate analysis started at the beginning of October.

Overall, the research is proceeding timely, data collection just started and will continue through the winter. This report provides further details about the methods and experimental design, but preliminary data will be available only after completing one entire season of data collection.

1. Problem and its significance:

The California cherry industry struggles with warm winters, limiting the amount of accumulated chill necessary for an adequate bloom period and leading to low yields. Dormancy breaking products are used to mitigate lack of chill, but to be effective they must be applied at the right time. Timing of application is currently based on the accumulation of a certain level of chill, given a certain chill model. Currently available chill accumulation models are based on the use of air temperature as main predictor of dormancy stage. These models, excellent in predicting time for breaking agency application in normal years, lose precision during years with unusual temperature patterns recently driven by climate change. A more flexible and accurate characterization of winter dormancy status for cherry, based on the combination of different site-specific environmental and physiological factors will enable growers to better tailor spraying application on tree physiological status and maximize its efficacy under different climatic scenarios.

2. Objectives

Our objective is to improve the identification of tree winter dormancy status and the efficacy of dormancy breaking agency application, developing information on:

1) tree carbohydrate and water dynamics during winter;
2) the relative contribution of solar radiation and relative humidity, in addition to air temperature, on tree bark temperature.

Hypothesis 1) Temperature variations during winter affect carbohydrates (sugars and starch) content in the trees. These changes in carbohydrates are associated with changes in water content and dormancy stages. Studies on cherry reported sugars to increase during endodormancy, stabilize during ecodormancy and decrease before bloom, and tree water content to decrease during endodormancy and increase during ecodormancy (Fig.1).

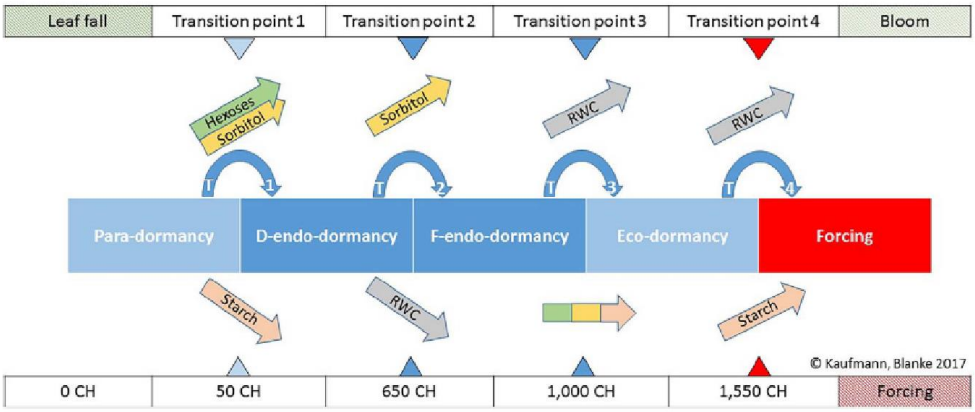

Fig. 3. Visualization of RWC, carbohydrate dynamics, chill accumulation and transition points.

Fig.1. Characterization of cherry dormancy stages based on carbohydrate and relative water content dynamic developed in Germany by Kaufmann and Blanke (2017)

Hypothesis 2) In humid environments radiation evaporates water from the soil that cools down the air, but direct solar radiation still heats up the trees, creating a discrepancy between temperature values used in chilling model and temperature sensed by trees (Fig. 2). We aim to create a model to include more environmental parameters (radiation, temperature and humidity) in the calculation of the chill portion to represent better tree temperature rather than air temperature.

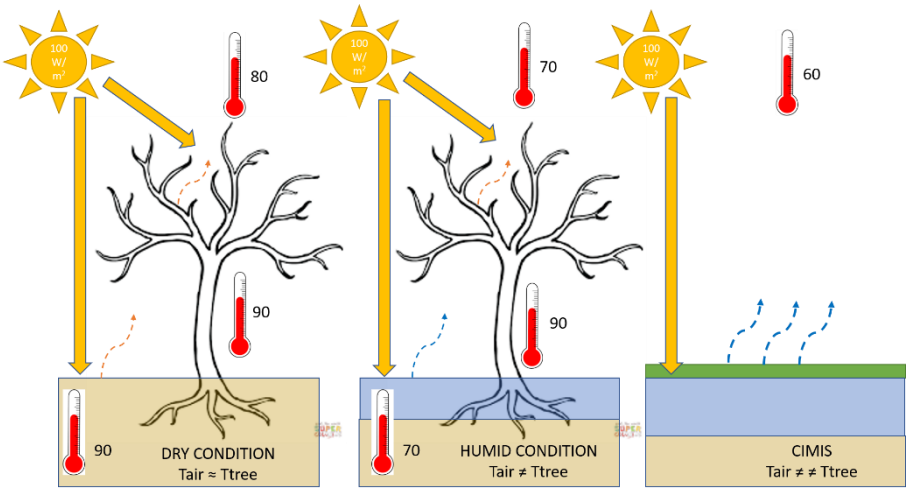

Fig. 2 - Examples of how air temperature can differ from tree temperature depending on the level of humidity in the measuring site.

3. Procedures and experimental setup

3.1 Study orchard characterization and experimental design

Five experimental location were selected at the beginning of August 2020, located within two main cherry production regions, Kern county and San Joaquin County, characterized by different climatic conditions (Fig. 3). The Northern location, close to Linden, reaches on the average 80 chill portions, as observed from the analysis of the data of the closest CIMIS station (#70, Manteca). In the southern location, close to Bakersfield, the chill portion accumulation calculated for the closest CIMIS station (#125, Arvin-Edison) is 13% lower (about 70 chill portion).

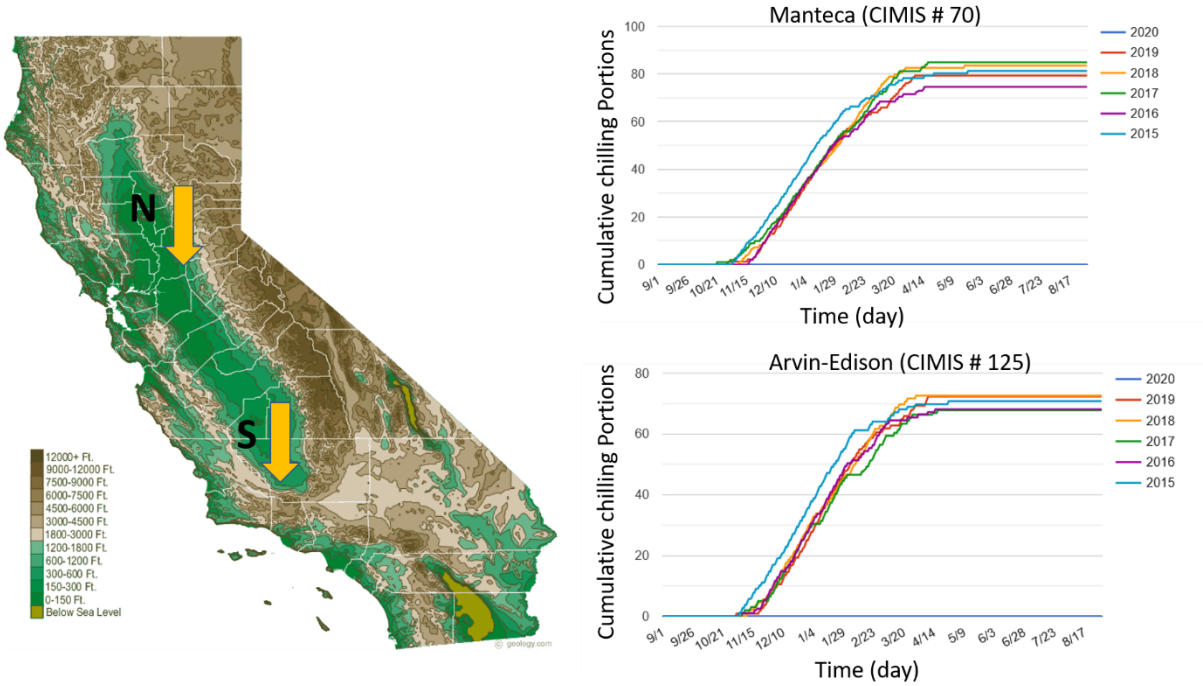

Fig. 3 – Localization of the North and South experimental sites and chill portion accumulation calculated from Fruit & Nut Research & Information Center for the CIMIS stations of Manteca and Arvin-Edison.

In the North location we selected 3 plots-orchards: the first one named is planted with Bing on Mazzard treated with CAN 17 (Fig. 4 a); the second one is planted with Coral on Mazzard and treated with Dormex (Fig. 4 b); the third one is planted with Bing on Mazzard and treated with Dormex (Fig. 4 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, 6 trees were selected, of which three will be our "Control" treatment, that will be acquired by bagging them just before spray to avoid contact with dormancy breaking agents, while the other three trees will be our "Treated" treatment, that will receive normal orchard practices (sprayed with dormancy breaking agents) (Fig. 2).

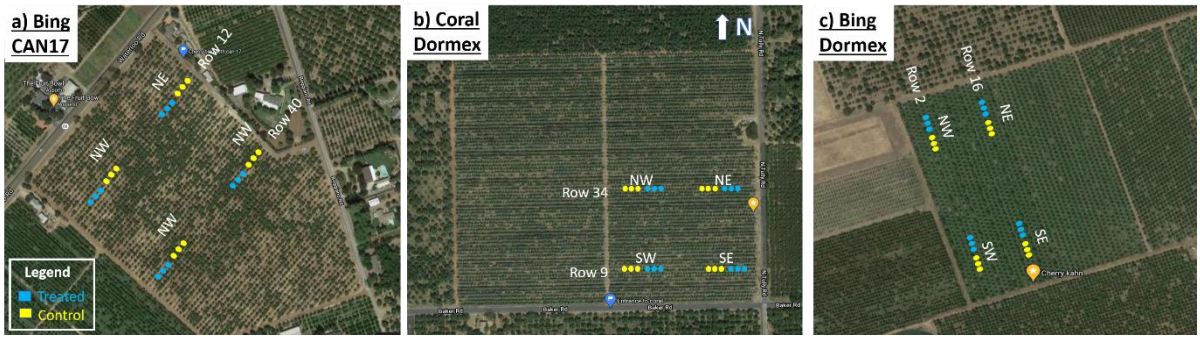

Fig. 4 – Experimental blocks and design in the 3 orchards in the Northern location (Linden).

In the South location, one orchard was selected planted with the cultivars Brooks and Coral on Mahaleb and treated with Dormex (Fig. 5). 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, located in the area treated only with CAN17 (South of the orchard), will be our "Control" (no treated) and "CAN17" treatment. The third plot, located in the North part of the orchard, will be the "Dormex" treatment. Within each plot, 12 trees per cultivar distributed on 4 different rows were selected and flagged for sampling. Each row will represent our replication block.

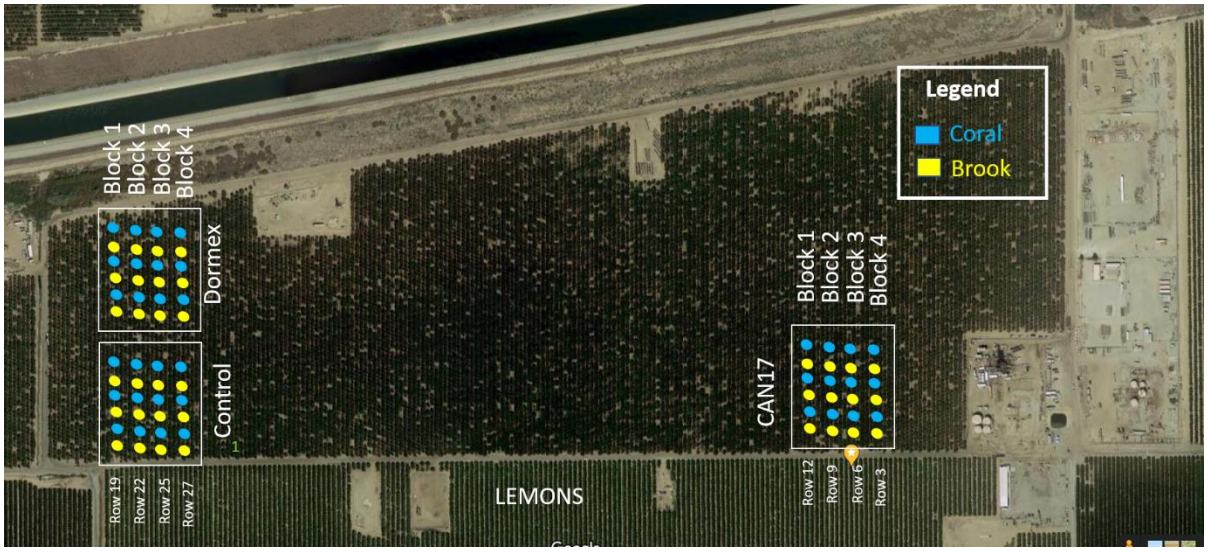

Fig. 5 – Experimental blocks and design for the orchard in the Southern location (Bakersfield).

This experimental set up will enable us to isolate the effect of 1) the genotype-specific chill requirement, comparing Coral to Bing (or Brooks) 2) the chemical treatment, comparing Bing, Coral and Brooks treated with CAN17 and Dormex, and 3) the environmental location, comparing Coral in the North and South location (see Fig. 6)

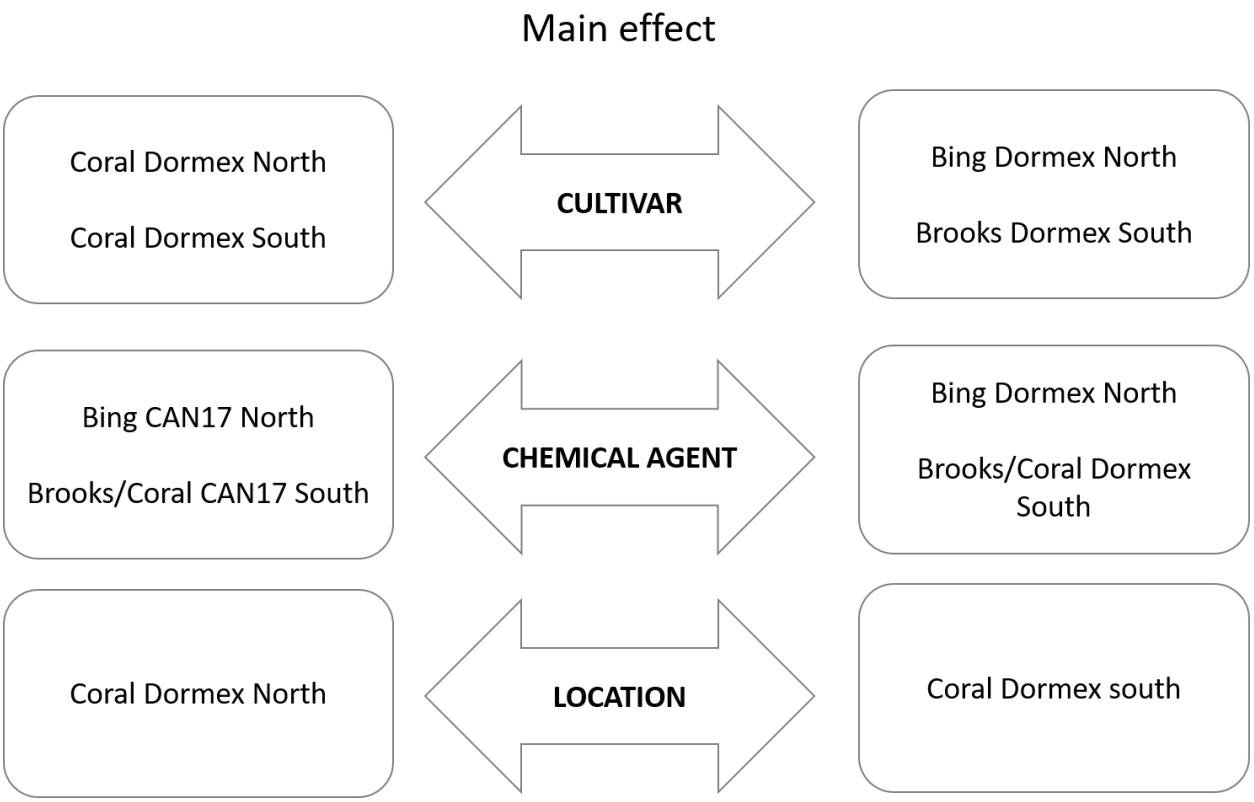

Fig. 6 – Main effects that will be observed with the selected experimental design comparing the orchards

3.2 Orchard instrumentation

Weather stations have been installed in the different experimental sites at the end of October 2020 (Fig. 7) to monitor continuously air temperature and humidity within canopy, incoming solar radiation, soil moisture, tree wood temperature, three phenology and tree water status in each experimental site. Information will be collected to characterize the impact of incoming radiation on bark temperature and will be integrated in the dynamic model to observe similarities or differences in the cumulative chill accumulation.

3.3 Sample collection

Twigs will be collected for carbohydrate (CHO) analysis starting October 1, 2020 to have a baseline level of CHO of the different plots before the beginning of winter chill accumulation and were delivered to Dr. Zwieniecki's laboratory at UC Davis. Sampling will proceed once per month (twice per month right before and after dormancy breaking agent applications), for characterizing CHO dynamics through the season as affected by the interaction of cultivar, environmental conditions and rest breaking agent applications.

Fig. 7 Bio-meteorological station installed in the orchards at the end of October (a), and details of the temperature and humidity sensor (b), phenocam (c), soil temperature and moisture sensors (d), dendrometer sensor (e), and bark temperature sensor (f).

4. Project timeline

Jul 2020	Aug 2020	Sep 2020	Oct 2020	Nov 2020	Dec 2020
Project starts	Orchard selection	Experimental setup Sensor purchasing	CHO sample Orchard instrumentation	CHO sample	CHO sample
Jan 2021	**Feb 2021**	**Mar 2021**	**Apr 2021**	**May 2021**	**Jun 2021**
CHO sample Bagging control trees	CHO sample	CHO sample	CHO sample	CHO sample Harvest South	CHO sample Harvest North
All other data than CHO will be frequently collected and analyzed along the course of the project.					

2nd PROGRESS REPORT ON THE SWEET CHERRY EVAPOTRANSPIRATION RESEARCH PROJECT

Prepared by Kosana Suvočarev, UC CE Biometeorology Specialist at the Department of Land, Air and Water Resources, University of California, UC Davis (ksuvocarev@ucdavis.edu) with the contribution of Richard L. Snyder (UC CE Biometeorologist Emeritus), Kari Arnold (UC CE Area Orchard and Vineyard Systems, Stanislaus County), Cayle Little (Associate Land and Water Use Scientist, California Department of Water Resources), Daniele Zaccaria (UC CE Agricultural Water Management Specialist), Khaled Bali (UC CE Irrigation Water Management Specialist).

MEASURING CHERRY EVAPOTRANSPIRATION AND DERIVING CROP COEFFICIENT (Kc) VALUES FOR USE IN IRRIGATION SCHEDULING

After the grant was awarded to study sweet cherry evapotranspiration, team members Richard Snyder, Daniele Zaccaria and Kari Arnold met with [redacted] in February 2019 and identified three sites at [redacted] commercial orchards for setting evapotranspiration measurements. These three sites are fully irrigated and well-managed orchards in Linden, CA (San Joaquin County in Northern San Joaquin Valley).

Two sites are adjacent orchards [redacted] with the mature trees, averaging 4 m in height, same variety (Bing) and soil type, but have different irrigation systems, rootstocks, row orientation and tree density (Figure 1) as described hereafter:

- Site 1: "[redacted]" block, Micro-sprinkler, Mazzard rootstock, NW-SE orientation, lower tree density (20 x 22 ft) and
- Site 2: "[redacted]" block, Drip irrigation, Mahaleb rootstock, NW-SE row orientation, higher tree density (16 x 16 ft).

Figure 1. *Site 1 (with red mark) and Site 2 (with green mark)*

The third site is in a different location ([redacted]) of the same commercial farming operation:

- Site 3: "[redacted]" block, Drip-irrigation, Mazzard rootstock. Lower tree density (20 x 20 ft) row orientation East-West

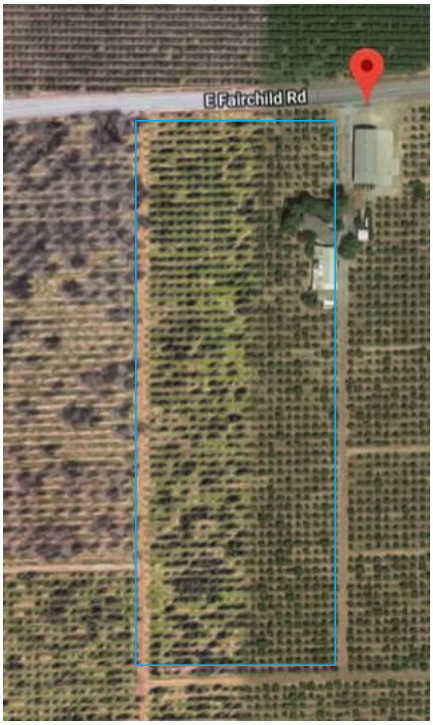

Figure 2. *Site 3*

Materials and methods

The project objectives of California sweet cherry evapotranspiration measurements and crop coefficients development is expected to be obtained through both on-site measurements and CIMIS station data use. We have so far collected about 2 months of data from the three orchard sites and identified the suitable site for the CIMIS station and are working toward its installation.

The hybrid surface renewal-eddy covariance measurements are one of the most direct, non-intrusive continuous measurement method available. We are using the sonic anemometer with thermocouple, net radiometer and soil heat flux measurements to quantify the energy balance components which help us deduce the latent heat flux as an energy equivalent of evapotranspiration. Once the crop evapotranspiration is deduced from our measurements, we can use the freely available data from the CIMIS network and compute the crop coefficients from the ratio of the crop evapotranspiration ($ET_{c,cherry}$) and CIMICs reference evapotranspiration ($ET_{o,CIMIS}$):

$$K_{c,cherry} = \frac{ET_{c,cherry}}{ET_{o,CIMIS}}$$

The knowledge on crop coefficients is useful for the growers where the direct measurements of this type are not possible. However, the CIMIS network (and the networks of this type) are well-distributed in the water-limited agricultural areas and the data is freely available for $ET_{o,CIMIS}$ for the computation of the $ET_{c,cherry.}$ Also, FAO Irrigation and drainage paper 56 provides the tabulated values for different crops, but they are not always representative of the different climates or local orchard systems management.

New CIMIS Station

On April 9th, 2019, Kari Arnold and Kosana Suvočarev visited Linden with Cayle Little, a project collaborator from the California Department of Water Resources (DWR) (Figure 3), in order to find a suitable location for establishing a new CIMIS automated weather station. [redacted] helped us identify a suitable site with well-watered irrigated pasture managed by [redacted] (38°03'56.8"N 121°04'19.7"W, coordinates are approximate). The new CIMIS weather station will be sited in the center of 40+ acre field bordered by open unirrigated ground on the west side and young walnut orchard on the North, East and South sides. The predominant wind is from north-west to west. Collecting local weather and ETo information is critical to quantify conditions of the orchards under our measurements and for developing reliable crop coefficient information for sweet cherries grown in this area.

Figure 3. *Site visit for the future CIMIS station*

Evapotranspiration measurements:

The research team established three measurement sites at the beginning of May 2019. Various sensors for micrometeorological measurements were installed on painter scaffolding above the cherry trees (Figure 4) to observe areas of interest of cherry trees and obtain average evapotranspiration values for each of the orchard management practice considered in this study.

Figure 4. *Evapotranspiration measurement above the cherry trees.*

The measurements taken so far cover the period between May 4th and July 3rd, 2019. In this period, trees are at full canopy and evapotranspiration rates are expected to be near peak. In our

preliminary analysis, the results for the three sites are similar (Figure 5). Most of the daily ET values were between 4 and 8 mm (0.16-0.30 in). Average daily values for this period suggest that the highest crop water use was measured at the first station "[redacted]" with 6.6 mm (0.26 in), then second station "[redacted]" with 6.4 mm (0.25 in), while the lowest average daily water use of 6.0 mm (0.24 in) was measured at the third station, "[redacted]". However, differences among the average daily water use at the study orchards are small and probably reflect the highest rate of ET where micro-sprinkler system is used (Site 1, "[redacted]") where larger ground area is wetted, while the second highest average daily ET rate is at the orchard "[redacted]" where the tree density is the highest (Site 2), and the least average daily water use is at the orchard "[redacted]" with medium tree density and drip irrigation. The research team will keep collecting ET measurements for the remainder of the growth season, which will allow understanding how evapotranspiration rates relate to orchard characteristics and management practices, and whether ET values can be considered similar enough to represent average water use of sweet cherry orchards grown in the San Joaquin County.

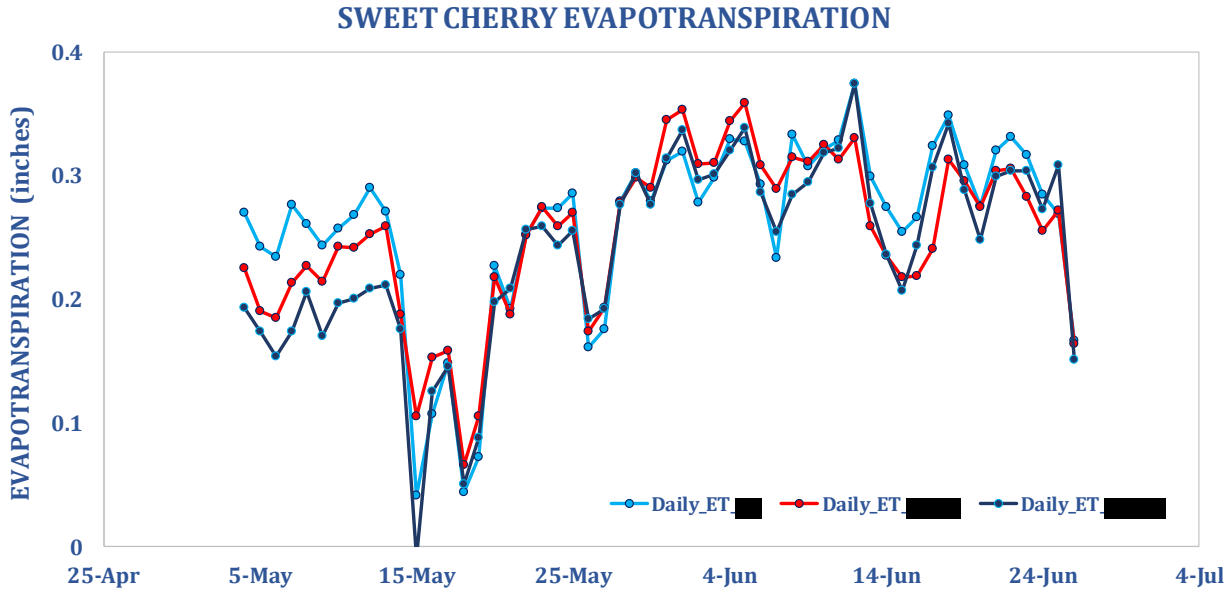

Figure 5. *Daily Evapotranspiration values for the three study orchards in Linden, Ca.*

Crop coefficients:

Crop coefficients were computed using the directly measured actual cherry evapotranspiration ($ET_{c,cherry}$) and nearest CIMIS station, from Manteca (17 miles away) values for the reference evapotranspiration (ET_o).

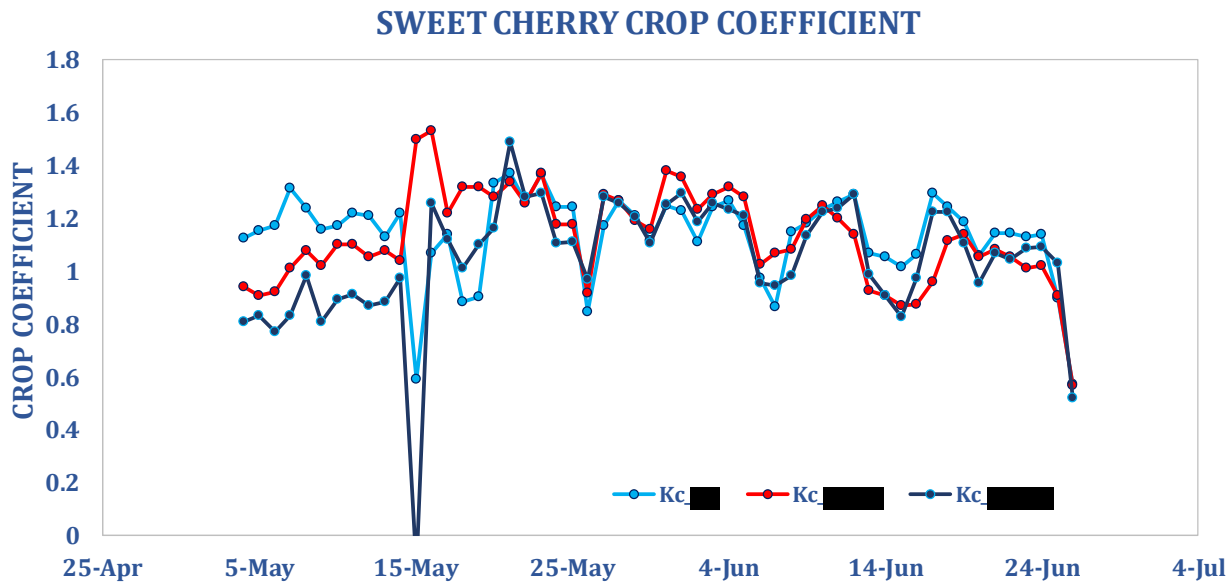

Figure 6. *Crop coefficient values for the three study orchards in Linden, Ca*

The Kc values obtained from field measurements were mostly within the range of 0.8 to 1.2 and are similar to crop coefficients of other stone fruit crops reported from literature in Mediterranean environments. The CIMIS data for reference evapotranspiration was chosen from the nearest site, but the new CIMIS station, located in the close proximity of our experimental orchards will probably provide higher accuracy and reliability of Kc values.

It is important to note here that the crop coefficients are usually related to well-managed crop with optimal water status and no water restrictions. To ensure that the crop was optimally irrigated, we are doing weekly monitoring of the stem water potential and comparing the measured values to the recommendations by the University of California, Division of Agriculture and Natural Resources. Check the section below on stem water potential measurements.

Applied irrigation water:

The research team installed magnetic flowmeters (Sensus iPEARL, Raleigh, NC) along the irrigation tubing (laterals) at the different study orchards with the aim of measuring the applied irrigation water volumes and compare them with the evapotranspiration measurements (Figure 7).

Figure 7. *Installation of magnetic flowmeters along the irrigation tubing (laterals) at the cherry study orchards.*

The site 1 ("[redacted]") flowmeter cover 14 trees, which is an area of 6160 foot…

The flowmeter readings (Table 1) so far show that the applied water at Site 2, "[redacted]", and Site 3, "[redacted]", are similar to the highest evapotranspiration values observed through field measurements. The applied irrigation water at the Site 1, [redacted]", orchard is the lowest. Since the precipitation amounts in the spring of the current year are higher than the long-term average, cherries at the Site 1, "[redacted]", were harvested first, we believe that the trees used the stored soil moisture from both rainfall and irrigation applications in this period critical for the crop water use. Unfortunately, late precipitation (second half of May) was also damaging to the fruit skin (Figure 8).

Table 1. Flowmeter and depth of water applied for the three sites in m3 and mm per day in the top of the table and in gallons and inches per day in the bottom of the table.

Date	Site 1: Flow	Site 1: Depth	Site 2: Flow	Site 2: Depth	Site 3: Flow	Site 3: Depth
	(m^3)	(mm)	(m^3)	(mm)	(m^3)	(mm)
6 Jun	30.454	-	-	-	63.428	-
14 Jun	45.732	4.1	125.536	-	63.428	0
26 Jun	76.337	4.8	206.676	9.5	100.798	9.3
3 Jul	99.272	6.2	256.886	10	119.638	8.0
19 Jul	150.74	6.55	348.55	8.03	155.43	6.68
25 Jul	178.58	9.46	392.52	10.27	174.91	9.71
31 Jul	180.05	0.5	441.75	11.5	197.19	11.11
14 Aug	241.84	8.99	511.5	6.98		
21 Aug	241.91	0	546.18	6.94	238.2	5.84
5 Sep	291.1	6.68	638.36	8.61	278.94	8.12
9 Sep	315.79	7.20	685.27	9.39	297.21	7.80

20 Sep	321	1.32	719.46	6	310.12	4.83
27 Sep	348.51	8.02	751.68	6.45	322.82	5.42
4 Oct	373.25	7.2	758.92	1.45	322.82	0
18 Oct					333.1	2.2
	(gals)	**(in.)**	**(gals)**	**(in.)**	**(gals)**	**(in.)**
6 Jun	8045.1	-	-	-	16756	-
14 Jun	12081	0.15	33163	-	16756	0.00
26 Jun	20166	0.20	54598	0.37	26628	0.37
3 Jul	26225	0.26	67862	0.39	31605	0.31
19 Jul	39822	0.26	92077	0.32	41059	0.26
25 Jul	47175.89	0.37	103692	0.404	46205	0.38
31 Jul	47564.06	0.02	116698	045	52093	0.44
14 Aug	63886	0.35	135124	0.27		
21 Aug	63904.73	0	144284	0.27	62926	0.23
5 Sep	76901	0.26	168638	0.34	73689	0.32
12 Sep	83422	0.28	181029	0.37	78515	0.31
20 Sep	84794	0.05	190061	0.24	81926	0.20
27 Sep	92066	0.32	198572	0.25	85280	0.21
4 Oct	98602.6	0.28	198572	0.05	85280	0
18 Oct					87996	0.09

Figure 8. *Adverse effects (fruit cracking) of late spring precipitation events on cherries at the study orchards*

Stem Water Potential measurements:

The water status of cherry trees at the three study orchards was appraised with periodic measurements of midday stem water potential (bars) and following the recommendations provided by the University of California, Division of Agriculture and Natural Resources (UC ANR) on target values for mature stone fruit trees under optimal water management (http://fruitsandnuts.ucdavis.edu/pressure_chamber_prunes/).

Table 2 - Periodic measurements of midday stem water potential (bars) at the study orchards:

Date	Site 1, "[redacted]"	Site 2 "[redacted]"	Site 3, "[redacted]"
2019-05-31	-7.5	-7.4	-5
2019-06-06	-5.8	-6.7	-6.1
2019-06-14	-7.9	-15.2	-9.5
2019-06-26	-7.6	-6.7	-7.5
2019-07-03	-8.9	-6.7	-7.5
2019-07-19	-8.3	-6.8	-9
2019-07-25	-12.1	-10	-9.5
2019-07-31	-11.4	-6.4	-7.9
2019-08-14	- 10.8	-9	
2019-08-21	-10.8	-10	-9
2019-09-06	-10	-8.7	-10
2019-09-12		-7.4	-9.3
2019-09-20	-8.3	-7.2	-7.2
2019-09-27	-6.5	-6	-7
2019-10-04	7.4	8	8.1

Based on the extensive research in Mediterranean climates and the UC ANR's recommendations for prunes, our periodic measurements of midday stem water potential indicate that there was little to no water stress with the scheduled irrigation frequencies and amounts. There was just one value at Site 2 (in red) that was below the lower limit (threshold) of water stress in prunes for the central part of the crop growing season. The Table 3. lists the suggested values of midday steam water potential (in bars) during different months of the growing season in prunes and for mature trees (http://fruitsandnuts.ucdavis.edu/pressure_chamber_prunes/):

Table 3. Target values recommended by UC Davis Fruit & Nut Research & Information Center for stem water potential of mature prune tress during the growing season

Season	Month						
	March	April	May	June	July	Aug.	Sept.
Early-	-6	-8	-9	-10	-12	-13	-14
Mid-	-6	-8	-9	-11	-12	-13	-15
Late-	-7	-9	-10	-11	-12	-14	-15

Leaf sampling and lab analysis:

Leaves were sampled according to Washington State Extension recommendations (http://treefruit.wsu.edu/orchard-management/soils-nutrition/leaf-tissue-analysis/?print-view=true). Each of the three cherry orchards were sampled in two areas, therefore the Tables 4 and 5. List the two sampling results for each of the three sites.

Table 4. Leaf tissue Analysis – the same black color for all values is to mark that the levels are within the normal thresholds.

SAMPLE #	N (Total) [SOP 522.1] %	P (Total) [SOP 590.2] %	K (Total) [SOP 590.2] %
"[redacted]" 1	2.58	0.187	1.65
"[redacted]" 2	2.51	0.183	1.27
"[redacted]" 1	2.40	0.306	1.78
"[redacted]" 2	2.26	0.317	1.61
"[redacted]" 1	2.28	0.287	1.50
"[redacted]" 2	2.33	0.298	1.84

Table 5. Leaf tissue analysis continued – blues values are to mark lower then the recommenden threshold and the red values are for higher then recommended from (http://treefruit.wsu.edu/orchard-management/soils-nutrition/leaf-tissue-analysis/?print-view=true)

SAMPLE #	S (Total) [SOP 590.02] ppm	B (Total) [SOP 590.02] ppm	Ca (Total) [SOP 590.02] %	Mg (Total) [SOP 590.02] %	Zn (Total) [SOP 590.02] ppm	Mn (Total) [SOP 590.02] ppm	Fe (Total) [SOP 590.02] ppm	Cu (Total) [SOP 590.02] ppm
"[redacted]" 1	1440	74.3	1.91	0.906	16.6	51.5	74.1	6.5
"[redacted]" 2	1420	74.1	2.18	1.059	18.1	62.3	65.0	8.7
"[redacted]" 1	1450	97.0	2.38	0.606	37.4	70.8	82.1	9.5
"[redacted]" 2	1350	94.3	2.40	0.628	32.9	59.6	78.7	10.4
"[redacted]" 1	1340	86.4	2.20	0.629	16.1	58.8	86.0	7.7
"[redacted]" 2	1330	92.6	2.13	0.574	18.6	61.8	73.7	7.5

Pending tasks:

1. CIMIS station installation
2. Irrigation system evaluation
3. Installation of soil moisture monitoring units
4. Collect information on yield quantity and quality from the grower

Report year 2020

New CIMIS station was installed and it is functional since February 26, 2020. Our collaborator Mohamed Nouri is overseeing the site's measurements quality and helping mowing the grass, which is also important for maintaining the measurements to be representative of a reference grass. Please contact me (ksuvocarev@ucdavis.edu) if you have questions how to access the reference evapotranspiration data to be used for computing the actual evapotranspiration from cherry fields:

$$ET_a = Kc * ETo$$

Actual evapotranspiration measurements continued in 2020. Due to COVID-19 pandemic and request from UC Davis to downscale our research activities, measurements at two of the three stations were conducted. Because we have agreed on no-cost extension, there will be another full experimental year to quantify the evapotranspiration at all three orchards.

I have chosen for 2020 to monitor evapotranspiration at the highest tree density with drip irrigation ("[redacted]") and the lowest tree density with sprinkler ("[redacted]"). These two orchards differ in irrigation systems and row orientation.

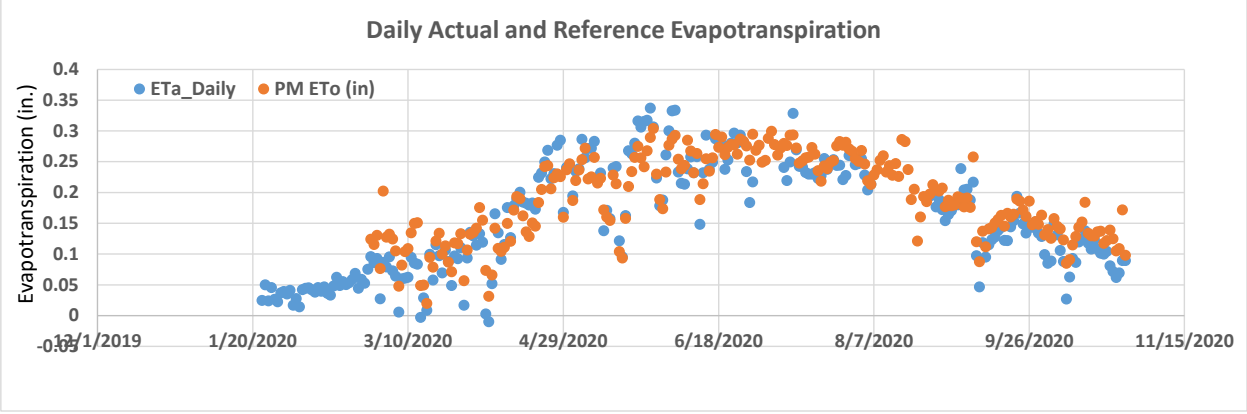

Figure 9. Daily Evapotranspiration values, Orange dots is CIMIS downloaded reference evapotranspiration); Blue dots are measured actual daily evapotranspiration at "[redacted]" orchard with sprinkler.

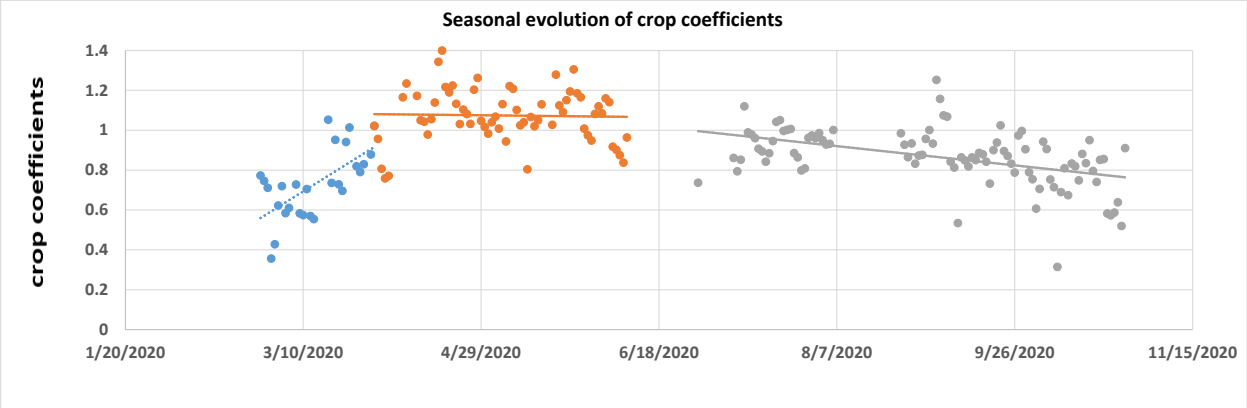

Figure 10. Daily crop coefficient values between February 27 and October 27, 2020. Initial period of development is marked with blue, orange is for mid-season values and gray for late season values at "[redacted]" orchard with sprinkler.

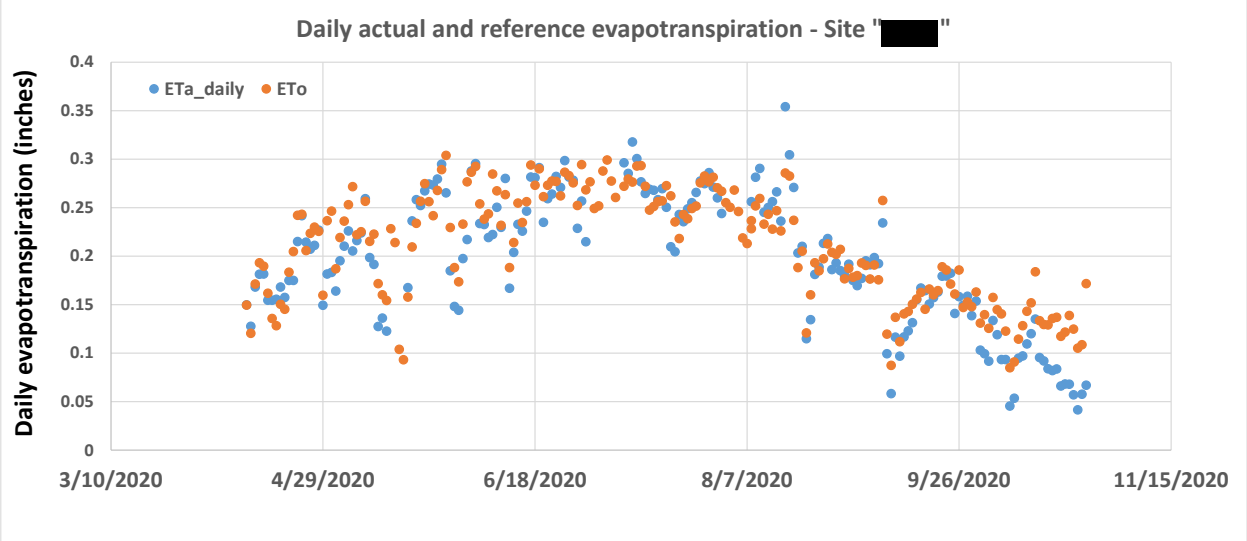

Figure 11. Daily Evapotranspiration values, Orange dots is CIMIS downloaded reference evapotranspiration); Blue dots are measured actual daily evapotranspiration at "[redacted]" orchard with drip irrigation.

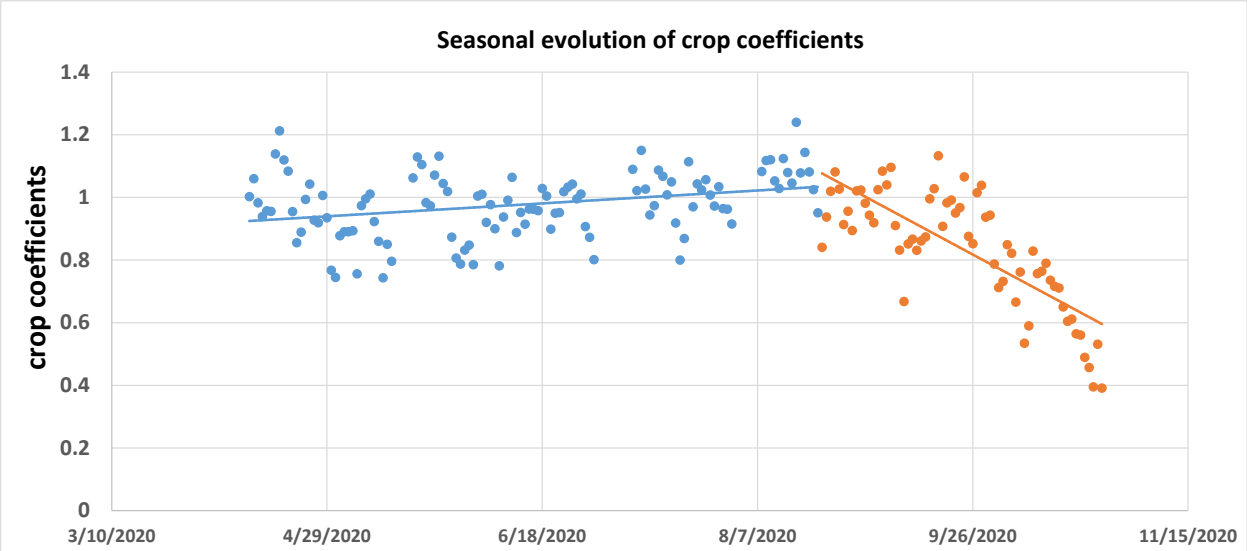

Figure 12. Daily crop coefficient values between February 27 and October 27, 2020. Initial period of development is marked with blue, orange is for mid-season values and gray for late season values at "[redacted]" orchard with drip irrigation.

We missed to capture the initial crop coefficient for developmental stage at the "[redacted]" site, since we could not install the stations before April 11th. In the year 2021 we will start measurements season earlier to fill the gap in this measurement. At the "[redacted]" site we did full winter measurements, and will continue in the incoming winter to couple this project with the project on chilling.

In this project year, we have started a collaboration with Dr. Mallika Nocco who uses drone flights to estimate evapotranspiration from the cherry crop. Based on our direct measurements of evapotranspiration, drone imaging can be adjusted better to estimate evapotranspiration. Drone flights were performed mostly on the days Landsat8 satellite passage was expected in order to compare remote sensing products and drone imaging to the direct measurements o analyze for the potential of different options to be used in the future where the direct measurements are not available. We are working on data analysis and will communicate results of comparison soon.

Figure 13. Dr. Mallika Nocco with her student Logan Ebert and Mohamed Nouri on the day first drone flight was performed.

With a similar motivation, we have started a collaboration with Arable Labs company to evaluate a performance of their device "Mark 2" to estimate evapotranspiration when it is placed near our measurements within the same orchard. We are currently comparing their results with our measurements and will report soon on this device performance as another option for evapotranspiration estimation where we do not have measurements possibilities.

Figure 14. Arable Mark 2 above a cherry tree in '[redacted]" orchard

California Cherry Board

Annual Report

1. Project Information

Project Title	Development of Nutrient Budget and Nutrient Demand Model for Nitrogen Management in Cherry	
Project leaders	- Patrick Brown, Professor, Department of Plant Sciences, UC Davis, One Shields Ave, Davis, CA 95616 - Douglas Amaral, Coop Ext Advisor, UC ANR, 680 Campus Dr. Ste. A, Hanford, CA 93230	
Grant Number	19-0954 (CCB-FREP-CDFA)	
Project Duration	Start Date: 01/01/2020	End Date: 12/31/2022
Email and Phone	(530) 219-8329, amaral@ucdavis.edu	
Report Type	Annual Report	
Reporting Period	Start Date: 01/01/2020	End Date: 06/30/2020

2. Abstract

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.

3. Project background

Matching fertilizer application with plant demand is important to maximize production as well as to minimize losses to the environment. Application of nitrogenous fertilizers to tree crops when not needed or in excess of crop demand, has resulted in leaching of N

to ground water (Weinbaum et al., 1992) with nitrate levels in ground water now exceeding the maximum contamination level of 45 ppm in many parts of the world (Burow et al., 2008; Viers et al., 2012). In response to evidence of widespread nitrate pollution of groundwater, the Central Valley Region Water Quality Control Board has adopted a regulatory widespread nitrate pollution of groundwater, the program to protect groundwater resources that requires growers to use best nitrogen (N) management practices to reduce nitrate leaching.

In annual crops, knowledge of crop nutrient demand and the application of fertilizers at rates and times consistent with crop demand has been central to the improvement of N use efficiency and the reduction in leaching potential (Fessehazion et al., 2011). Our knowledge of crop demand and patterns of nutrient uptake in deciduous perennial tree species is currently very poor and hence our ability to optimize fertilization strategies is limited. The large size and perennial nature of deciduous tree crops complicates the derivation of nutrient uptake patterns. The ability of perennials to store nutrients to meet a substantial proportion of the early demands of growing leaves and flowers/fruits further complicates the determination of seasonal uptake patterns (Millard and Grelet, 2010). The amount of stored nutrients also varies depending on environment, tree age and species (Weinbaum and Van Kessel,1998).

Understanding of the pattern of nutrient acquisition and demand in trees is relevant in an agricultural context when nutrients must be applied to maximize productivity and minimize wastage. Climate change is predicted to change the quantity and distribution of precipitation, while changing temperatures will alter the rate of soil microbial processes that in turn determine soil nutrient availability. Knowledge of the patterns of nutrient acquisition by tree species will enhance our ability to predict the impact of changes in climate on tree growth and productivity.

Efficient management of nutrients in agricultural production is essential for both economic sustainability and to minimize the movement of nutrients from the field where they may result in environmental degradation. According to the most recent California Agricultural Statistics Review publication, California sweet cherry production grossed $330,773,000 in revenues during the 2017 growing season. Albeit a relatively high-value crop, to date, no research has been conducted in California to evaluate either the seasonal demands or indicators of sufficiency of nutrients in sweet cherry cultivars.

Currently, cherry growers know little about efficiently supplying demand-driven nutrients. Historically, the management of nutrition of cherries has mostly been based on leaf sampling and subsequent contrast with established critical values (CVs). CVs are the nutrient concentration in a standard leaf sample at which growth is 90% of the maximum growth (Ulrich and Hills, 1967, 1990). While this has been a useful tool for diagnosis of nutrient deficiency or excess (Ulrich and Hills, 1967), it is now recognized that this approach does not provide sufficient information to define the rate and timing of fertilizer applications. Thus, knowledge of tree internal N dynamics is important to determine the timing and amount of N supply in spring and summer.

Recognizing the limitations of traditional leaf sampling as a mean of managing fertilization in high value crops such as cherries, several alternate approaches have been developed. Most promising among these is the use of seasonal nutrient uptake dynamics and demand estimations to construct a 'budget' approach to fertilizer management. For a wide range of annual and perennial field crop species, nutrient budgets have been developed to determine time and rate of fertilizer applications (Geraldson and Tyler, 1990; Ulrich and Hills, 1990; Benbi and Biswas,1999).

The development of nutrient budgets for mature trees is complex and costly requiring whole plant excavation and determination of the nutrient concentration and biomass of the individual plant organs to calculate the nutrient accumulation rate over the season and hence has rarely been performed (Weinbaum et al., 2001; Muhammad et al, 2015). Nutrient demand curves have not been developed for cherry and those available for other deciduous tree species were generally limited in scale. The objective of this project is to develop and extend nutrient Best Management Practices (BMP) to optimize N use efficiency in cherry cultivars with the outcome of reducing N leaching.

4. Activities performed

4.1. 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.

The specific objectives of the project are:

1. Develop nutrient demand curves to guide the quantity and time of fertilizer application in cherry. Repeat for most representative cultivars and production systems.
2. Develop and extend nutrient Best Management Practices (BMP) for cherry cultivars.

Objective #: 1.1
Tasks activities and accomplishments: Finalize grower agreements to carry out the research on grower's farm.
Results for each task: 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.
Objective #: 1.2

Tasks activities and accomplishments: Soil analysis and irrigation water measurements.
Results for each task: Samples of soils from all locations are being collected at every 6-month periods. The samples will be analyzed for Organic Matter (OM), soluble and mineralizable N pools and other critical parameters.
Objective #: 1.3
Tasks activities and accomplishments: Nutrient and Carbohydrate accumulation in annual and perennial organs.
Results for each task: We have been monitoring three replicated blocks of trees (4 trees per block, totaling 12 trees per orchard) for each cherry cultivar (“Bing”, “Coral”, and “Rainier”) in the California Central Valley 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. Samples collected are being processed for analysis.
Objective #: 1.4
Tasks activities and accomplishments: Tree excavations to determine tree biomass at the beginning and end of season.
Results for each task: Highly productive groves of cherries were selected in the California Central Valley. Trees that represent optimum leaf N concentrations and not showing any deficiency of other nutrients were excavated. Samples collected are being processed for analysis.

4.2 Outreach

The first year of this experiment was assigned to establish the trial and to collect samples, thus no outreach activities have been performed.

5. Challenges

Describe any challenges or delays that occurred during this reporting period and the corrective actions and/or changes to the project as a result. Include lessons learned based on feedback from outreach events. Add more rows as needed. Enter N/A when applicable.

Challenge	Corrective Action and/or Project Change/lessons learned
Delay on sampling at the beginning of the season due to COVID-19.	Project change: delay on sample processing and analyses.

6. Additional information

The first year of this project was assigned to stablish the experiment and to collect samples, thus no data have been analyzed.

7. Activities for next reporting period

Describe activities you plan to complete during the next reporting period. Add more rows as needed.

Activity	Anticipated Completion Date
Sample processing and data analyses	12/2021
Soil analysis and irrigation water measurements	12/2021
Collect samples to determine nutrient accumulation in annual and perennial organs	12/2021

8. Figures, tables, and supporting documents

N/A

9. References

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Annual Report - 2020

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
Cooperators:	Dr. H. Förster, D. Thompson, C. O'Fallon, and L. Wade

SUMMARY

In 2020, we continued our efforts in developing new management strategies for major foliar and fruit diseases of sweet cherry in California including bacterial blast caused by *Pseudomonas syringae* pv. *syringae*, powdery mildew caused by *Podosphaera clandestina*, blossom blights and fruit rots caused by *Monilinia* and *Botrytis* spp., as well as postharvest decays and Phytophthora root and crown rots.

1) **Bacterial blast** caused by *Pseudomonas syringae* pv. *syringae*:
   a. In a field study on cv. Coral Champagne with a low incidence of blast, Nisin + Manniplex Zn, Kasumin, Mycoshield, and the essential oil compounds ET91 and BacStop significantly reduced the incidence of disease. Kasumin and Mycoshield were most effective in reducing bacterial canker.
   b. Kasumin obtained full registration on cherry in California and the United States in the spring of 2018. A registration of oxytetracycline on sweet cherry is currently pursued with support of the registrant through the IR-4 program and is pending at EPA after the PRIA data was postponed again. New formulations of the antimicrobial food additives nisin and ε-poly-L-lysine are being developed in collaboration with a chemical company.

2) In a **powdery mildew** study in San Joaquin Co., the experimentals pyraziflumid, Miravis Duo and Miravis Prime, as well as the registered Luna Sensation and Merivon were highly effective on leaves inside the canopy and on the outside. The new powdery mildew fungicide Gatten (flutianil) as well as the essential oil compounds EF-400 and Gargoil showed moderate efficacy.

3) For **brown rot blossom and gray mold blight**, all of the registered and experimental (e.g., Cevya, GWN 10570, Miravis Duo, Miravis Prime, Fervent, UC-2) compounds evaluated were highly effective as pre- and post-infection treatments in laboratory studies.

4) A field study was conducted on the efficacy of **fungicide treatments applied 7 days preharvest**.
   a. **Brown rot**: In wound-inoculations of harvested non-washed and washed fruit, Cevya was the most effective in reducing the incidence of brown rot. Other effective treatments included Quadris Top, Miravis Duo, and UC-2. In non-wound inoculations, all treatments were highly effective.
   b. For **gray mold**, high efficacy on non-washed and washed, non-wound-inoculated fruit was obtained using pyraziflumid, GWN 10570, Quadris Top, Miravis Duo, and Miravis Prime.

5) **Postharvest studies** focused on the evaluation of new biologicals, three formulations of natamycin, and registered conventional postharvest fungicides.
   a. Among biological treatments, organic formulations of polyoxin-D (Ph-D, Oso), GWN 10474, and Howler significantly reduced the incidence of brown rot. Only polyoxin-D was highly effective against gray mold, and none of these biological treatments provided Rhizopus rot control.
   b. Teb at 8 oz was very effective against the three major postharvest decays, but Mentor at 4 fl oz (the highest label rate) was less effective against gray mold and Rhizopus rot. Thus, Mentor (propiconazole) is best used in mixtures or as the pre-mixture Chairman. In our previous studies, Chairman at rates between 8 and 16 fl oz reduced the three decays to zero levels. This pre-mixture of fludioxonil and propiconazole has a broad spectrum and high activity (brown rot, gray mold, Rhizopus rot, sour rot).
   c. Three formulations of natamycin were all very effective against brown rot, gray mold, and Rhizopus rot. The mixture of BioSpectra and Ph-D showed very good efficacy against brown rot, gray mold, and Rhizopus rot With increasing emphasis on food safety and consumer concerns, natamycin and

polyoxin-D with 'exempt from tolerance status' may become important components of postharvest decay management in the future. Natamycin will also have a role in resistance management because resistance in filamentous fungi has never been reported. It will be best used in mixture with low rates of Scholar or another fungicide.

6) Laboratory and field studies were conducted on the evaluation of new fungicides for management of **Phytophthora root and crown rot**.
   a. ***Phytophthora*** and *Phytopythium* isolates from cherry were all sensitive to the new Oomycota fungicides oxathiapiprolin, fluopicolide, and ethaboxam, but mandipropamid was only highly toxic to *Phytophthora* spp.
   b. In two trials at UC Davis where trees in newly established orchards were inoculated with *Phytophthora* spp., a treatment with the new fungicides in the spring of 2020 resulted in significantly better tree health ratings in the fall as compared to the control. In a trial in a commercial orchard with natural inoculum, Orondis significantly reduced the incidence of trees with symptoms of crown rot. These trials are ongoing, and additional treatments and evaluations will be done. Oxathiapiprolin (Orondis) was accepted into the IR-4 residue program on sweet cherry in Sept. 2020.

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. Canker symptoms develop weeks to months later with gumming around the infected, sunken bark tissue. 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 treated with rest-breaking treatments that bloom earlier and may experience cooler, wet spring environments. Bud death and spots on leaves and fruit are additional symptoms of the blast phase of the disease.

Copper resistance in the pathogen populations is widespread in California, therefore, we have been 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.

Additional treatments need to be identified that could be used in rotations and mixtures. We evaluated numerous other compounds, including 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, and other novel bactericides such as a nano-particle zinc compound, and Cinetis, a nutritional stress reducer. In 2019, two GRAS antibacterial food additives (i.e., nisin and ε-poly-L-lysine) showed promising results, and these were evaluated again in 2020. Because the efficacy of these compounds has been effective in some but not in other studies on bacterial diseases of fruit trees, we are currently collaborating with a chemical company to develop agrochemical formulations. In 2020, we also evaluated additional natural products and biocontrols.

Management of powdery mildew, blossom blight, and fruit rot. Powdery mildew of sweet cherry is an ongoing problem for growers in California, especially in southern production areas (e.g., Tulare and Kern Co.). Warm temperatures with low rainfall and high humidity from dews or irrigation are highly favorable for disease development. Flower sepals, leaves, and fruit may be infected. In some export markets, powdery mildew is a quarantine disease, 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

Table 1: Fungicides and bactericides used in 2020 studies*.

FRAC group	Trade name	Active ingredient
Single active ingredients		
3	Cevya	mefentrifluconazole
3	Mentor	propiconazole
3	Procure	triflumizole
3	Quash	metconazole
3	Rally	myclobutanil
3	Regev	difenoconazole + tea oil
3	Teb	tebuconazole
4	Ridomil Gold	mefenoxam
7	Fontelis	penthiopyrad
7	Pyraziflumid	pyraziflumid
12	Scholar	fludioxonil
13	Quintec	quinoxyfen
17	Elevate	fenhexamid
19	Ph-D, Oso	polyoxin-D
22	Intego	ethaboxam
40	Revus	mandipropamid
43	Presidio	fluopicolide
48	Orondis	oxathiapiprolin
49	BioSpectra, Cerafruta, Natamicina	natamycin
P07 (33)	ProPhyt	potassium phosphite
U13	Gatten	flutianil
Bactericides	ε-Poly-L-lysine	food additive
	Kasumin	kasugamycin
	Mycoshield	oxytetracycline
	Nisin	food additive
Miticide	Magister	fenazaquin
Experimentals	EXP-19A	not disclosed
	F-4406-3	not disclosed
	GWN 10570	not disclosed
	UC-2	not disclosed
Biologicals	AllPhase	potassium sorbate and sodium lauryl sulfate
	BacStop	essential oils
	Blossom Protect	*Aureobasidium pullulans*
	Double Nickel	*Bacillus amyloliquefaciens*
	Ecoswing	extract of *Swinglea glutinosa*
	EF400	essential oils
	ET91	essential oils
	Gargoil	garlic oil
	GWN 10474	not disclosed
	Howler	*Pseudomonas chlororaphis*
	LifeGuard	*Bacillus mycoides*
	Serenade ASO	*Bacillus amyloliquefaciens*
	Serifel	*Bacillus amyloliquefaciens* strain MBI600
	TDA-NC-1	chlorine dioxide generator
	Thymox	essential oils
	Timorex	tea tree oil
	un-named	*Bacillus subtilis*
Premixtures		
3 + 11	Quadris Top	difenoconazole + azoxystrobin
7 + 3	Luna Experience	fluopyram + tebuconazole
7 + 3	Miravis Top (Miravis Duo)	pydiflumetofen + difenoconazole
7 + 11	Fervent	tebuconazole + isofetamid
7 + 11	Luna Sensation	fluopyram + trifloxystrobin
7 + 11	Merivon	fluxapyroxad + pyraclostrobin
7 + 12	Miravis Prime	pydiflumetofen + fludioxonil

* - Sorted by Fungicide Resistance Action Committee (FRAC) code or mode of action.
Some fungicides were used with adjuvants such as Breakthru or DyneAmic.

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 2020, excellent control was again obtained using the experimentals Miravis Duo, Miravis Prime, and pyraziflumid, whereas the new powdery mildew fungicide Gatten (flutianil) showed intermediate efficacy. The essential oil compounds EF400 and Gargoil also showed very good efficacy. All these compounds will need to be continued to be evaluated to possibly obtain new rotation alternatives not only for powdery mildew, but also for other bloom, petal fall, and preharvest diseases. Fungicides evaluated in 2020 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 the QoIs, DMIs, anilinopyrimidines, phenylpyrroles, hydroxyanilides, SDHIs, and polyoxins to be effective. The pre-mixtures Quadris Top, Pristine, Merivon, Luna Experience, and Luna Sensation 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. Some of the newer fungicides such as Miravis (pydiflumetofen) and Cevya (mefentrifluconazole; UC-1) 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. Information on the preventative and post-infection activity of fungicides is helping to develop our delayed bloom fungicide application model for improved timing in low- to moderate-disease pressure years and for optimizing fungicide treatments. Although DMI fungicides are highly effective against brown rot, they have to be complemented with other materials to obtain high efficacy against gray mold. Pre-screenings of additional fungicides and biological products that potentially can be used as preharvest treatments in the field was done in laboratory studies. For this, fruit were treated, air-dried and then inoculated.

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. In 2020, we also evaluated the efficacy of several new biological treatments that included a biocontrol, essential oils, and plant extracts.

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 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 for managing Phytophthora root and crown rot diseases on 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 also established field trials in 2019/20 in newly planted orchards at UC Davis where trees were inoculated and in collaboration with growers in naturally infested fields. These new compounds all have different modes of action, and our goal is to develop efficacy data for them so they can be registered simultaneously. 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, Ɛ-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 Duo, 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 and crown 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. Selected new antibacterial compounds were evaluated for their direct toxicity against *Pss.* For this, bacteria were exposed to the test compound solution for 30 min and were then plated onto nutrient agar. For the controls, water was added to the reaction mixture instead of the test compounds. Viability of bacteria (i.e., the number of colonies formed) was assessed after 2 days of growth.

A trial on blossom blast was done on cv. Coral cherry at UC Davis just before a 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 3-25-20. Bactericide applications were made using a hand sprayer. After air-drying for 2 h, flowers were inoculated with *P. syringae* (2×10^6 cfu/ml) by hand-spraying. Inoculated branches were covered with white plastic bags overnight. The incidence of disease (based on the number of diseased flowers per total number of

flowers) was evaluated after 8 days. In another study, newly emerged leaves were treated on 4-1-20. After air-drying, leaves were spray-inoculated with *Pss* ($2x10^8$ cfu/ml) and bagged overnight. Disease was evaluated after 8 days, and the number of lesions per leaf was assessed.

In a study on bacterial canker, branches of cv. Coral cherry trees were puncture-wounded laterally in Jan. 2020 using a nail to expose the cambium and wood. Wounds were spray-treated with bactericides and inoculated with *Pss* (approximately 4 x 10^7 cfu/ml) after air-drying. Branches were evaluated for gumming and canker formation in late-April 2020.

Evaluation of new fungicides for control of powdery mildew. In a field trial in San Joaquin Co., treatments were done on 3-10-20 (petal fall) for protection from primary inoculum (ascospores from overwintering chasmothecia) and were followed by treatments on 4-1 and 4-21-20 for protection from secondary infection from conidia. Single fungicides, mixtures, pre-mixtures, and two rotation programs were evaluated. The incidence of powdery mildew was evaluated on 20 leaves from four random shoots each from inside the tree or from the outer tree perimeter for each of the four single-tree replications on 5-19-20. Severity was rated using a scale: 0 = healthy, 1 = 1-3 lesions, 2 = <25%, 3 = up to 50%, 4 = >50% of leaf area affected. Data were expressed as disease intensity (the multiplication product of incidence and severity) and analyzed using analysis of variance and mean separation procedures of SAS 9.4.

Evaluation of new fungicides for control of brown rot and Botrytis blossom blight and fruit decay. Laboratory experiments were conducted to evaluate the pre- and post-infection activity of fungicides against brown rot and gray mold blossom blight. Flowers were collected at white bud and allowed to open in the laboratory. For evaluation of the pre-infection activity, flowers were treated using a hand sprayer, air-dried, and inoculated with a spore suspension of *M. fructicola* or *B. cinerea* (30 K/ml) until water droplets formed on anther filaments. For post-infection activity, flowers were inoculated, incubated at 22 C, >95% relative humidity, and treated after 15 h. Disease incidence was evaluated as the number of stamens infected divided by the total number of stamens per flower after 4-5 days of incubation at 20 C. Three replications of 8 flowers were used for each treatment, and data were analyzed using analysis of variance and mean separation procedures (SAS 9.4).

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 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 5-7 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.

Additional fungicides and biological products were screened as pre-infection treatments in laboratory studies. This was done to determine their potential as preharvest treatments in the field. For this, fruit were spray-treated, air-dried, and then wound- or non-wound-inoculated using procedures 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 on the comparative evaluation of several new biological treatments that included biocontrols (i.e., *Bacillus subtilis, Pseudomonas chororaphis*- Howler), essential oils (i.e., EF400, Timorex, ET91), plant extracts (i.e., EcoSwing), and a mixture of potassium sorbate and sodium lauryl sulfate. The efficacy of three formulations of natamycin (i.e., BioSpectra, Cerafruta, Natamicina) was compared in another study. We also compared the efficacy of several treatments when applied in water or in a diluted fruit coating. In these studies, fruit were wound-inoculated with *M. fructicola, B. cinerea,* or *R. stolonifer* as described above and treated after 8 to 17 h by spraying using an air-nozzle sprayer. After treatment, fruit were incubated for 4-7 days at 20 C, >95% RH. Incidence of decay was determined as the number of infected fruit of the total fruit evaluated. Data were analyzed using analysis of variance procedures of SAS 9.4.

Evaluate new fungicides for managing Phytophthora root rot and crown rot of cherry. Surveys were initiated on causal agents of cherry trees declining from apparent *Phytophthora* spp. infection in three commercial orchards. Root, crown, and rhizosphere soil from symptomatic cherry trees from orchards with a history of Phytophthora diseases 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.

In vitro sensitivities of collected isolates to oxathiapiprolin, mandipropamid, fluopicolide, and ethaboxam were determined using our standard spiral gradient dilution method. Fungi were grown on strips of hydrophilic cellophane, and strips were placed onto agar media with fungicide concentration gradients (Förster et al. 2004). This fungicide gradient is established by spiral-plating selected stock concentrations. EC_{50} values were calculated using a computer program.

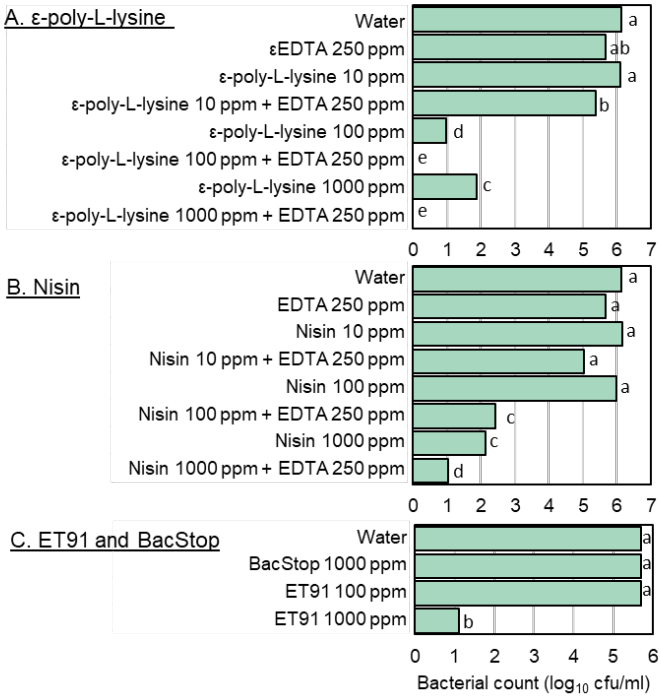
Greenhouse studies with potted Bing cherry on Krymsk or Mazzard rootstocks were initiated on the efficacy of new fungicides against Phytophthora root and crown rots. The soil of each pot was inoculated with a *Phytophthora*-colonized mixture of rice, vermiculite, and V8 juice. A mixture of *P. cambivora, 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 ethaboxam (10 fl oz/A) was applied to each pot after 7 days.

Field studies on the evaluation of new fungicides were initiated in two newly planted orchards at UC Davis. At UC Davis, trees (Mahaleb rootstock with Bing or Coral scions) were planted in Jan. 2020. In Study A, treatments were done on 4-28-20 by pouring the fungicide solution around the base of the tree and over the lower trunk. Treatments were then watered in by irrigation. Trees were inoculated on the same day with a mixture of *P. citricola, P. cactorum*, and *P. cambivora* (all obtained from cherry trees) that were grown on a rice-vermiculite-V8C juice mixture as described above for the greenhouse studies. In Study B, trees were first inoculated, and treated the following day. Treatments in these two studies consisted of single fungicides (i.e., Orondis, Revus, Presidio, ethaboxam, Ridomil Gold), a premixture (i.e., Orondis Ultra = Orondis + Revus), and mixtures (i.e., Orondis + Ridomil Gold, Presidio + ethaboxam). There were 8-10 trees for each treatment. Tree health was rated on Sept. 20, 2020 using a scale from 0 (= healthy, vigorous) to 4 (= tree dead). Another study was done in a commercial orchard with a history of the disease and trees showing symptoms of Phytophthora root rot and where river or district water is used for irrigation. In this plot, trees were re-planted into naturally infested soil and showed symptoms when Orondis was applied in mid-May. Trees were rated for disease on Oct. 8, 2020.

RESULTS AND DISCUSSION

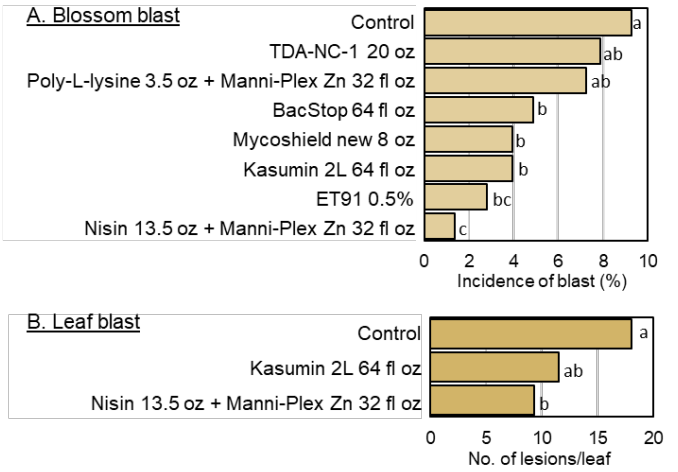
Evaluation of treatments for control of bacterial blast and canker. In in vitro studies, ε-poly-L-lysine at 100 and 1000 ppm as well as nisin at 1000 ppm were toxic to *Pss* and significantly reduced viability after 30-min direct exposures (Fig. 1A,B). The addition of the chelator EDTA (250 ppm) significantly increased toxicity, and no colonies developed in mixtures with 100 or 1000 ε-poly-L-lysine. For nisin that by itself did not reduce viability of *Pss* at 100 ppm, the addition of EDTA resulted in a 3.7-$\log_{10}$ reduction in colonies. These studies support the potential of these two food preservatives as highly effective agricultural bactericides by developing formulations with the addition of certain compounds. In direct

Fig. 1. In vitro toxicity of antibacterial food preservatives against *P. syringae* pv. *syringae*



Bacteria were exposed to the test compound solution for 30 min and were then plated onto agar media. Viability (i.e., the number of colonies formed) was assessed after 2 days of growth.

Fig. 2. Efficacy of antibacterial treatments against bacterial blast of cv. Coral cherry in small-scale field studies at UC Davis



Stamens were cut off, and flowers were treated on 3-25-20. After air-drying, flowers were spray-inoculated with *Pseudomonas syringae* ($2x10^6$ cfu/ml) and bagged overnight. Disease was evaluated after 8 days, and dark, wilted flowers with brown peduncles were counted.

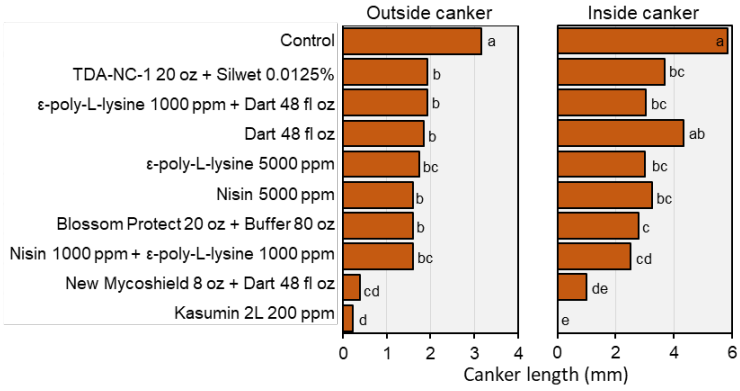
Newly emerged leaves were treated on 4-1-20. After air-drying, leaves were spray-inoculated with *Pseudomonas syringae* ($2x10^8$ cfu/ml) and bagged overnight. Disease was evaluated after 8 days, and the number of lesions per leaf was assessed.

exposure assays with two essential oil products, ET91 at 1000 ppm reduced colony formation by 4.6 log_{10}, but BacStop was not effective (Fig. 1C).

In field studies, inoculations of treated, injured flowers with *Pss* resulted in 9.3% blossom blast incidence in the water-treated control. Flowers wilted and had dark peduncles. The most effective treatment was Nisin mixed with ManniPlex Zn, and this treatment significantly reduced the incidence to 1.4% (Fig. 2A). Treatments with intermediate efficacy includee Mycoshield, Kasumin, BacStop, and ET91. Developing leaves that were inoculated with *Pss*, showed spotting and had a shot hole appearance. Nisin mixed with ManniPlex Zn again significantly reduced the severity of the disease (Fig. 2B).

In a study on bacterial canker, where twig wounds were treated and inoculated with *Pss* in January, disease severity (canker length) was very low. Still, efficacy data could be obtained, and Kasumin and

Fig. 3. Evaluation of antibacterial treatments for protection of cv. Coral branches from bacterial canker in 2020

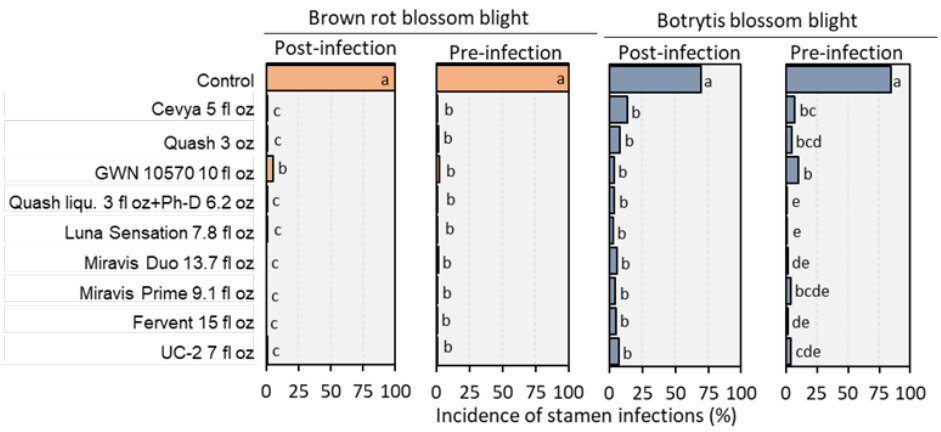


Branches were puncture-wounded laterally in Jan. 2020 using a nail. Wounds were spray-treated with bactericides and inoculated with *P. syringae* (approximately 4 x 10^7 cfu/ml) after air-drying. Branches were evaluated for gumming and canker formation in late-April 2020.

Mycoshield mixed with Dart were the most effective treatment in reducing canker size (Fig. 3). Most of the other treatments evaluated also showed some efficacy.

In our experiments over several years, 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. 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 that was postponed for a second time. The two food additives (nisin, poly-L-lysine) that we are developing gave promising results as unformulated active ingredients in 2019 and 2020, and we are working with a chemical company to obtain agricultural formulations that may improve their performance. With widespread copper resistance in the pathogen *Pss*, new effective treatments are needed. 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.

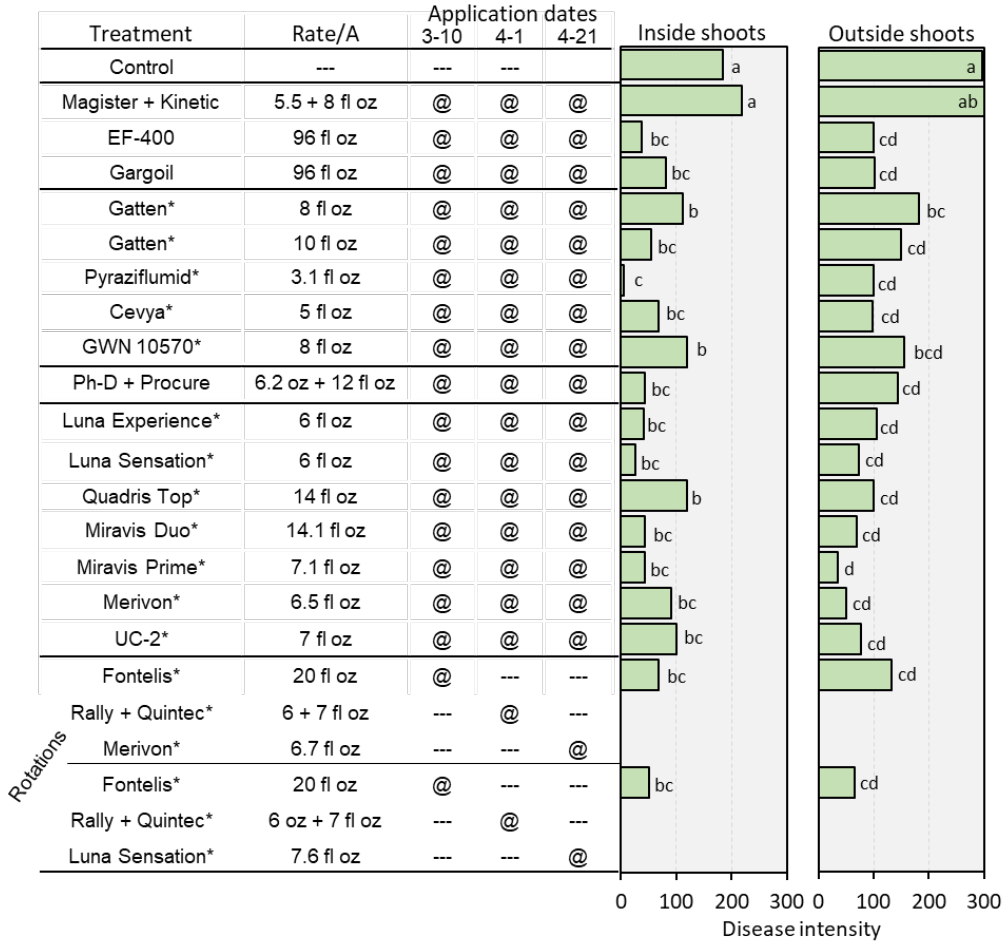
Fig. 4. Efficacy of pre- and post-infection treatments for control of brown rot and Botrytis blossom blight of Coral cherry – Laboratory studies 2020



For evaluation of the pre-infection activity, closed blossoms were collected in the field on 3-10-20, allowed to open, treated in the laboratory using a hand sprayer, air-dried, and inoculated with a spore suspension of *M. fructicola* or *B. cinerea* (30 K/ml). For post-infection activity, blossoms were inoculated, incubated at 22 C, and treated after 15 h. Blossoms were evaluated for stamen infections after 4-5 days of incubation at 20 C.

Efficacy of new fungicides for control of brown rot and Botrytis blossom blight. Selected fungicides were evaluated for their pre- and post-infection activity on detached, open flowers in laboratory studies. For both brown rot and Botrytis blossom blights, all of the registered and experimental compounds evaluated were highly effective, including the new Cevya, Miravis Duo, Miravis Prime, Fervent, GWN 10570 and

Fig. 5. Evaluation of preharvest fungicide treatments for management of powdery mildew of Bing cherries in San Joaquin Co. 2020



Applications were done using an airblast sprayer at 100 gal/A starting at petal fall. Treatments with an * were applied in combination with DyneAmic (6 fl oz /A). For evaluation on 5-19-20, 20 leaves from 4 random shoots each from inside or outside of the tree were sampled. The rating scale was: 0=healthy, 1=1-3 lesions/leaf, 2=<25%, 3=26-50%, 4 = >50% of leaf area diseased. Disease intensity is the multiplication product of disease incidence and severity.

UC-2 (Fig. 4). Post-infection treatments for brown rot resulted in reductions from 100% incidence in the control to between 5% (i.e., GWN 10570) and 0% (i.e., Miravis products, Fervent), and treatments for Botrytis blossom blight reduced the incidence of stamen infections from 69.7% in the control to 13.7% (i.e., Cevya) to 2.4% (i.e., Luna Sensation). Pre-infection treatments for brown rot resulted in reductions from 99.8% incidence in the control to $\leq$2.2% for all treatments evaluated, and treatments for Botrytis blossom blight reduced the incidence of stamen infections from 84.6% in the control to 10.1% (i.e., GWN 10570) to 1.3% (i.e., Luna Sensation and a mixture of Quash and Ph-D). Thus, treatments with excellent activity for management of blossom blight caused by both pathogens are currently available and include Luna Experience and Quadris Top, and new treatments are in development like Miravis Duo in 2021.

Due to the good pre- and post-infection activity of most conventional fungicides, the practice of a single delayed-bloom application when environmental conditions are not favorable for disease development is an excellent strategy for obtaining highly effective blossom disease management and result in a minimal number of bloom treatments on sweet cherry. Selected biological treatments were identified previously (Botector, Serenade Opti) that are not as effective as conventional fungicides but can benefit disease management in organic production systems.

Evaluation of new fungicides for control of powdery mildew of sweet cherry. Our epidemiological studies have shown that mildew sequentially develops on: 1) leaves of inside shoots (water sprouts); 2) leaves of outer shoots; 3) green stems of fruit; and 4) on ripening fruit (fruit with color). The disease has not been found on epi- or mesocarp tissue of green fruit, and young leaves are more susceptible than old leaves. The efficacy of new fungicides and pre-mixtures was evaluated in a trial in San Joaquin Co. Three applications were done in approximately three-week intervals starting at petal fall. Environmental conditions were favorable for powdery mildew development at our trial site in the spring of 2020. At evaluation in mid-May, 75% of leaves on water shoots inside the canopy and 71.7% of leaves in the outer canopy showed symptoms of powdery mildew.

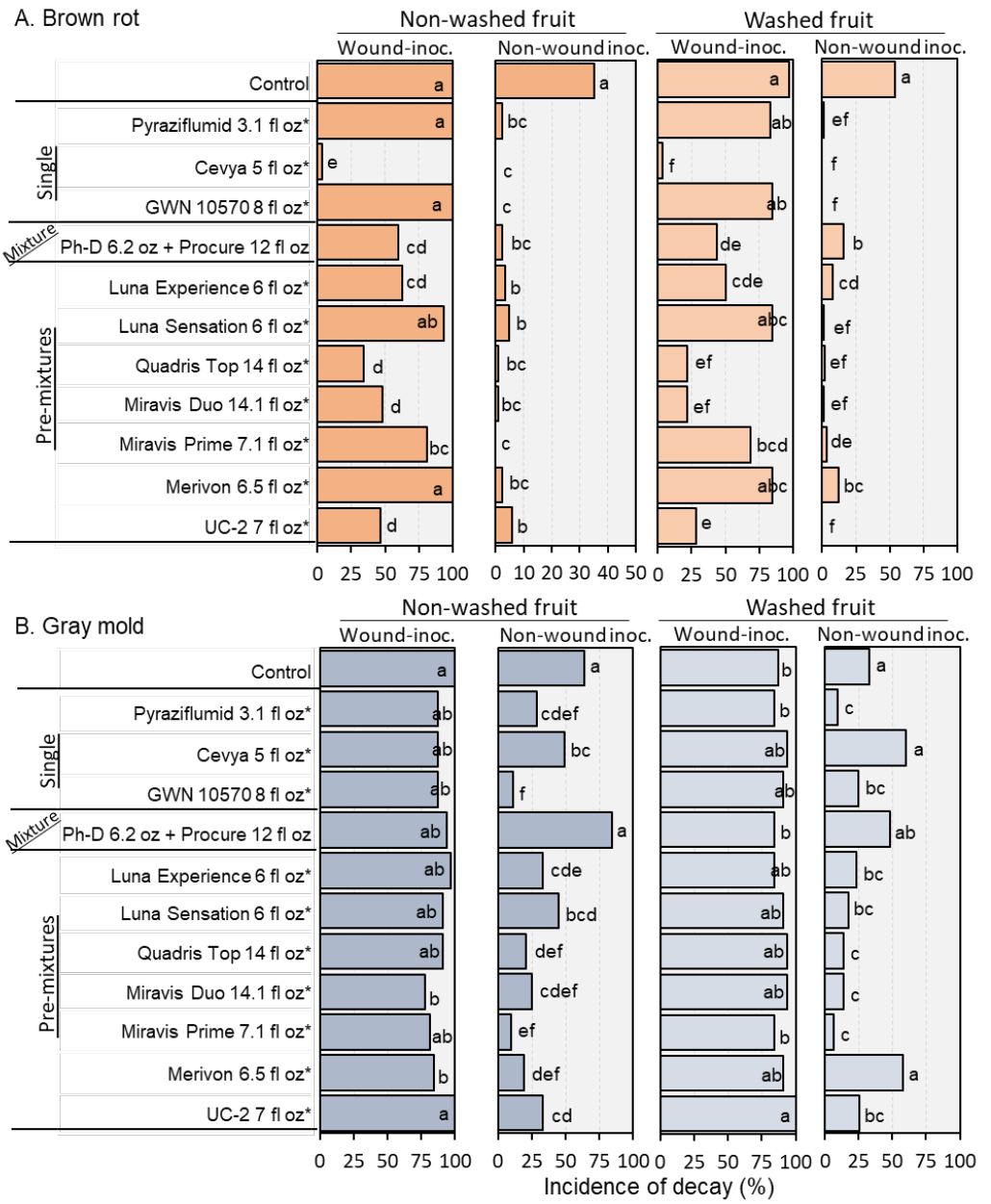
Based on disease intensity (the multiplication product of incidence and severity), all treatments except the miticide Magister significantly reduced the disease as compared with the control (Fig. 5). As in previous years, the experimentals Miravis Duo and Miravis Prime, as well as the registered Luna Sensation and Merivon were highly effective on inside and outside leaves. These contain DMI, SDHI, and/or QoI compounds which are known to have high activity against powdery mildews. Several treatments including the essential oil product EF400 and the fungicides pyraziflumid, Luna Experience, the Ph-D-Procure mixture, and the new powdery mildew fungicide Gatten (flutianil) at the 10-fl oz rate only showed high efficacy on inside leaves and moderate efficacy on outside leaves. A rotation of Fontelis, Rally + Quintec, and Luna Sensation reduced the disease to low levels on inside and outside leaves. 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 demonstrated 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 FRAC numbers) 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 have 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 FRAC code 3 fungicides like Procure for effective suppression of the disease.

Evaluation of preharvest treatments for management of fruit decays. Preharvest treatments to Bing cherries applied 7-days PHI were evaluated in a commercial orchard. In wound-inoculations of harvested fruit with *M. fructicola*, Cevya with 3.1% incidence was significantly the most effective in reducing the incidence of brown rot from the control where all fruit decayed (Fig. 6A). Other effective treatments included Quadris Top (34.4%), Miravis Duo (47.9%), and UC-2 (46.9%). These four treatments that all include a FC 3 compound were also highly effective when fruit were washed after harvest in a simulated hydrocooler treatment. In non-wound inoculations where 35% of control fruit developed decay, all treatments were highly effective and reduced the incidence to between 0 (i.e., Cevya, GWN 10570, Miravis Prime) and 5.8% (i.e., UC-2) (Fig. 6A) on non-washed fruit and to between 0 (i.e., Cevya, GWN 10570, UC-2) and 15.8% (i.e., Ph-D + Procure) on washed fruit.

In wound-inoculations of non-washed and washed fruit with *B. cinerea*, none of the treatments was very effective in reducing gray mold decay (Fig. 6B) although in 2019, Miravis Duo and Miravis Prime showed high efficacy even when applied 12 days before harvest. In laboratory studies where fruit were first treated and then wound-inoculated with *B. cinerea* (this sequence simulating a preharvest treatment), however, Miravis Prime and Elevate-Teb completely suppressed gray mold development (Fig. 7A). Thus, certain environmental conditions in 2020 possibly could have contributed to reduced persistence of Miravis Prime in 2020. Still, in non-wound-inoculations with *B. cinerea* of non-washed and washed

Fig. 6. Efficacy of 7-day preharvest fungicide treatments for management of postharvest brown rot and gray mold of Bing cherries - San Joaquin Co. - 2020

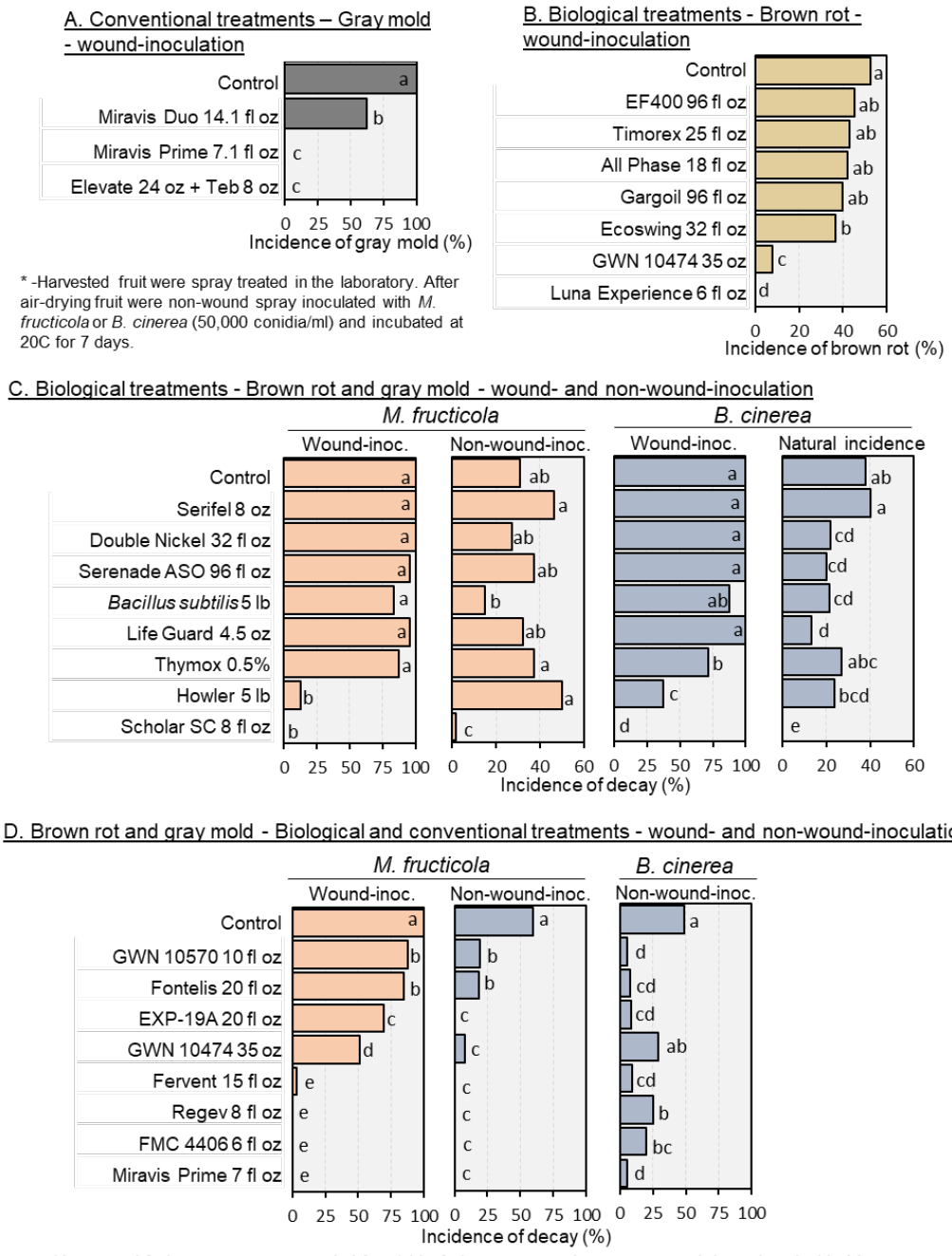


Treatments were applied on 5-19-20 using an air-blast sprayer at a rate of 100 gal/A. Treatments with * were applied in combination with DyneAmic. Fruit were washed by gently agitating in water for 2 min. Fruit were wound-inoculated with *M. fructicola* (50,000 spores/ml) or *B. cinerea* (30,000 spores/ml) 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.

fruit in 2020, several fungicides were highly effective, and these included pyraziflumid, GWN 10570, Quadris Top, Miravis Duo and Miravis Prime (Fig. 6B).

In laboratory studies, additional treatments were evaluated as pre-infection applications to determine their potential as preharvest treatments. Among twelve biological treatments, Howler and GWN 10474 were very effective against brown rot and significantly reduced decay after wound-inoculation (Fig. 7B, C, D). The natural incidence of gray mold was significantly reduced from the control after treatment with Double Nickel, Serenade ASO, *Bacillus subtilis*, and LifeGuard (Fig. 7C). Thus, GWN 10474 with an undisclosed active ingredient and Howler that both were also evaluated in postharvest studies (see below) were identified as the most promising biological treatments in these studies. Among conventional

Fig. 7. Efficacy of biological and conventional treatments for control of brown rot and gray mold of Bing cherry fruit in simulated laboratory preharvest studies 2020



Harvested fruit were spray-treated. After 14 h, fruit were wound- or non-wound inoculated with *M. fructicola* (50,000 spores/ml) or wound-inoculated with *B. cinerea* (300,000 spores/ml). Gray mold also developed naturally.

fungicides, Fervent, Regev, Miravis Prime, and FMC 4406 showed high efficacy in wound- and non-wound inoculations with *M. fructicola* (Fig. 7D) and in non-wound inoculations with *B. cinerea*.

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 may remove residues of many fungicides from fruit although in our studies, the efficacy of most fungicides after 2-min wash treatments of fruit was similar to non-washed fruit. 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.

Fig. 8. Efficacy of postharvest biological treatments for postharvest decay control of inoculated Bing cherry fruit in laboratory studies 2020

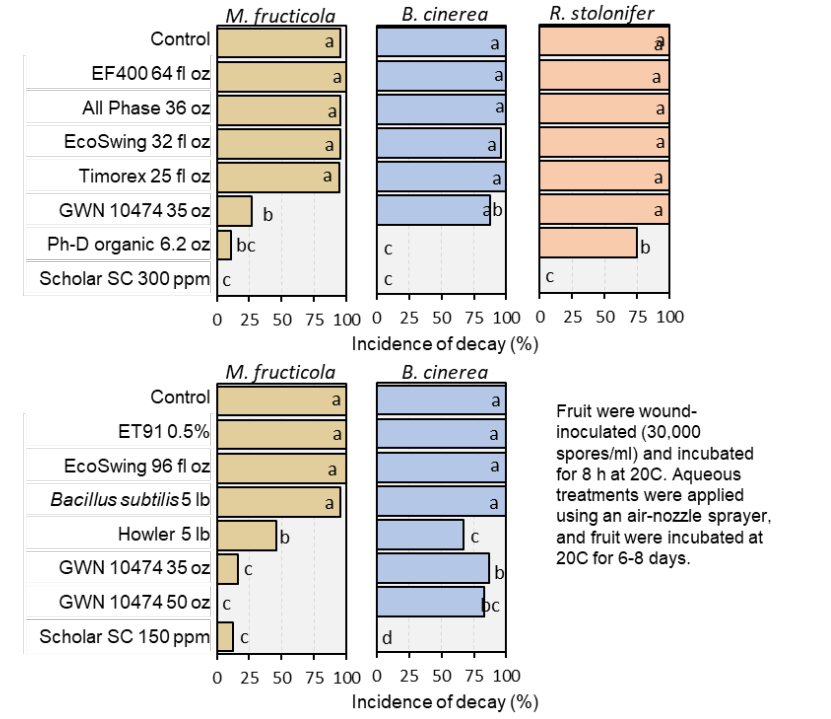
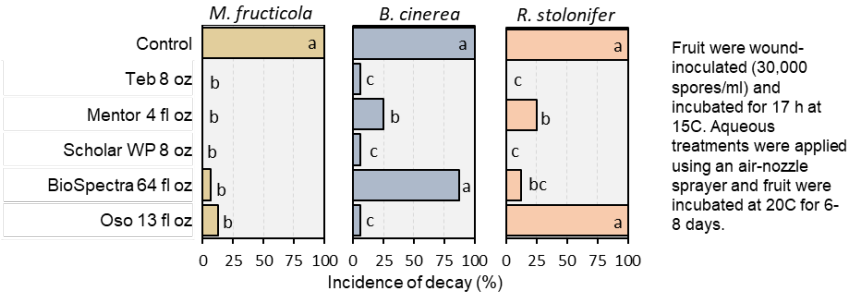


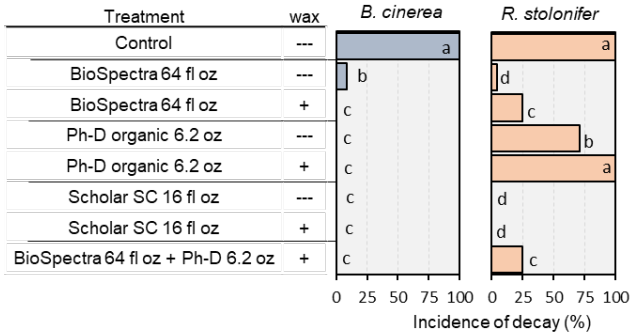
Fig. 9. Postharvest treatments of inoculated Bing cherry fruit with registered fungicides in laboratory studies 2020



Efficacy of new postharvest treatments for managing brown rot, gray mold, and Rhizopus rot of sweet cherry. Postharvest studies where treatments were applied to fruit 8 to 17 h after inoculation focused on comparative evaluations of new biological treatments, three formulations of natamycin, and registered conventional postharvest fungicides. Among biological treatments, organic formulations of polyoxin-D (Ph-D, Oso), GWN 10474, and Howler significantly reduced the incidence of brown rot (Fig. 8; additional results for Oso are shown in Figs. 9-11). Only polyoxin-D was highly effective against gray mold (Figs. 8-11), and none of these biological treatments provided Rhizopus control (Figs. 8-11).

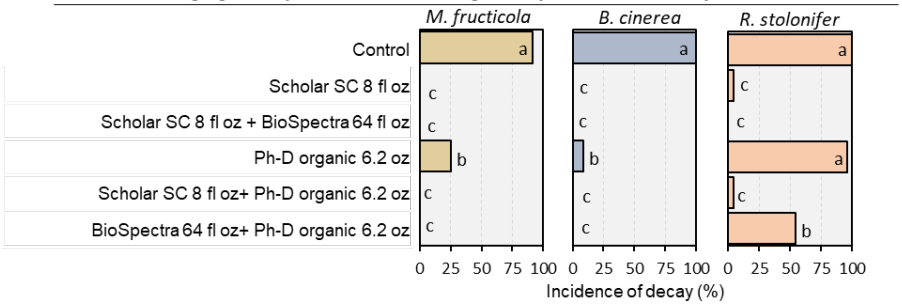
Teb at 8 oz was very effective against the three major postharvest decays, but Mentor at 4 fl oz (the highest label rate) was less effective against gray mold and Rhizopus rot (Fig. 9). Thus, Mentor (propiconazole) is best used in mixtures or as the pre-mixture Chairman. In our previous studies, Chairman at

Fig. 10. Efficacy of postharvest fungicides in combination with a fruit coating for managing decays of inoculated Bing cherry fruit in laboratory studies 2020



Fruit were wound-inoculated (30,000 spores/ml) and incubated for 14 h at 20C. Treatments were applied using an air-nozzle sprayer. A diluted mineral oil-based fruit coating was used. Fruit were incubated at 20C for 6-8 days.

Fig. 11. Efficacy of postharvest fungicides in combination with a fruit coating for managing decays of inoculated Bing cherry fruit in laboratory studies 2020

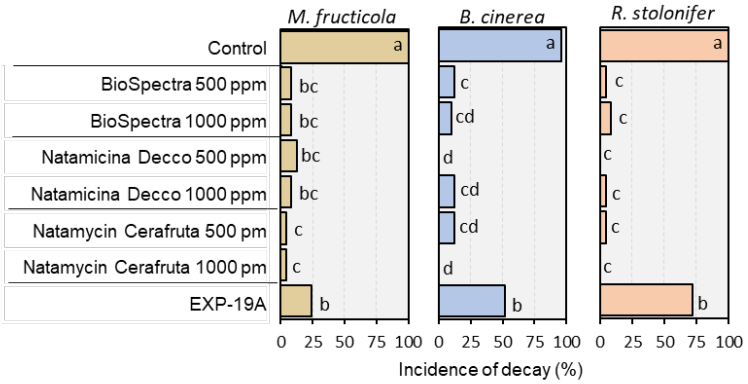


Fruit were wound-inoculated (30,000 spores/ml) and incubated for 15 h at 20C. Treatments were applied in combination with a diluted mineral oil-based fruit coating using an air-nozzle sprayer, and fruit were incubated at 20C for 6-8 days.

rates between 8 and 16 fl oz reduced the three decays to zero levels. This pre-mixture of fludioxonil and propiconazole not only has a broad spectrum of activity (brown rot, gray mold, Rhizopus rot, sour rot), but also is highly effective at low rates.

The efficacy of BioSpectra, Ph-D, and Scholar was compared as treatments prepared in water or in a diluted mineral oil-based fruit coating. The effectiveness of BioSpectra against gray mold was slightly improved when applied in fruit coating but was reduced in the evaluation for Rhizopus rot control (Fig. 10). In this study, Ph-D showed weak efficacy against Rhizopus rot when applied as an aqueous treatment but had no efficacy in an application in fruit coating. Scholar was highly effective against both decays using either application method. The mixture of BioSpectra and Ph-D showed very good efficacy against brown rot, gray mold, and Rhizopus rot (Figs. 10, 11). Polyoxin-D is already available as an organic formulation. Thus, if the

Fig. 12. Postharvest treatments of inoculated Bing cherry fruit in laboratory studies – comparison of three natamycin formulations and a new experimental 2020



Fruit were wound-inoculated (30,000 spores/ml) and incubated for 11 h at 20C. Aqueous treatments were applied using an air-nozzle sprayer and fruit were incubated at 20C for 6-8 days.

natural product natamycin is organically approved, this mixture could be an effective treatment option for organic marketing of cherry fruit. Three formulations of natamycin provided very similar high efficacy against the three major postharvest decays of sweet cherry (Fig.12). The new experimental fungicide EXP-10A, however was not very effective in this and previous studies.

Our studies indicate that postharvest decays of sweet cherry can be effectively and economically managed using Scholar or BioSpectra by themselves or by using selected mixtures or the pre-mixture Chairman. All these treatments have been registered based on our efficacy studies. For propiconazole MRLs have been established and FATs were approved in June 2018 and thus, Chairman can be used for cherries (and other stone fruits) exported to Japan. Propiconazole is registered as a preharvest fungicide and thus, its postharvest use is within established tolerances.

MRLs have not yet been established for natamycin in many international markets. It's exempt from residue tolerance status is only approved in the United States. This limits its current use to domestic markets (including Canada). Natamycin, however, is an exciting compound because resistance has never been reported in filamentous fungi. Therefore, natamycin 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 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, sanitation of fruit and packinghouse equipment, and not marketing over-ripe fruit. Some new biological compounds evaluated in 2020 were mostly effective against brown rot. 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 2021 in cooperation with commercial packinghouses.

Studies on the management of Phytophthora root and crown rots with new fungicides.

Surveys of California cherry orchards to determine the causal species of Phytophthora root and crown rot. A total of 31 soil, root, and crown samples from three orchards in the main cherry production area in California were collected in early May 2020, focusing on trees with root and crown rot symptoms. *Phytophthora syringae* was recovered in soil pear baitings and its species identification was confirmed by DNA sequencing of rDNA and cytochrome c oxidase (cox) genomic regions. Oomycota organisms other than *Phytophthora* spp. were recovered consistently from samples from untreated and fungicide-treated trees with most of the isolates coming from untreated samples. These unknown isolates were identified as *Phytopythium vexans* (previously *Pythium vexans*) by molecular methods. Greenhouse inoculation studies are ongoing to determine the virulence of this species in comparison with known root rot pathogens of cherry. Because the success of *Phytophthora* spp. isolations is known to be seasonal, samples were re-collected from these orchards in September 2020, and results are pending. Root flushes occur in early spring and fall, and these are times when the pathogens are more likely to be recovered.

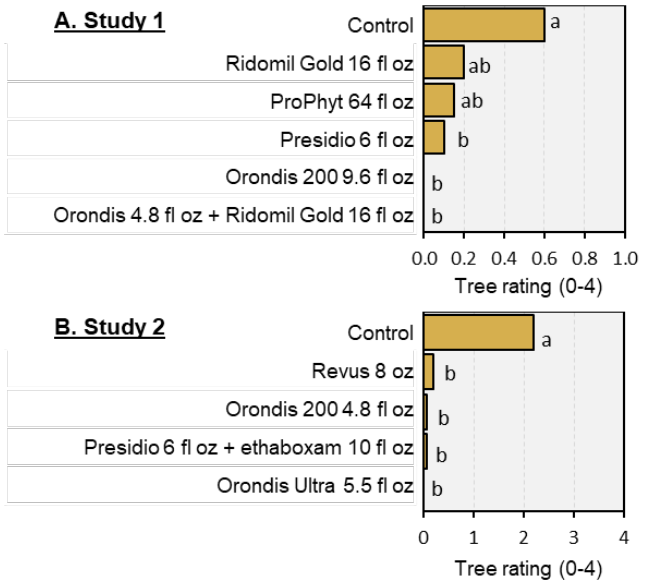
In vitro sensitivity against new Oomycota fungicides. The in vitro sensitivity against oxathiapiprolin, ethaboxam, fluopicolide, and mandipropamid was determined for four isolates of *Ph. vexans* and for *Phytophthora* spp. collected from cherry (Table 2). Oxathiapiprolin was highly toxic to all isolates evaluated, and EC_{50} values for inhibition of mycelial growth were <0.0006 ppm. The four *Phytophthora* species were inhibited by mandipropamid with EC_{50} values of <0.007 ppm, but *Ph. vexans* was not inhibited by concentrations of >40 ppm. EC_{50} values for fluopicolide for the *Phytophthora* and *Phytopythium* spp. evaluated ranged from 0.023 (i.e., *Ph. vexans*) to 0.104 ppm (i.e., *P. cactorum*) and those for ethaboxam from 0.015 (i.e., *Ph. vexans*) to 0.251 ppm (i.e., *P. citricola* complex). Thus, all Oomycota organisms, except for *Ph. vexans* against mandipropamid, were highly susceptible to the four new fungicides, and inhibitory values were in a similar range as for mefenoxam. *Phytophthora* spp. isolated in future surveys will be evaluated similarly. The goal is to determine if natural resistance is present in the pathogen populations and to establish baseline sensitivity ranges.

Table 2. In vitro sensitivities of *Phytophthora* and *Phytopythium* species from sweet cherry against five Oomycota fungicides.

Species	EC_{50} values for mycelial growth (ppm)				
	Mefenoxam	Oxathiapiprolin	Mandipropamid	Ethaboxam	Fluopicolide
Phytophthora cactorum	0.009	0.0006	0.007	0.033	0.104
P. citricola complex (2 isolates)	0.094 - 0.098	0.0005 - 0.0006	0.003	0.207 - 0.251	0.028
P. cambivora	0.011	0.0003	0.003	0.021	0.035
P. syringae	0.002	0.0002	0.001	0.017	0.035
Phytopythium vexans (4 isolates)	0.014 – 0.034	0.0012 – 0.0028	>40	0.015 - 0.074	0.023 – 0.054

Field studies on the evaluation of new fungicides. Two newly planted orchards on Mahaleb rootstock at UC Davis that were inoculated with *Phytophthora* spp. were evaluated in Sept. 2020 for tree health. Two control trees in the second orchard died. Isolates of the *P. citricola* complex that was one of the species used for inoculation was re-isolated from diseased tissues. In both orchards, a treatment applied with the new fungicides in the spring of 2020 resulted in significantly lower tree ratings (i.e., trees looked more healthy) as compared to the control and there was no significant difference among these treatments (Fig. 13). Ratings for Ridomil Gold and ProPhyt were not significantly different from the control due to high variability among the eight single-tree replications.

Fig. 13. 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 2020



Trees were planted in Jan. 2020. In Study A, treatments were done on 4-28-20 by pouring the fungicide solution around the base of the tree and over the lower trunk. Trees were inoculated on the same day with a mixture of *P. citricola*, *P. cactorum*, and *P. cambivora* (all obtained from cherry trees) that were grown on a rice-vermiculite-V8C juice mixture. In Study B, trees were first inoculated, and treated the following day. There were 8-10 trees for each treatment.

Tree health was rated on Sept. 20, 2020 using a scale from 0 (=healthy, vigorous) to 4 (= tree dead).

Some trees in the commercial orchard showed symptoms of crown rot and gumming along the trunks, and some trees had died by Sept. 2020. Isolations from infected trees are ongoing. Among untreated trees, 41.2% showed symptoms, whereas 25% of trees treated with Orondis were symptomatic. Isolations from these trees are being done. Trees had been treated in May, several months after planting. Because they were planted at sites where trees previously had died from root rot, planting sites were not fumigated, and fungicide treatments were applied to trees with early symptoms of Phytophthora crown rot. These factors contributed to the relatively high incidence of treated, diseased trees. As for the UC Davis trials, we will continue to treat the orchard using a spring and fall application strategy and monitor for disease. Oxathiapiprolin (Orondis) was nominated and accepted into the IR-4 residue program on sweet cherry in Sept. 2020. This initiated the registration process on sweet cherry in the United States.

Annual report 2020

Project Title: INTEGRATED MANAGEMENT OF FUNGAL CANKER DISEASES OF SWEET CHERRY

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Interpretive summary

Canker diseases caused by plant-pathogenic fungi *Calosphaeria pulchella*, *Eutypa lata* and *Cytospora sorbicola* are some of the main factors limiting productivity and longevity of sweet cherry trees in California. These diseases affect the wood, killing branches, scaffolds and trunks of cherry trees, causing important yield losses. In order to improve management of fungal canker diseases, we developed an integrated, preventive approach to improve the quality of planting material and minimize risks of field infection of sweet cherry trees by canker pathogens. In the last two years, we evaluated the sanitary status of cherry planting materials from three nurseries. In 2020, we evaluated the pathogenicity of isolates recovered from nursery stocks, including *Cytospora sorbicola, Phomopis/Diaporthe* spp., *Fusarium proliferatum* and *Cadophora viticola* associated with cankers, as well as *Trametes* and *Schizophyllum* spp. associated with wood decay symptoms. Our results indicated that isolates of *Diaporthe australafricana*, *Diaporthe ambigua*, *Trametes versicolor* and *Cadophora viticola* can be considered canker pathogens of sweet cherry trees, causing wood lesions significantly longer than control inoculations in Bing branches after 1-year incubation in the field. This highlights the necessity to work closely with nurseries to elaborate propagation protocols less amenable to infections by canker pathogens. In 2020, we did not evaluate the possibility of spreading canker diseases within orchards with pruning tools due to the public health crisis and work time limitations. Nonetheless, we further evaluated the efficacy of 12 compounds to protect pruning wounds from infections by canker pathogens by acquiring a third year of field data. Our results from 2020 confirmed Topsin M and Quilt Xcel performed best against *Eutypa lata* and *Cytospora sorbicola*, allowing 65 to 80% disease control. The biological, Trichoderma-based product Vintec provided the same range of disease control and can be considered an efficient alternative to Topsin M and Quilt Xcel in organic cherry production systems. Based on field surveys from 2019, *Calosphaeria pulchella*, *Cytospora sorbicola* and *Eutypa lata* were found commonly in spurs and shoots expressing dieback symptoms in the absence of pruning wounds. Thus in 2020 we investigated the potential role of fruits, leaf and/or bud scars as infection courts for canker pathogens. Our field inoculations with these three pathogens of leaf scars in November 2019 and bud scars in March 2020 did not result in infections. Results from fruit scar inoculations are pending. In 2020 we repeated experiments evaluating differences in the seasonal susceptibility of pruning wounds to *Calosphaeria pulchella*. Winter pruning was unfavorable to pruning wounds infection when compared to summer pruning. These findings were supported by our previous *in*

vitro temperature studies indicating that *Calosphaeria pulchella* requires warm temperatures (30° C) for ascospores germination and optimal mycelial growth. This suggests that pruning during cold (and dry) winter weather should prevent infection of pruning wounds by *Calosphaeria pulchella*, which is particularly relevant in counties where Calosphaeria canker represent the main canker disease. Knowledge acquired from the present study are permitting the development of integrated disease management strategies that together can mitigate the impact of canker diseases in sweet cherry.

Objective 1: Determine latent infection of canker pathogens in nursery stocks

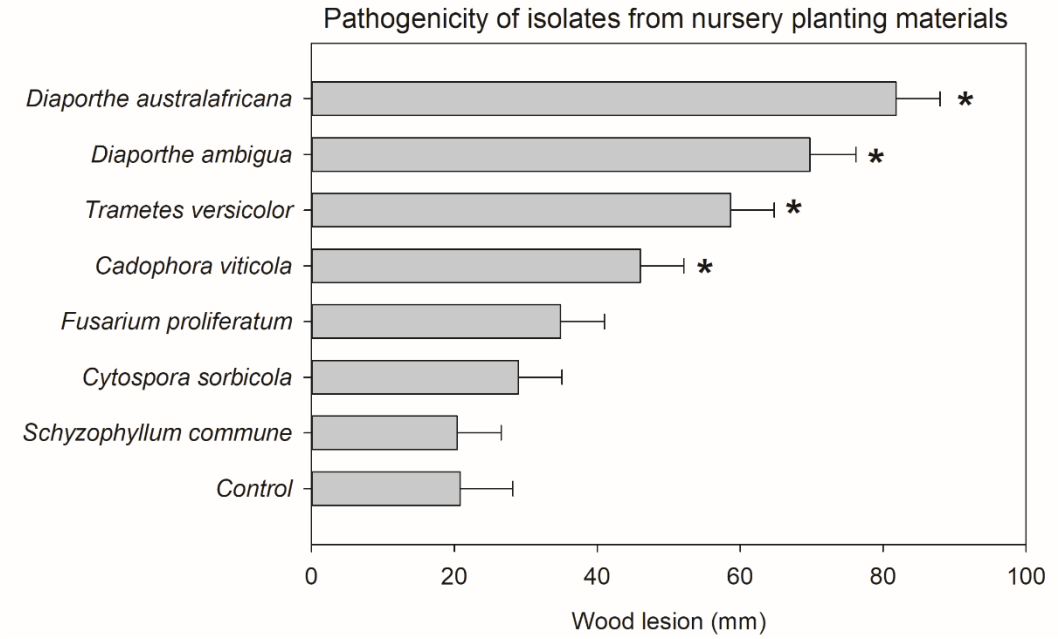
Material and methods

In 2020, we acquired our first year of data after field inoculation of sweet cherry Bing branches with fungal isolates recovered from nursery stocks. We used a row of Bing trees grafted onto Colt and planted in 2011 at the experimental station of the Department of Plant Pathology in Davis. We selected ten trees, and each tree received 8 inoculation treatments on 8 different branches. For each branch (~2cm in diameter), a 3-mm wound was made using a power drill and a flame sterilized 3-mm drill bit. A 3-mm-diameter mycelial plug from a 7-day-old Potato Dextrose Agar (PDA) culture was aseptically inserted into the wound, sealed with petroleum jelly, and then protected with Parafilm. The 8 treaments consisted of either a uncolonized, sterile PDA plug (control) or of a plug of mycelium of *Diaporthe australafricana*, *Diaporthe ambigua*, *Trametes versicolor*, *Cadophora viticola*, *Fusarium proliferatum*, *Cytospora sorbicola* or *Schyzophyllum commune*. Wood lesions developing around the inoculation sites were recorded one year after inoculation as a measure of fungal pathogenicity. Lesion length was compared among the different fungal isolates to determine which isolates were pathogenic. Re-isolations were carried out as previously described. ANOVAs were performed in SAS 9.2 with the GLM procedure and isolate pathogenicity was evaluated based on the length of wood lesions. Pairwise mean differences compared to the non-inoculated control were analyzed with Dunnett's test ($P = 0.05$).

Results and Discussion

Using mycelium inoculations, after 11 months incubation in branches of 'Bing' trees, 4 of 7 isolates had greater average wood lesions ($P < 0.05$; Dunnett's test; **Figure 1**) than that of the control plants (20.8 mm; n = 10 branches), suggesting that these 4 isolates were pathogenic. Isolates of *D. ambigua*, *D. australafricana*, *T. versicolor* and *C. viticola* were considered pathogenic. In contrast, isolates of *F. proliferatum*, *C. sorbicola* and *S. commune* were not considered pathogenic based on no significant differences in length of wood lesions from the controls. The two Diaporthe species were considered the most pathogenic, with an average length of wood lesions of 81.8 mm and 70 mm for *D. australafricana* and *D. ambigua*, respectively, followed by *T. versicolor* (58.6 mm). Isolations from these wood lesions showed that all isolates were recovered from inoculated branches, except *C. sorbicola* (0% recovery), with recovery rates ranging from 30% for *D. australafricana* to 100% for *S. commune*. No pathogen were recovered from control branches.

Figure 1. Length of wood lesions developing into branches of cultivar "Bing" 11 months after inoculations with mycelial plugs of 7 fungal species recovered from nursery stocks. Means and standard errors are presented (n = 10). Means significantly different from the control inoculations are denoted with an asterisk (Dunnett test, $p < 0.05$).



This pathogenicity experiment was repeated in February 2020 and the results will be obtained in January 2021. In the meantime, these preliminary results confirm that fungal isolates found in cherry nursery trees can harbor some canker pathogens (*D. ambigua*, *D. australafricana* and *C. viticola*) and wood decay fungi (*T. versicolor*). The introduction of diseased planting material is a serious threat to the good establishment of sweet cherry orchards. Trees planted with developing symptoms of wood decay or canker diseases, as encountered in our study, are likely to collapse within the first few years following planting. It has been documented previously that canker pathogens can be present within planting material of almond and grapevine, suggesting that infections of these crops with canker pathogens can take place during the plant propagation processes (Gramaje and Armengol, 2011; Themis Michailides, personal communication). Planting infected material has been detrimental to the grapevine industry in Europe and most management efforts have shifted toward early prevention of infection at the nursery and during propagation of grapevine plants. In the absence of curative control methods, introducing canker and wood decay diseases into new orchards via planting material would make further disease control efforts inefficient. Hence, production of healthy trees at the nursery is crucial to the successful establishment and sustainability of orchards. Detection of wood pathogens prior to planting also is critical to assure longevity of newly established orchards. We have initiated collaborations with nurserymen and we submitted a new proposal to the CCB for the funding cycle 2020-2021 in order to implement production practices that minimize the risk of nursery stocks contaminations by canker and wood decay fungi.

Objective 2: Investigate the role of pruning tools on canker disease transmission

No data from 2020 are available for this annual report.

Objective 3: Test the efficacy of various compounds for the protection of pruning wounds

Material and methods

After two years of field trial conducted in Davis, first from February to May 2018, second from January to May 2019, we repeated this experiment from January 2020 to May 2020. Lignified branches (2nd to 3rd year wood) of 13-year-old cherry trees were pruned in order to make a flat wound. 24 hours after pruning, wounds were treated with either sterilized water (negative control) or one of the 12 compounds listed in **Table 1**. Applications were made with hand-held spray bottles at the label rate, and wounds were sprayed until runoff.

Approximately 24 hours after wounds were treated with fungicidal products, wounds were misted with sterilized water to provide high relative humidity and these wounds were inoculated with 100 µL of a spore suspension of the fungal canker pathogens *Eutypa lata* and *Cytospora sorbicola* at a concentration of 1,000 spores per wound. 13 replicates per treatment (treatment = protection product + fungal isolate) were established in a randomized complete block design.

Treated branches were collected approximately 14 weeks after inoculation and brought to the laboratory for fungal isolations. Presence (1) or absence (0) of the inoculated fungal species was recorded for each treatment. Low rates of fungal recovery were correlated with high product efficacy.

A linear mixed logistic regression model was used to predict the probability that the event "fungal recovery = 0" (dichotomous, dependent variable of interest) will occur as a function of the independent variable, fixed effect "product". The variable "year" was considered random effect. Generalized linear mixed models were implemented in the SAS® System, Version 9.4, using the GLIMMIX procedure from the [SAS/STAT] product, which utilizes the logit link function to accommodate binomial data. In case of significant main effect of the fixed effect "product" (α = 0.05), pairwise mean comparisons among least square means (LS means) were conducted using the Tukey-Kramer method. In the case of the logistic regression model implemented here, the inverse of the LS-mean values for each product are equivalent to the predicted probabilities of "fungal recovery = 0" (high efficacy) and these values and 95% confidence limits were used to graphically visualize product efficacies for each pathogen. Products efficacy was represented as the mean percentage of recovery (MPR) of *C. sorbicola* or *E. lata*. The mean percent of disease control (MPDC) was calculated as the reduction in MPR as a proportion of the inoculated negative control branches.

As winter inoculation of *Calosphaeria pulchella* fail to cause branch infection, another fungicide trial was initiated in September 2020 for the testing of fungicide efficacy to protect pruning wound against Calosphaeria canker.

Results and Discussion

The results presented here involve *Eutypa lata* and *Cytospora sorbicola* over three dormant seasons (2018, 2019 and 2020).

Eutypa lata: On average, 90% of pruning wounds treated with water and inoculated with spores of *Eutypa lata* had successful infections, providing high infection rates in our control treatments. Of the 12 compounds tested against *E. lata*, Topsin M, Vintec and Quilt Xcel

performed best, allowing 79%, 64% and 64% disease reduction, respectively (**Table 1**). Of the 12 products tested, in addition to Topsin M, Vintec and Quilt Xcel, 5 products [Quash (58% disease reduction), Luna Experience (56%), Merivon (50%), Quadris Top (50%), and RootShield Plus WP (48%)] provided significant reductions in infection by *E. lata*.

Cytospora sorbicola: On average, 45% of pruning wounds treated with water and inoculated with spores of *Cytospora sorbicola* had successful infections, providing moderate infection rates in our control treatments. Of the 12 compounds tested against *E. lata*, Luna Sensation (100% disease reduction), Vintec (79%), Topsin M (78%) and Quilt Xcel (78%) allowed the best disease control for this pathogen (**Table 1**). However, there were no significant differences among the different products, which is likely a consequence of moderately low infection rates in control branches.

Table 1. Efficacy of treatments applied by handheld spray bottles to cherry pruning wounds in pruning wound protection experiments followed by inoculation with 1,000 spores of *Eutypa lata* or *Cytospora sorbicola* in 100-µl droplets to each wound, respectively. [A] Product efficacy is based on the mean percent of recovery (MPR) of *E. lata* or *C. sorbicola* from inoculated branches over 3 years of experimentation. Values followed by distinct letters are significantly different from each other (Tukey-Kramer test; $P < 0.05$). [B] Mean percent of disease control (MPDC) was calculated as 100 × [1 – (MPRtreatment/MPR control)].

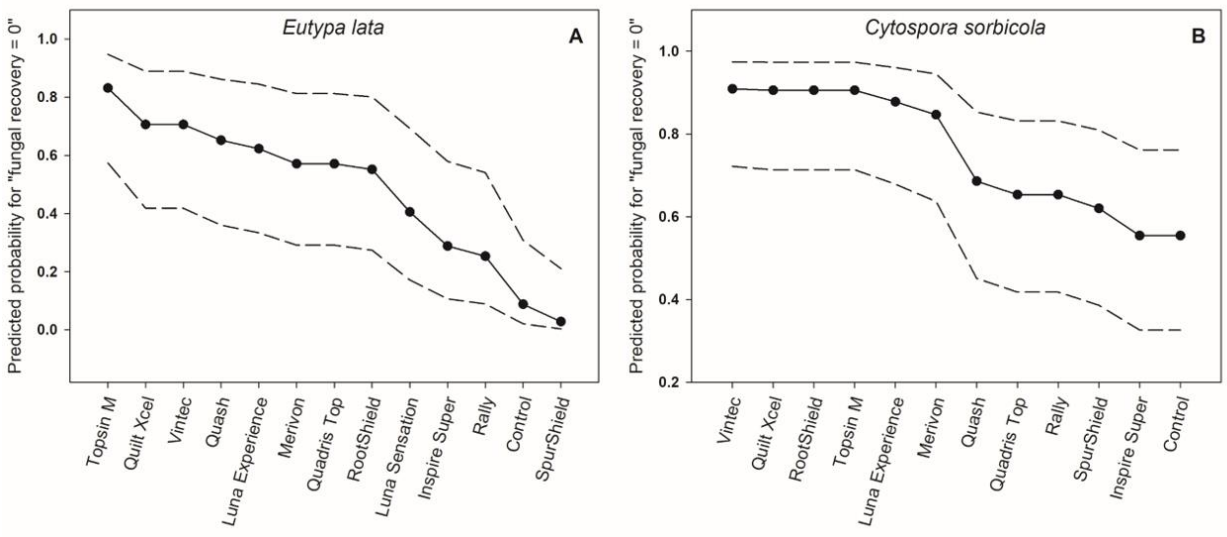
Product	**Active Ingredient**	**Application Rate**	***E. lata***		***C. sorbicola***	
			MPR	**MPDC**	**MPR**	**MPDC**
Inoculated control	water	...	90D	…	45	…
Topsin M	thiophanate-methyl	1.8 g/L	19A	79	10	78
Quilt Xcel	propiconazole/azoxystrobin	1.1 ml/L	32AB	64	10	78
Vintec	*Trichoderma atroviride* SC1	2.0 g/L	32AB	64	10	79
Quash	metconazole	0.3 g/L	38ABC	58	32	29
Luna Experience	fluopyram/tebuconazole	0.8 ml/L	40ABC	56	13	71
Merivon	pyraclostrobin/fluxapyroxad	0.5 ml/L	45ABC	50	16	64
Quadris Top	difenoconazole/azoxystrobin	1.1 ml/L	45ABC	50	35	21
RootShield Plus WP	*Trichoderma harzianum* T-22 and *T. virens* G-41	0.6 g/L	47ABC	48	10	78
Luna Sensation	fluopyram/trifloxystrobin	0.6 ml/L	60ABCD	34	0	100
Inspire Super	difenoconazole/cyprodinil	1.6 ml/L	71BCD	21	45	0
Rally	myclobutanil	0.6 ml/L	74CD	18	35	21
SpurShield	polymer of cyclohexan, 1 methyl-4 (1-methylethyl)	25mL/L	97D	0	39	14

Overall and following three-year experiments, the products Topsin M, Vintec and Quilt Xcel provided the best reductions in pruning wound infections against the two pathogens. In contrast, the products Inspire Super, Rally and SpurShield did not provide any significant disease reduction. The efficiency of Topsin M for pruning wound protection against *Eutypa lata* has been demonstrated in the past for grapevines (Rolshausen et al., 2010) so our findings in cherry confirmed the efficacy of this product against the same pathogen in cherry. The efficacy of Vintec for almond pruning wound protection by canker pathogens has recently been demonstrated by our

lab; the present findings support the use of Vintec not only in almond orchards but also in cherry orchards. However, further evaluations including protection of summer pruning wounds are necessary, in particular for *Calosphaeria pulchella*. Our preliminary results described hereafter and in previous annual reports suggest that *Calosphaeria pulchella* ascospores germination, infection and growth occurs mainly during warm weathers. Accordingly, pruning wound protection trials have been initiated in the summer 2020 to elucidate control of Calosphaeria canker.

For each pathogen, the products efficacies can be depicted on **Figure 2**: protectants are sorted from the most efficient to least efficient at preventing cherry pruning wound infections based on the predicted probabilities of the event "fungal recovery = 0" which is synonymous to "no infection". For instance, the 6 products working best against *Cytospora sorbicola* can be easily visualized on Figure 2B as the products with the highest predicted probabilities.

Figure 2. Predicted probabilities of the event "fungal recovery = 0" (i.e. no infection) for (A) Eutypa lata and (B) Cytospora sorbicola, when inoculated onto cherry pruning wounds after applications of various pruning wound protectants. Values were obtained from a linear mixed logistic regression model and based on three field trials. The solid black lines with black dots represent the predicted probabilities and the dashed lines represent the 95% confidence limits around the means. For each pathogen, protectants are sorted from the largest to smallest predicted probability values.



Objective 4: Determine main infection pathways of fungal canker pathogens:

Material and methods

Field surveys and tree sampling conducting by our laboratory during the summer 2019 suggested multiple entry sites for infection by canker pathogens. Indeed, *C. pulchella* and *C. sorbicola* were recovered abundantly in our survey from dead spurs and terminal shoot dieback with no apparent pruning wounds. This implies that pruning wounds are not the sole infection site for canker pathogens and we hypothesized that fruits, leaf and/or bud scars in trees may act as additional

infection sites for these pathogens. Additionally, we investigated the potential for dormant buds to serve as reservoirs of canker pathogens and potential infection courts.

Survey of Buds

Surveys of the incidence of common fungal canker pathogens in buds of sweet cherry in Californian orchards were conducted across three different counties (Yolo, San Joaquin, and Stanislaus) to assess the bud's potential for latent infection. Buds (before bud break) with no apparent symptoms (n=100 per orchard) were collected at random from two different experimental orchards in Yolo County (Yolo 1a and 1b) during January 2020 and another experimental orchard (Yolo 2) during February 2020; two commercial orchards in Stanislaus County (Stanislaus 1 and 2) during February 2020; and 3 commercial orchards in San Joaquin county (San Joaquin 1, 2 and 3) during February 2020.

All field samples were processed on the same day of collection, or between 2 to 7 days following storage at 4°C in a cold room. Bud samples were placed in small cassettes and surface-sterilized by submerging in a 0.1 dilution of commercial bleach (sodium hypochlorite) for 60 seconds followed by two rounds of washing in sterile DI water and allowed to dry over clean paper towel. Each surface-sterilized set bud samples were plated on Potato Dextrose Agar amended with tetracyclin (PDAtet) petri dishes and incubated in clear crisper boxes at laboratory ambient temperature (22-24°C) and light. Cultures were checked every two days for growth of prospective canker-pathogen colonies, which were then transferred to fresh PDAtet petri dishes and allowed for growth as pure cultures. The identity of isolates was determined by colony morphology. The incidence of canker pathogens was determined as the percentage of samples with recovery of the pathogen out of total samples processed for each orchard.

Field Inoculations of scars and re-isolation

Inoculations of leaf, bud and fruit scars were performed to determine their susceptibility to infection by fungal canker pathogens (*C. pulchella, C. sorbicola, and E. lata*). Scars occurred through natural processes such as leaf drop (leaf senescence), bud break or due to harvest (fruit scars).

C. pulchella ascospore, *C. sorbicola* conidial, and *E. lata* ascospore suspension concentrations were obtained from fruiting bodies occurring naturally on diseased wood, and estimated using a standard hemocytometer and adjusted with sterile deionized water to 10^5 spores·mL^{-1}. Each spore suspension was then transferred to a 100 ml spray bottle that outputs about 0.13 ml per spray, which equates to 13,000 spores per spray. Spray bottles were then used to apply inoculum onto the various plant parts in field experiments.

Leaf scars were inoculated in December 2019 when leaves naturally senesced. Senescing leaves were removed by light, but not forceful pulling. Starting from shoot tips, each of 8 leaf scars per fungal pathogens were treated with 1 spray of spore suspension. Bud scars were inoculated in March 2020 after the green tip of a shoot had emerged from each bud, eventually creating small mechanical damages. Bud scars were inoculated on branches using 8 bud scars and 1 spray of each fungal pathogen inoculum. Inoculations for leaf and bud scars were replicated on 15 trees (Coral Champagne grafted onto Maxma14). Fruit scars were inoculated in July 2020, following harvesting of fruit clusters and using the same method as described above. Each treatment had 8 repetitions and replicated on two trees (1 Rainier and 1 Bing).

After 3-4 months following inoculations, all branches that has received the leaf and bud scar inoculations were collected (fruit scar collection pending) and assessed for disease incidence

following re-isolation of inoculated tissue for fungal canker pathogens. For each sample, the bark was removed and a vertical cross-section along the branch through the point of inoculation (leaf scar and bud scar) and any discoloration was noted. Ten 3 mm x 3 mm x 3 mm wood fragments collected below the inoculated area (bud and leaf scars) were surface sterilized with diluted 10% bleach suspension as described above and plated onto PDAtet. Every two days, prospective fungal colonies of canker pathogens were transferred to fresh PDAtet. Growing fungal colonies were identified using colony morphology. The susceptibility of each type of tissues to infection was determined by the proportion of samples out of all samples (out of 8) of that type that have its respective fungal pathogen re-isolated from at least one wood fragment.

Results and Discussion

Survey of Buds

Among the major fungal canker pathogens in California, only *Cytospora sorbicola* was recovered from asymptomatic buds and present in 5 of 8 orchards sampled across all three counties (**Table 2**). Most notable result was *C. sorbicola* being found in the highest incidence at 50% in Yolo 1A buds, but less than 3% incidence in Yolo 1B, Stanislaus 1, San Joaquin 1, and San Joaquin 2. No major fungal canker pathogens were found in Yolo 2, Stanislaus 2, and San Joaquin 3. Other potential fungal canker pathogens such as *Botryosphaeria* spp., *Diaporthe* spp., and *Fusarium* spp. were also recovered from asymptomatic cherry buds.

The recovery of *C. sorbicola* and other canker-causing pathogens such as *Botryosphaeria* spp., *Diaporthe* spp., and *Fusarium* spp. from asymptomatic cherry buds suggests a potential for these fungi to persist latently into cherry buds from where they may infect the trees at bloom or during shoot expansion from these buds. The high incidence of *C. sorbicola* found in Yolo 1A buds compared to other Yolo county sites and commercial fields in other counties might be explained by the high abundance of canker diseases as well as inoculum sources in this orchard. In orchards with a relatively low rate of fungal fruiting bodies (Yolo county orchards 2 and commercial orchards from Stanislaus and San Joaquin county), the survey data suggests low occurrence of latent infection in buds in well maintained commercial fields. Interestingly, there was no recovery of *C. pulchella* nor *E. lata* from dormant buds in all orchards sampled, which contrasts with the high incidence of *C. pulchella* (and some *E. lata*) in symptomatic spurs, shoot tips, and branches based on previous surveys.

Field Inoculations of scars and re-isolation

There were no observed recovery of *C. pulchella, C. sorbicola, and E. lata* from neither leaf nor bud scar inoculations. These findings suggest that these 3 pathogens do not infect cherry trees from bud scars nor leaf scars. However, this experimentation should be repeated during the next growing season to draw any firm conclusions. Also, it should be noted that high winds were present during the weeks of bud scar and leaf scar inoculations of young cherry trees (2 years-old Coral Champagne trees). High winds could cause rapid drying of the inoculum, which may not be non-conducive for spore germination and infection, which can explain the lack of recovery of inoculated pathogens. Data for fruit scar inoculations will be completed by December 2020.

Table 2: Survey of fungal canker pathogens in asymptomatic cherry buds. Asymptomatic buds (N=100) were collected at random from 8 different orchard spanning across three counties. The incidence of canker pathogen is reflected as a value out of 100. Other potential fungal canker pathogens were detected (Diaporthe sp., Botryosphaeria sp.) and noted from survey.

County/ Orchard Designation	Date Sampled	Fungal isolation	Incidence % (out of 100 buds)
Yolo 1A	1/22/2020	*C. sorbicola*	50
		Diaporthe sp.	4
		Botryosphaeria sp.	7
		Non-canker-forming fungi	39
Yolo 1b	1/30/2020	*C. sorbicola*	2
		Diaporthe sp.	7
		Botryosphaeria sp.	4
		Fusarium sp.	2
		Non-canker-forming fungi	83
Yolo 2	2/4/2020	*Diaporthe* sp.	2
		Botryosphaeria sp.	5
		Non-canker-forming fungi	92
Stanislaus 1	2/8/2020	*C. sorbicola*	2
		Diaporthe sp.	1
		Non-canker-forming fungi	97
Stanislaus 2	2/8/2020	*Diaporthe* sp.	0
		Botryosphaeria sp.	3
		Fusarium sp.	1
		Non-canker-forming fungi	96
San Joaquin 1	2/13/2020	*C. sorbicola*	1
		Diaporthe sp.	1
		Botryosphaeria sp.	1
		Fusarium sp.	2
		Non-canker-forming fungi	95
San Joaquin 2	2/13/2020	*C. sorbicola*	1
		Diaporthe sp.	2
		Botryosphaeria sp.	1
		Non-canker-forming fungi	96
San Joaquin 3	2/13/2020	Non-canker-forming fungi	100

Objective 5: Determine the effect of temperatures on spore germination of *Calosphaeria pulchella* and *Eutypa lata*

Materials and Methods

In 2019, we showed that spores of *Calosphaeria pulchella* did not germinate at temperatures below 15°C after 36 hours. In 2020, we thus investigated the effect of low temperatures on *Calosphaeria pulchella* ascospores and conidia over longer incubation times.

Calosphaeria pulchella spore germination: The effect of temperatures on spore germination was studied using one representative strains of *Calosphaeria pulchella*. Ascospores were collected from fruiting bodies found under the periderm of infected sweet cherry branches. Pieces of dead wood containing perithecia were affixed to a plastic petri lid with Vaseline and were submerged in deionized water for 1 hour. Water was then poured out and perithecia were blotted dry before placing them back over a clean petri dish for spore discharge. After 2 hours, the bottom of plates was washed with deionized water to collect ascospores and mix them into a suspension adjusted to 3×10^5 ascospores mL^{-1}. Conidia were harvested from pure cultures grown in the laboratory and the spore suspensions was adjusted to 3×10^5 ascospores mL^{-1}. Four, 10µL droplets of either the ascospore suspension or the conidia suspension were pipetted onto three, 2% water agar plate replicates and incubated at each temperature of 5, 10, and 15°C. After 12, 24, 48, 72, 96, 120 and 144 hours, spore germination was assessed under 200x magnification light microscope by counting the number of spores germinated out of 100 spores counted for each repetition. Average germination rates were obtained for all temperatures and isolates. Experiments were conducted twice.

Eutypa lata ascospore germination: The effect of temperatures on ascospore germination was studied using natural sources of *Eutypa lata* ascospores collected from fruiting bodies found in the bark and woody tissue of symptomatic *Nerium oleander*. To access ascospores, a thin razor blade was used to cut tangentially along the wood surface to remove thin layers of wood to expose the internal perithecia cavity. Sterile DI water was sprayed over these openings to generate the production of ascospores. With the aid of a dissecting microscope and a needle, the spore matrix was carefully collected and transferred to a 1.5 ml Eppendorf tube containing 1000 uL of sterile DI water. The resulting mixture was vortexed for 2 minutes to evenly suspend ascospores. Spore inoculum concentration was adjusted to 3×10^5 spores mL^{-1}. Three, 10µL droplets of the ascospore suspension were pipetted onto three, 2% water agar plate replicates and incubated at each temperature of 5, 10, 15, 20, 25, 30, and 35°C. After 12, 24, 36 and 48 hours of incubation, ascospore germination was assessed under 200x magnification light microscope by counting the number of spores germinated out of 100 spores counted for each repetition. Average germination rates were obtained for all temperatures and isolates. Experiments were conducted twice.

Results and Discussion

Calosphaeria ascospores and conidia were able to germinate at low temperatures (5, 10, and 15°C). At 5°C there were no germination of either type of spores. At 10°C, it took a minimum of 72 hours and 48 hours for *Calosphaeria* ascospores and conidia to germinate, respectively. Faster and higher germination rates were achieved at 15°C (**Figure 3**). In complement to our results from last year, this year data confirmed that low temperatures in the range tested are not favorable for *Calosphaeria* spores germination under natural winter conditions of California. This supports our assumptions that cherry tree infections by this pathogen are minor during the dormant season.

Eutypa ascospores exhibited the highest germination rate (90%) at 25°C while no germination occurred at 35°C nor at 5°C after 36 hours incubation (**Figure 4**). Germination rate at 10°C after 48 hours reached above 20%. These data indicate a preferential growth of *Eutypa lata* at temperatures between 20 and 25°C, and limited growth at higher temperatures (~35°). *Eutypa lata* tolerated lower temperatures, in relation to *C. pulchella*, to initiate germination (10°C vs

15°C). This suggests that *E. lata* infection in the field can occur in winter, but also is more likely to occur during fall and spring months when average temperature is around 20 to 25°C (**Figure 5**). This contrasts with results from previous years on *Calosphaeria pulchella* spore germination and mycelial growth temperature studies indicating optimum *C. pulchella* temperatures at 30 to 35°C, corresponding to the hotter summer months in CA. Therefore, careful timing of pruning should be considered to mitigate chances of infection by these two pathogens.

Figure 3. *Calosphaeria pulchella* ascospore and conidia germination rates after incubation 12, 24, 48, 72, 96, 120 and 144 hours at temperatures of 5, 10 and 15°C (low temperatures). Values show an average of two repeated trials.

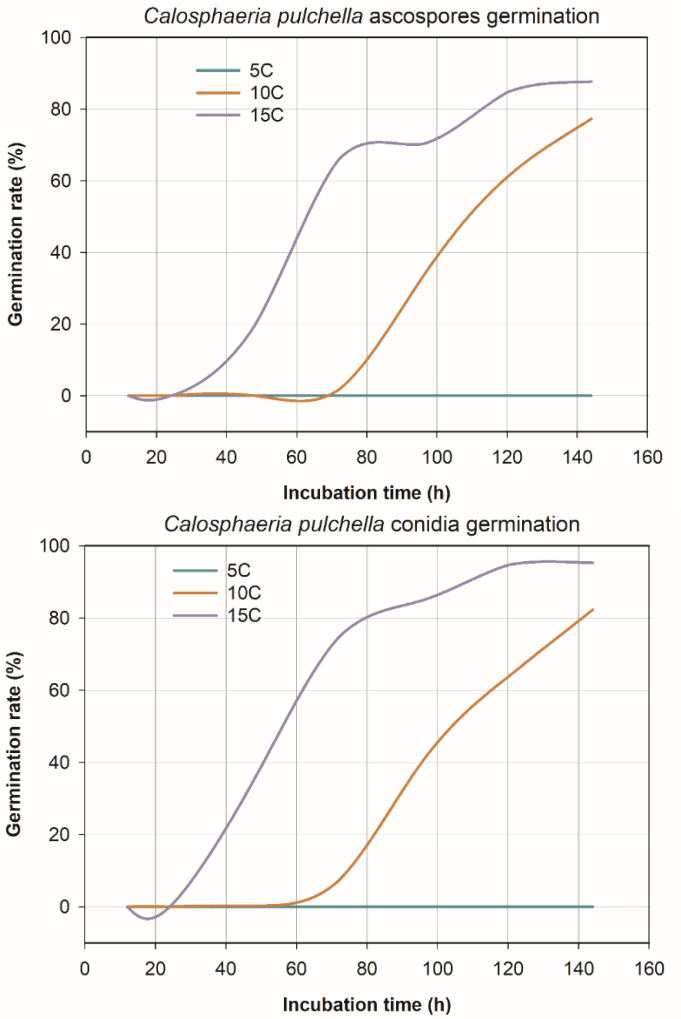


Figure 4. *Eutypa lata* ascospore germination rates after incubation for 12, 24, 36 and 48 hours in a range of temperatures. Values show an average of two repeated trials.

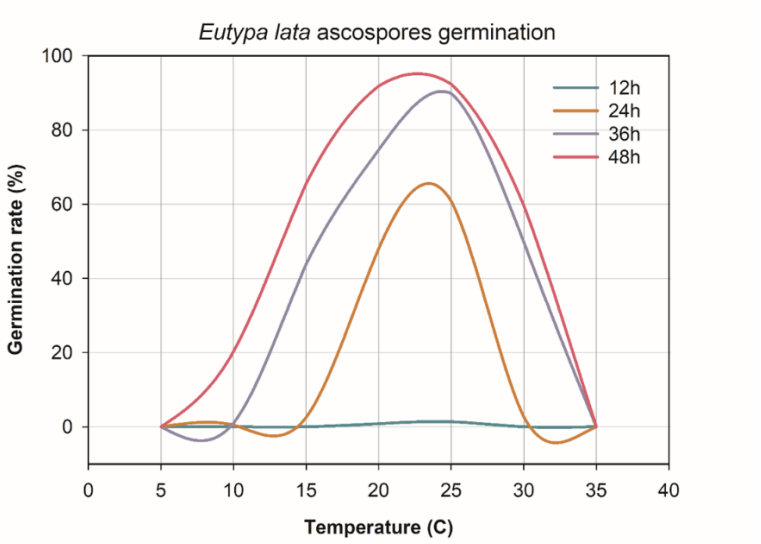
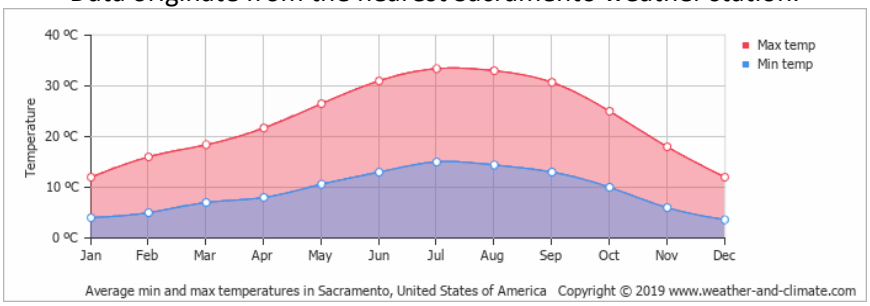


Figure 5. Estimated maximum and minimum average temperatures throughout the year 2018 in Davis. Data originate from the nearest Sacramento weather station.



Objective 6: Determine the seasonal susceptibility of pruning wounds to infection by *Calosphaeria pulchella*:

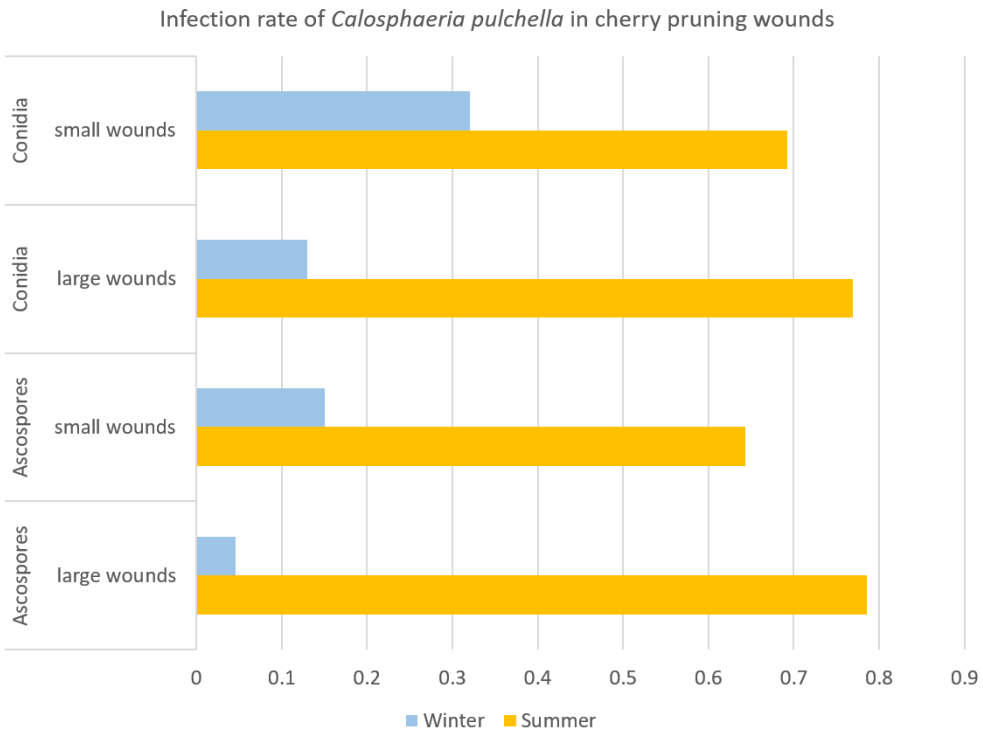
Materials and Methods

Field trials were set up to assess pruning wound susceptibility according to the time of pruning (summer vs. winter). Mature trees located in an experimental orchard in Davis were selected and pruned in January 2019, July 2019, January 2020 and September 2020, respectively. At each pruning time, branches with different diameters were selected to include a set of 1 cm diameter branches (small wounds) as well as a set of 2-3 cm branches (large wounds). Pruning cuts were made into healthy branches using loppers and wounds were immediately inoculated with 100 µL of a 1×10^4 spores mL-1 spore suspension of *Calosphaeria pulchella*. Twelve branch replicates were used for each treatment in this study. Four months after each inoculation time (summer vs. winter), branches were collected and brought to the laboratory to proceed with fungal isolation, assess the percent fungal recovery and determine the susceptibility of pruning wounds according to the time of pruning.

Results and Discussion

Experiments conducted during 2 winters (January 2019 and January 2020) and data from one summer (2019; data from summer 2020 are pending) revealed differences in the seasonal susceptibility of pruning wounds to *Calosphaeria pulchella*. Infection rate in branches (all diameters combined) pruned and inoculated in January was less than 17%, whereas branches (all diameters combined) pruned and inoculated in July yielded up to 72% infection of pruning wounds (**Figure 6**). These results are consistent with results from our *in vitro* temperatures studies indicating *Calosphaeria pulchella* optimal temperatures for ascospores germination and growth is 30° C. This suggests that pruning during cold (and dry) winter weather may suffice to prevent infection of pruning wounds by *Calosphaeria*, which is particularly relevant in counties where Calosphaeria canker represent the main canker disease, and where inoculum of *Calosphaeria pulchella* abounds (Trouillas et al. 2012). Finally, this work explains previous failure to infect pruning wounds in our fungicide trials conducted in winter. Our objectives are to continue investigating the effect of temperature on the biology of *Calosphaeria pulchella* as well as the seasonal susceptibility of cherry trees to *Calosphaeria pulchella* and other fungi in order to determine best pruning timing.

Figure 6. Susceptibility of pruning wounds in different diameter branches (small = 1 cm diameter; large = 2-3 cm diameter) to *Calosphaeria pulchella* according to the time of pruning (January for winter vs. July for summer)



Objective 7: Investigate the resistance of sweet cherry main scion cultivars to canker diseases:

Materials and Methods

We conducted experiments to determine the resistance/tolerance of scion cultivars to Eutypa, Cytospora and Calosphaeria canker diseases. One cherry orchard was established at Kearney Agricultural Research and Extension Center using four cultivars (Rainier, Bing, Santina and Benton). For each cultivar, three branches on each of 20 trees were selected and inoculated with one isolate of each of *Calosphaeria pulchella*, *Eutypa lata* and *Cytospora sorbicola* in November 2020. The outer bark at the inoculation area was disinfected by spraying with 70% ethanol and a 5-mm wound was made using a sterilized cork borer. A 5-mm-diameter mycelium plug from a 7-day-old PDA culture was aseptically inserted into the wound, sealed with petroleum jelly, and then protected with Parafilm. Scion susceptibility data will be recorded one year after inoculation by measuring the length of wood discoloration above and below the point of inoculation. Lesion length will be compared among the different cultivars to determine which cultivars are most tolerant to fungal canker diseases. Re-isolations will be carried out as previously described. A two-way ANOVA will be performed to determine significant differences of susceptibility among the sweet cherry scion cultivars.

Results and Discussion

No data from 2020 trials are yet available for this annual report.

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Project Title: Exploring New and Alternative Insecticides for Resistance Management of Spotted Wing Drosophila in Cherries

Project Leader: Jhalendra Rijal, Area IPM Advisor-Northern San Joaquin Valley (UCCE Stanislaus)

Collaborators: Frank Zalom (UC Davis), Kari Arnold (UCCE Stanislaus), Mohamed Nouri (UCCE San Joaquin), Sudan Gyawaly (UCCE Stanislaus)

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. This progress report covers the first year of the 2 years project on SWD control in cherries. In this, we tested several new insecticides against SWD adults using insecticide-treated cherries in a series of studies under laboratory conditions. 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. Along with a few new ones, these insecticides will be tested in semi-field experiments in the year 2021. The results will be disseminated to the growers via multiple educational events and meetings.

Introduction

Spotted Wing Drosophila (SWD), *Drosophila suzukii*, is one of the major pests of sweet cherry in California. Unlike many other related vinegar flies that attack overripe fruits, SWD larvae can attack soft, ripening fruits. SWD females are equipped with a unique ovipositor that enables them to deposit eggs inside fruits where larvae feed and develop. SWD has high fecundity, multiple generations per season, and a broad host range. Larval feeding inside the soft-skinned fruits, such as cherry, causes severe damage to the crop. The economic threshold of SWD in cherry is practically 'zero,' and, thus, cherry production in California heavily relies on insecticide applications. 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 (i.e., when cherry fruit changes its color from green to straw-like) to the harvest (Van Steenwyk 2014; http://ipm.ucanr.edu/PDF/MISC/2014_Cherry_Spotted_Wing_Drosophila.pdf). 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 major 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 harvest 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 based on not only the efficacy but also 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 some 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 as a part of the 2-year project.

Objectives

1. To evaluate new active ingredients against cherry in the laboratory using various combinations of choice and no-choice bioassays.
2. To conduct preliminary testing of orchard-collected spotted wing drosophila for potential insecticide resistance.

Study methods

In 2020, we screened several conventional and organic insecticides approved by the CA Cherry Board Research Committee. Moreover, as per the Research Committee's feedback based on studies conducted in Oregon, we included erythritol alone and etrythritol+sucrose in our trial in 2020. Insecticide active ingredients or the materials tested against SWD under laboratory conditions in 2020 are presented in Table 1.

Two sets of trials were conducted because of the lack of enough SWD and all the insecticides simultaneously. The first set of trials included eight insecticides (insecticide listed from 1-8 in Table 1) and untreated control. The second trial contained two insecticides (insecticides listed from 9-10 in Table 1) and untreated control. All insecticide efficacy bioassays on SWD was conducted using the SWD adults obtained from the laboratory colony maintained at UCCE Stanislaus, where SWD are reared in a Jazzmix-based fly diet. The colony was initially obtained from Dr. Joanna Chiu lab at UC Davis.
Cherry fruits used in the study had the same level of ripeness visually and were obtained from stores. Fruits were washed thoroughly to remove any external dirt, and other potential contamination and air dry for ~20 min, under room temperature. Cherries with peduncle were used. Cherry fruits were dipped singly in the respective treatment solutions for 5 seconds, left to dry on paper towels for about 45 minutes, and then placed in plastic cups (12oz.) with a screened lid top. The control sets were treated with distilled water. Then, 10 SWD flies (age: <7 days old) were released into the container to expose them to the treated fruit. A cotton flug soaked in water was also placed in the container to increase the relative humidity in the container. Each set of the trial had ten replicates for each treatment and control. The containers were examined for fly mortality in 6 hours first and every 24 hours then after.

Table 1. Insecticides used to conduct SWD insecticide studies-2020

SN	***Treatments***	***Active Ingredient***	***Rate/Acre***
1.	Exirel	cyantraniliprole	16 oz
2.	Minecto Pro	cyantraniliprole+abamectin	12 oz
3.	Pyganic 1.4 EC	pyrethrin	2 qt
4.	Venerate	*Burkholderia* spp. strain A396	4 qt
5.	Grandevo	*Chromobacterium subtsugae*	3 lbs
6.	Warrior II	lambda-cyhalothrin	2.56 oz
7.	Erythritol	-	0.5 M
8.	Erythritol + Sucrose	-	(1.5 M) + (0.5 M)
9	Movento	spirotetramat	9 fl oz
10.	Verdepryn	cyclaniliprole	11 fl oz

Study Results

In the first set of trials, there was a significant effect of treatments on fly mortality (F = 79.77, df= 8, 81, P< 0.001). Also, there was a significant effect of treatments on fly mortality (F = 59.25, df= 2, 27, P< 0.001) in the second set of trial. Overall, the laboratory study showed that, in addition to industry-standard (Warrior II, avg. mortality 98%), Exirel (93%), Verdepryn (88%), Minecto Pro (84%), and Pyganic (84%) caused significantly higher SWD mortality than other treatments (P< 0.001) and are highly effective against SWD adults at 48 hours after exposure (Fig. 1 and 2). The SWD mortalities for the rest of the insecticides were at par with the control mortality.

We did not find significant effects of sugar compounds in SWD adult mortality in our laboratory bioassays. In 2021, we are interested in exploring more about these sugar compounds, especially looking at SWD fly behavior and oviposition activities.

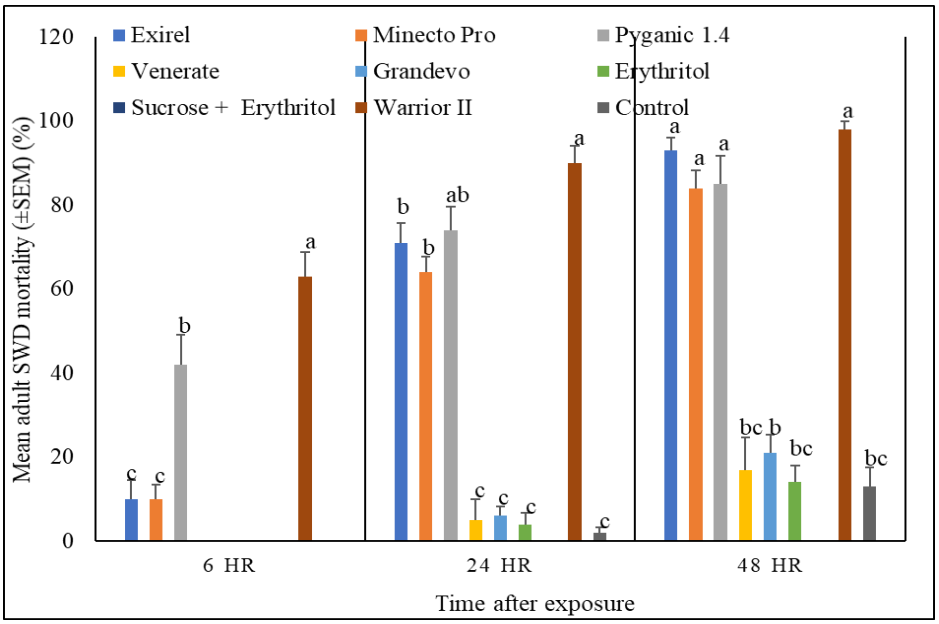

Fig. 1. Effect of insecticide treatments on SWD adult mortality. Means within the same sampling period with the same letters are statistically not different (ANOVA, p > 0.05).

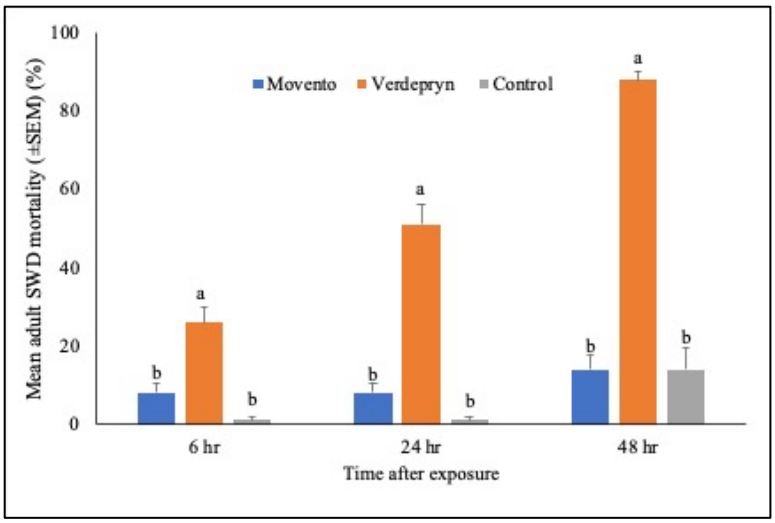

Fig. 2**.** Effect of insecticide treatments on SWD adult mortality. Means within the same sampling period with the same letters are statistically not different (ANOVA, $p > 0.05$).

Plans for 2020-2021

Insecticide field study.
A field study will be conducted to determine the efficacies of insecticides that have shown promising result in our laboratory bioassay in 2020, Exirel (93%), Verdepryn (88%), Minecto Pro (84%), and Pyganic (84%), a positive control (Warrior II), and negative control (untreated control). We did not see the effect of Movento in SWD mortality in lab bioassays. However, it is important to test this product field condition as its mode of action is systemic and relatively slow-moving. The insecticides will be prepared at a standard rate of 100 gallons/acre. We plan to test these insecticides in commercial cherry orchards using one tree as an experimental unit and replicated five times. A power backpack sprayer (Stihl SR 200) will be used to spray the insecticides after the cherry fruit begins to develop its color as the fruit becomes susceptible to SWD attack from that stage. 100 fruit samples will be collected at 1, 3, 7 days after the spray from individual treated trees. The fruits will be evaluated for the number of oviposition stings and the larvae in the fruit.

Insecticide efficacy laboratory bioassays.
We will continue to carry out laboratory bioassays to screen new commercial products (e.g., neem-based insecticide, Rango) in 2021. Briefly, insecticide bioassay in the laboratory will involve treating individual cherry fruits with each treatment and placing it in the small plastic cups (12 oz.) with a screened lid. The fruits for control treatment will be treated with distilled water. Ten adult SWD flies (age: 7-10 days old) will be released into the container to expose them to the treated fruit. There will be 10 replications for each bioassay. The mortality of adults will be assessed for 7 days.

Erythritol effect on SWD oviposition.
We are interested in designing and conducting studies to explore further the potential role of erythritol in SWD egg-laying behavior and fruit damage in 2021. Briefly, this bioassay will involve treating individual cherry fruits with erythritol solution and hanging it on the lid of the small plastic cups (12 oz.) with a screened lid. Then, 5 (2 males and 3 females) adult SWD (age: 7-10 days old) will be released in the cup. The oviposition marks ‘stings’on individual fruits will be evaluated after 24 hours, and then, the fruits will be transferred to a different container to allow larval growth for two weeks. We will count the number of larvae that emerged from each fruit using the floating technique. My lab has successfully used

this technique for testing the efficacy of other neem-based products against SWD in the past (Rijal and Grant, California Cherry Board Report-2017).

Testing for insecticide resistance.
We will explore the status of SWD flies collected from one cherry orchard where there was potential resistance to commonly used insecticides in the 2019 season and a few other locations. We will collaborate with Dr. Frank Zalom's lab at UC Davis to test field-collected flies using a relatively more straightforward technique to identify the potential resistance. Van Timmeren et al. 2018 (Pest Manag Sci.75: 1782–1793) developed and tested a simple, effective, and reliable method for monitoring the susceptibility of SWD to different insecticides. In this study, they conducted insecticide bioassays by preparing and using the stock solutions of five other insecticides in 20mL glass vials. Different concentrations for each insecticide were tested by diluting the stock solution and exposing the SWD flies to both field-collected and susceptible populations to insecticides. Following a similar protocol, Gress and Zalom (2018) tested field-collected SWD from one of the major caneberry production areas (i.e., Monterey county) in California for resistance spinosyn products. In collaboration with the Zalom lab, we plant to test SWD that we will collect from 2-3 cherry orchards in San Joaquin Valley.

Expected outcomes

In the 2021 season, we will conduct the field screening of the selected insecticides that have shown promising results in 2020 bioassays in the field. If the insecticides are found effective under field conditions, this study will provide a few more alternatives to the insecticides used for SWD management. We will continue to explore an opportunity to collaborate with UCD Entomologist (Drs: Zalom) in testing field-collected SWD for insecticide resistance. Research results will be shared with growers and pest control professionals during several extension events, including annual CA Cherry Research meetings.

CONTINUING PROJECT REPORT **YEAR**: 2 of 3

Project Title: Engineered transgenic *D. suzukii* for wild population suppression

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Objectives: Spotted wing Drosophila, *D. suzukii*, is a major worldwide crop pest of various soft-skinned fruits. A highly promising approach to *D. suzkuii* control that could complement existing control methods is genetic pest management, which includes strategies such as gene drive and precision-guided sterile insect technique (pgSIT)[1,2]. SIT has been a successful technology for insect population suppression, which is achieved by introducing large number sterile males into a target population. While the classic irradiation-based SIT presents an environment-friendly method of a local population suppression, it is not technically feasible or scalable for the control of most insects. PgSIT, on the other hand, is a simplified way to generate sterile males and should be less expensive and labor intensive than irradiation-based SIT even at scale.

We also propose to engineer *D. suzukii* gene drive strains, which can be utilized to more rapidly spread desirable genes (e.g., susceptibility to a novel bio-friendly pesticide) throughout, or to entirely suppress/eradicate, wild *D. suzukii* populations. Such an approach is catalytic, with release of only modest numbers of engineered insects required to spread desirable genes or achieve population suppression. Additionally, since such a system relies on only a few releases of transgenic insects to do all of the work on an ongoing basis, it is affordable as compared to the use of insecticides, which need to be applied regularly. Finally, such an approach is environmentally friendly and entirely insect-specific and would have no effect on crops or on beneficial organisms.

Our objective is to therefore engineer *D. suzukii* gene drive strains that could be utilized as part of current integrated pest management programs to control wild *D. suzukii* populations. Specifically, out of the multiple types of gene drive systems that can be utilized in a genetic pest management program, we aim to develop a pgSIT system in *D. suzukii* using the design principles we have optimized in *D. melanogaster*[2]. We also aim to develop synthetic *Medea* elements that can be used to suppress wild *D. suzukii* populations[1]. Ultimately, our goal is to develop a product (a genetically modified *D. suzukii*) that can be mass-reared and deployed into the wild to catalytically suppress, and completely eliminate, the wild populations of this significant pest.

Objective A - Refinement of a *Medea* drive system for *D. suzukii* population suppression. We have developed a synthetic *Medea* gene drive system for population suppression[6]. Engineered *Medea* systems rely on a *Medea* element consisting of a toxin-antidote combination. The toxin consists of a miRNA that is expressed during oogenesis in *Medea*-bearing females, disrupting an embryonic essential gene. A linked antidote is expressed early during embryogenesis and consists of a recoded version of the target gene that is resistant to the miRNA. This combination results in the survival of half of the embryos originating from a *Medea*-bearing heterozygous female, as those that do not inherit the *Medea* element perish. If a heterozygous *Medea* female has mated with a heterozygous *Medea* male, the antidote from the male will also take effect in the embryo, resulting in 3/4 of the embryos surviving. Therefore, *Medea* will rapidly spread through a population, carrying any linked genes with it.

We have already engineered a first-generation *Medea* system in *D. suzukii*[1], which is the first functional gene drive developed in this pest. We had rigorously tested it in laboratory cage populations and had characterized it in different genetic backgrounds to determine effectiveness and fecundity. We found that this first-generation *Medea* system was capable of biasing Mendelian inheritance rates with up to 100% efficiency and could maintain itself at high frequencies in a wild population; however, drive resistance, resulting from naturally occurring genetic variation and associated fitness costs, was present and could hinder the spread of such a drive. Therefore, since mathematical modeling indicates that our *Medea* drive system could spread to fixation if resistance was reduced[1], we need to engineer a second-generation *Medea* system that should obviate the specific resistance that we observed. To safeguard, reduce risk, and mitigate the spread of the *D. suzukii Medea* system into wild populations, we also aim to develop a reversal *Medea* (RM) system that can be used to replace the original *Medea* in case a recall is necessary. Finally, in order to use *Medea* to bring about population suppression, we need to link it to a cargo gene capable of killing *D. suzukii* under specific conditions to bring about a population crash. We have already identified several promising putative cargo genes and are testing them in *D. melanogaster*, a closely related species to *D. suzukii* that is easier to work with and provides a useful testing platform for transgenes. However, we will still need to build and test them in *D. suzukii*. Successful completion of the above objectives would lead to the development of a genetically modified *D. suzukii* strain (carrying a synthetic *Medea* element) that can be mass-reared and deployed into the wild to catalytically suppress, and completely eliminate, wild populations of *D. suzukii*.

Objective B: Precision guided sterile insect technique (pgSIT) for *D. suzukii* population suppression. The sterile insect technique (SIT) is an alternative, proven pest management approach that could complement existing control methods. SIT involves the mass-production and release of sterile males, and has historically been used to control, and eradicate, insect pest populations dating back to the mid-1930s[10–14]. Traditional SIT methodologies have relied on DNA-damaging agents for sterilization, substantially reducing overall fitness and mating competitiveness of released males. A next-generation highly efficient technology that can be used for biocontrol of *D. suzukii* is precision guided SIT (pgSIT). PgSIT functions by exploiting the precision and accuracy of CRISPR to simultaneously disrupt genes essential for either female viability or male fertility. It utilizes a simple breeding scheme requiring two homozygous strains - one expressing Cas9 and the other expressing double guide RNAs (dgRNAs). A single mating between these strains mechanistically results in synchronous RNA-guided dominant biallelic knockouts of both target genes throughout development, resulting in the complete penetrance of desired phenotypes in all progeny. We have previously built pgSIT in *Drosophila melanogaster*, a model organism that is closely related to *D. suzukii*, and shown that it is extremely robust at genetically sexing and simultaneously sterilizing resulting progeny reproducibly with 100% efficiency, and that pgSIT sterile males are fit and can compete for mates[2]. We therefore aim to develop pgSIT technology in *D. suzukii* (Objective B). Successful development of this technology would produce a genetic-based sterile insect strain that can be mass-reared and released to reduce populations of *D. suzukii* in a straightforward manner with respect to regulations.

Significant Findings:

Objective A:

- We have developed a modified version of our original *Medea* system that is designed to reduce resistance to the drive. We are currently rigorously testing this second-generation *Medea* element and planning for longer term population cage studies.
- We have developed a second-generation "reversal" *Medea* system that should be more robust in the face of genetic diversity in general and could be used to replace the original *Medea* in case a recall is necessary. We are currently testing this system and planning for longer term population cage studies.

- We have identified several promising putative cargo genes that could be spread with the *Medea* gene drive to cause population suppression. Multiple genes have been tested in *D. melanogaster* as proof of principle and are now being transitioned to *D. suzukii*.

Objective B:

- Established six transgenic gRNA lines targeting both *sxl* and ***β****-tub* simultaneously.
- Generated homozygous pgSIT lines that consistently produce sterile males when crossed (**Figure 1**) and identified the *vas-Cas9* line has been identified as the best *Cas9* line for *D. suzukii* pgSIT system.
- We also assessed the pgSIT (double knockout) efficiency and fitness (ease of maintenance) of six different $gRNA^{\beta Tub,Sxl}$ lines, as well as confirmed that each established line was homozygous.
- We found that the fitness of pgSIT males are relatively high facilitating the effective application of released pgSIT males for suppression of wild populations.
- We are also developing comprehensive methods to assess the pgSIT population suppression using population cage studies. We established a strategy to assess the pgSIT males population suppression in discrete-generation cage populations.
- We have started preparations for semi-field trials of the pgSIT in Corvallis, Oregon: https://www.youtube.com/watch?v=AkVfcrj0zkI&feature=emb_logo
- We developed a new temperature inducible pgSIT (TI-pgSIT) system to eliminate the need for gRNA and Cas9 crosses. We engineered different TI-pgSIT systems, injected into *D. suzukii* embryos, and generated a few *D. suzukii* transgenic lines harboring TI-pgSIT systems. We have been able to stably maintain *D. suzukii* TI-pgSIT lines under the permissive temperature and started homozygouse these lines for pure-breeding.

Methods:

Objective A - Refinement of a *Medea* drive system for *D. suzukii* population suppression. We have developed the first proof of concept *Medea* drive in *D. suzukii*[6]. Given our observations regarding resistance and its effect on *Medea* function, we now need to engineer improved *Medea* systems that could reduce the chances of resistance acting as an impediment to spread. So far, we have performed some sequencing-based characterization of naturally occurring genetic variation in various geographically distinct target populations to help guide selection of target sites that are well conserved across all populations in which the drive is intended to function. We then designed a modified version of the original *Medea* system that targeted different, conserved sequences (still in the 5'UTR of the *myd88* target gene), reasoning that such a *Medea* element should function very similarly to the original element but not be impeded by the resistance we previously observed. We are now obtaining transgenic lines for this improved *Medea* element, and preliminary data indicates that it works better than the original *Medea*, producing 100% inheritance bias. We are continuing to rigorously test this second-generation *Medea* element to characterize its function and ability to bias inheritance 100% in geographically distinct populations. We also will need to perform multiple long term multi-generational population cage experiments to determine whether this *Medea* can drive robust population replacement.

Additionally, we hypothesized that to reduce resistance, miRNA target site selection could be limited to the coding DNA sequence regions of a genome, which tend to be strongly conserved, as opposed to regions such as the 5'UTR, which canonically have higher tolerance for sequence variation. We have therefore also developed a second-generation "reversal" *Medea* system in *D. suzukii* that should be more robust in the face of genetic diversity in general (because it targets coding DNA regions as opposed to the 5'UTR) and could be used to replace the original *Medea* in case a recall is necessary. Specifically, to reduce risk and mitigate the spread of the *D. suzukii Medea* system into wild populations, it is important to develop a reversal Medea (RM) system and demonstrate that it can function as predicted. We have finished designing and building a reversal *Medea* system capable of spreading on its own and of replacing the first *Medea* described above and are in the process of

obtaining transgenic *D. suzukii* individuals containing this *Medea.* Once we have transgenic lines for this construct, we need to rigorously test them for their ability to bias inheritance in both wild type and original *Medea* backgrounds. We will then need to perform multiple long term multi-generational population cage experiments to determine whether this *Medea* can actually spread and replace the original *Medea*.

Identification of Putative "Cargo" Genes: For *D. suzukii*, elimination of the pest populations is ultimately the goal. An engineered *Medea* system could achieve this by spreading a "cargo" gene proffering susceptibility to a particular pesticide, or a conditional lethal gene that would be activated by some substance or environmental cue such as high temperature or diapause. One promising type of candidate "cargo" gene is a thermally activated TRPA1 cation channel. Specifically, TRPA1 is an ion channel located on the plasma membrane of many human and animal cells and is finely tuned to detect specific temperatures ranging from extreme cold to noxious heat. Upon exposure to a critical "threshold" temperature, this cation channel can "open" and modulate Ca2+ and Mg2+ entry into the cell[16]; when TRPA1 is overexpressed in an exogenous tissue (such as the fly brain, for example), this "opening" can lead to total fly paralysis and death. We therefore have started to engineer *D. suzukii* to express a specific TRPA1 channel in the brain, so that exposure of the engineered individuals to a threshold temperature (determined by the specific TRPA1 channel used) would paralyze/kill the flies.

Developing a field-ready strain: Similar to the other suppression drives, when we build an optimized *Medea* drive, we will also need to conduct laboratory and caged field trials to determine mating competitiveness, longevity, and fitness of these strains. This data will be used and fed into mathematical models to predict the numbers of flies we will need to release to achieve suppression.

Objective B: Precision guided sterile insect technique (pgSIT) for *D. suzukii* population suppression. In order to construct a pgSIT system, we need functional Cas9 tools (including gRNA lines that target genes essential for female viability and male sterility and Cas9 expressing lines (Figure 2) in *D. suzukii*. We have now developed multiple transgenic lines that express Cas9 (*bicC-cas9, dhd-cas9, vasa-cas9, nanos-cas9, ubiq-cas9*). Also, essential to building a pgSIT system are guide RNA (gRNA) lines that target genes essential for female viability and male fertility. We have previously identified genes essential for female viability or male fertility in *D. melanogaster* and have shown that disrupting these genes via CRISPR/Cas9 produces the desired results (e.g., female death or conversion of females into sterile intersex individuals for the former group, male sterility for the latter. Since *D. melanogaster* is closely related to *D. suzukii*, we reasoned that disruption of these same genes would have a similar effect in *D. suzukii* and are focusing our efforts on these validated target genes. Specifically, to disrupt female viability, we are targeting several sex-specifically alternatively spliced sex-determination genes including *sex lethal* (*sxl*), *transformer* (*tra*), and *doublesex* (*dsxF*), as well as *zero population growth* (*zpg*), a germline-specific gap junction gene. So far, we have identified *D. suzukii* homologues of all of these genes and have carefully selected two gRNA target sites in each gene that are highly conserved and thus unlikely to harbor sequence variation. We have generated multiple transgenic lines for each gRNA target and we are currently in the process of crossing each one separately to our five Cas9 strains to see whether the combinations of Cas9+gRNA will produce female lethality and male sterility. So far, we have multiple gRNA lines that generate the expected 100% sterile male phenotype (**Table 1**). We are now rigorously testing these strains to ensure these results are reproducible over many replicates. We are also conducting male competition and fitness studies to ensure the sterile males are fit to compete in field conditions.

Developing a field-ready strain: Once all of these components are individually validated, we can proceed to assemble a single transgene that, coupled with a Cas9 strain, can be used to generate a pgSIT strain ready for use in the field for *D. suzukii* biocontrol. Laboratory and caged field trials will also be conducted on this strain to determine mating competitiveness, longevity, and fitness compared to wild flies. This data will be used and fed into mathematical models to predict the introduction frequencies we will need to use to achieve suppression. Gene drive experiments will be initiated at various introduction frequencies to characterize the population suppression dynamics. Modeling work

will occur in collaboration with Dr. John Marshall (UC Berkeley), a mathematical biologist with whom we have worked on a number of modeling studies.

Since the ultimate goal here is to develop a product (a genetically modified *D. suzukii*) that can be mass-reared and deployed into the wild to suppress, and completely eliminate, the wild populations of *D. suzukii*, we will need regulatory bodies to permit such releases. In brief, we have a field cage study permit from USDA-APHIS BRS/PPQ and we have a permit for a BRS 2000 (Application for Permit or Courtesy Permit for Movement or Release of Genetically Engineered Organisms), which has been uses in past and ongoing SIT programs. Our commercial collaborator, Agragene, has started preparations for a semi-field trial of the pgSIT technology in Corvallis, Oregon. A video of these preparations can be found here: https://www.youtube.com/watch?v=AkVfcrj0zkI&feature=emb_logo.

Results and Discussion: Objective A: We have developed a modified version of our original *Medea* system that is designed to reduce resistance to the drive. Given our observations regarding resistance and its effect on Medea function, we set out to engineer improved Medea systems that could reduce the chances of resistance acting as an impediment to spread. Specifically, we performed some sequencing-based characterization of naturally occurring genetic variation in various geographically distinct target populations to help guide selection of target sites that are well conserved across all populations in which the drive is intended to function. We then designed a modified version of the original *Medea* system that targeted different, conserved sequences (still in the 5'UTR of the myd88 target gene), reasoning that such a *Medea* element should function very similarly to the original element but not be impeded by the resistance we previously observed. We have obtained transgenic lines for this improved Medea element, and preliminary data indicates that it works better than the original *Medea*, producing 100% inheritance bias. We are currently rigorously testing this second-generation *Medea* element and planning for longer term population cage studies.

We have developed a second-generation "reversal" *Medea* system that should be more robust in the face of genetic diversity in general and could be used to replace the original *Medea* in case a recall is necessary. We have finished designing and building a Reversal Medea system capable of spreading on its own and of replacing the first *Medea* described above and are in the process of obtaining transgenic *D. suzukii* individuals containing this *Medea* and of rigorously characterizing this system. We are currently testing this system and planning for longer term population cage studies.

We have identified and are characterizing several promising putative cargo genes that could be spread with the *Medea* gene drive to cause population suppression. We are exploring TRPA1 channels with different activation temperatures (including rattlesnake TRPA1, python snake TRPA1, boa snake TRPA1 and fruit fly TRPA1) in *D. melanogaster* as a proof of principle, and has preliminary data indicating that at least some of the tested TRPA1 channels, when expressed in the fly brain, work as expected. Once we know which TRPA1 channel appears most promising, we will insert it into our best Medea element and begin testing this approach in *D. suzukii*. However, multiple genes have been tested in *D. melanogaster* as proof of principle and are now being transitioned to *D. suzukii*.

Objective B: We generated homozygous *D. suzukii* pgSIT lines that consistently produce sterile males when crossed. Both homozygous *Cas9* and *gRNA* lines have been established for pure-breeding. In this reporting period, we confirmed the efficiency of our *Cas9* lines by scoring the knockouts of two phenotypic genes, *white* and *yellow* loci, in both somatic and germ cells induced by different *Cas9* lines. Four *Cas9* lines (*BicC-*, *Ubiq-*, *vas-*, and *nos-Cas9*) were crossed to each $gRNA^{w}$ and $gRNA^{y}$ in both reciprocal directions and their progeny was followed over two generations to assess germline knockouts. This analysis was replicated five times. Each tested *Cas9* line induced robust somatic knockout in the F_1 (trans-heterozygous) progeny and further supported the pgSIT technology. *Vas-Cas9* supported the most robust knockout in both somatic and germ line cells. Therefore, the *vas-Cas9* line has been identified as the best *Cas9* line for *D. suzukii* pgSIT system.

We also assessed the pgSIT (double knockout) efficiency and fitness (ease of maintenance) of six different $gRNA^{\beta Tub,Sxl}$ lines, as well as confirmed that each established line was homozygous. The

gRNA$^{\beta Tub,Sxl}$ line #52 supports the robust knockout and is easy to maintain in the laboratory. Therefore, it suits best for the production of pgSIT (trans-heterozygous) males. The combination of *vas-Cas9* and *gRNA*$^{\beta Tub,Sxl}$ #52 results in 100% sterile trans-heterozygous F_1 progeny. It is notable that when numbers of scored F_1 progeny exceeded thousands of flies, we noticed that around 1% of F1 progeny were intersexes, which were found to be 100% sterile.

We proceeded to assess the fitness of generated pgSIT (*gRNA*$^{\beta Tub,Sxl}$/+; *vas-Cas9*/+) males. The fitness of released pgSIT males as their ability to compete with *wt* males for secure mating with *wt* females in the field is exceeding important for the efficient application of the pgSIT technology. First, we tested the mating competitiveness of pgSIT males with *wt* males *and* found that the pgSIT males were able to efficiently court and mate with *wt* females in the presence of *wt* males (**Figure 1**). Notably, a 1:1 release ratio of pgSIT males, which is much lower than a recommended 1:20 release ratio of classic SIT males, induced the significant drop in female fecundity, measured as an egg hatching rate (from 86% to 62% , **Figure 1**). Second, we measure the longevity of pgSIT males relative to *wt* males (**Figure 2**) and found that the pgSIT males live only a slightly shorter life than *wt* males (medial longevity 43 days vs 49 days, respectively, **Figure 2**). The shorter life expectancy of pgSIT males relative to *wt* males is expected, and can be easily compensated by increasing the pgSIT release ratio from 1:1 to 1:3 or 1:5. In summary, we found that the fitness of pgSIT males are relatively high facilitating the effective application of released pgSIT males for suppression of wild populations.

We are also developing comprehensive methods to assess the pgSIT population suppression using population cage studies. We established a strategy to assess the pgSIT male population suppression in discrete-generation cage populations. Three experimental and three control cage populations are currently used in the laboratory to assess the efficiency of pgSIT suppression under the established protocol.

We have stopped developing genetic sex-sorting for pgSIT lines. The previously engineered constructs for marking the female sex, which were based on the female-specific splicing *tra* intron inside the coding sequence a fluorescent protein, were injected. However, generated transgenic flies did not provide the consistent and strong fluorescent signal sufficient for automated sex-sorting. In addition, the development of Temperature-Inducible pgSIT (TI-pgSIT) genetic system had sex-sorting obsolete.

We recently demonstrated the TI-pgSIT proof-of-concept in *Drosophila melanogaster*. Now, we are developing multiple TI-pgSIT systems for *D. suzukii*. TI-pgSIT address one shortcoming of the pgSIT, i.e. requirement to maintain two lines and their sex sorting to generated F_1 eggs in the lab. The TI-pgSIT relies on the maintenance of a single transgenic line and Temperature-Inducible activation of the pgSIT system. We engineered different TI-pgSIT systems, injected into *D. suzukii* embryos, and generated a few *D. suzukii* transgenic lines harboring TI-pgSIT systems. We have been able to stably maintain *D. suzukii* TI-pgSIT lines under the permissive temperature and started homozygousing these lines for pure-breeding. Our ability to maintain these lines over multiple generations suggests the TI-pgSIT is indeed not-active under the lower temperature. We will test the activation of TI-pgSIT as soon as the generated lines will be expanded.

Additional Items:

References: 1. Buchman, A., Marshall, J. M., Ostrovski, D., Yang, T. & Akbari, O. S. Synthetically engineered Medea gene drive system in the worldwide crop pest Drosophila suzukii. *Proc. Natl. Acad. Sci. U. S. A.* 115, 4725–4730 (2018). **2.** Kandul, N. P. *et al.* Transforming insect population control with precision guided sterile males with demonstration in flies. *Nature Communications* vol. 10 (2019).

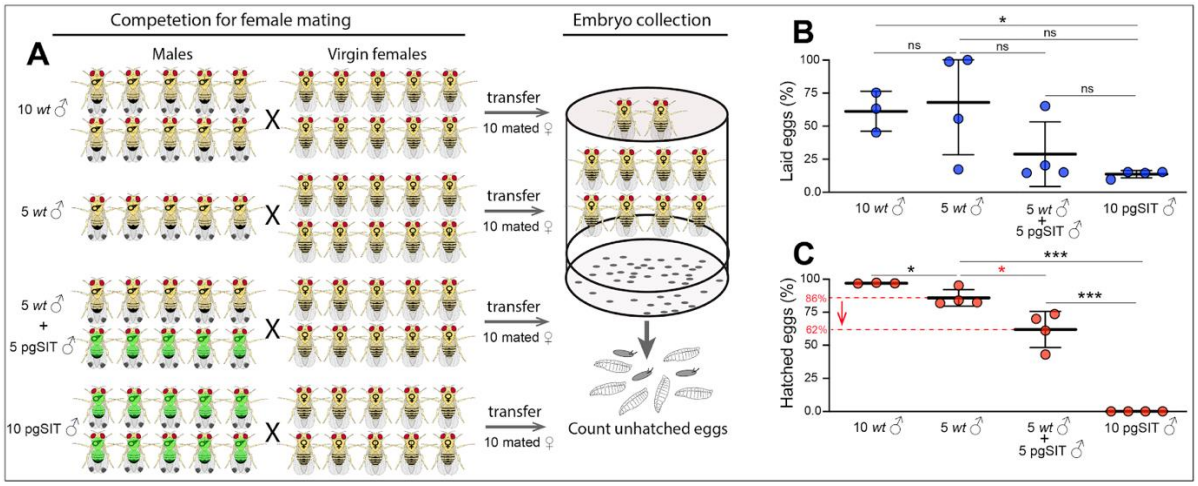

Figure 1: *D. suzukii* pgSIT males compete with *wt* males for mating with females. (**A**) An experimental setup to estimate the mating competitiveness of sterile pgSIT males (*gRNA*$^{\beta Tub,Sxl}$/+; *vas-Cas9*/+; marked with green) competing against *wt* males to secure matings with *wt* virgin females. A mated female is resistant to the next mating for around 24 H, and the mating success of sterile males was evaluated by fertility decrease (aka. increase of unhatched egg rate). (**B-C**) Bars graph percentages of laid and hatched eggs. (**B**) Numbers of laid egg (%) were normalized to the highest number of laid eggs (595 eggs). Notably, the females mated to 10 pgSIT males laid fewer eggs that the females mated with 10 *wt* males (13.7±2.8% vs 61.2±15.1%, *P*=0.030*). (**C**) The presence of 5 sterile pgSIT males competing with 5 *wt* fertile males resulted in a significant decrease in female fertility from 86.0±6.3% to 61.9±13.7% (*P*=0.031*) that could not be accounted by removal of 5 *wt* males (97.0±4.5% vs. 86.0±6.3%, *P*=0.039*). Bars represent means ± SD for three/four replicates. $P > 0.05^{ns}$, $P < 0.05$*, $P < 0.001$*** by a *t* test assuming unequal variance.

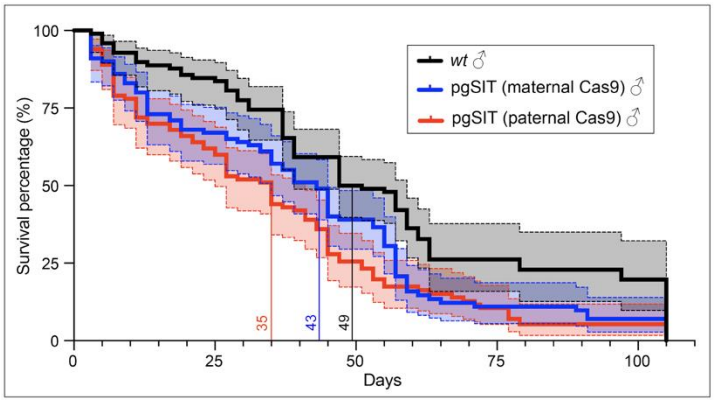

Figure 2: The survival curve of *D. suzukii* pgSIT males is comparable to *wt* males. Survival curves of *wt* males (black line) and two types of pgSIT (*gRNA*$^{\beta Tub,Sxl}$/+; *vas-Cas9*/+) males generated by reciprocal crosses with *vas-Cas9* inherited from female parents (maternal Cas9, blue line) or by male parents (paternal Cas9, red line). Survival curves show nonparametric maximum likelihood estimates for five male groups, along with 95% confidence intervals in light shade. The y-axis shows the estimated survival percentage. The median survival for each male type is presented on the x-axis. Both types of pgSIT males lived slightly shorter than *wt* males (median survival of 43 vs 49 days, $P < 0.001$), while no significant difference was found between two types of pgSIT males (median survival of 35 vs 43 days, $P = 0.106$). The Log-rank (Martel-Cox) and Gehan-Breslow-Wilcoxon tests were used to assess statistical significance.