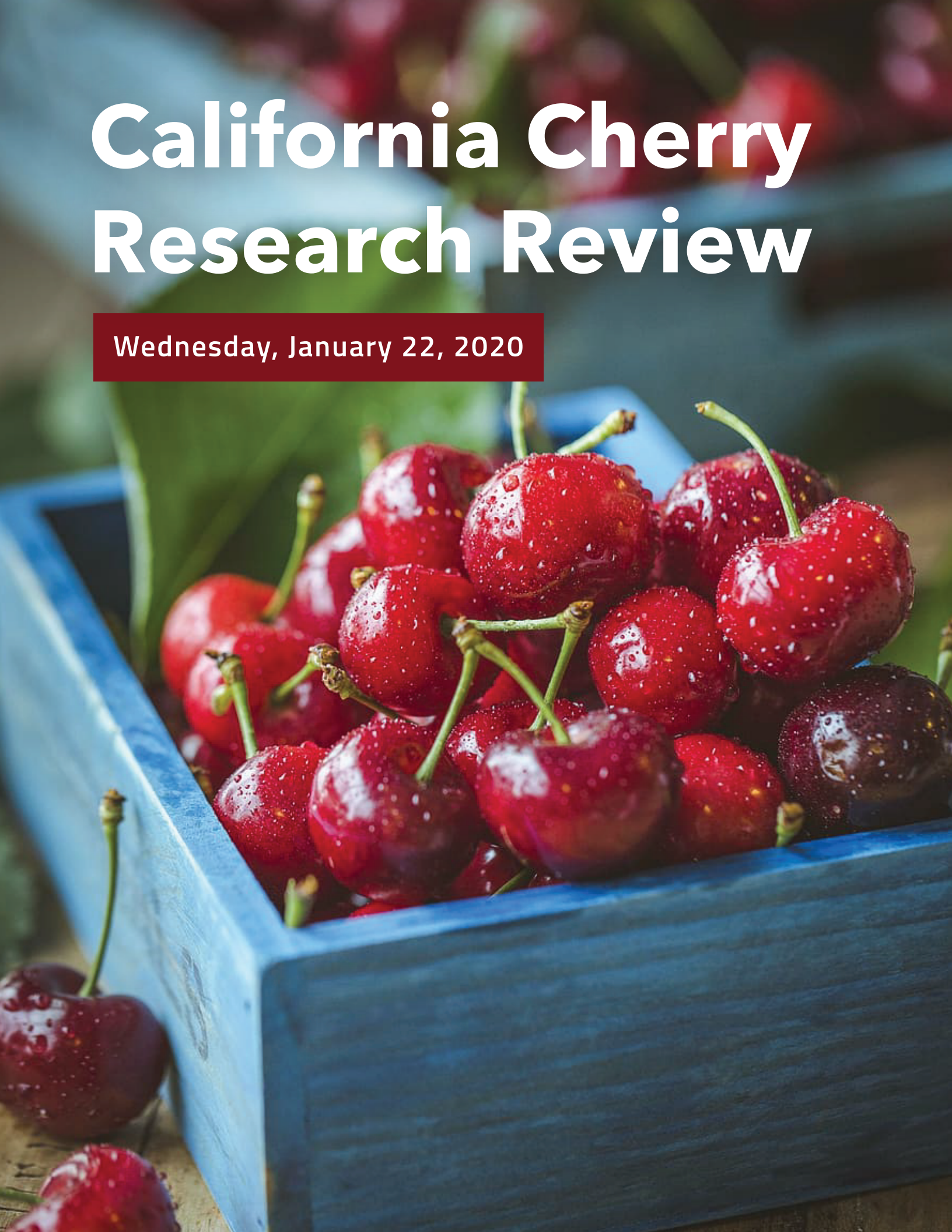
California Cherry Research Review

Wednesday, January 22, 2020



CALIFORNIA CHERRY RESEARCH REVIEW
Wednesday, January 22, 2020

Evelyn Costa Assembly Room
San Joaquin County – Office of the Agricultural Commissioner
2101 E. Earhart Avenue, #100, Stockton, California 95206
Sponsored by the University of California and California Cherry Board

9:00	**Welcome** Scott Brown, Production Manager, Morada Produce Tyler Rood, California Cherry Board
9:10	**The investigation into dormancy breaking agents and the dynamic chill portions model in CA cherries via carbohydrates and solar radiation** Dr. Kari Arnold, UCCE Orchard and Vineyard Systems Advisor, Stanislaus County
9:30	**Cherry buckskin disease review: x-disease phytoplasma** Dr. Mohamed Nouri, UCCE Orchard Systems Advisor, San Joaquin County
10:00	**Measuring cherry evapotranspiration and deriving crop coefficient (Kc) values for use in irrigation scheduling** Dr. Kosana Suvočarev, Dept. of Land, Air & Water Resources, UC Davis
10:20	**Engineered transgenic *Drosophila suzukii* for wild population suppression & eradication: production, performance assessment and effective wild releases** Dr. Nikolay Kandul, Div. of Biology Sciences, UC San Diego
10:50	**Break**
11:10	**Improved management of fungal canker diseases of sweet cherry** **Investigating the cause of sudden decline of sweet cherry in California** Dr. Renaud Travadon, Dept. of Plant Pathology, UC Davis, Kearney Agricultural Research & Extension Center, Parlier, CA
12:00	**Management and epidemiology of pre- and postharvest diseases of sweet cherry** Dr. James Adaskaveg, Dept. of Plant Pathology, UC Riverside
12:30	**Lunch** (Courtesy of California Cherry Board)
1:30	**Adjourn**

CDPR CE CREDITS AVAILABLE

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CALIFORNIA CHERRY BOARD -- RESEARCH COMMITTEE BUDGET WORKSHEET
YE: April 1, 2019 - March 31, 2020

	Project Leader	Project Title	Status	Project Objectives	2019 Projects Requested Funding	2019 Projects Approved Funding	Supplemental Information
1	Trouillas	Improved management of fungal canker diseases of sweet cherry	On-going (yrs. 2 of 3)	**Obj. 1**: Latent infection of canker pathogens in nursery stock; **Obj. 2**: role of pruning tools on disease transmission; **Obj. 3**: efficacy of various compounds for the protection of pruning wounds; **Obj. 4**: main infection pathways of Calosphaeria and Cytospora cankers; **Obj. 5**: seasonal susceptibility of pruning wounds on infection by Calosphaeria pulchella; **Obj. 6**: relative susceptibility of main scion cultivars to canker diseases.	$ 68,460.00	$ 58,460.00	Determine latent infection of canker pathogens in nursery stock and improve management of fungal canker diseases. This work has indicated a high incidence of wood decay fungi and canker pathogens from trees sampled before or right after planting. Investigators have proposed to work closely with nurseries to improve the quality of nursery stocks by preventing introductions of canker diseases and wood decay fungi via planting material.
2	Suvočarev	Measuring cherry evapotranspiration and deriving crop coefficient (Kc) values for use in irrigation scheduling	New (yrs. 1 of 2)	Year 1: $41,000; Year 2: $21,000 = $62,000; **Obj. 1**: Develop updated water use information based on field measurements of three orchards, for a mature, well-watered and high-yielding cherry orchard grown in the San Joaquin Valley under the typical California production practices; **Obj. 2**: Outreach the developed information to the cherry production community and to water agencies in California; **Obj. 3**: Address growers' concerns related to water management practices for typical cherry production practices.	$ 41,000.00	$ 41,000.00	Update water use information (ie. ET, Kc, and SWP (stem-water-potential) and best management practices for growers. Orchards selected for trial: Drip + Bing + Mahaleb; Impact Sprinkler + Bing + Mazzard; Drip + Bing + Mazzard. Research team will apply for a CDFA grant to trial additional growing regions of sweet cherry in California.
3	Brown	Develop nutrient budget and early spring nutrient prediction model for nutrient management in cherry	New (yrs. 1 of 3)	Year 1: $50,000; Year 2: $50,000 = $100,000; **Obj. 1**: Develop nutrient demand curves to guide the quantity and time of fertilizer application in cherry: repeat for most representative cultivars and production systems; **Obj. 2**: Develop and extend nutrient Best Management Practices (BMP) for cherry cultivars.	$ 75,000.00	$ 50,000.00	Develop nutrient demand curves, early season leaf sampling predicition models and best management practices that guide the quantity and time of fertilizer. Funding was made contingent on Brown securing CDFA FREP funds. CDFA FREP has since approved the proposal in full ($150,000). Excavations and non-destructive sampling will begin in February of 2020.
3(b)	Brown	Develop nutrient budget and early spring nutrient prediction model for nutrient management in cherry	New (yrs. 1 of 3)	Funds are purposed with reimbursing trial participants for losses in revenue via yields and for expenses related labor and equipment required for tree excavation.	$ -	$ 9,000.00	Orchard sites (3) x scion varieties (2) x excavated trees (3) x reimbursement per tree excavated ($500) = $9,000. Excavations will begin in February of 2020.
4	Adaskaveg	Management and epidemiology of pre- and postharvest diseases of sweet cherry	New (yrs. 1 of 3)	**Obj. 1**: Evaluate new products against bacterial blast in flower inoculation studies and against canker in twig inoculation studies; **Obj. 2**: Evaluate under field conditions bloom and preharvest applications of new compounds for control of brown rot and Botrytis blossom blight, powdery mildew, and preharvest brown rot and gray mold fruit decay; **Obj. 3**: Evaluate new fungicides as postharvest treatments; **Obj. 4**: Evaluate new fungicides for managing Phytophthora root rot of cherry.	$ 51,000.00	$ 51,000.00	Evaluate efficacy of new treatments against bacterial blast and canker; new and experimental bloom and preharvest sprays; efficacy of new postharvest fungicides; new Phytophthora root and crown rot fungicides. Kasugamycin (Kasumin) approved by CDPR for control of walnut blight in walnuts and bacterial blast and canker in cherry in January of 2018.
5	Akbari	Engineered transgenic *Drosophila suzukii* for wild population suppression & eradication	New (yrs 1 of 3)	**Obj 1**: Development of a D. suzukii Medea-based gene drive system for population suppression; **Obj. 2**: Development of CRISPR/Cas9-based drive systems in D. suzukii; **Obj. 3**: Development of pgSIT in D. suzukii.	$ 80,000.00	$ 80,000.00	Engineering genetic solutions for the wild population suppression and eradication of spotted wind drosophila (SWD). The pgSIT technology is closest to field release and has the clearest regulatory pathway to market. The homing-based, Medea, and Y-Drive systems are versatile technologies with the potential to more swiftly and affordably suppress D. suzukii populations than the pgSIT technology, but their technical development will require more refinement and the regulatory pathway is less clear. The California Blueberry Commission, Washington Tree Fruit Research Commission and Oregon Sweet Cherry Commission allocated $15,000, $34,957 and $11,652 towards the project, respectively.
6	CA Dept. of Water Resources	CIMIS station: Linden district	On-going (yrs. 1 of 1)	While spatial CIMIS may be used to estimate Eto for the Suvočarev project, a CIMIS station near trial sights will stregthen the accuracy of ET and Kc measurments. In addition, considering all CIMIS stations in the Morada and Linden districts were no-longer functioning or online, Research Committee members felt a CIMIS station needed to be made available to the industry: a minimum service for growers who cannot rely on their own weather station data.	$ -	$ 10,000.00	Lawrence Sambado helped to identify a suitable site for hosting a CIMIS station in Linden, CA: 40+ acres of irrigated pasture. Mohamed Nouri – San Joaquin County farm advisor – has agreed to serve the "Cooperator" and will help to maintain the CIMIS station. Funds have been allocated from the Suvočarev project to construct fencing around the station.
				Totals:		$ 299,460.00	
				Net:		$ 284,460.00	

California Cherry Board
RESEARCH COMMITTEE (RC) & RESEARCH ADVISORY COMMITTEE (RAC) ROSTERS

Last Updated: Thursday, November 7, 2019

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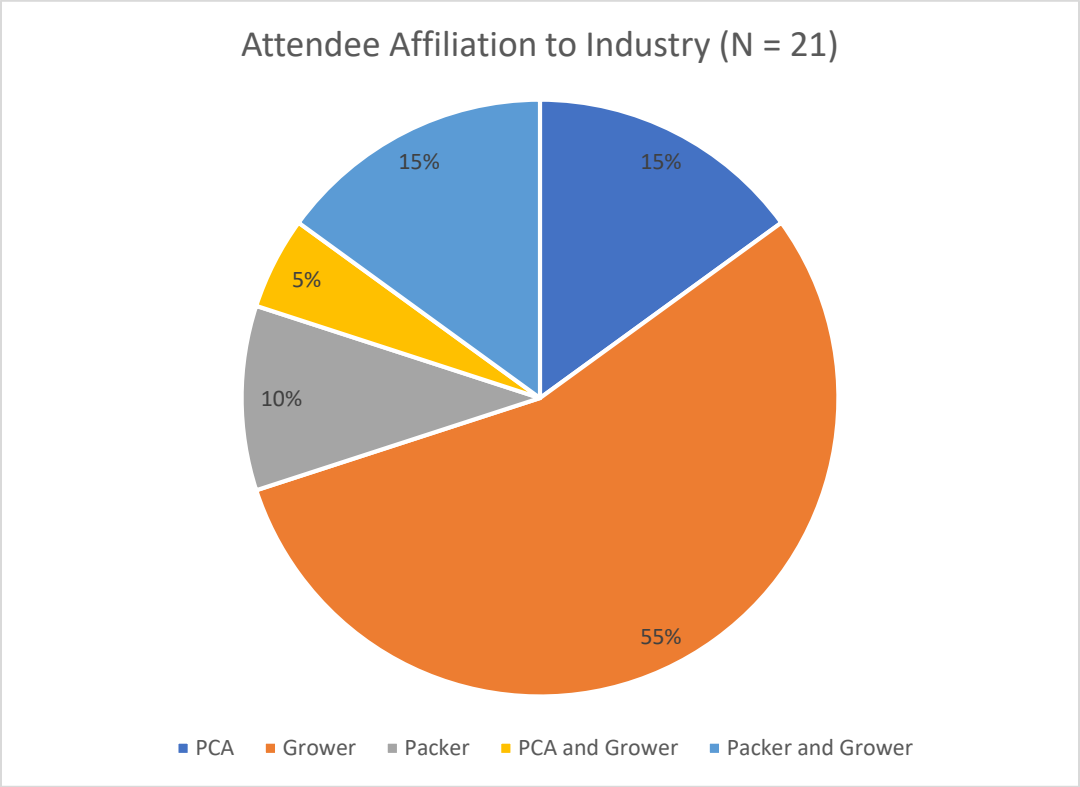
California Cherry Research Review: a report on the "Attendee Survey"

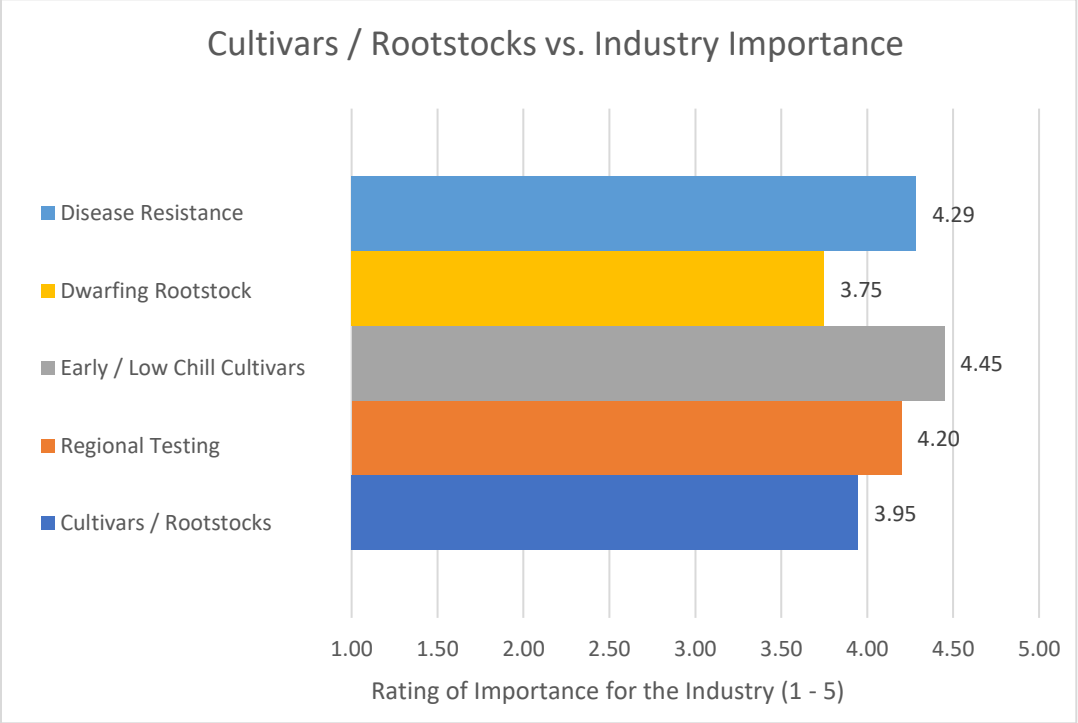
Wednesday, January 16, 2019: 9:00a – 1p

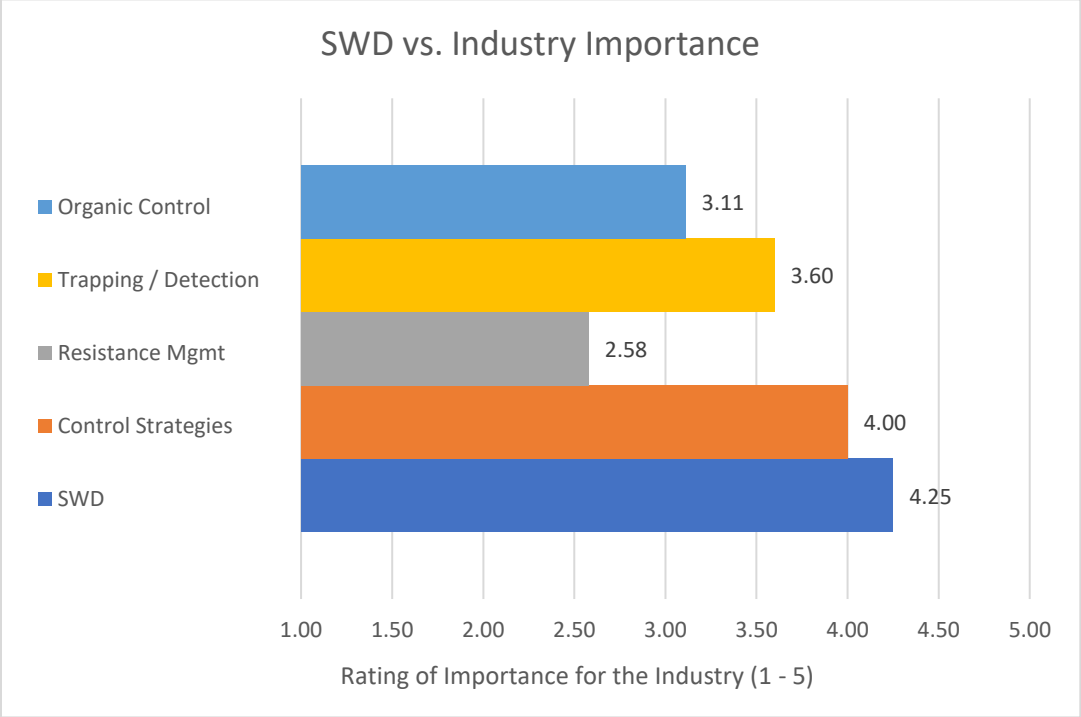
San Joaquin County Agricultural Commissioner
2101 E Earhart Ave #100, Stockton, CA 95206

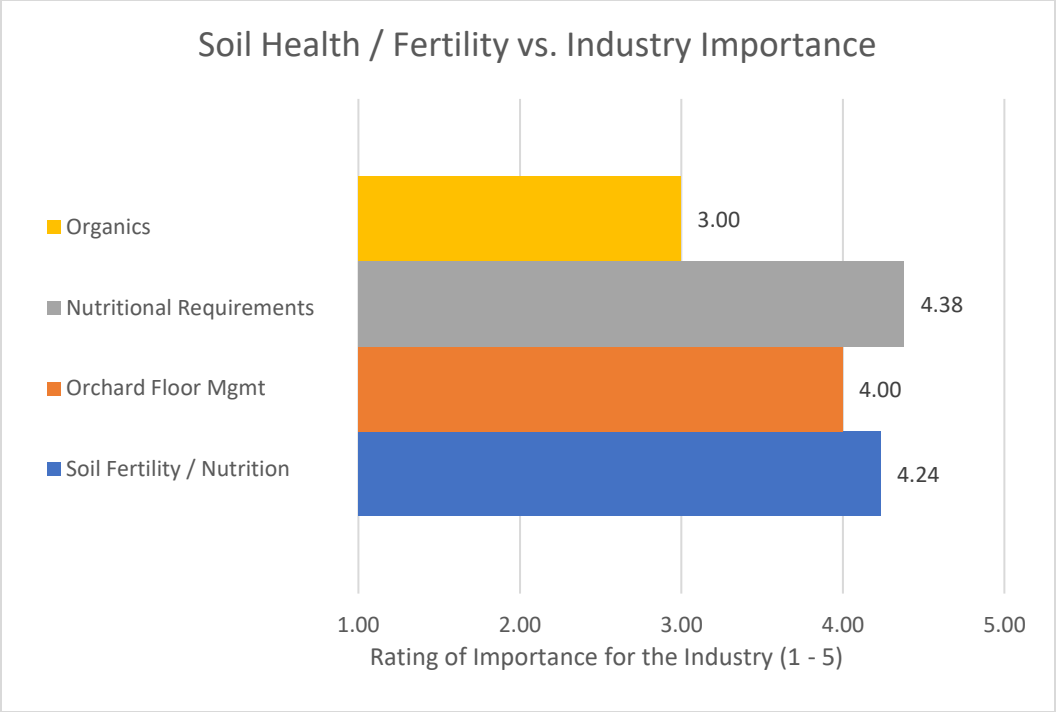
A. Please describe your affiliation with the California sweet cherry industry:

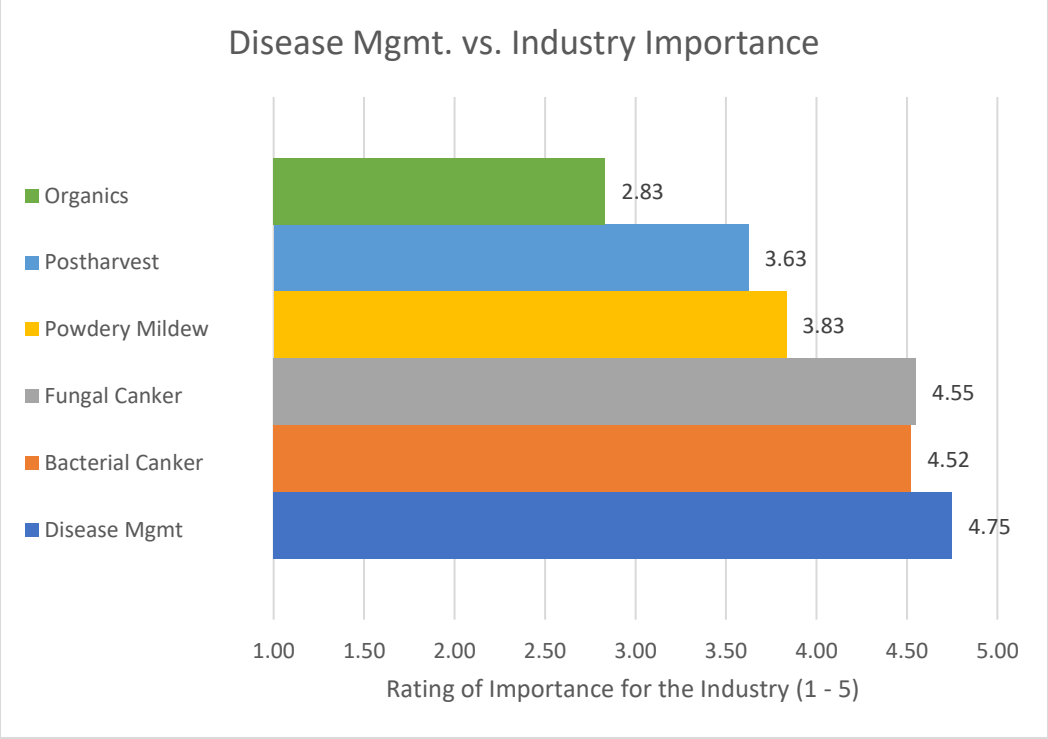


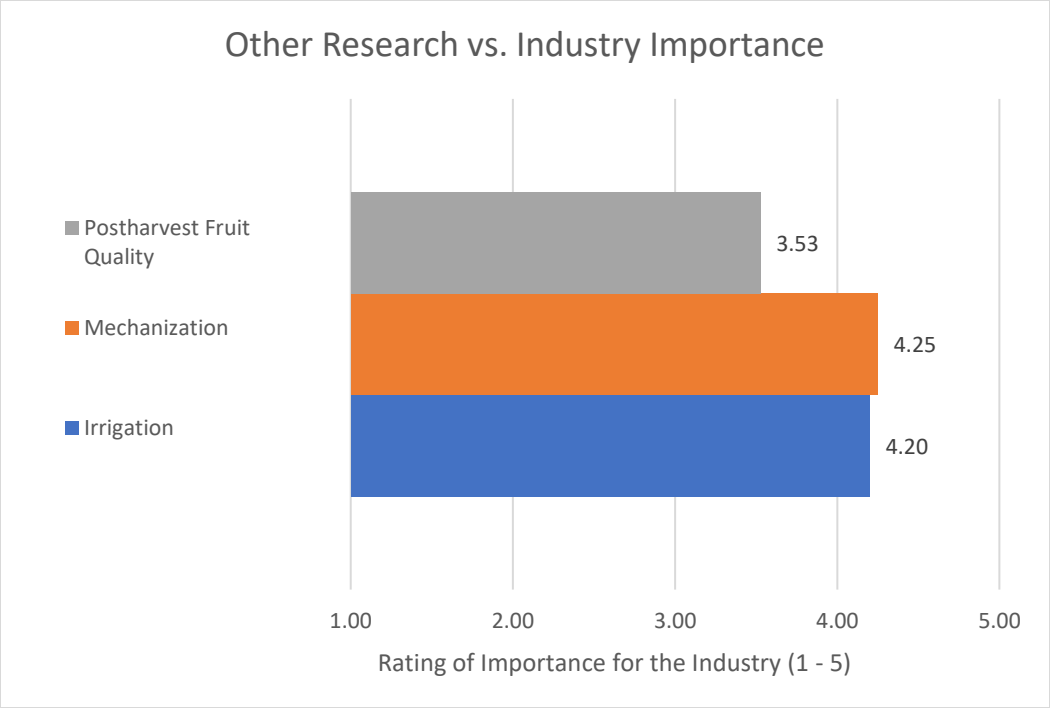
B. Funding research to address the following category and/or sub-category is important for the California sweet cherry industry:






Soil Health / Fertility vs. Industry Importance
Organics
Nutritional Requirements
Orchard Floor Mgmt
Soil Fertility / Nutrition
3.00
4.38
4.00
4.24
1.00
2.00
3.00
4.00
5.00
Rating of Importance for the Industry (1 - 5)


Disease Mgmt. vs. Industry Importance
Organics
Postharvest
Powdery Mildew
Fungal Canker
Bacterial Canker
Disease Mgmt
2.83
3.63
3.83
4.55
4.52
4.75
1.00
1.50
2.00
2.50
3.00
3.50
4.00
4.50
5.00
Rating of Importance for the Industry (1 - 5)



C. Please list any research priorities that are not identified as a category and/or sub-category above and should be considered by the California Cherry Board for funding:

1. Chill requirements and window (start – end); when to use rest breaking products
2. New products and MRLs for existing products
3. Potassium requirements and its association(s) to size and/or quality
4. Stemless cherry promotion and handling
5. Packing house food safety and sanitation

D. The subjects presented on during the California Cherry Research Review were of interest to me: = 3.9/5

E. Any other comments and/or suggestions on how we can improve the California Cherry Research Review

1. More breaks between presentations
2. More CE units
3. Expand the event and make it more comprehensive
4. Include more discussion on postharvest fungicides and pathogens
5. Include presentations on food safety and impending regulations (county representative)

1st 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 Lawrence Sambado in February 2019 and identified three sites at A. Sambado & Sons 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 (38°01'28.7"N 121°12'16.1"W) 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: “Go” block, Micro-sprinkler, Mazzard rootstock, NW-SE orientation, lower tree density (20 x 22 ft) and
- Site 2: “Kahn” 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 (38°00'51.1"N 121°10'41.3"W) of the same commercial farming operation:

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

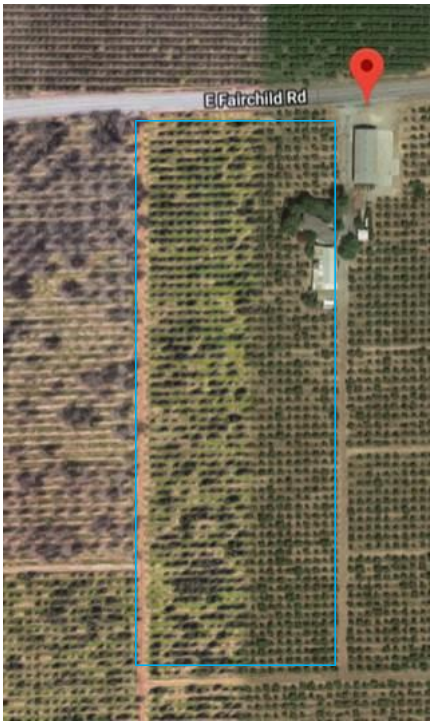


Figure 2. *Site 3*

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. Lawrence Sambado helped us identify a suitable site with well-watered irrigated pasture managed by farmer Mark Lewallen (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.

Currently we are working on land use agreement with the farmer who offered the pasture and will soon proceed with building the fence for the CIMIS measurement station to be installed in a protected area from cows.

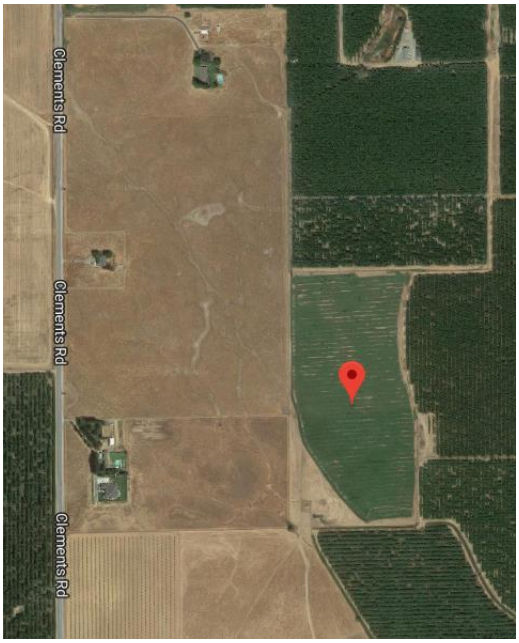


Figure 3. *Site visit for the future CIMIS station*

<u>Evapotranspiration measurements:</u>

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 October 18th, 2019. Most of this period trees are at full canopy and evapotranspiration rates are expected to be near peak. Also, in the fall, it is visible how evapotranspiration values start to decrease. 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 Site 2 "Khan" with 6.8 mm (0.27 in), then Site 1 "Go" with 6.5 mm (0.25 in), while the lowest average daily water use of 6.2 mm (0.24 in) was measured at the Site 3, "Dasso". However, differences among the average daily water use at the study orchards are small and probably reflect the high rate of ET where micro-sprinkler system is used and the grass cover between the rows contributes to the ET (Site 1, "Go") and larger ground area is wetted, while the highest average daily ET rate is at the orchard "Kahn" where the tree density is the highest (Site 2), and the least average daily water use is at the orchard "Dasso" with medium tree density and drip irrigation. The cumulative values of the evapotranspiration for the period May 3rd through September 12th, also show that the most evapotranspiration was recorded at the "Khan" site (34.86 in), then at the "Go" site (33.60 in) and the least at the "Dasso" site (31.67 in). The selected period is for comparison reasons only until September 12th because the last data collection was not performed in one of the three sites due to herbicide application during our last field visit on October 18th. 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. Also, additional study year would be beneficial to account for the 'typical' year, since the year 2019 had abnormally high precipitation values in the spring.

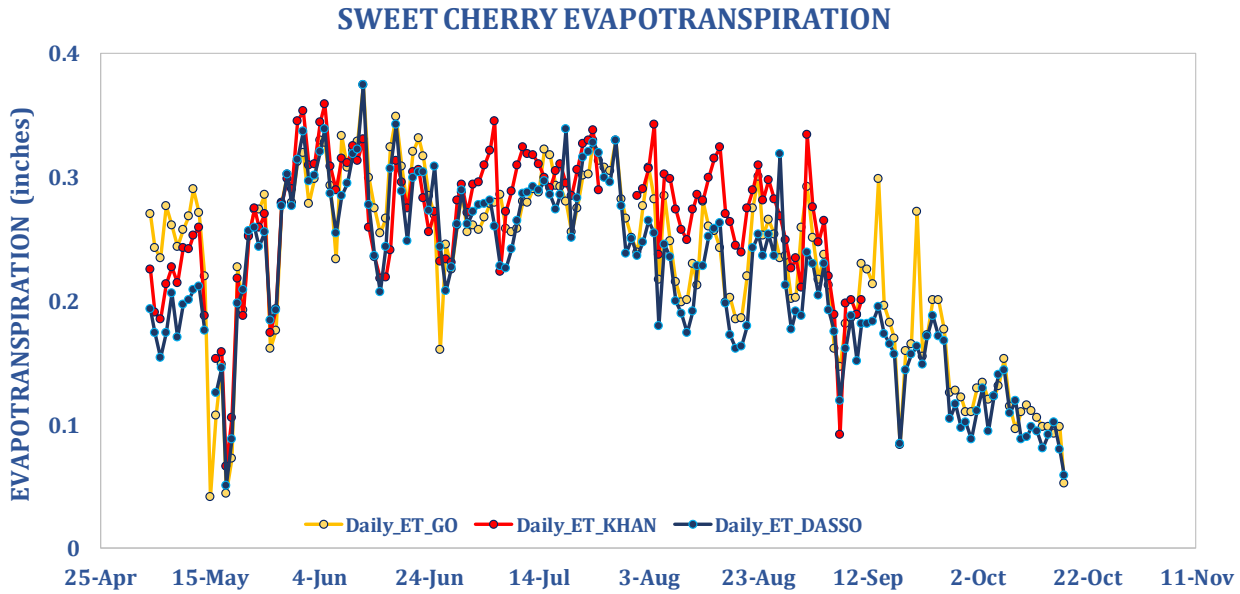


Figure 5. *Daily Evapotranspiration values for the three study orchards in Linden, Ca.*

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).

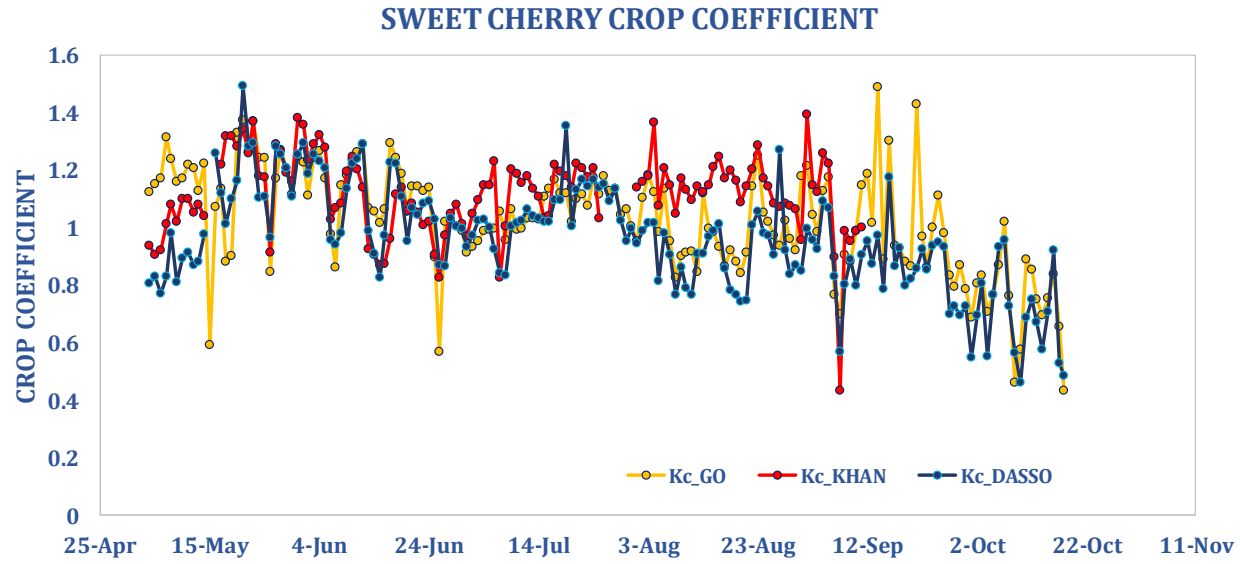


Figure 6. *Crop coefficient values for the three study orchards in Linden, Ca*

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 average values are found to be for “Go” site 1.07, “Khan” 1.12 and “Dasso” 1.01. 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 (“Go”) flowmeter cover 14 trees, which is an area of 6160 ft^2, The site 2 (“Khan”) flowmeter measures the water applied to 30 trees and covers the area of 7680 ft^2 and the site 3 (“Dasso”) flowmeter covers only 9 trees and the area of 3600 ft^2. Therefore the gallons of water read from the flowmeter are not directly comparable. The values in inches of water depth are normalized to the

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

Or the period May 3rd through September 12th cumulative values of applied water were compared to the cumulative evapotranspiration values. The Site 1, "Go" cumulative measured evapotranspiration was 33.60 in, while 25.12 in was measured as applied water; the Site 2, "Khan" cumulative measured evapotranspiration was 34.86 in, while 37.65 in was measured as applied water; and Site 3, "Dasso" cumulative evapotranspiration was 31.67 in while 34.65 was applied water. We will assure the functionality of the Site's "Go" flowmeter and monitor if the evapotranspiration values match better the applied water.

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, "Go"	Site 2 "Kahn"	Site 3, "Dasso"
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] %
"Go" 1	2.58	0.187	1.65
"Go" 2	2.51	0.183	1.27
"Khan" 1	2.40	0.306	1.78
"Khan" 2	2.26	0.317	1.61
"Dasso" 1	2.28	0.287	1.50
"Dasso" 2	2.33	0.298	1.84

Table 5. Leaf tissue analysis continued – blues values are to mark lower than the recommended 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
"Go" 1	1440	74.3	1.91	0.906	16.6	51.5	74.1	6.5
"Go" 2	1420	74.1	2.18	1.059	18.1	62.3	65.0	8.7
"Khan" 1	1450	97.0	2.38	0.606	37.4	70.8	82.1	9.5
"Khan" 2	1350	94.3	2.40	0.628	32.9	59.6	78.7	10.4
"Dasso" 1	1340	86.4	2.20	0.629	16.1	58.8	86.0	7.7
"Dasso" 2	1330	92.6	2.13	0.574	18.6	61.8	73.7	7.5

Yield

Since the precipitation amounts in the spring of the current year are higher than the long-term average, cherries at the Site 1, "Go", 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). Therefore, we have not collected the data on fruit yiled quantity and quality, since it would not be representative of a 'typical' year. This task should be performed during the next study year.

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

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

Spotted Wing Drosophila 2019 Progress Report

Project Title: Engineered transgenic *Drosophila suzukii* for wild population suppression and eradication.

Principal Investigator
Omar S. Akbari, University of California, San Diego

OBJECTIVES (2019)

Objective A- *Medea*-based drive system:

Previously, we have described a functional population replacement system in *D. suzukii* termed *Medea*, and have shown that it is capable of working in diverse genetic backgrounds and of maintaining itself at high frequencies in a population. We have now molecularly developed a second-generation optimized *Medea* system that can spread to fixation quickly and can be used to replace the first population should a recall ever be necessary, and are working on testing this system in flies (to be completed within a year). We are also working, in collaboration with the Montell Lab at UCSB, to test effector genes capable of bringing about conditional lethality in *D. melanogaster*, which should also be completed within a year. Once such genes are characterized, we can then link them with our optimized *Medea* system to generate a fully functional gene drive system capable of population suppression.

Objective B- CRISPR/Cas9-based drives:

Y-drive: We are also working on engineering a second type of suppression system termed Y-drive that relies on CRISPR/Cas9 to bias sex ratios by shredding the X chromosome, leading to an all-male population crash. We have already developed several components required to generate Y-drive: we have engineered multiple Cas9 strains, optimized transgenic gRNA designs, demonstrated efficient CRISPR/Cas9 function in *D. suzukii*, and developed a method to dock transgenes on the Y chromosome of flies[16]. We have also been testing X chromosome-targeting gRNAs in *D. melanogaster*. We now plan to combine these components to attempt to generate *D. suzukii* gRNA transgenes capable of shredding the X chromosome, and to express said transgenes from the Y chromosome.

Homing drive: We propose to engineer a Cas-9-mediated suppression homing drive. This system has a self-replicating (i.e., homing) Cas9-based transgene that targets a region within a gene necessary for female fertility, which over time would facilitate the sterilization of all females in a target population thereby resulting in a population collapse. To engineer a Cas9-mediated suppression homing drive, we need to introduce the coding sequence for Cas9 and gRNA into the genomic site targeted by the Cas9/gRNAs to generate a self-replicating transgene that could continuously mutate a target gene every generation and/or carry a transgene into the population. We have identified and characterized several promising candidate target genes, including *dsx*, *tra*, and *sxl*, which are all essential for female development in fruit flies.

Objective C- pgSIT:

We propose to create a precision guided sterile insect technique SIT (pgSIT) as a new, genetic-based methods 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) (Figure 9). 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.

PROGRESS TO DATE

I. Objective A - *Medea*-based drive system

Previously, we had developed the first D. suzukii functional replacement gene drive system termed Medea, had rigorously tested it in laboratory cage populations, and had characterized it in different genetic backgrounds to determine effectiveness and fecundity (our results on this project were published in PNAS last year). 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 indicated that our *Medea* drive system could spread to fixation if either its fitness costs or toxin resistance were reduced, we have developed a modified version of this same system that should obviate the specific resistance that we observed. We are currently finishing the crosses to confirm reduced resistance in the new design. We have also developed a second-generation *Medea* system in *D. suzukii* 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. Finally, we have identified several promising putative cargo genes that could be spread with the *Medea* gene drive to cause population suppression, and have started testing them in *D. suzukii*.

Summary of Objective A:

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.

B. 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.

C. We have identified several promising putative cargo genes that could be spread with the *Medea* gene drive to cause population suppression. To achieve this, we are working to leverage data from the Montell lab (UCSD), which is developing this technology for mosquito control. The Montell lab is currently testing several 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*

II. Objective B- CRISPR/Cas9-based drives

CRISPR/Cas9 technology has great applicability to the development of genetic pest management approaches, and can be used to build various gene drives - including Y-chromosome drive and Cas9-mediated homing-based drive - that can be employed to suppress and eliminate pest populations. We have made significant progress in developing the tools needed to engineer both of these types of gene drives in *D. suzukii*. Specifically, we have developed and characterized multiple Cas9 transgenes in *D. suzukii* that are highly functional and enable efficient Cas9-mediated mutagenesis in this pest. We have also developed several ways to efficiently express gRNAs from the *D. suzukii* genome. Together, these tools enable efficient CRISPR/Cas9-based manipulations of the *D. suzukii* genome, and provide the basis for building Cas9-based gene drives. Furthermore, we have developed/optimized several components needed to build Y-gene drive, including identifying *D. suzukii* X and Y chromosome regions, identifying putative X chromosome specific target sites, and efficiently engineering the Y chromosome of flies. Additionally, we have also taken steps towards engineering Cas9-based suppression gene drive, including: identifying promising candidate genes to be targeted by this drive; finding *D. suzukii* homologues of, and selected suitable gRNA target sites within, these genes; designing gRNA-expressing transgenes to test our ability to target these genes; and building a proof of principle Cas9-based homing system in the white gene to test its ability to self-replicate. We can now begin putting these components together to generate functional suppression gene drives in *D.suzukii*.

A. Last year, we developed and characterized multiple Cas9 transgenes in *D. suzukii* that are highly functional and enable efficient Cas9-mediated mutagenesis. So far this year, we have expanded and optimized multiple Cas9 expression lines with female germline specific promoters (*BicC* and *Dhd*), male and female germline specific promoters (*vasa* and *nanos*) or strong full body expression (*ubiq*).
B. Last year, we developed several ways to efficiently express gRNAs from the *D. suzukii* genome and we have used these systems to build and evaluate multiple gRNA expression systems. Now that we have a highly functional gRNA expression configuration, we have started to clone X chromosome-targeting gRNAs into our gRNA expression cassettes and test them.
C. Previously, we developed/optimized several components needed to build Y-gene drive including identifying X and Y chromosome regions, putative X chromosome specific target sites and we have efficiently engineered the Y chromosome of flies. We are now testing whether we can reliably insert, and detect expression from, Cas9-containing transgenes at these same Y chromosome locations, as we will need to be able to express Cas9 cassettes from the Y in order for the Y gene drive approach to work.

D. We have developed/optimized several components needed to build Cas9-based suppression gene drive, including:

1. Building a proof of principle Cas9-based homing system in the *white* gene to test its ability to self-replicate. Specifically, we demonstrated that a genetically encoded, PolIII U6:3 promoter-driven gRNA targeting *white* produces up to 100% mutated (white and mosaic-eyed) progeny when crossed to a Cas9 expressing line. These experiments allowed us to determine whether we can dock transgenes in a site-specific location using CRISPR/Cas9 and observe the efficiency of self-replication/homing of this Cas9-based transgene in *D. suzukii*.

2. We have designed and generated multiple gRNA-expressing transgenes targeting one or more essential genes (threshold dependent split drive) and dual gRNA and Cas9 expression lines (full drive). We are expanding these lines and have started testing their drive capabilities in small population studies. Our preliminary full and split suppression drives targeting the *doublesex* (*dsx*) gene have a reduced female to male ratio, but not all females are eliminated from the population; however, the viable females were all intersex and sterile, so these results may still be useful for a suppression. We are testing these lines further to determine the reproducibility of these results and we plan to optimize these lines and our others to achieve the female killing phenotype.

III. Objective C- pgSIT

In order to construct a pgSIT system, we previously created functional Cas9 tools (including gRNA lines that target genes essential for female viability and male sterility and Cas9 expressing lines) in *D. suzukii*. 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 chose to evaluate whether targeting these same genes in *D. suzukii* would have a similar result. Specifically, we are building constructs to disrupt female viability by 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. 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 constructed double-gRNA transgenes targeting each candidate gene, and have begun generating and testing *D. suzukii* lines harboring these transgenes. Each of these lines are being crossed separately to our Cas9 strains to see whether the combinations of Cas9+gRNA will produce female lethality and male sterility. As soon as we identify the gRNA sets that produce the desired phenotypes, we can proceed to combine best sets of gRNAs to produce a single transgene that crossed with a Cas9 strain kills female and sterilize male progeny.

A. Designed (>20) and injected (>10) constructs that express gRNAs targeting the female viability genes and *beta tubulin* (***β*-*tub***), a male fertility gene. We are expanding these lines and will test them in crosses to multiple Cas9 expression lines to determine the most efficient gRNA and Cas9 line combinations to generate sterile male progeny.
B. Established six transgenic gRNA lines targeting both *sxl* and ***β*-*tub*** simultaneously; Exciting preliminary results indicate that there are **no female transheterozygote progeny** from gRNA and vasa-Cas9 lines crosses (Table 1), which indicates females that inherit these transgenes are killed as we expected!!!! Additionally, when the transheterzygote progeny were crossed with wildtype (WT) females, as expected they **produced no viable progeny** (Table 2). Taken together, these data strongly indicate that

we may **have a functioning pgSIT system in *D. suzukii***, however we need to continue to assess these lines in more replicates and also expand and homozygose these lines and measure fitness and mating competitiveness.

Table 1	**1056H L.3 ♂ x vas-Cas9 ♀**	**1056J L.2 ♂ x vas-Cas9 ♀**	**1056J L.3 ♂ x vas-Cas9 ♀**
WT M	36	16	34
WT F	-	-	-
Inherited 1056 M	3	12	12
Inherited 1056 F	-	-	-
Inherited Cas9 M	77	63	62
Inherited Cas9 F	-	-	-
Transhet Females	0	0	0
Intersex	2	0	3
Transhet Males	**23**	**42**	**9**
n=	141	133	120

Table 1. Preliminary results from vasa-Cas9 and *sxl* and *β-tub* gRNA line crosses demonstrate female killing. The table depicts the number of resulting progeny of crosses between three different gRNA lines that simultaneously target *sxl* and *β-tub* (genes required for female viability and male fertility, respectively) and a vasa-Cas9 line. The highlighted row shows that no transheterozygous (transhet) females were generated from these crosses demonstrating that females inheriting both transgenes are efficiently killed.

Parental Genotype		**Replicate 1**			
		Genetic Cross			
Female	***Male***	***# of WT Females***	***# of transhet Males***	***Embryo Count***	***Emerged***
WT	**1056H L.3 ; vasa-Cas9**	15	5	385	0
WT	**1056J L.2 ; vasa-Cas9**	15	5	364	0
WT	**1056J L.3 ; vasa-Cas9**	15	5	in progress	in progress
WT	**1056J L.5 ; vasa-Cas9**	15	5	in progress	in progress
WT	**1056K L.1 ; vasa-Cas9**	15	5	194	0
WT	**1056K L.3 ; vasa-Cas9**	15	5	279	0

Table 2. Preliminary results from crosses between male transheterozygote progeny and wildtype (WT) females demonstrate male sterility. The transheterozygote male progeny generated from crosses between the gRNA and Cas9 lines (e.g. Table 1) were crossed to WT females to assess male fertility. No males resulted from these crosses (highlighted column) indicating that the male progeny are sterile.

Final report 2019

Project Title: INVESTIGATING THE CAUSE OF SUDDEN DECLINE OF SWEET CHERRY IN CALIFORNIA

Project leader: Florent Trouillas, Assistant C.E. Specialist, Dept. of Plant Pathology, UC Davis, flotrouillas@ucanr.edu

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Interpretive summary
During the springs and summers of 2015 and 2016, cherry orchards expressing symptoms of sudden decline were detected in Fresno, Kern, Kings and San Joaquin Counties, the main sweet cherry producing counties in California. Symptoms included wilting of the entire tree followed by tree death. Comprehensive examinations of affected trees revealed reduced amounts of fine roots as well as root rot, suggesting that the causal agent(s) may be soil-borne. Isolations from diseased fine roots revealed the occurrence of common soil-borne fungal pathogens from the genera *Fusarium*, *Ilyonectria* (synonym: *Cylindrocarpon*), *Macrophomina* and *Neocosmospora*. Morphological and molecular identifications refined identification of these pathogens to the species *Fusarium oxysporum*, *Neocosmospora solani* (syn. *Fusarium solani*), *Ilyonectria liriodendri* (syn. *Cylindrocarpon liriodendri*) and *Macrophomina phaseolina*. These fungi are known soil-borne pathogens of other crops in California, including Charcoal rot of strawberry and Black foot disease of grapevines. This is, however, the first report of *Macrophomina phaseolina* affecting sweet cherry in California. One-year long pathogenicity assays were conducted in duplicated experiments in a lath-house using three important cherry rootstocks, Mahaleb, Colt and Krimsk5, to determine the pathogenicity of these fungal species. Our results indicated that all fungal species tested could cause root rot in these cherry rootstocks, with *Macrophomina phaseolina* being the most aggressive pathogen. Rootstock inoculations using a mixture of the four fungal species revealed synergistic effects, exacerbating rootstock mortality and reducing plant growth. Not all three rootstocks reacted similarly to all pathogens, suggesting differential rootstock tolerance to specific pathogens. Some rootstocks may be better suited to local pathogens, potentially attenuating tree mortality as a result of this emerging disease.

Objective 1: Surveys of sweet cherry orchards affected by sudden decline and identification of the causal agents

Material and methods

Surveys and sampling of cherry orchards with symptoms of sudden decline were conducted during three growing seasons from 2015 to 2017 in collaboration with UCCE farm advisors and Pest Control Advisors. As a result, we identified multiple orchards expressing sudden decline in Fresno, Kern, Kings and San Joaquin Counties. Affected trees were backhoed to examine their root systems and roots showing symptoms of root rot and necrosis were collected

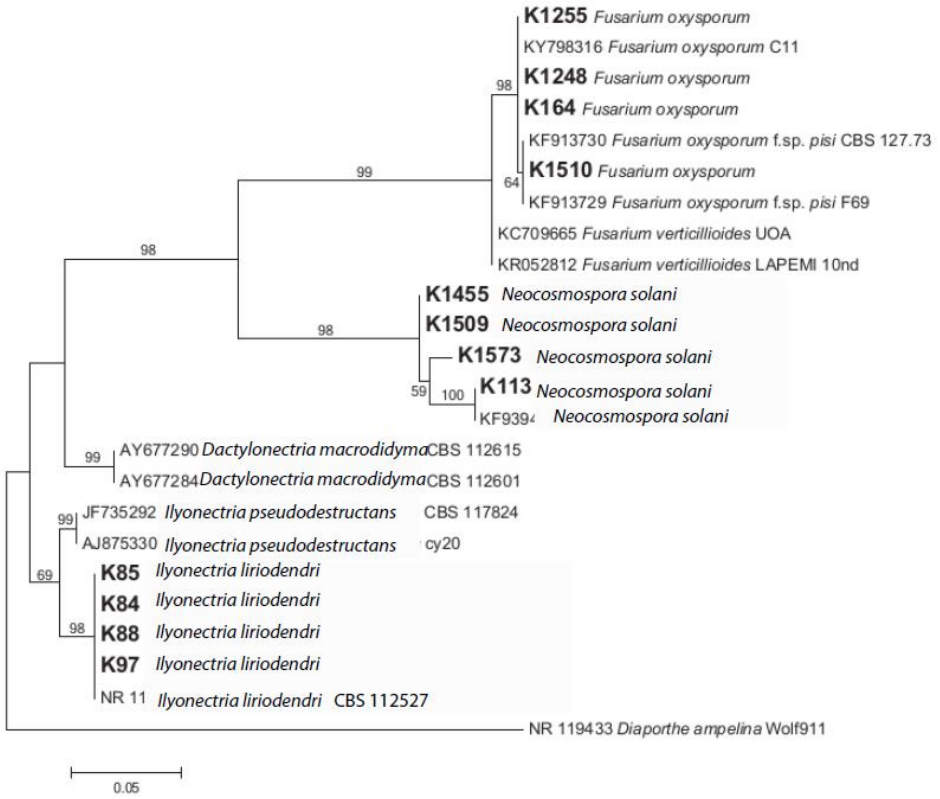
and taken to the laboratory for microbiological isolations. Pieces of roots were surface disinfested by immersion for 2 mins in a 1.5 % sodium hypochlorite solution and rinsed twice with sterile distilled water. Small pieces from the margin between healthy and necrotic tissues were placed onto Petri dishes filled with potato dextrose agar (PDA) amended with 100 ppm tetracycline (PDA-tet) for isolation of fungi, the suspected causal agents.

Pure cultures of fungal colonies were established, and their DNA was extracted. Molecular refinement of pathogen identification was performed through PCR amplification and sequencing of the internal transcribed spacer region (ITS) of the ribosomal DNA using primers ITS1 and ITS4. The ITS sequences generated were compared to DNA sequences present in public databases using the nucleotide query algorithms BLAST in GenBank. Phylogenetic analyses were furthered used for unequivocal taxonomic placement among fungal members of related species.

Results and Discussion

Isolations from diseased roots consistently yielded fungal colonies with morphological characteristics of the genera *Fusarium*, *Neocosmospora* (synonym *Fusarium*), *Ilyonectria* (synonym *Cylindrocarpon*) and *Macrophomina*. Phylogenetic placement of representative isolates revealed that a large proportion of fungal pathogens belonged to the species *Fusarium oxysporum*, *Neocosmospora solani* (synonym *Fusarium solani*), and *Ilyonectria liriodendri* (**Figure 1**).

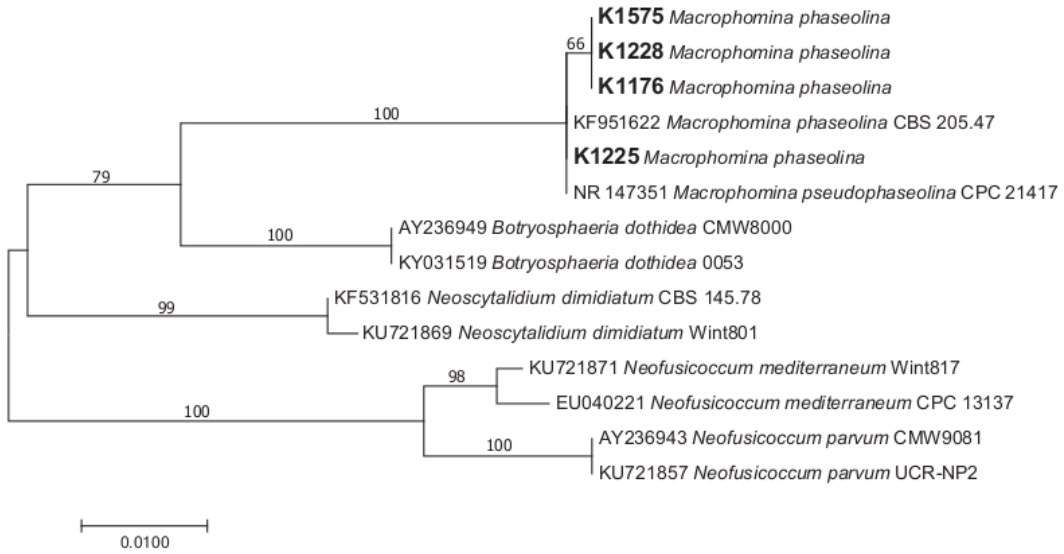
Figure 1. Phylogenetic analysis of ITS sequences of representative isolates (in bold) of the genera *Fusarium*, *Ilyonectira* and *Neocosmospora* recovered from diseased cherry roots. Isolates belong to the species *Fusarium oxysporum*, *Ilyonectria liriodendri*, and *Neocosmospora solani*.



Fusarium oxysporum was recently shown to be responsible for a root and crown rot disease of sweet cherry in British Columbia; it is very likely that this species and the related species *Neocosmospora solani* are also causing root damage to cherry trees in California. *Ilyonectria* (syn. *Cylindrocarpon*) species have been associated with Black foot disease of grapevine in California, with roots of symptomatic grapevines showing black, sunken, necrotic lesions. Grape leaves appear scorched and the stunting and decline of the entire vine frequently follows. *Ilyonectria liriodendri* role in sudden cherry decline is plausible and under investigation.

Another large proportion of fungal isolates belonged to the genus *Macrophomina* from the family Botryosphaeriaceae, which is known to include aggressive pathogens of perennial plants. Phylogenetic analyses revealed that these isolates belonged to the species *Macrophomina phaseolina* (**Figure 2**).

Figure 2. Phylogenetic analysis of ITS sequences of representative isolates (in bold) of the genus *Macrophomina* recovered from diseased cherry roots. Isolates belong to the species *Macrophomina phaseolina*.



Macrophomina phaseolina is the causal agent of Charcoal rot of strawberry roots in California. It is also known to cause root rot in other crops such as corn, cotton, sunflower, potato and sorghum. In California, charcoal rot appears to be the most important current concern for the strawberry industry due to its steady increase over the past 10 years (Koike 2008). Every year additional new fields are infested, and the disease has now been found in all of the major strawberry producing counties in the state. The fungus is known to produce microsclerotia, survival structures below ground, which production increases under low water potentials that occurs during drought. Despite little reports of *Macrophomina phaseolina* affecting perennial woody crops, we have isolated it recently also from declining table grapes and pistachio trees in California. The role of *Macrophomina phaseolina* in root diseases of perennial crops is under further investigation.

Objective 2: Determine the pathogenicity of the isolated fungi in cherry rootstocks

Material and methods

A first pathogenicity assay using the putative pathogens was initiated in November 2017 and the pathogenicity assay was repeated in April 2018. We selected three commercially important cherry rootstocks: Mahaleb Performer, Colt and Krimsk5. Some of the surveyed orchards with sudden decline symptoms were grafted onto these rootstocks. Pathogenicity tests were initiated to perform Koch's postulates and to establish a causal relationship between the various fungi and the sudden decline of sweet cherry. Four fungal species recovered from the roots of diseased cherry trees were used for pathogenicity assays (**Table 1**).

Table 1. Isolates used in pathogenicity studies.

Isolate	Genus	species	Host	Rootstock	Isolation date	Location
K 84	Ilyonectria	liriodendri	Cherry	Colt	4/1/2015	Reedley
K 85	Ilyonectria	liriodendri	Cherry	Colt	4/1/2015	Reedley
K 88	Ilyonectria	liriodendri	Cherry	Colt	4/1/2015	Reedley
K 97	Ilyonectria	liriodendri	Cherry	Colt	4/1/2015	Reedley
K 164	Fusarium	oxysporum	Cherry	Colt	4/1/2015	Reedley
K1248	Fusarium	oxysporum	Cherry	Mahaleb	6/17/2016	San Joaquin County
K1255	Fusarium	oxysporum	Cherry	Krymsk5	6/17/2016	San Joaquin County
K1510	Fusarium	oxysporum	Cherry	Mahaleb	8/12/2016	Brentwood
K 113	Fusarium	solani	Cherry	NA	4/10/2015	Reedley
K1455	Fusarium	solani	Cherry	Mahaleb	6/17/2016	San Joaquin County
K1509	Fusarium	solani	Cherry	Mahaleb	8/12/2016	Brentwood
K1573	Fusarium	solani	Cherry	Maxma14	10/6/2016	Fresno
K1176	Macrophomina	phaseolina	Cherry	Mahaleb	6/17/2016	San Joaquin County
K1225	Macrophomina	phaseolina	Cherry	Krymsk5	6/17/2016	San Joaquin County
K1228	Macrophomina	phaseolina	Cherry	Mahaleb	6/17/2016	San Joaquin County
K1575	Macrophomina	phaseolina	Cherry	Maxma14	10/6/2026	Fresno

For each species, a mixture of four isolates was used as inoculum. In addition, a known and aggressive pathogen of cherry trees (*Phytophthora cambivora*) was used as a positive control. The plant growth media (UC potting mix) was inoculated either using millet seeds (ascomycetes) or sand-bran (*P. cambivora*). Sterile water (125mL) was added to millet seeds (250g) in 1 L flasks and steeped for 12 hours. Flasks were autoclaved at 120C for 20 min 24 h apart. Autoclaved millet seeds were inoculated with 10, 5-mm diameter mycelial plugs from 10-day-old PDA cultures of each isolate (2 flasks per isolate, 8 flasks per ascomycete species) and incubated for 14 days in the dark at 25C and shaken every other day. Control bottles were inoculated with plugs of PDA only. The sand-bran inoculum of *P. cambivora* consisted of 200g of sterile river sand, 20 g of wheat bran and 30 mL of sterile water in Schott bottles autoclaved at 120C for 20 min 24 h apart. Each bottle was inoculated with 10, 5-mm mycelial plugs from a 10-day-old *P. cambivora* CMA (Corn Meal Agar) whereas control bottles were inoculated with 10, 5-mm uncolonized CMA plugs. Bottles were incubated for 14 days in the dark at 25C and shaken every other day. Millet seed and sand-bran inoculum were added at a rate of 10% (v/v) in the plant growth media, using pots of 880 ml (MT38BT; Stuewe and Sons, Tangent, OR) held in trays.

One-year-old cherry rootstock plants were obtained from a commercial nursery in California and grown in the greenhouse for eight weeks before inoculations. The pathogenicity of each fungal species was evaluated using nine replicates per assay, each replicate corresponding to a tree in a single pot.

The experimental design consisted of 7 treatments: 4 fungal species each corresponding to 4 treatments, a treatment consisting of the four suspected fungal species mixed in equal proportions into the soil, a positive control consisting of *Phytophthora cambivora* inoculum, and a negative control consisting of sterile soil. There were 9 plant replicates per treatment per rootstock with 3 rootstocks tested. The experiment was repeated once; hence we examined a total of 378 plants (7 treatments x 9 plants x 3 rootstocks x 2 experiments = 378 trees).

The experiment was set up as a complete random block design. Inoculated trees were grown in a lath-house at the Plant Pathology field experiment station in Davis. Inoculated trees were examined after a 12 months incubation period, in November 2018 for the first assay and April 2019 for the second assay.

After 12 months in the lath-house, each inoculated tree was examined visually for symptoms of sudden decline resembling those observed in affected orchards, namely decline, wilting of leaves and mortality. The diameter of each tree stem was measured with a caliper and the weight of the aerial part of each tree (i.e., top fresh weight) was measured with a digital scale. Following these measurements, the root system of each tree was washed under running water to remove the soil and placed in a hermetic plastic bag to maintain moisture and prevent desiccation before being taken to the laboratory. Root mass fresh weight was then measured, and percent root rot was visually estimated. In order to prove that the observed root rot was caused by the inoculated fungi, pieces of roots were plated onto PDA as previously described and the identity of fungal colonies emerging on cultures were confirm based on colony morphologies.

A second set of measures was taken using the software WinRhizo Pro, allowing the automated measurements of a set of root parameters based on photographs of each root system taken with a scanner. To this aim, each root system was cut into smaller independent sections with scissors to avoid the clustering and overlapping of roots and all root sections from a single plant were placed into a plastic tray containing water, as illustrated in **Figure 3**. The tray was placed on a scanner connected to a desktop computer with the program WinRhizo Pro running.

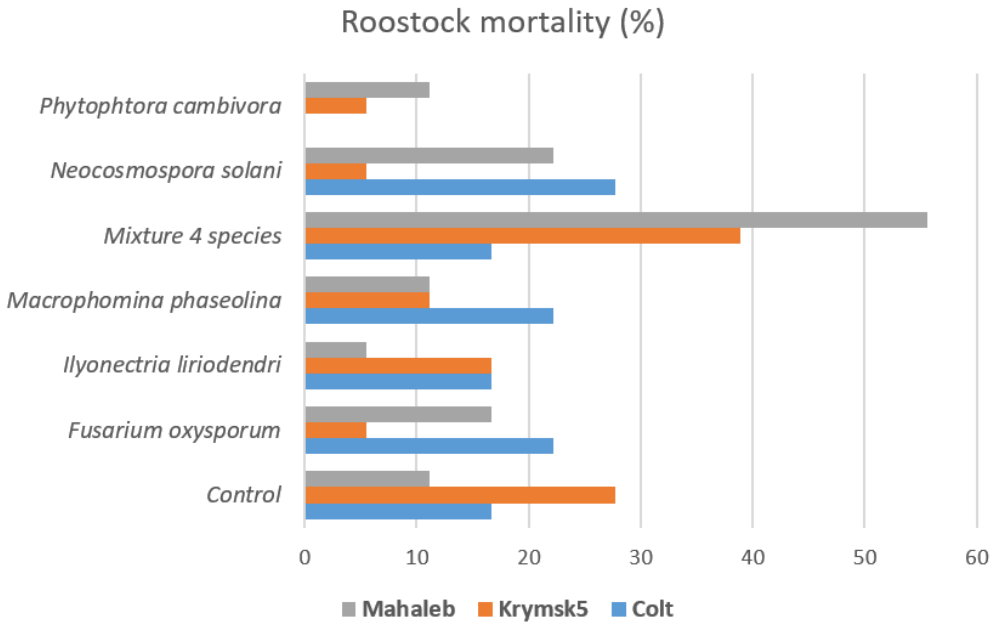
Figure 3. Image analyses of root systems using the program WinRhizo Pro.

This automated procedure allowed the estimation of the total length of each root system as well as the total length of the fine root systems (roots with a diameter < 0.3mm), as we suspected the degradation of fine roots by fungi to be a potential cause of the sudden decline symptoms observed in the field. To assess the effect of treatment on each measured variable (% root rot, top fresh weight, stem diameter, root weight, total root length and total fine root length), linear mixed models were performed using the MIXED procedure in SAS. Mean comparison with control treatment was performed using a Dunnett test to evaluate the effect of each treatment on each variable, relative to the negative control treatment (non-inoculated trees).

Results and Discussion

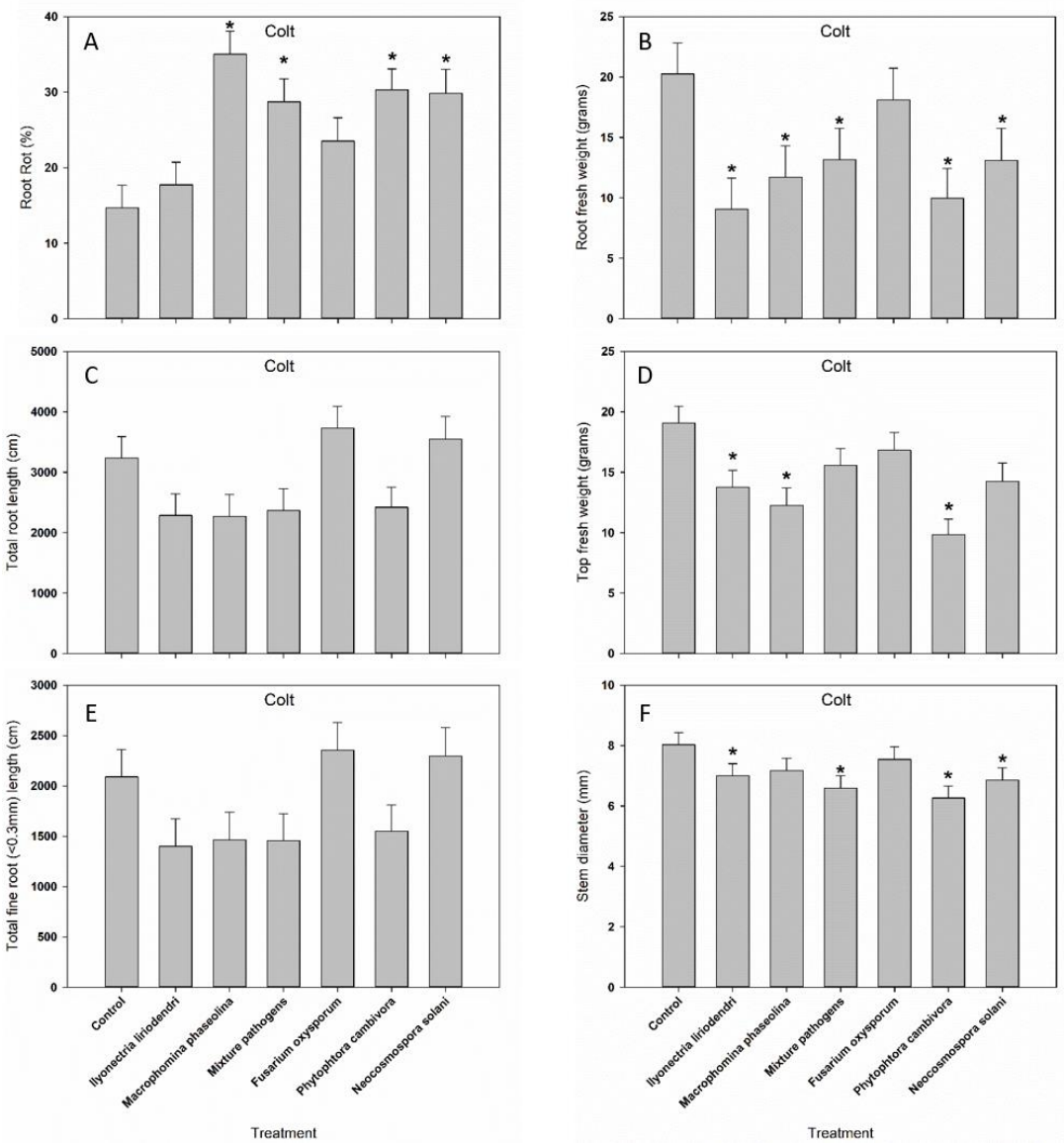
After a one-year incubation period in the lath-house, mortality was highest for cherry rootstock plants inoculated with the mixture of four pathogens (*Fusarium oxysporum*, *Ilyonectria liriodendri*, *Neocosmospora solani* and *Macrophomina phaseolina*) with a mortality rate of 37% averaged across rootstocks and across the two experiments. The maximal percent mortality was 56% for the Mahaleb plants inoculated with this combination of pathogens. In comparison, mortality for the control, non-inoculated plants averaged 18% across rootstocks. The mortality rate for rootstock plants inoculated with individual pathogens ranged from 5 to 18% (**Figure 4**). Symptoms observed on the dead/dying plants included wilting and desiccation of the aerial parts, like the symptoms observed in orchards affected by sudden decline. However, the same symptoms were also observed on the non-inoculated, control plants that were dead and thus prevented drawing a firm link between inoculation treatment and symptoms. The only striking observation was the higher mortality rate observed in the rootstock plants inoculated with a mixture of pathogens, suggesting that a combination of fungal pathogens amended within the soil of potted rootstocks exacerbated mortality, relative to the other treatments.

Figure 4. Percent mortality of plants after a one year incubation period in the lath-house after each inoculation treatment. For each rootstock/treatment combination, values are based on 18 plants.



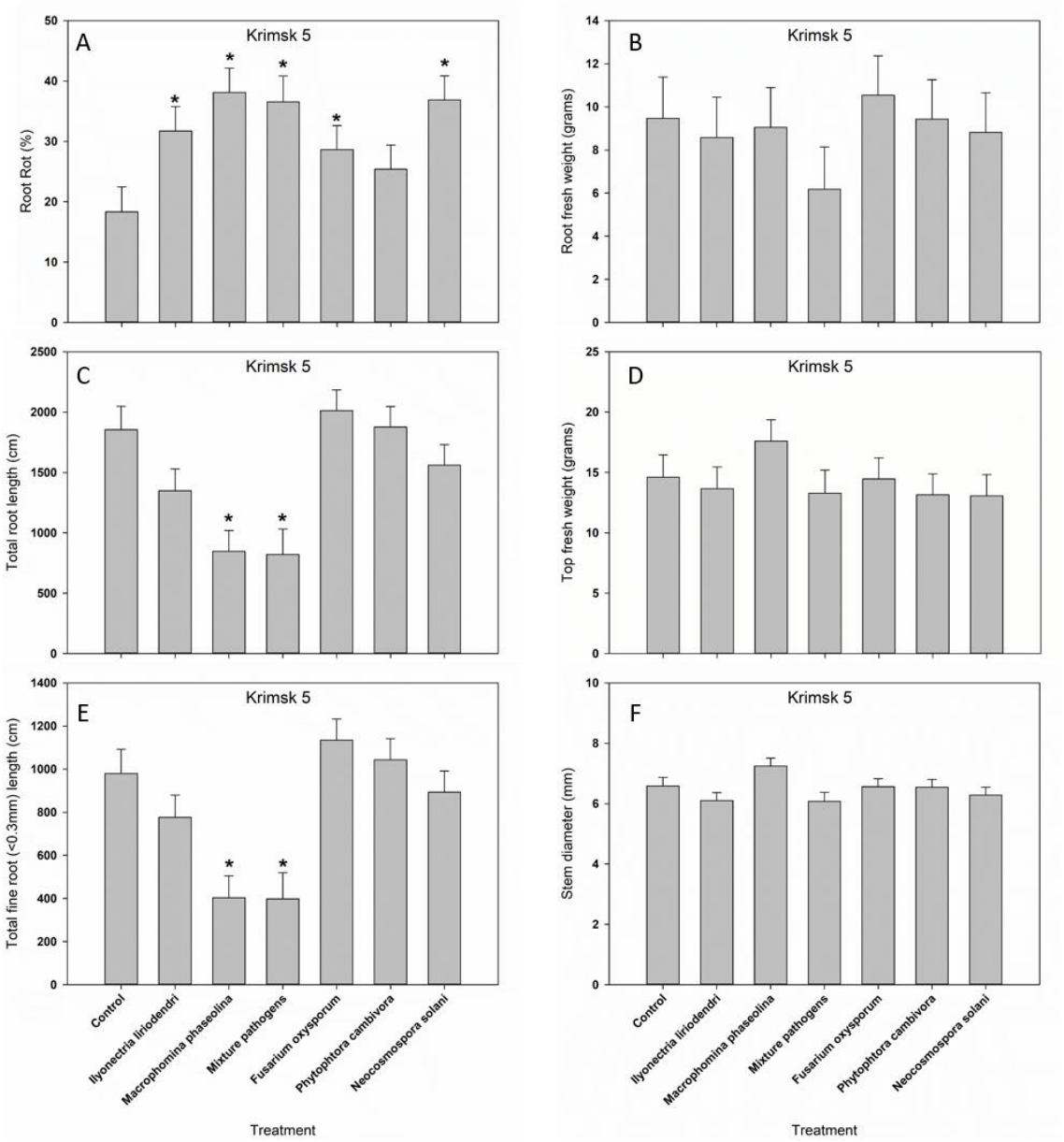
Low levels of root rot (15%) developed in non-inoculated, control Colt rootstock plants. Colt plants inoculated with *M. phaseolina*, a mixture of four pathogens, *P. cambivora* and *N. solani* developed the highest amount of root rot (from 29 to 35%) and these values were significantly higher than in the control treatment *(P* < 0.003; **Figure 5**). While there were no significant differences in total root length or total fine root length between the control and fungal treatments, there was a consistent trend in reduced root weight, top fresh weight and stem diameter for Colt rootstock plants inoculated with *I. liriodendri*, *M. phaseolina* and *P. cambivora*, indicating that these three fungal species decreased plant growth in Colt.

Figure 5. Assessment of fungal pathogenicity on Colt rootstock plants one year after inoculations as measured by percent root rot (A), root fresh weight (B), total root system length (C), top fresh weight (D), total length of fine roots (<0.3mm in diameter; E), and stem diameter (F). Histograms denoted with an asterisk indicate a significant difference with the non-inoculated, control treatment (Dunnett's test; *P* < 0.05). Values are averaged across 18 plants per treatment. Means and standard errors are presented.



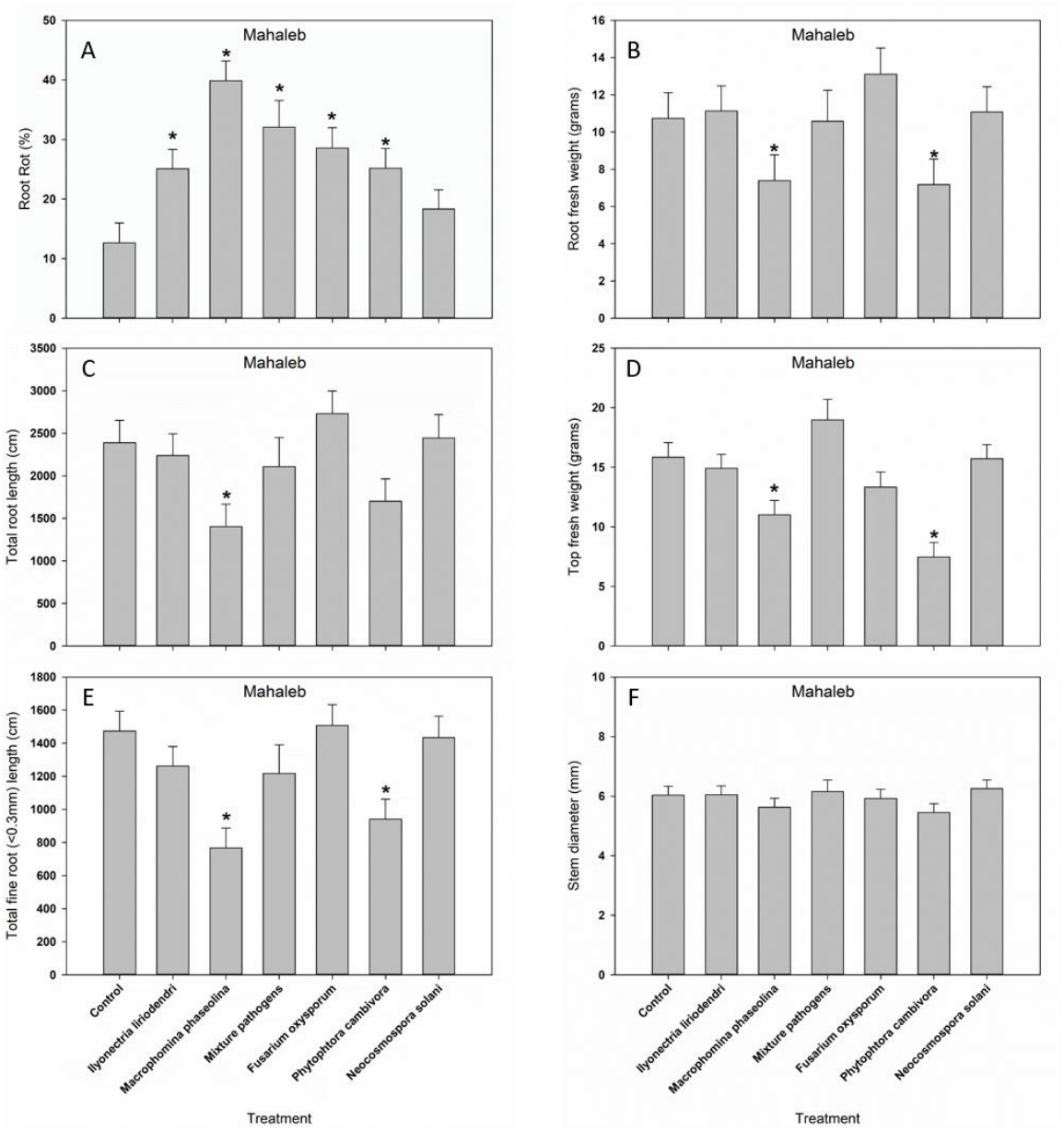
Root rot in non-inoculated, control Krimsk5 plants averaged 18% and this value was significantly lower than average root rot values for any other inoculation treatment ($P < 0.016$), except for plants inoculated with *P. cambivora* (average of 25%; $P = 0.16$). Noticeably, Krimsk5 plants inoculated with *M. phaseolina* and the mixture of four pathogens displayed a significant reduction in the length of their root systems, including the total length of their fine roots, relative to the control treatment. *Macrophomina phaseolina* and the mixture of four pathogens appeared to have the most detrimental effects on the root systems of Krimsk5 plants (**Figure 6**).

Figure 6. Assessment of fungal pathogenicity on Krimsk5 rootstock plants one year after inoculations as measured by percent root rot (A), root fresh weight (B), total root system length (C), top fresh weight (D), total length of fine roots (<0.3mm in diameter; E), and stem diameter (F). Histograms denoted with an asterisk indicate a significant difference with the non-inoculated, control treatment (Dunnett's test; $P < 0.05$). Values are averaged across 18 plants per treatment. Means and standard errors are presented.



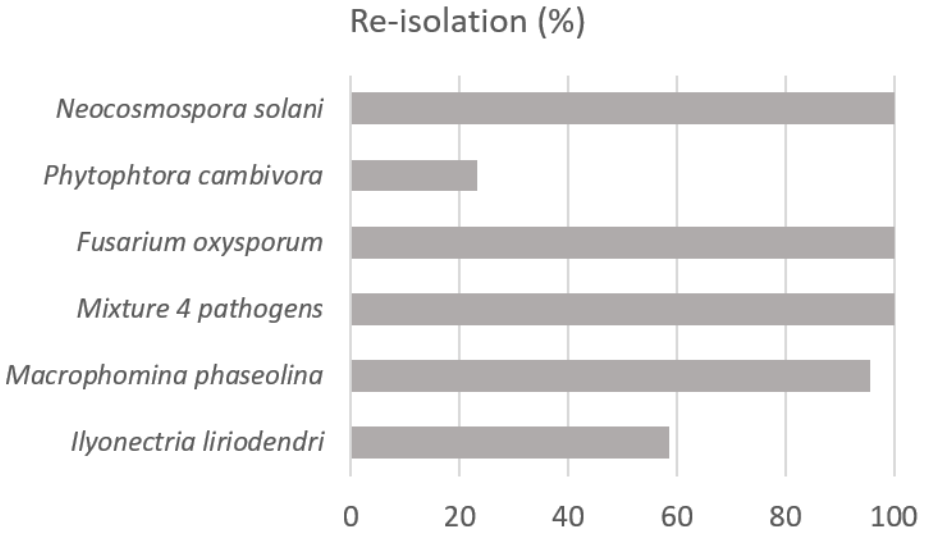
Root rot in non-inoculated, control Mahaleb plants averaged 13% and this value was significantly lower than average root rot values for any other inoculation treatment ($P < 0.012$), except for plants inoculated with *N. solani* (average of 18%; $P = 0.52$). The two treatments with the most detrimental effects on Mahaleb plant growth were *M. phaseolina* and *P. cambivora*, with significant reductions of root fresh weight, top fresh weight and total length of fine roots for plants inoculated with these two pathogens, relative to the control treatment ($P < 0.039$; **Figure 7**).

Figure 7. Assessment of fungal pathogenicity on Mahaleb rootstock plants one year after inoculations as measured by percent root rot (A), root fresh weight (B), total root system length (C), top fresh weight (D), total length of fine roots (<0.3mm in diameter; E), and stem diameter (F). Histograms denoted with an asterisk indicate a significant difference with the non-inoculated, control treatment (Dunnett's test; $P < 0.05$). Values are averaged across 18 plants per treatment. Means and standard errors are presented.



Isolations from the diseased roots of inoculated plants allowed the recovery of the inoculated fungi for all inoculation treatments (**Figure 8**). The lowest recovery was for *Phytophthora cambivora* with 26% re-isolation, whereas we obtained 95% recovery for the *Macrophomina phaseolina*, 100% recovery for the species *Neocosmospora solani*, *Fusarium oxysporum* and for the mixture of four pathogens.

Figure 8. Percent re-isolation per fungal species. For the mixture of four pathogens, positive re-isolation of one fungal species was considered a positive recovery. Values are averaged across the three rootstocks and two experiments.



Overall our findings are consistent with soil-borne pathogenic fungi contributing to the cherry sudden decline observed in orchards throughout the main cherry producing counties in California. A fungal origin to this emerging disease is supported by (*i*) a high mortality rate of rootstock plants inoculated with a combination of fungal species recovered from field trees suffering sudden decline, (*ii*) our observations of significant root rot in plants inoculated under controlled conditions, (*iii*) the observed reductions in plant growth of these inoculated plants, (*iv*) the degradation of the root systems of these inoculated plants, as exemplified by the decrease in the number of fine roots for Krimsk5 and Mahaleb plants in the presence of fungal pathogens, and (*v*) the positive recovery of all the inoculated fungi from the inoculated roots.

Some fungal species appear more virulent than others, with *Macrophomina phaseolina* being particularly aggressive on all three rootstocks tested. Furthermore, our results suggest that the presence of several of these fungi in the vicinity of the root systems of cherry trees might worsen disease symptoms.

It is possible that rootstocks not tested in this study may tolerate better the presence of these fungi in the soil surrounding their root systems. In addition, soil disinfestation methods used in other perennial crops might provide satisfactory control for cherry sudden decline, but the examination of these potential control methods was outside the scope of the present work.

Annual report 2019

Project Title: IMPROVED 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. Sampling of trees recognized the presence, in nursery stocks, of canker pathogens including *Eutypa lata, Cytospora sorbicola, Phomopis/Diaporthe* spp. associated with cankers, as well as *Trametes* and *Schizophyllum* spp. associated with wood decay symptoms. Planting with such infected material would doom an orchard to low productivity and reduced longevity. Work has been initiated with nurseries to prevent the introduction of plant pathogenic fungi via planting material and improve the quality of planting stocks. We assessed also the possibility of spreading canker diseases within orchards with pruning tools. Our results showed that *Calosphaeria pulchella*, *Eutypa lata* and *Cytospora sorbicola* could be transmitted to fresh pruning wounds by pruning tools following cutting through a dead branch carrying fungal fruiting bodies of theses pathogens. These findings highlighted the necessity of disinfecting pruning tools in order to minimize canker disease transmission during pruning. Deccosan 321, a quaternary ammonium compound used for equipment disinfestation, provided only partial disinfection of pruning tools. Additional sanitizing products are currently under evaluation. Furthermore, we evaluated the efficacy of 12 compounds to protect pruning wound from infections by canker pathogens. Of the 12 fungicidal compounds tested, Topsin M and Quilt Xcel performed best against *Eutypa lata* and *Cytospora sorbicola*, allowing up to 90% disease control. Biological, Trichoderma-based products provided significant protection and can be considered promising candidates in integrated disease management programs against cherry canker diseases. New field surveys and tree sampling also were conducted to investigate the main infection pathways (entry points) for canker pathogens in sweet cherry trees. Surprisingly, *Calosphaeria pulchella* and *Cytospora sorbicola* were found commonly in spurs and shoots expressing dieback symptoms in the absence of pruning wounds. This implies that pruning wounds are not the sole infection sites for canker pathogens and we hypothesize that fruits, leaf and/or bud scars may serve as additional infection sites, which, if confirmed, will

require different approaches to disease control. In addition, last year field experiments revealed significant 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 *in vitro* temperatures 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. Finally, we conducted experiments to determine the resistance/tolerance of Benton, Santina, Bing and Rainier cultivars to Eutypa, Cytospora and Calosphaeria canker diseases. While none of these cultivars appeared to be resistant to canker diseases, Santina was the least susceptible. On the other hand, Benton appeared highly susceptible to all three canker pathogens, suggesting this cultivar should be avoided in locations at risk for canker diseases. 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

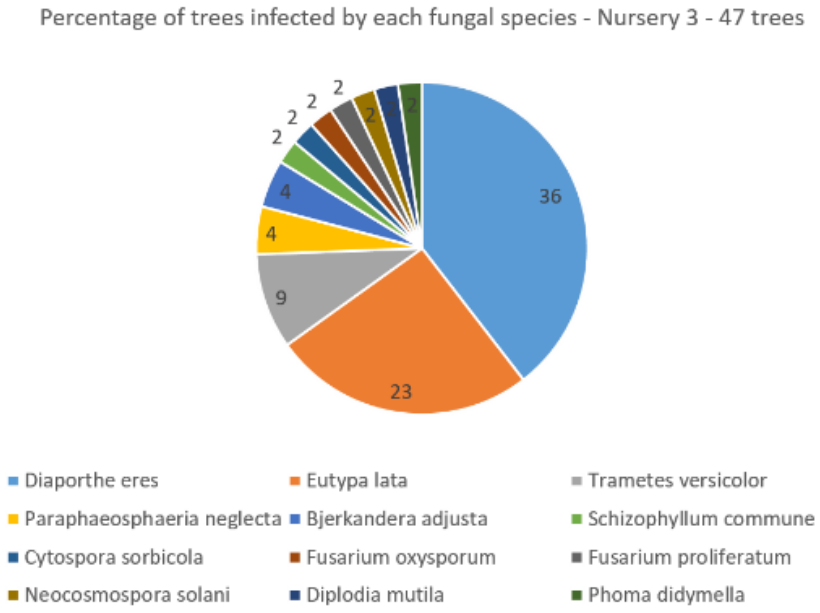
In 2019, we examined 47 plants originating from one commercial nursery (hereafter "Nursery 3") in order to determine the sanitary status of nursery stocks before planting. All 47 trees were sectioned transversally and longitudinally in order to examine the presence of vascular discolorations, wood rot and wood cankers at four sampling locations: within the rootstock, just below the graft union, just above the graft union, and within the scion. In addition, we sampled apparently healthy wood tissues in the scion and rootstock to detect putative latent infection. From all sampled tissues, approximately 10 wood pieces were surface-sterilized by immersion for 2 mins in a 1.5 % sodium hypochlorite solution and washed twice with sterile distilled water. Small pieces of healthy as well as necrotic tissues were selected and placed onto petri dishes filled with potato dextrose agar (PDA) amended with 100 ppm tetracycline (PDA-tet) for isolation of fungi. Fungal identification was conducted using DNA based techniques including the polymerase chain reaction (PCR), amplification and sequencing of the internal transcribed spacer region (ITS) of the rDNA using primers ITS1 and ITS4.

Results and Discussion

From the 47 trees examined from Nursery 3, all had symptoms of fungal infections, namely wood decay, cankers and/or vascular discolorations developing in the rootstock section of trees below pruning wounds, or in the scion. From these diseased trees, we principally isolated the fungal pathogen *Diaporthe eres* (36% of diseased trees) but also *Eutypa lata* (23% of trees) (**Figure 1**). From 9% of these diseased trees, we isolated *Trametes versicolor*, a Basidiomycete fungus known to be responsible for white rot and wood degradation in many tree crops (**Figure 1**). These results confirm our findings from previous years in that cherry nursery trees can harbor many canker pathogens as well as wood decay fungi. 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 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 to implement production practices that minimize the risk of nursery stocks contaminations by canker and wood decay fungi. We are testing various pruning wound protectants to prevent infections of cuts occurring during grafting of nursery trees.

Figure 1. Sanitary status of cherry planting material (47 trees from one nursery) expressed as the % of trees with fungal infections.



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

Material and methods

This experiment was designed to evaluate the possibility of transmitting canker pathogens from diseases branches to healthy branches while cutting with pruning shears through a canker or through fruiting bodies of canker pathogens present on dead branches. Included in these experiments was the evaluation of Deccosan 321 (Decco US, Monrovia, CA), a quaternary ammonium compound used for equipment disinfestation in other crops, as an effective method for pathogen disinfection of pruning tools. Pruning experiments were conducted in January 2019 in

one orchard located in Davis. For each of the 3 pathogens (*Calosphaeria pulchella*, *Eutypa lata* and *Cytospora sorbicola*) 5 treatments were tested and applied to 6 branches (6 repetitions):

1. Canker transmission: non-disinfected pruning shears are first used to make a cut through a wood canker in a branch affected by Calosphaeria, Eutypa or Cytospora canker before making a new cut into a healthy branch.
2. Canker transmission + Deccosan 321: same as #1 except the pruning shears are sprayed with Deccosan 321 before the second cut into a healthy branch.
3. Fruiting body transmission: non-disinfected pruning shears are first used to make a cut through a dead branch carrying fruiting bodies of either Calosphaeria, Eutypa or Cytospora pathogens before making a new cut into a healthy branch.
4. Fruiting body transmission + Deccosan 321: same as #3 except the pruning shears are sprayed with Deccosan 321 before the second cut into a healthy branch.
5. Positive control: artificial inoculations of clean pruning cuts using spore suspensions of the three pathogens.

Following pruning, wounds were protected with Parafilm to prevent natural infection. Six months after pruning, pruned branches were brought to the laboratory for examination of wood lesions from pruning cuts and recovery of canker fungi from these lesions. For fungal isolations, 10 wood pieces per pruned branches were selected approximately 1 cm below the pruning cut. These wood pieces were placed onto petri dishes filled with potato dextrose agar (PDA) amended with 100 ppm tetracycline (PDA-tet) for isolation of fungi. Successful recovery of *Calosphaeria pulchella*, *Eutypa lata* and *Cytospora sorbicola* were evaluated based on colony morphology.

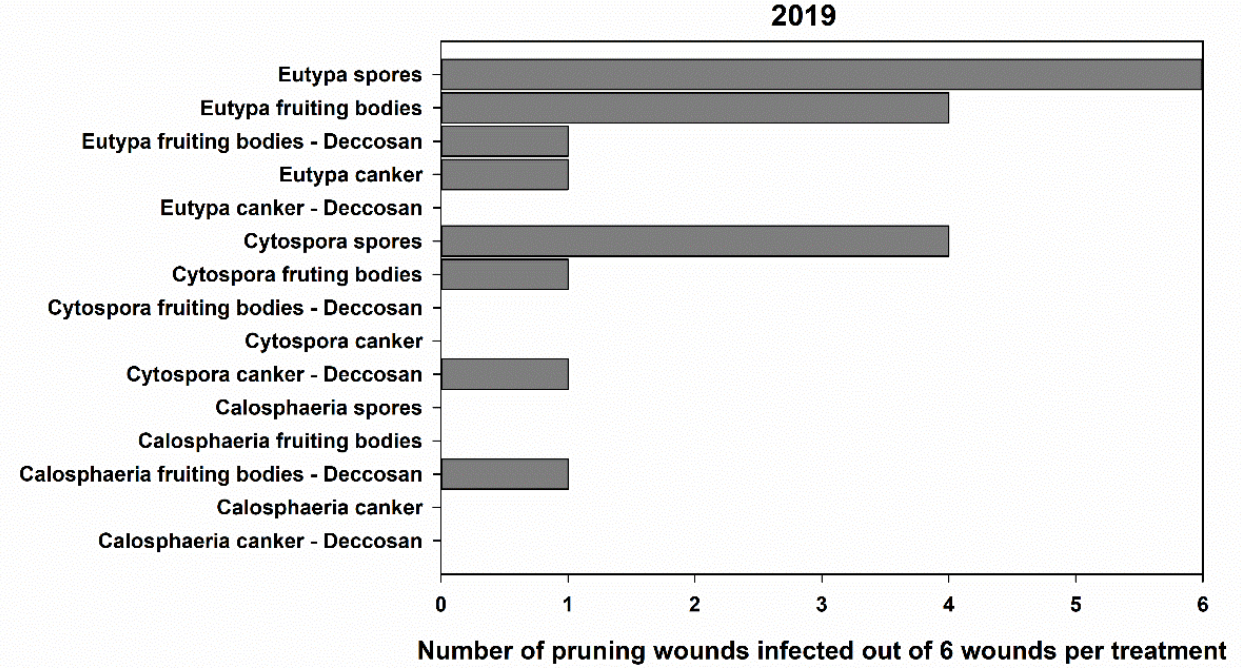
In June 2019, all six branches corresponding to each of the 15 treatments (3 pathogens, 5 treatments per pathogen applied to six pruning cuts) were collected to determine if the disease was successfully transmitted by pruning tools. Pruned branches were brought to the laboratory for examination of wood lesions from pruning cuts and recovery of canker fungi from these lesions.

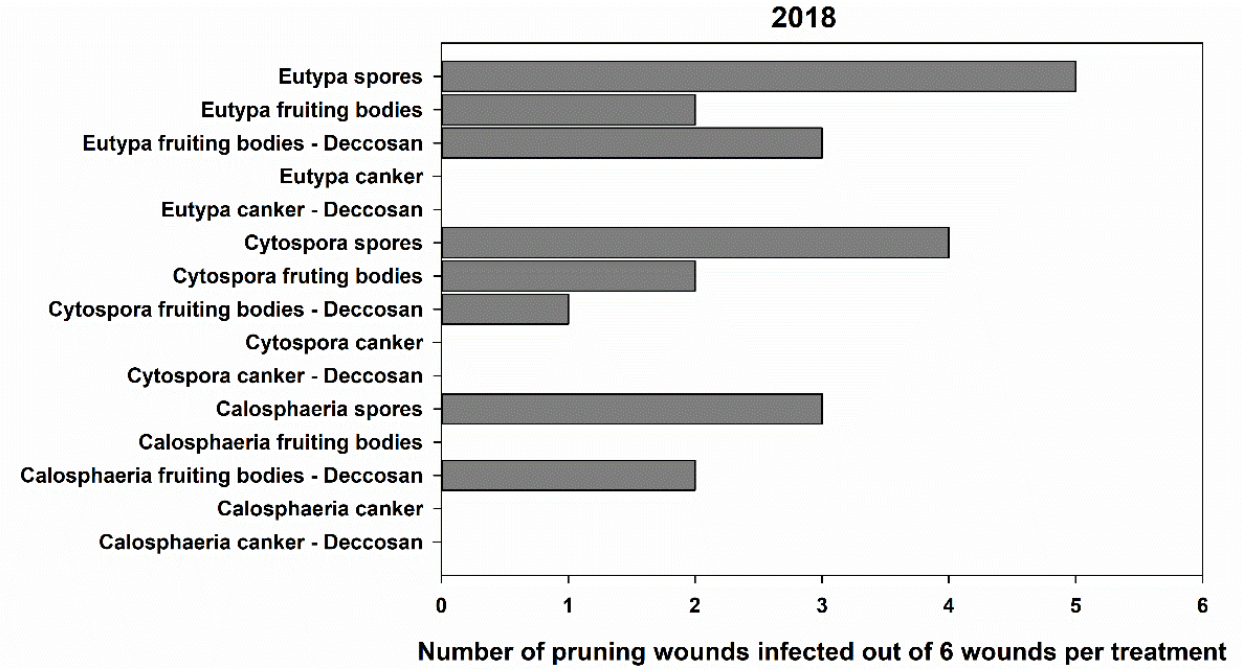
Results and Discussion

For *Eutypa lata* and *Cytospora sorbicola*, spore inoculations (positive control) resulted in infections of fresh pruning cuts (6/6 for Eutypa, 4/6 for Cytospora), whereas no infections were successful for *Calosphaeria pulchella* (**Figure 2**). Disease transmission with pruning tools was somewhat achieved when cutting through branches carrying pathogen fruiting bodies: 4/6 for Eutypa and 1/6 for Cytospora but 0/6 for Calosphaeria. The spray-disinfestation of pruning blades with Deccosan 321 reduced but did not prevent disease transmission to new, clean pruning wound for Eutypa and Calosphaeria. Cutting through cankers first and making a new cut into a healthy branch did not transmit efficiently these diseases (1/6 for Eutypa was the only positive infection) (**Figure 2**). Our findings regarding the possibility of transmitting canker diseases with pruning tools confirm last year results as well as previous findings regarding the transmission of canker pathogens of sweet cherry and other canker diseases of grapevine (Agustí-Brisach et al., 2015, Berbegal and Armengol, 2018). Pruning of sweet cherry trees occurs yearly in California and fruiting bodies of *Cytospora sorbicola* and *Calosphaeria pulchella* are very common and widespread in cherry orchards. Our findings raised the question of the importance of pruning tool disinfestation following pruning through dead wood, where most fungal fruiting structures are present. However, pruning tool disinfestation with Deccosan 321 does not seem very effective

following our duplicated experiments in 2018 and 2019. Our research will look next into the efficacy of other disinfectants to provide efficient tool sanitation strategies to growers.

Figure 2. Number of branches out of 6 branches tested with successful transmission of canker pathogens following the various treatments.





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

Material and methods

After a first field trial was conducted in Davis from February to May 2018, we repeated this experimentation from January to May 2019. Lignified branches (2nd to 3rd year wood) of 12-year-old cherry trees were pruned in order to make a flat wound. 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 48 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 *Calosphaeria pulchella*, *Eutypa lata* and *Cytospora sorbicola* at a concentration of 1,000 spores per wound. Nine 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 and averaged for each treatment. Low rates of fungal recovery were correlated with high product efficacy. Infection rate was calculated as the percentage of pruning wounds from which the pathogen was recovered, out of the total number of inoculated pruning wounds. To assess the effect of each product on infection rate, generalized linear mixed models were performed using the GLIMMIX procedure in SAS, which utilizes the *logit* link function to accommodate binomial data (0/1). Mean comparison with control treatment was performed using a Dunnett test in SAS. Mean percent disease control (MPDC) was calculated as the reduction in Mean Percent Recovery (MPR) as a proportion of the inoculated control (MPDC = 100 × [1 – (MPRtreatment/MPRI control)]).

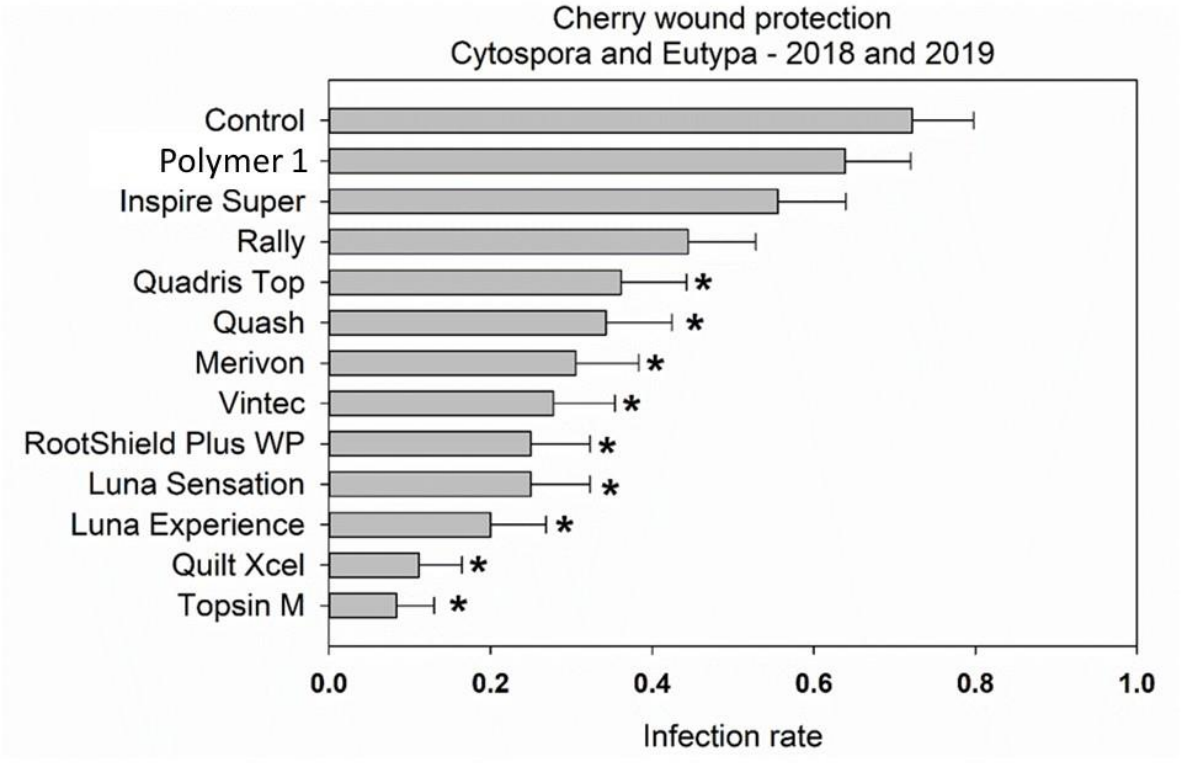
Table 1. Compounds tested for preventing cherry pruning wound infections by spores of *Calosphaeria pulchella, Eutypa lata and Cytospora sorbicola*.

Compound	Active ingredient
Trichoderma sp.	Trichoderma atroviride
Rally	myclobutanil
Topsin M	thiophanate-methyl
Quash	metconazole
Quadris Top	azoxystrobin + difenoconazole
Inspire Super	difenoconazole + cyprodinil
Quilt Xcel	azoxystrobin + propiconazole
Luna Experience	fluopyram + tebuconazole
Merivon	fluxapyroxad + pyraclostrobin
Luna Sensation	fluopyram + trifloxystrobin
Polymer 1	polymer of cyclohexane
Trichoderma spp.	T. harzianum + T. virens
Control (water)	Water

Results and Discussion

The efficacy of fungicidal products against Calosphaeria canker could not be assessed due to the lack of positive infection of pruning wounds by *Calosphaeria pulchella* experienced during the 2018 and 2019 winter experiments. The results presented here involve *Eutypa lata* and *Cytospora sorbicola*. On average, 72% of pruning wounds treated with water and inoculated with spores of *Cytospora sorbicola* and *Eutypa lata* had successful infections, providing high infection rates in our control treatments. Of the 12 compounds tested, Topsin M and Quilt Xcel performed best, allowing 92% and 89% disease reduction, respectively (**Figure 3**). Overall and following two-year experiments, these two products provided approximately 85% disease control. Luna Sensation, Luna Experience and RootShield Plus WP also provided significant control (65 to 72% disease control; **Figure 3**). Only 3 compounds did not provide significantly lower infections than in the control wounds: Polymer 1, Inspire Super and Rally. 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. Our results from 2019 overall confirmed our findings from 2018. However, further evaluations including protection of summer pruning wounds are necessary to provide strong support for the adoptions of pruning wound protectants. As observed in 2018, the lack of positive infection of *Calosphaeria pulchella* in pruning wounds has raised questions about the disease epidemiology. Our preliminary results described hereafter suggest that ascospores germination, infection and growth of *Calosphaeria pulchella* at pruning wounds occurs mainly during warm weathers. Accordingly, pruning wound protection trials should be conducted also in summer months to elucidate control of Calosphaeria canker.

Figure 3. Infection rates of cherry pruning wounds by Eutypa and Cytospora depending on the fungicide applied. Data from 2018 and 2019 were combined.



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

Material and methods

We questioned the main infection pathways, in addition to pruning wounds, associated with Calosphaeria and Cytospora cankers. Field surveys and sampling were conducted to investigate alternative points of entry into cherry trees for infection by canker pathogens in addition to pruning wounds. Cherry wood samples exhibiting canker and dieback symptoms (wood discoloration, gumming, sunken tissue, and defoliation) were collected from three different orchards (1 in Yolo Co. and 2 in San Joaquín Co.). Samples collected included:

1. Spurs that exhibited dieback and canker symptoms in the absence of pruning wounds (20 samples/orchard).
2. Shoots that exhibited dieback and canker symptoms in the absence of pruning wounds (20 samples/orchard).
3. Branches that exhibited canker symptoms developing at pruning wounds (20 samples/orchard).

Wood pieces from the margin of discolorations were surface sterilized with 10% bleach for 90 seconds followed by 2 washes with sterile deionized water. Ten wood pieces were plated on potato dextrose agar amended with 2 ppm tetracycline for isolation. Successfully recovery of canker-causing pathogens was annotated, and fungal pathogens were identified based on colony morphology.

Results and Discussion

From the Yolo County cherry orchard, we were able to recover *Calosphaeria pulchella* from 19% of dying spurs, 27% of shoots with dieback, and 26% of cankers developing at pruning wounds in branches (**Figure 4**). *Cytospora sorbicola* was recovered from 23% of sampled spurs, 9% of shoots with dieback, and 18% of cankers at pruning wounds in branches (**Figure 4**). Additionally, other canker-causing pathogens (*Eutypa lata*, *Botryosphaeria* and *Diaporthe*/*Phomopsis*) were recovered also from spurs and shoots dieback, and at pruning wounds in branches showing a canker. *Diaporthe/Phomopis* spp. were found commonly in spurs with dieback symptoms. In the San Joaquin Orchard 1, recovery of *Calosphaeria pulchella* was10% from dead spurs, 20% from shoots with dieback and cankers at pruning wounds in branches (**Figure 5**). Recovery rate for *Cytospora sorbicola* was 65% from spur dieback, 25% from shoot dieback and 20% from pruning wound cankers (**Figure 5**). As with the Yolo county orchard, *Botryosphaeria* and *Diaporthe*/*Phomopsis* were also recovered from these symptoms. In the San Joaquin Orchard 2, recovery of *Calosphaeria pulchella* was observed from 27% of cankers sampled below pruning wound in branches and the pathogen was not isolated from spurs and shoots with dieback (**Figure 6**). *Cytospora sorbicola* was isolated from 5% of dead spurs, 15% of shoots with dieback, and 14% of cankers at pruning wounds in branches (**Figure 6**). Again, *Botryosphaeria* and *Diaporthe*/*Phomopsis* accounted for additional canker pathogens that were commonly isolated from the various tissues sampled. Overall, *Calosphaeria pulchella* and *Cytospora sorbicola* were recovered abundantly in our survey from dead spurs and shoot dieback in the absence of pruning wounds. This implies that pruning wounds are not the sole infection site for canker pathogens and we hypothesize that fruits, leaf and/or bud scars in trees can act as additional infection sites for these pathogens.

Figure 4. Recovery of canker-causing fungi from spurs and shoots showing dieback (no pruning wound), and from branch cankers below pruning wounds (Yolo Co orchard 1)

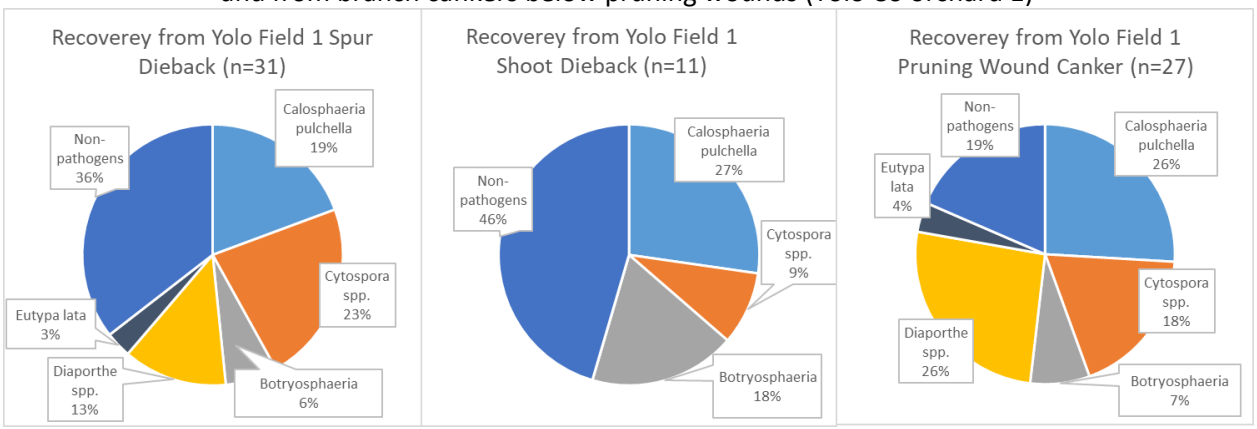


Figure 5. Recovery of canker-causing fungi from spurs and shoots showing dieback (no pruning wound), and from branch cankers below pruning wounds (San Joaquin Co orchard 1)

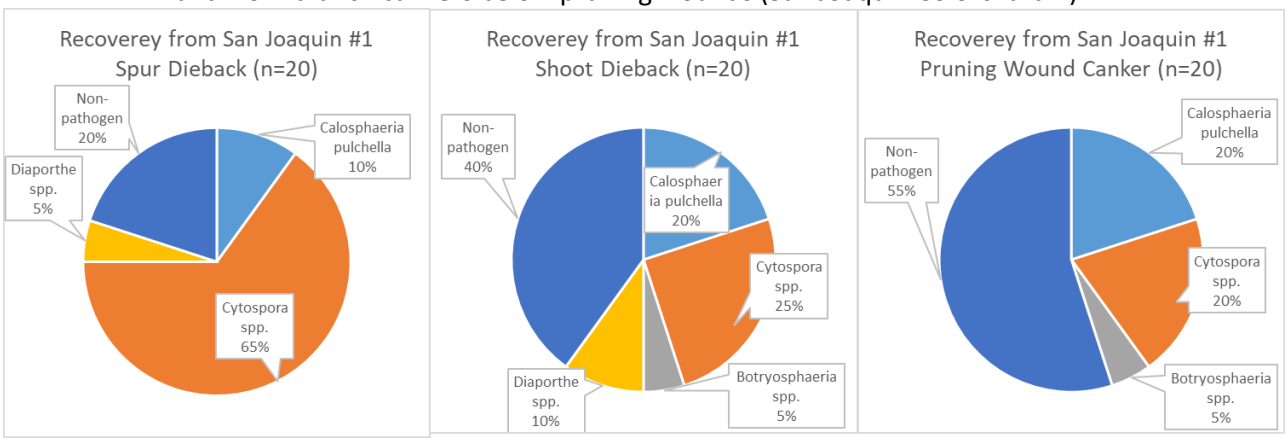
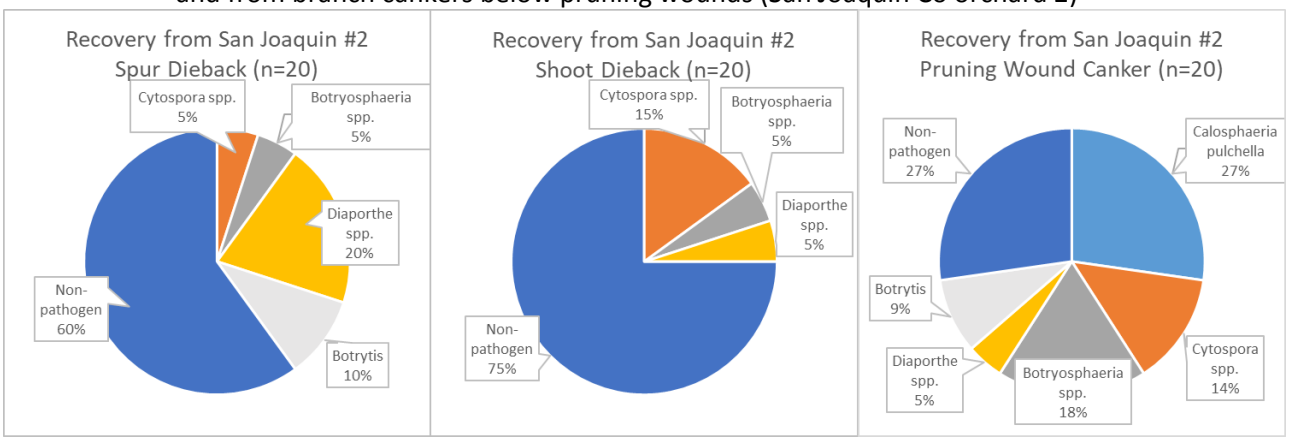


Figure 6. Recovery of canker-causing fungi from spurs and shoots showing dieback (no pruning wound), and from branch cankers below pruning wounds (San Joaquin Co orchard 2)



Objective 5: Determine the effect of temperatures spore germination and mycelial growth of *Calosphaeria pulchella*:

Materials and Methods

Ascospore Germination: The effect of temperatures on spore germination was studied using four 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 x 10^5 ascospores mL-1. Four, 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, 35, and 40°C. After for 37 hours, 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.

Mycelial Growth: The effect of temperatures on fungal growth was studied using four representative strains of *Calosphaeria pulchella*. A 5-mm mycelial plug taken from the margin of an actively growing colony was placed in the center of an 85-mm diameter PDA Petri dish. Cultures of four replicates for each isolate were incubated at temperatures ranging from 5°–35°C in five-degree increments. Plates were incubated in the dark and two measurements of colony diameter at right angles to each other were taken after 4, 8 and 12 days of growth. Colony diameters were averaged for each of the seven temperatures tested (5°, 10°, 15°, 20°, 25°, 30° and 35°C) and the optimum temperature for growth was determined. Experiments were conducted twice.

Results and Discussion

Calosphaeria ascospores exhibited the highest germination rate (90%) at 30°C while no germination occurred at 40°C and at 15°C and below after 37 hours incubation (**Figure 7**). Mycelial growth and colony growth rate were optimal at temperatures between 25 and 30°C with relatively slow growth at 40°C and at 15°C and below (**Figure 8**). These data indicate a preferential growth of *Calosphaeria pulchella* at higher temperatures (~30°C) and limited growth at lower temperatures (~15°C and below). This suggests that *Calosphaeria pulchella* infection in the field is more likely to occur during early or late summer months when average temperature is around 30°C. These finding may explain the lack of recovery of *Calosphaeria pulchella* from our pruning wound protection trials which were performed during winter (January), where average and highest temperatures generally remain below 15°C (**Figure 9**). This also suggests that pruning wound protection trials for *Calosphaeria pulchella* should be conducted in early to late summer to favor pruning wound infection with this pathogen.

Figure 7. Ascospore germination rates after incubation for 37 hours in a range of temperatures. Values show an average of two repeated trials.

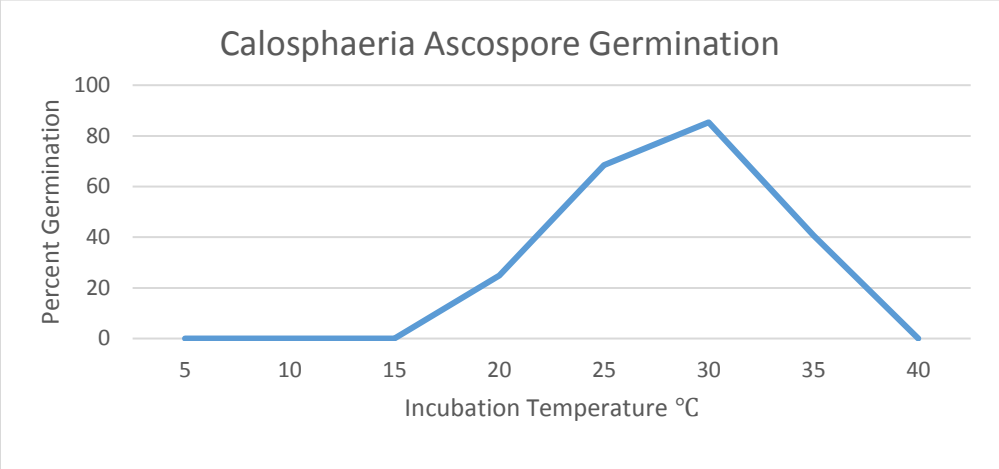


Figure 8. Mycelial growth after 4-, 8- and 12-day incubation in a range of temperatures. Values show an average of four *Calosphaeria pulchella* isolates from two repeated trials.

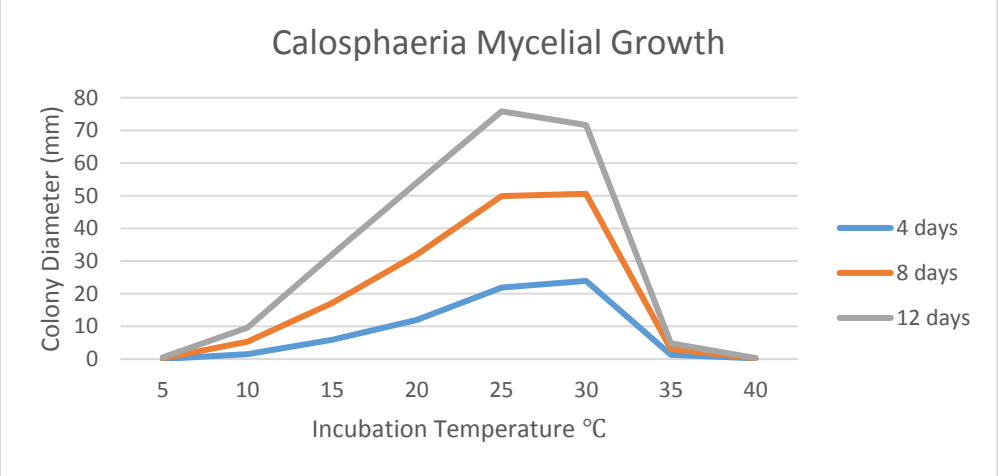
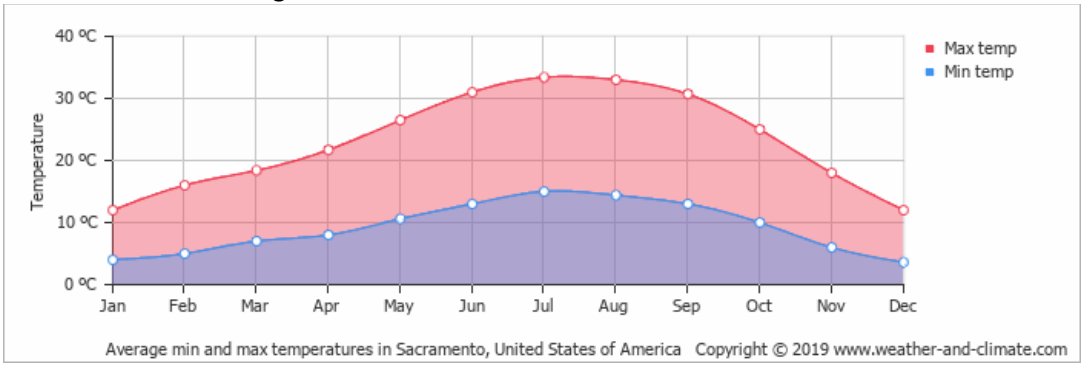


Figure 9. Estimated maximum and minimum average temperatures throughout the year 2018 in Davis. Data originate from the nearest Sacramento weather station.



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

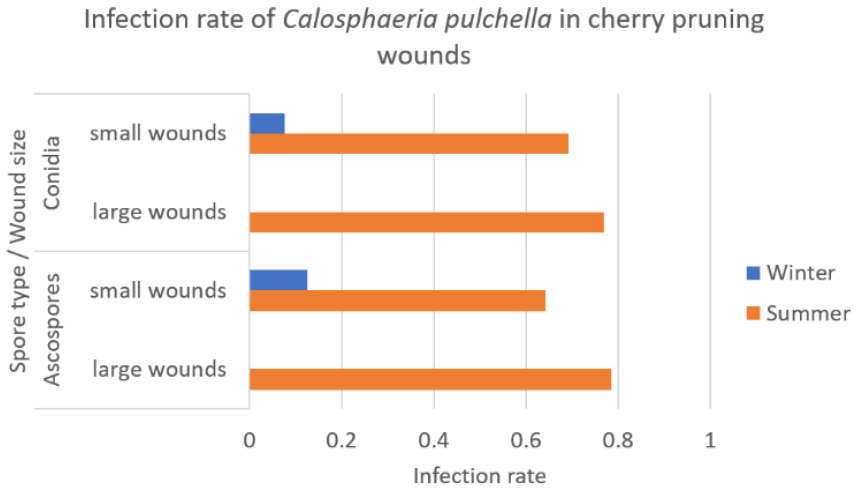
Materials and Methods

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 and July 2019, 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.* Twenty branch replicates were used for each treatment in this study. Four months after each inoculation time (January vs. July), 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. Data were analyzed in the statistical software R.

Results and Discussion

Experiments revealed significant 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 10%, whereas branches (all diameters combined) pruned and inoculated in July yielded up to 75% infection of pruning wounds (**Figure 10**). 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 10. 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 vs. July)



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

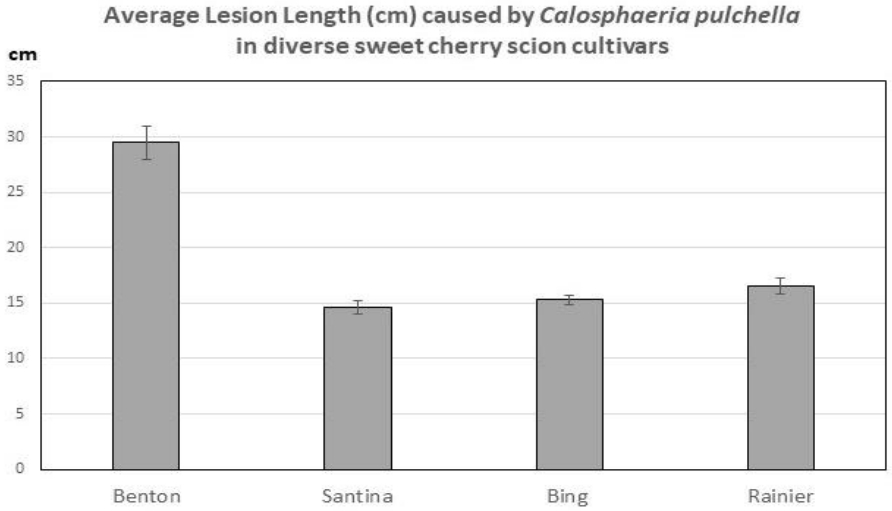
Materials and Methods

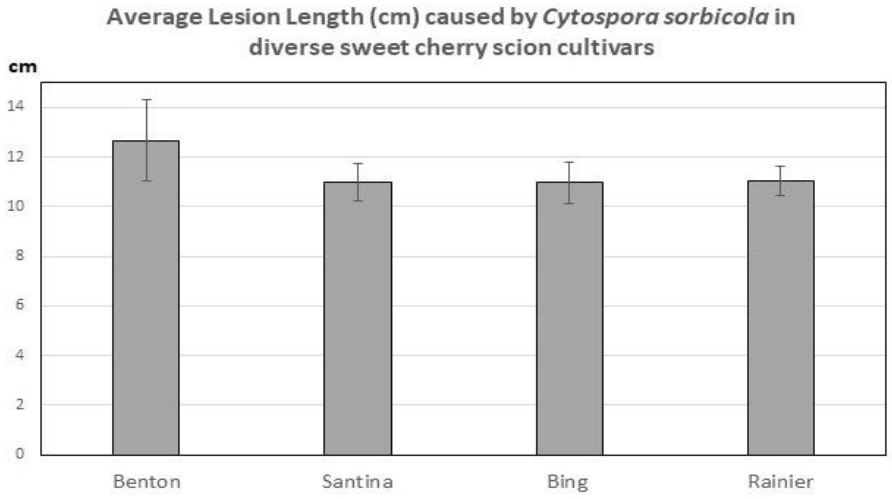
We conducted experiments to determine the resistance/tolerance of scion cultivars to Eutypa, Cytospora and Calosphaeria canker diseases. One cherry orchard was established at Kearney Agricultural Research and Extension Center using four cultivars (Rainier, Bing, Santina and Benton). For each cultivar, 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 August 2018. 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 were recorded one year after inoculation by measuring the length of wood discoloration above and below the point of inoculation. Lesion length was compared among the different cultivars to determine which cultivars are most tolerant to fungal canker diseases. Re-isolations were carried out as previously described. A two-way ANOVA was performed to determine significant differences of susceptibility among the sweet cherry scion cultivars.

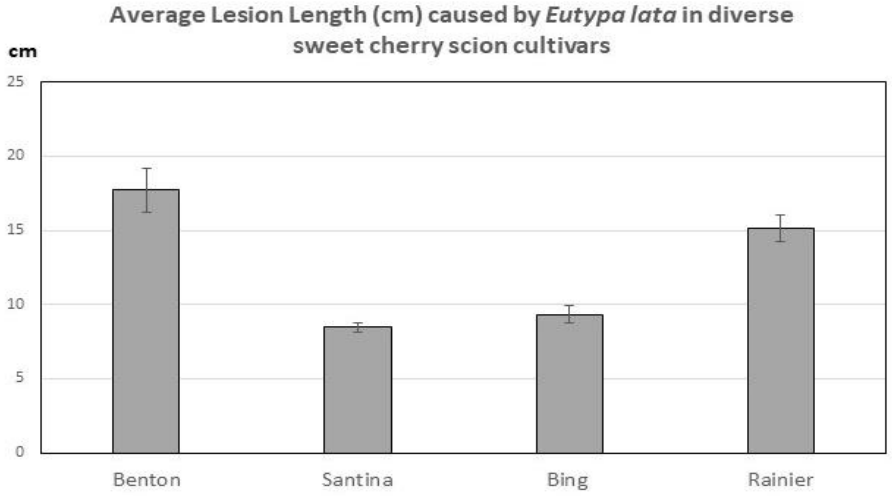
Results and Discussion

The experiment revealed significant differences in scion cultivars' susceptibility to canker pathogens. Nevertheless, no cultivar appeared resistant to canker disease, although cv. Santina developed smaller lesions overall than all the other cultivars. On the other hand, Benton appeared highly susceptible to all three canker pathogens. Lesion caused by *Calosphaeria pulchella* in cv. Benton averaged 29.5 cm in length while they were approximately 15 cm for all remaining cultivars (**Figure 11**). Lesion caused by *Eutypa lata* averaged 17.7 cm in length in cv. Benton and were approximately 8.5 cm in cv. Santina (**Figure 11**). All cultivars appeared equally susceptible to Cytospora canker with lesion length ranging between 11 and 12 cm. Although no cultivar appeared resistant to fungal canker diseases, this research revealed a higher susceptibility of cv. Benton, suggesting this cultivar should be avoided in locations at risk for canker diseases. This work also suggests the need to integrate additional cultivars in this study. Gaining knowledge on the relative susceptibility/resistance of sweet cherry scion cultivars to canker diseases and promoting the establishment of orchards using resistant cultivars would constitute an efficient and sustainable strategy to manage canker diseases.

Figure 11. Susceptibility of different sweet cherry scion cultivars to *Calosphaeria pulchella*, *Eutypa lata* and *Cytospora sorbicola* expressed by the lesion length produced after artificial inoculation in stems of







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Annual Report - 2019

Prepared for the California Cherry Advisory Board

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

In 2019, 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 brown rot, gray mold, and Rhizopus rot.

1) **Bacterial blast** caused by *Pseudomonas syringae* pv. *syringae*:

   a. In a field study on cv. Coral Champagne with a low incidence of blast, no disease developed using Kasumin (kasugamycin). Treatments with oxytetracycline or the antibacterial food preservatives Nisin and ε-poly-L-lysine reduced the disease by at least 70%.

   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.

2) In a **powdery mildew** study in San Joaquin Co., the disease developed at high incidence on untreated leaves of water sprouts inside the canopy and on leaves of terminal branches in the outside canopy.

   a. The most effective treatments included the SDHI pyraziflumid, pre-mixtures that contain DMI, SDHI, and/or QoI compounds such as Luna Sensation, and Merivon, as well as the experimental fungicides UC-2, EXP-AD, and EXP-AF. The new powdery mildew fungicide Gatten (flutianil) showed moderate efficacy, and the biocontrol Serifel significantly reduced disease intensity only on outside leaves.

3) For **brown rot blossom blight**, all of the registered and experimental (e.g., Cevya, pyraziflumid, V-10424, EXP-19A, EXP-AD, EXP-AF, F-4406-3) compounds evaluated were highly effective as pre- and post-infection treatments in laboratory studies. For **gray mold blossom blight**, pyraziflumid, Quash + Sercadis, and EXP-AF were the most effective in both inoculation-treatment timings, whereas Fontelis, V-10424, and EXP-AD were among the most effective only in pre-infection treatments.

4) Two field studies were conducted on the efficacy of **preharvest fungicide treatments**.

   a. **Brown rot**: In studies with 0-, 6-, or 12-day PHI applications, Procure, Rhyme, Quadris Top, the Procure-Ph-D, Elevate-Teb, and Quash + Sercadis mixtures, Luna Experience, and Luna Sensation, as well as the experimentals Cevya, EXP-AD, and UC-2 provided excellent protection in wound- and non-wound-inoculations.

   b. For **gray mold**, Elevate-Teb, and the experimentals EXP-AF, EXP–AD, and UC-2 were most effective. Pyraziflumid and Merivon-Serifel also consistently reduced decay.

5) **Postharvest** studies on the evaluation of **fungicides** had an emphasis on BioSpectra (natamycin) used by itself or in mixtures with Scholar or Mentor.

   a. In aqueous drench and spray applications, BioSpectra was highly effective against brown rot and Rhizopus rot, and also very effective against gray mold at rates as low as 125 ppm (= 16 fl oz/100 gal). Decay incidences were zero or near zero when BioSpectra was mixed with Scholar (as low as 75 ppm = 4 fl oz/100 gal) or Mentor (as low as 62.5 ppm = 2 oz). Mentor by itself was least effective against Rhizopus rot. With increasing emphasis on food safety and consumer concerns, natamycin with

'exempt from tolerance status' will likely become an important component 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.

b. Chairman was highly effective against the three major decays after wound-inoculation at rates between 8 and 16 fl oz.

6) All ***Phytophthora*** isolates (*P. niederhauseri, P. syringae, P. citricola* complex, *P. megasperma, P. cactorum*) were most sensitive to oxathiapiprolin with EC_{50} values for mycelial growth inhibition of $\leq$0.001 mg/liter. A rather narrow range of EC_{50} values (0.001 to 0.01 mg/liter) among all isolates was also found for mandipropamid. No resistance was detected to any of the fungicides evaluated. Field trials were established at UC Davis and in commercial orchards with a history of Phytophthora root and crown rot and where river or district water is used for irrigation. These trials are ongoing, and treated trees will be compared to non-treated trees.

INTRODUCTION

Management of bacterial blast and canker. Pseudomonas syringae pv. *syringae* 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.

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 also 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. We are currently collaborating with a chemical company to develop agrochemical formulations of these two compounds.

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 but 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 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 3 (DMI) Procure (triflumizole), the FRAC Code 7 (SDHI) fungicides (e.g., fluopyram, fluxapyroxad, and penthiopyrad), and the pre-mixtures Luna Sensation (fluopyram/ trifloxystrobin), Merivon (fluxapyroxad/pyraclostrobin) (FRAC Code 7/11), and Quadris Top (azoxystrobin/ difenoconazole) (FRAC Code 3/11), as well as polyoxin-D (FRAC Code 19). In 2019, excellent control was obtained using the experimentals pyraziflumid, UC-2, EXP-AD, and –AF, whereas the new powdery mildew fungicide Gatten (flutianil) was not very effective. These 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 2019 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, 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 FRAC Code 3 and 7 fungicides. Still, more new fungicides are being developed. They generally belong to the same FRAC codes 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.

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, Rhizopus rot, as well as powdery mildew lesions from field infections. Currently, seven postharvest fungicides, Tebucon (tebuconazole, FRAC 3), Mentor (propiconazole, FRAC 3), Scholar (fludioxonil, FRAC 12), Chairman (fludioxonil/propiconazole, FRAC 3/12), Penbotec (pyrimethanil, FRAC 9), and the biofungicide BioSpectra (natamycin, FRAC 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. All 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.

Objectives

1. Evaluate new products against bacterial blast and canker in inoculation studies.
   a. Biologicals/natural products (e.g., GA-142, Blossom Protect, nisin, Ɛ-poly-L-lysine, isoprenoid).
   b. Antibiotics – kasugamycin, oxytetracycline – large-scale trials under favorable environments and trials to improve penetration into plant tissue.
   c. Continue to evaluate wound susceptibility of branches and antibiotic protection over time to prevent bacterial canker.
2. Evaluate bloom and preharvest applications of new compounds (e.g., Miravis, Cevya), premixtures (e.g., Luna Sensation, Merivon, Quadris Top, EXP-AD, EXP-AF, UC-2, pyraziflumid, Fervent, and biologicals for control of brown rot and Botrytis blossom blight, powdery mildew, and pre- and postharvest brown rot and gray mold fruit decay.
   a. Evaluate new powdery mildew fungicides using different rates and timings and develop a powdery mildew fungicide program that integrates new materials with single- and multi-site mildew fungicides.
   b. Evaluate new brown rot and gray mold materials including new DMIs, SDHIs, polyoxins, and pre-mixtures.
3. Evaluate new fungicides as postharvest treatments and develop cost-effective application methods:
   a. Continue to evaluate registered fungicides as well as mixtures including Chairman and Scholar with natamycin or EXP-19A for approved or pending food additive tolerance (FAT) in Japan.

b. Continue to develop EC_{50} values, baseline sensitivities, and monitor resistance in target pathogen populations to newly developed fungicides.
c. Continue to evaluate 'exempt from tolerance' bio-fungicides (natamycin and EXP-19A).

4. Studies on new Phytophthora root rot fungicides
   a. Develop baseline sensitivity data for oxathiapiprolin, mandipropamid, fluopicolide, and ethaboxam for *Phytophthora* spp. that occur on cherry and other fruit tree crops.
   b. Initiate field studies in naturally infested orchards or in experimental, inoculated orchards.

MATERIALS AND METHODS

Evaluation of treatments for control of bacterial blast. A trial was done on cv. Coral cherry at UC Davis. 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 3-26-19. 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 for 18 h. The incidence of disease (based on the number of diseased flowers per total number of flowers) was evaluated after approximately 2 weeks.

Evaluation of new fungicides for control of powdery mildew. In a field trial in San Joaquin Co., treatments were done on 3-28-19 (petal fall) for protection from primary inoculum (ascospores from overwintering chasmothecia) and were followed by treatments on 4-17 and 5-9-19 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 6-5-19. 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).

To evaluate preharvest fungicide applications for control of fruit decay, an experimental orchard at UC Davis and a commercial orchard in San Joaquin Co were used. Treatments were applied 6 and 12 days (UC Davis) or 0 days (commercial orchard) 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* or *B. cinerea* (30,000 conidia/ml) or were non-wound drop-inoculated with *M. fructicola* (200,000 spores/ml) or *B. cinerea* (300,000 spores/ml in cherry juice. 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.

Efficacy of new and registered postharvest treatments for managing brown rot, gray mold, and Rhizopus rot of sweet cherry. Postharvest studies focused on the comparative evaluation of BioSpectra by itself and in mixtures with Scholar or Mentor. Fruit were wound-inoculated with *M. fructicola, B. cinerea,* or *R. stolonifer* as described above and treated after 12 to 14 h by spraying or drenching. For spraying, an air-nozzle sprayer was used. Drenches were applied by pouring the aqueous fungicide solution over the fruit. 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.

Table 1: Fungicides used in 2019 studies*.

FRAC group	Trade name	Active ingredient
Single active ingredients		
2	Rovral	iprodione
3	Cevya	mefentrifluconazole
3	Mentor	propiconazole
3	Procure	triflumizole
3	Quash	metconazole
3	Rally	myclobutanil
3	Rhyme	flutriafol
7	Fontelis	penthiopyrad
7	Pyraziflumid	pyraziflumid
7	Sercadis	fluxapyroxad
12	Scholar	fludioxonil
13	Quintec	quinoxyfen
17	Elevate	fenhexamid
19	Ph-D	polyoxin-D
22	Intego	ethaboxam
24	Kasumin	kasugamycin
40	Revus	mandipropamid
41	Mycoshield	oxytetracycline
43	Presidio	fluopicolide
48	Orondis	oxathiapiprolin
49	BioSpectra	natamycin
U13	Gatten	flutianil
Experimentals	EXP-AD	not disclosed
	EXP-AF	not disclosed
	UC-2	not disclosed
	NS1	not disclosed
	HML Silco	not disclosed
	Nisin	food additive
	ε-Poly-L-lysine	food additive
	V-10424	not disclosed
	EXP-19A	not disclosed
	F-4406-3	not disclosed
Biologicals	Serifel	*Bacillus amyloliquefaciens* strain MBI600
Premixtures		
7 + 11	Luna Sensation	fluopyram + trifloxystrobin
7 + 3	Luna Experience	fluopyram + tebuconazole
7 + 11	Merivon	fluxapyroxad + pyraclostrobin
3 + 11	Quadris Top	difenoconazole + azoxystrobin
12 + 3	Chairman	fludioxonil + propiconazole

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

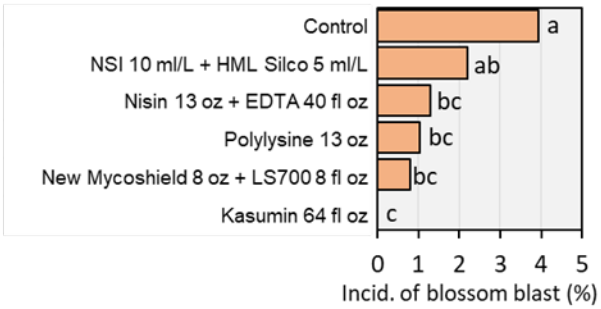
Initiate studies on the management of Phytophthora root rot with new fungicides. An orchard was planted at UC Davis in spring of 2019 with Bing or Coral cherry on Mahaleb rootstock. The soil around the base of the tree trunks was inoculated with a mixture of *P. cactorum, P. cambivora,* and *P. citricola* that were grown in an oats-vermiculite-vegetable juice substrate. Soil treatments were applied on the same day and consisted of Orondis (oxathiapiprolin), Revus (mandipropamid), Orondis Ultra (a mixture of oxathiapiprolin and mandipropamid), and Presidio (fluopicolide) using labeled rates for other tree crops. Trees were evaluated weekly for treatment effects based on tree health.

RESULTS AND DISCUSSION

Evaluation of treatments for control of bacterial blast. Inoculations of treated, injured flowers with *P. syringae* pv. *syringae* resulted only in a very low incidence (3.9%) of blossom blast. Still, significant treatment effects were present (Fig. 1). Kasumin prevented all infections, whereas Mycoshield (oxytetracycline) and the two antibacterial food additives Nisin and ε-poly-L-lysine showed intermediate efficacy. In previous experimental and commercial field trials with much higher disease incidence in the controls, Kasumin was shown to be very effective and also significantly reduced the severity of bacterial

cankers. Kasumin was registered on sweet cherry in 2018. Registration of oxytetracycline is currently pursued with support of the registrant through the IR-4 program and is pending at EPA. The two food additives look promising, and we are working with a chemical company to obtain agricultural formulations that may improve their performance. Another food-grade antibacterial was identified and we plan to evaluate this compound in the future. With widespread copper resistance in the pathogen *P. syringae* pv. *syringae*, new effective treatments are needed to manage bacterial canker and blast. These are important diseases of sweet cherry that can impact cherry production in seasons with favorable environmental conditions and can also have long-term effects on tree health.

Fig. 1. Evaluation of antibacterial treatments for protection of cv. Coral cherry flowers from bacterial blast in 2019

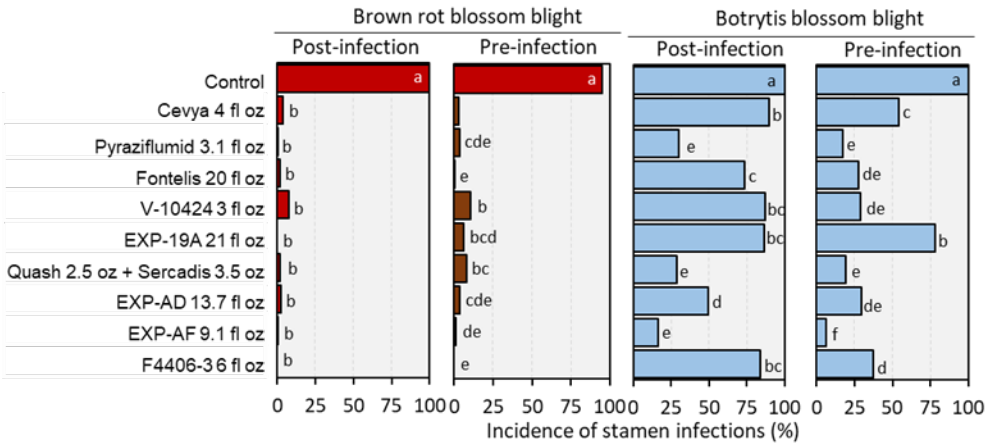


Flowers stamens were cut off with scissors, and flowers were treated using a hand sprayer on 3-26-19. After air-drying, flowers were spray-inoculated with *Pseudomonas syringae* (2x10^6 cfu/ml) and bagged overnight. Disease was evaluated after 8 days, and dark, wilted flowers with brown peduncles were counted.

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 opened flowers in laboratory studies. For brown rot blossom blight, all of the registered and experimental compounds evaluated were highly effective, including the new Cevya, pyraziflumid, V-10424, EXP-19A (that belongs to a new FRAC Code), EXP-AD, EXP-AF, and F-4406-3 (Fig. 2), For gray mold blossom blight, pyraziflumid, Quash + Sercadis, and EXP-AF were most effective in both inoculation-treatment timings, whereas Fontelis, V-10424, and EXP-AD were among the most effective only in pre-infection treatments. 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.

Due to the good pre- and post-infection activity of most of the conventional fungicides that was demonstrated previously, 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

Fig. 2. Efficacy of pre- and post-infection treatments for control of brown rot and Botrytis blossom blight of Coral cherry – Laboratory studies 2019



For evaluation of the pre-infection activity, closed blossoms were collected in the field on 3-26-19, 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.

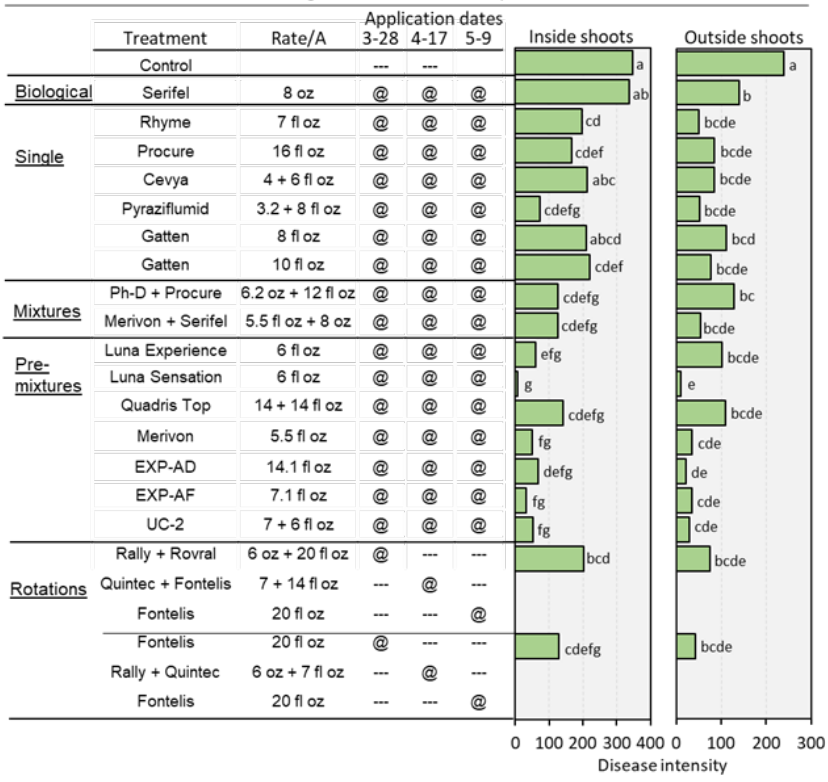
biological treatments were identified previously (Botector, Serenade Opti) that are not as effective as conventional fungicides but can benefit disease management in organic production systems.

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 highly favorable for powdery mildew development at our trial site in the spring of 2019. At evaluation in early June, 98.9% of leaves on water shoots inside the canopy and 87.2% of leaves in the outer canopy showed symptoms of powdery mildew.

Based on disease intensity (the multiplication product of incidence and severity), all (outside leaves) or most (inside leaves) treatments significantly reduced the disease as compared with the control (Fig. 3). As in previous years, treatments that were highly effective on inside and outside leaves included the experimentals pyraziflumid, EXP-AD, EXP-AF, and UC-2, as well as the registered Luna Sensation and Merivon. These contain DMI, SDHI, and/or QoI compounds which are known to have high activity against powdery mildews. The new powdery mildew fungicide Gatten (flutianil) showed moderate efficacy, and the biocontrol Serifel significantly reduced disease intensity only on outside leaves. Quintec (FRAC 13) that was highly effective in the first years after its registration on cherry was used in mixtures in two rotation programs in 2019, and these treatments mostly showed moderate efficacy. Reduced sensitivity to Quintec is still localized. 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

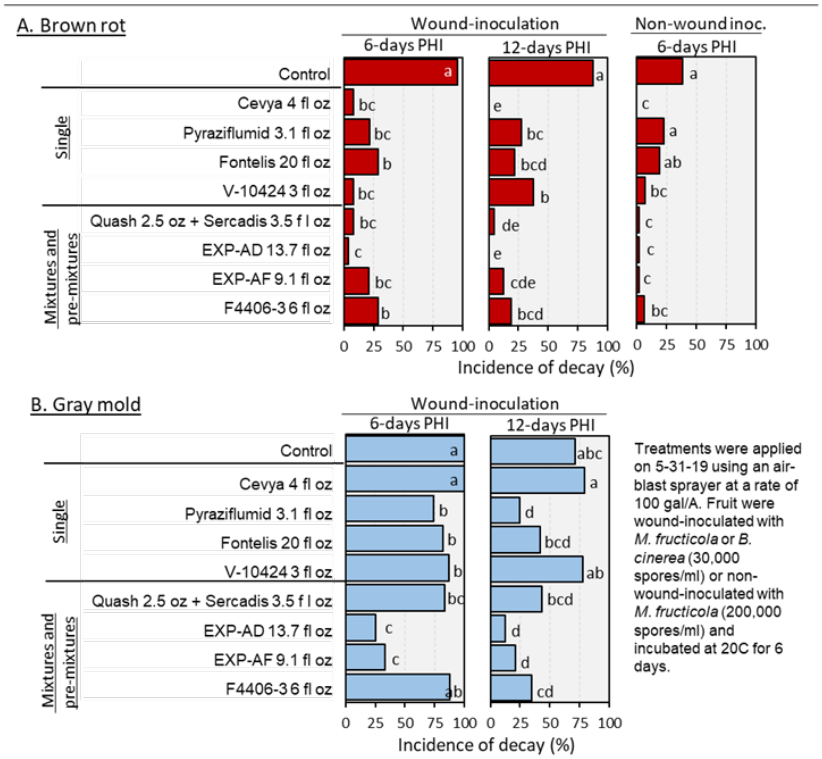
Fig. 3. Evaluation of preharvest fungicide treatments for management of powdery mildew of Bing cherries in San Joaquin Co. 2019



Applications were done using an airblast sprayer at 100 gal/A. For evaluation on 6-5-19, 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.

applications. Because of the potential of resistance to single-site mode of action fungicides, pre-mixtures or tank mixtures of FRAC 3, FRAC 7, FRAC 11, and FRAC 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 selection pressure for selecting for fungicide resistance. Limiting the number of applications of any one mode of action (i.e., FRAC) 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.

Fig. 4. Efficacy of 6- and 12-day preharvest fungicide treatments for management of postharvest brown rot and gray mold of Bing cherries - UC Davis - 2019

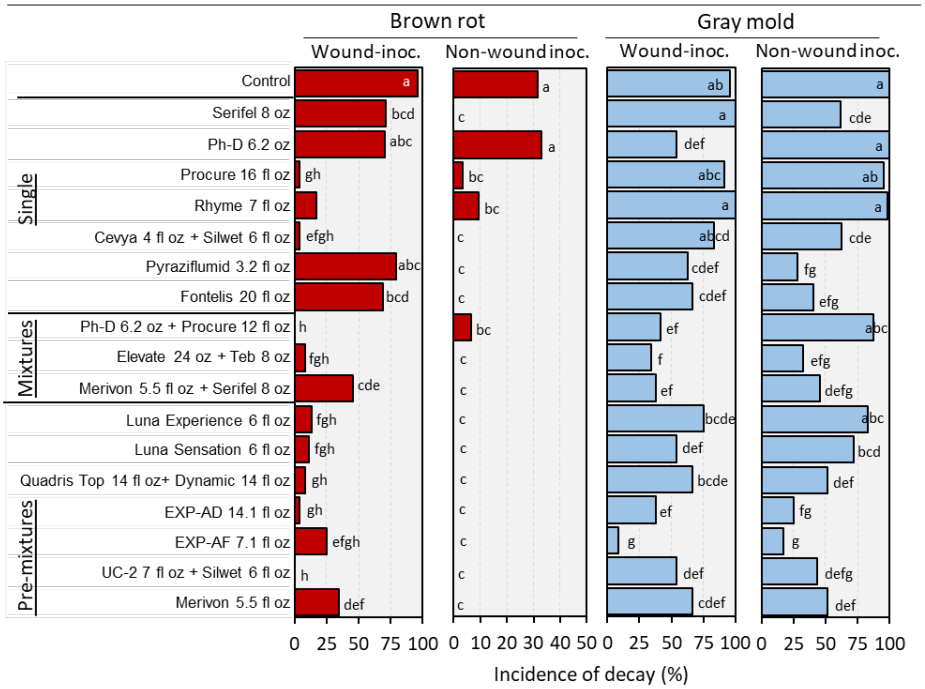


Evaluation of preharvest treatments for management of fruit decays. Rainfall during the cherry harvest season at our trial locations in 2019 resulted in a high incidence of fruit cracking and decay development. As a result, pre- and postharvest studies on decay control were minimized because of the limited and highly susceptible crop available. Thus, preharvest intervals for field applications were shorter, no postharvest fruit washes of field-treated fruit could be done, and only three postharvest studies were conducted.

Preharvest treatments to Bing cherries at UC Davis were applied at 6- and 12-days PHI. In wound-inoculations of harvested fruit with *M. fructicola*, all treatments significantly reduced the incidence of brown rot from the control (Fig. 4A). Cevya, Quash + Sercadis, and EXP-AD were the most effective at both preharvest intervals, whereas V-10424 was only highly effective in 6-day PHI treatments. Still, pyraziflumid, Fontelis, EXP-AF, and F4406-3 were also very effective. In non-wound-inoculations, all fungicides except pyraziflumid and Fontelis were highly effective. In wound-inoculations with *B. cinerea*, the experimental EXP-AF and –AD were highly effective at both preharvest intervals, and some of the other treatments were more effective at the 12-day than at the 6-day-PHI timing (Fig. 4B).

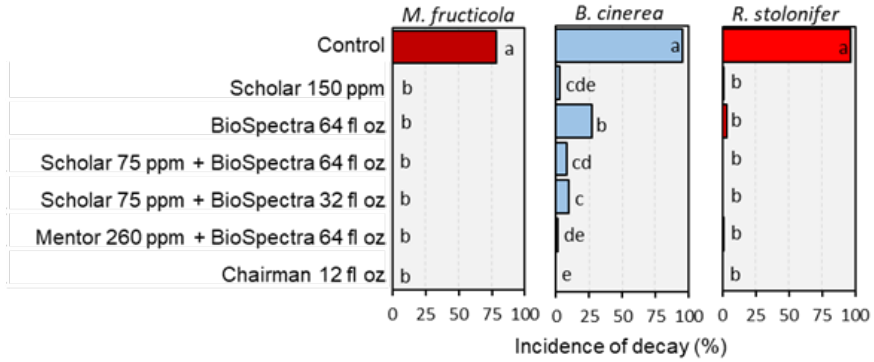
At the commercial trial site with 0-day PHI applications, the registered Procure, Rhyme, Quadris Top, the Procure-Ph-D and Elevate-Teb mixtures, Luna Experience, and Luna Sensation, as well as the experimentals Cevya, EXP-AD, and UC-2 provided excellent protection against brown rot when fruit were wound- or non-wound-inoculated (Fig. 5). The biocontrol Serifel and the fungicides pyraziflumid,

Fig. 5. Efficacy of 0-day preharvest fungicide treatments for management of postharvest brown rot and gray mold of Bing cherries - San Joaquin Co. - 2019



Treatments were applied on 5-30-19 using an air-blast sprayer at a rate of 100 gal/A. Fruit wound-inoculated (30,000 spores/ml) or non-wound drop-inoculated with *M. fructicola* (200,000 spores/ml) or *B. cinerea* (300,000 spores/ml in cherry juice). Fruit were then incubated for 5-10 days at 22C.

Fig. 6. Efficacy of postharvest laboratory drench treatments for postharvest decay control of inoculated Bing cherry fruit 2019

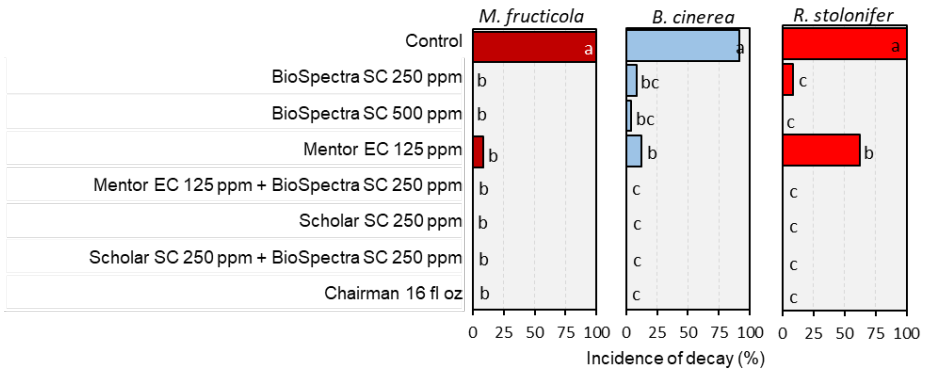


Fruit were wound-inoculated (30,000 spores/ml each pathogen) and incubated for 12 h at 20C. Aqueous drench treatments were applied by pouring over the fruit. 64 fl oz of BioSpectra = 500 ppm. Fruit were then incubated at 20C for 6-8 days.

Fontelis, Merivon, and EXP-AF were only highly effective in non-wound-inoculations, but Merivon and EXP-AF still showed good control in wound-inoculations. Gray mold after wound- or non-wound-inoculation was most effectively reduced using EXP-AF. Pyraziflumid, Fontelis, Elevate-Teb, Merivon-Serifel, UC-2, and EXP-AD were moderately effective, and Procure and Rhyme showed no gray mold activity (Fig. 5).

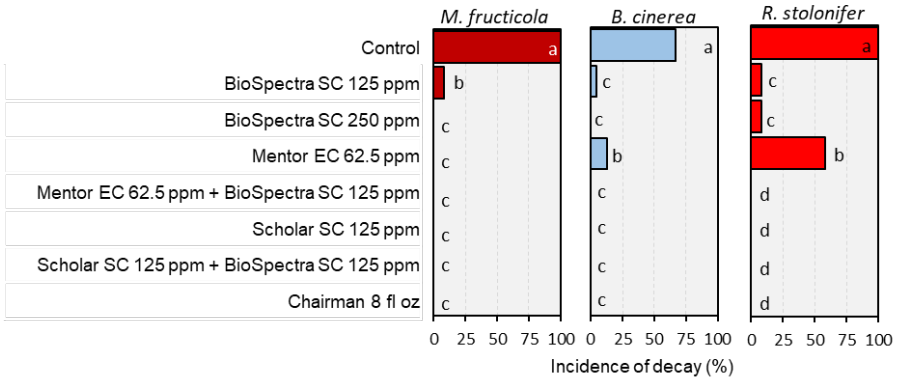
Our studies demonstrate that some preharvest treatments can effectively protect fruit from infections before and during harvest. Some treatments such as EXP-AF and EXP-AD were very effective against the two major decays, brown rot and gray mold, even when applied 12 days before harvest (see UC Davis trial). Based on these studies, these two experimentals are scheduled for registration on sweet cherry. Even with highly effective treatments like these, postharvest decays can still develop due to injuries

Fig. 7. Postharvest treatments of inoculated Bing cherry fruit with new fungicides in laboratory studies 2019



Fruit were wound-inoculated (30,000 spores/ml) and incubated for 13-14 h at 20C. Aqueous treatments were applied using an air-nozzle sprayer and fruit were incubated at 20C for 6-8 days.

Fig. 8. Postharvest treatments of inoculated Bing cherry fruit with new fungicides in laboratory studies 2019



Fruit were wound-inoculated (30,000 spores/ml) and incubated for 13-14 h at 20C. Aqueous treatments were applied using an air-nozzle sprayer and fruit were incubated at 20C for 6-8 days.

occurring during bulk handling of fruit if the fungicides lack locally systemic action. Additionally, hydrocooling removes residues of many fungicides from fruit as we demonstrated in many of our previous studies. This emphasizes that postharvest fungicides are still warranted for shipping and marketing fruit to distant markets.

Efficacy of new postharvest treatments for managing brown rot, gray mold, and Rhizopus rot of sweet cherry. Three postharvest studies focused on the comparative evaluation of BioSpectra by itself and in mixtures with Scholar or Mentor. In aqueous drench and spray applications, BioSpectra was highly effective against brown rot and Rhizopus rot, and also very effective against gray mold (Figs. 6, 7, 8) even at a low rate of 125 ppm (= 16 fl oz/100 gal). Decay incidences were zero or near zero when BioSpectra was mixed with Scholar (as low as 75 ppm = 4 fl oz/100 gal) or Mentor (as low as 62.5 ppm = 2 fl oz). As indicated previously, Mentor was least effective against Rhizopus rot. Chairman at rates between 8 and 16 fl oz reduced the three decays to zero levels in the three studies. This pre-mixture of fludioxonil and propiconazole not only has a wide spectrum of activity (brown rot, gray mold, Rhizopus rot, sour rot), but also is highly effective at low rates.

In summary, 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 with the help of our studies. MRLs have not been established yet for natamycin because it is exempt from residue tolerance 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. Additionally, with increasing emphasis on food safety and consumer concerns, natamycin with 'exempt from tolerance status' will likely become an important component of postharvest decay management in the future. MRLs have been established and FATs for propiconazole were approved in June 2018 and thus, Chairman can be used for cherries (and other stone fruits) exported to Japan. The registered use of propiconazole for both pre- and postharvest use will also provide numerous options for the packinghouse manager and marketing teams. We will continue our evaluations of these treatments in 2020 in cooperation with commercial packinghouses.

Studies on the management of Phytophthora root rot with new fungicides. We continued to collect isolates from cherry in 2019. All isolates were most sensitive to oxathiapiprolin with EC_{50} values for mycelial growth inhibition of $\leq$0.001 mg/liter. A rather narrow range of EC_{50} values (0.001 to 0.01 mg/liter) among all isolates was also found for mandipropamid, whereas for ethaboxam and fluopicolide generally higher rates were needed. All isolates were also inhibited by mefenoxam, but higher rates were needed. Still, no resistance was detected to any of the fungicides evaluated.

Newly planted trees in our field trial proved to be highly susceptible to the three species of *Phytophthora* that were used for inoculation at the time of planting using high levels of each pathogen. Many trees, including treated ones, died indicating that our inoculation methods need to be adjusted. Still, the number of surviving trees was significantly higher for all treatments as compared with the control where 90% of the trees died and was highest for Presidio and Orondis Ultra with approximately 50% survival.

Field trials with oxathiapiprolin were also established in commercial orchards with a history of Phytophthora root and crown rot and where river or district water is used for irrigation. These trials are ongoing, and treated trees will be compared to non-treated trees in the same orchard over the course of several years using a spring and fall application strategy.

FINAL REPORT

Project Title: Electronic sensors to capture spatiotemporal population density of SWD

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Organization: University of California Davis
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Email: jcchiu@ucdavis.edu

Cooperators: Eamonn Keogh (UC Riverside, Dept. of computer science and engineering)

Budget:Year 1: $31,384

Percentage time per crop: Cherry: 100%

Budget
Organization Name: UC Davis **Contract Administrator: Yang Yeh**
Telephone: (530) 752-3794 **Email address:** ypyeh@ucdavis.edu

Item	2017-18 2018-19 (no cost extension)	(type additional year if relevant)	(type additional year if relevant)
Salaries	$16,016		
Benefits	$8,168		
Wages	-		
Benefits	-		
Equipment	-		
Supplies	$5,200		
Travel	$2,000		
Miscellaneous			
Plot Fees	-		
Total	$31,384		

Footnotes:
Salaries and Benefits are for one SRAI (technician) for sensor testing and insect collection (33.3% time)
Supplies include funding to construct 20 sensors for testing ($4000) and for insect capture and maintenance ($1,200)
Travel funds ($2,000) are requested for SRAI to travel to Washington or Oregon to conduct field sensor testing

JUSTIFICATION

Sensor technologies and automated insect identification models were developed for the control of insects that spread human diseases. Our cooperator Dr. Keogh, a computer scientist at UC Riverside, has recently developed inexpensive pseudo-acoustic opto-electronic sensors and accompanying classification algorithm that can accurately classify multiple species of mosquitoes that vector pathogens such as Zika and West Nile virus (Chen et al. 2014) by using wing-beat frequencies, daily activity patterns, and geographical distribution. The ability to remotely capture real-time measurements and forecast insect density in a spatiotemporal manner allows for efficient and precise insect control response that could prevent public health crisis. ***The overall goal of this proposal was to adopt and translate this technology to optimize insect pest management programs and benefit agricultural stakeholders.*** We proposed to develop and ultimately deploy opto-electronic sensors that can accurately identify Spotted Wing Drosophila (SWD) and differentiate it from other insect inhabitants of cherry orchards.

SWD is a highly invasive pest species that cause up to $500 million in annual losses in the western United States because they oviposit into marketable, ripening fruit (Goodhue et al. 2011, Wiman et al. 2016). An insect sensor utilizing wing beat frequency for classification can theoretically be applied to identify any flying insect, but the substantial economic loss caused by SWD warrants the prioritization of optimizing this new technology for its control. It is important to stress that the electronic sensor technology we proposed to develop and optimize for SWD was not simply a modernized version of insect traps currently used for population monitoring. Besides supplanting conventional monitoring tools and greatly reducing the time necessary to process trap contents, we anticipated that the capability of the sensors to classify insects in real-time will revolutionize pest management research and lead to developments in precision agriculture. For example, current monitoring tools lack spatial and temporal resolution as conventional traps do not provide time-stamps for insect catches. Our sensors on the other hand can ultimately be connected to a central network and were capable of reporting real-time movement between crop and non-crop host plants, providing opportunities to target SWD for sprays at times when they are at maximum density in non-crop plants. This can reduce insecticide residues on crops, a major concern for export markets.

OBJECTIVES

Objective 1:

Measure wing beat frequency and circadian activity pattern of SWD to improve insect identification algorithm. Opto-electronic sensors will be installed in insect cages that house SWD to measure wing beat frequency and daily activity patterns simultaneously. Since biological parameters, e.g. sex, age, and seasonal morphology, may alter wing beat frequency and activity patterns, we plan to evaluate male and female SWD, different ages of SWD, and summer and winter forms of SWD. Various abiotic factors can also affect wing beat frequencies so we will evaluate recordings in a range of environmental conditions.

Objective 2:

Field recording to assess opto-electronic sensor and insect identification algorithm. We will deploy opto-electronic sensors housed in McPhail traps to assess the capability of the sensors to accurately identify SWD from other inhabitants of Cherry orchards.

METHODS

Objective 1: Refining insect identification algorithm using opto-electronic sensors

Overview: In order to automate the process of insect identification based on wing beat frequency,

an algorithm was created and refined to take into account biotic and abiotic factors that may result in changes to insect wing beat frequency and activity pattern. Our cooperators have previously created an algorithm to accurately identify insects down to species and sex using wing beat frequency in controlled environments, which they have tested on mosquito species (Chen et al. 2014). To refine this algorithm for SWD and use in the field, we recorded wing beat frequency of SWD and other insects commonly found in cherry orchards different environmental conditions (temperature, light cycle, humidity, etc.). The data acquired from these species in controlled environments were then incorporated in insect identification models to enable refinement of the algorithm.

Collection of data for insect identification algorithm refinement

Flies of a known species and sex (N=60) were placed into a modified McPhail trap outfitted with an opto-electronic sensor ring and connected to a recording device. This setup was then placed into a Digitherm incubator (Tritech Research) that allowed us to control the environmental conditions. Using this setup, we recorded wing beat frequency data in different temperatures, humidity, light-dark cycles with different photoperiods, etc. as well as wing beat frequency of different species and sexes. The data collected in these controlled environments were visualized using analysis programs using MATLAB (Mathworks). General trends were visualized using these analysis tools. Comparison between SWD and the closely related *Drosophila melanogaster* in controlled conditions showed distinct wing beat frequency patterns. Based on live capture in field in CA, we identified several closely related *Drosophila* species such as *D. simulans*, *D. biarmipes* and *D. tristis*. Recording using these different species and other relevant species present in cherry orchards were generated in order to refine the algorithm and improve identification accuracy.

Refinement of insect identification algorithm

Previously our cooperators have created an insect classification algorithm which they have used to accurately identify disease carrying mosquito species based on wing beat frequency alone (Chen et al. 2014). When more species were added or environmental conditions were changed the classification model was less accurate. Due to the large diversity of species present in the field and the heterogeneity of environmental conditions, it is important to have accurate classification established on a wide range of fluctuating parameters and species to mimic field scenarios. By creating a training dataset using the data we collected from *Drosophila* flies in various conditions, we were able to "train" the classification model to accurately identify insect pests in vastly different environments. We have already "trained" the insect classification model based on geographical and circadian rhythm data to increase the accuracy of the model in identifying mosquitos down to the species level. By "training" the insect classification model to correctly identify insects using a larger number of variables we were able to increase the accuracy of our identification process in the field. This was an iterative process of testing and refinement.

Objective 2: Assessment of insect identification algorithm and field deployment of sensors

Overview: With current monitoring methods, it is extremely time consuming to monitor insect pest species in the field because it requires the presence of a specialist to manually identify individuals. In addition, the time lapse between trapping and identification constitutes an important limitation to initiate a quick and appropriate response to slow down crop infestation. Our goal in refining the insect identification algorithm was to develop an automated identification process that is easier and faster to identify insect pests compared to current pest capture and identification processes. We assessed the ability of the sensors to correctly identify and monitor pest species both spatially and temporally in and around SWD habitats in CA.

Deployment of insect sensors in the field

Once the classification algorithm was found to be highly accurate (>99%), we deployed our system in SWD habitats. We used baited McPhail traps outfitted with sensors in the opening at the bottom to record the wing beat frequency and relevant environmental variables (temperature, humidity, time, etc.) of any insect that enters the trap and identifying them in real time. By deploying multiple trap/sensor setups, we were able to track the movements of SWD throughout the day, e.g. from crop to non-crop hosts. We envision this will allow for the development of more precise strategies of pest management than are possible through conventional monitoring techniques using traps and manual identification. The automated process of insect identification also means that there will be far less processing time required to identify flies allowing growers and researchers to respond to the presence of pests as soon as they arrive and are detected in their fields.

RESULTS:

Objective 1:

Hardware optimization for insect sensors

We successfully went through several iterations of design and testing of the sensors. We have converged on a solution that we feel is robust, maintainable and cheap to produce in large numbers. Briefly, we use IR emitters and phototransistors working at a wavelength of 940nm, which is outside the visible light spectrum. Our emitters (OSRAM SFH 4043) and phototransistors (Everlight PT19-21C) use around ~20mA. There may be some other low power emitters and phototransistors out there that we can used to further improve our design in the future. We are currently using a cortex M4 MCU, which runs at 80Mhz. This chip has 32K of flash and 2K of RAM. It consumes around 5.5mA when running at 8Mhz but we can put it to sleep when there is no activity, in sleep mode it only consumes few micro amps. For transmission we are using Long Range Wide Area Network (LoRaWAN) technology. LoRaWAN is a wireless standard designed for long range communications at a low bit rate on a very low power budget. We use Semtech SX1272 LoRa module which has a range of 2 miles in non-line-of-sight environment and up to 15 miles in line-of-sight environment. It can achieve data rates up to 50 kbps. SX1272 consumes ~15mA while transmitting/receiving and a negligible power (1.5 uA) in idle state. We have started to install solar panel to the sensor unit, so that the sensors can be left unattended in the field for weeks at a time (Figures 1 to 3 and Figure 5).

Development of species ID algorithm

Since the completion of the activity and wing beat frequency recordings for 5 different *Drosophila* species (*D. simulans, D. tristis, D. suzukii, D. biarmipies, D. melanogaster*) at various temperature and photoperiod conditions (Figure 4), we have collected more than one million insect "encounters", in diverse conditions of light, temperature, humidity, life-stage, species, sex. Using this data, we have built the state-of-the-art classification model for insect classification, which is invariant to environmental conditions. For example, we can now train a model say in our dry hot California research station at ten meters above sea level and be confident that the model will generalize to the cooler humid conditions. Just a year ago, this environmental variability would cause our models to fail to generalize, drastically reducing our accuracy. Now the accuracy of the resulting species ID algorithm is easily over 90% accuracy and will continue to improve as we continue to feed the algorithm with data collected in the field.

Objective 2:
Field deployment and testing

For the purpose of testing the power and communication modules, we leveraged a field setup that was already in place to monitor Navel Orange Worm (NOW). We can then easily adopt the same setup for use with SWD monitors. We outfitted the sensors with solar panel and cellular data transmission for remote sensing. A first field trial was performed in an almonds orchard located in CA from May to September 2019 to trap (Figure 5). NOW can be trapped using sticky card loaded with species specific pheromone allowing us to collect and validate information for a given species before increasing complexity by adding more species to the system. During this test we were able to successfully validate:

1) the autonomy of the sensors.
2) the ability of the sensors to remotely send information to the database.
3) the accuracy of the system to match insect counts recorded by the sensor with the number of insects collected on sticky trap.

Indeed, the solar panel efficiently ensured full autonomy of the system as it provided enough power to sustain the sensor during the 4 months of the trial without any intervention required from us to recharge the battery. Data were send remotely using cellular signal to an online database where insect counts as well as environmental parameters associated (T°C, Humidity, Pressure, Light cycles...) were readily accessible. Finally we observed a strong correlation between information collected from the sensor and number of insects on the sticky card attesting of the efficacy of the sensor to detect pest pressure in real time (Figure 6).

Sensors to monitor for SWD (Figure 7) are currently deployed in the UC Davis orchard of Wolfskill. Traps are filled up with yeast-sugar solution, an attractive lure for *Drosophilds* allowing us to validate the capability of the sensor to accurately identify the presence of SWD among other species of fruit flies. Given the current percentage of accuracy provided by the algorithm we are confident that SWD recordings will result in high accuracy identification. Results from this trial are expected to set the stage for field application in the upcoming growing seasons. We hope to offer growers and PCA unprecedent tools to optimize insect pest management programs. Users interested in testing the smart sensor in their own crop are invited to request a demo by contacting the PI Joanna Chiu (jcchiu@ucdavis.edu) or by visiting the Farmsense website (www.farmsense.io).

LITERATURE REVIEW:

There have been some efforts in identifying insects based on recordings of their wing beat frequencies and these attempts date back to the advent of commercially available computers and audio recording devices (Reed et al. 1942, Foster and Robinson 1991, Moore and Miller 2002, Raman et al. 2007). These attempts have not been successful in creating an automated and accurate identification process based on recordings of wing beat frequencies. In most studies, wing beat frequency has been recorded using acoustic microphones, which are susceptible to noise from the wind as well as any ambient noise in the environment (Reed et al. 1942, Mankin et al. 2006, Raman et al. 2007, Villarreal et al. 2017). This made it very difficult to get quality recordings of insect wing beat frequency with acoustic recording devices. Because of this difficulty, wing beat frequency data is sparse, low quality, and typically recorded in unnatural conditions (Moore et al. 1986). Despite the sparseness and low quality of available insect wing beat frequency data, some researchers have attempted to create insect identification models with 300 or less recordings (Moore 1991). It is

difficult to create models with such sparse data and this will cause the models to have very low accuracy in identifying insects (Banko and Brill 2001, Halevy et al. 2009). This is compounded by the fact that most attempts at classification of insects by recording wing beat frequency have used just one variable (wing beat frequency). Other environmental factors that cause wing beat frequency to change have also been ignored (Chen et al. 2014). By using pseudo-acoustic opto-electronic sensors, we will be able to record higher quality data. We will also be able to record larger volumes of data in more natural conditions than has been possible in the past, which will allow us to create a highly accurate insect classification model that can be used to identify SWD and differentiate it from other species in the field.

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EXECUTIVE SUMMARY

Spotted Wing Drosophila (SWD) is a highly invasive pest species that has now established itself as a keystone pest of U.S. fruit crops, including cherries. SWD oviposits into marketable, ripening fruits, leading to significant annual crop and economic losses. ***The overall goal of this proposal was to adopt electronic sensor technologies and develop automated insect identification models to enable remote and real-time SWD identification and monitoring to support management programs and IPM research efforts.*** Our cooperator Dr. Keogh, a computer scientist at UC Riverside, has previously developed inexpensive pseudo-acoustic opto-electronic sensors and accompanying classification algorithm that can accurately classify multiple species of mosquitoes that vector pathogens such as Zika and West Nile virus by using wing-beat frequencies, daily activity patterns, and geographical distribution. An insect sensor utilizing wing beat frequency for classification can theoretically be applied to identify any flying insect. We therefore proposed to optimize electronic sensor technologies for SWD identification. It is important to stress that the electronic sensor technology we proposed to develop and optimize for SWD is not simply a modernized version of insect traps currently used for population monitoring. Besides supplanting conventional monitoring tools and greatly reducing the time necessary to process trap contents, we anticipated that the capability of the sensors to classify insects in real-time will revolutionize pest management research and enable precision agriculture. For example, current monitoring tools lack spatial and temporal resolution as conventional traps do not provide time-stamps for insect catches. Our sensors on the other hand can ultimately be connected to a central network and were capable of reporting real-time movement between crop and non-crop host plants, providing opportunities to target SWD for sprays at times when they are at maximum density in non-crop plants. This can reduce insecticide residues on crops, a major concern for export markets.

Key products of project:

1. Software: We have collected more than one million insect “encounters” in diverse conditions of light, temperature, humidity, life-stage, species, sex, and completed the development of SWD species ID algorithm.
2. Hardware: We have successfully validated the solar power and remote communication modules of the sensor.
3. Field trials: We have initiated field testing of the SWD system and will continue repeated iterations of trial and optimization cycles. Measurements of field encounters will continue to improve the species ID algorithm. Demo units can now be requested.

KEY WORDS:

Spotted Wing Drosophila, *Drosophila suzukii*, remote sensing, insect identification

FIGURES

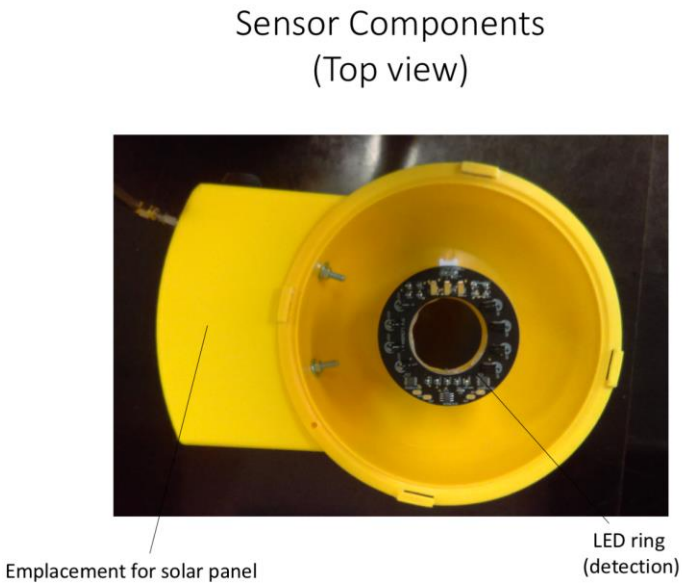

Figure 1: Top view of the modified Mcphail trap outfitted with the LED sensor ring, showing emplacement for solar panel.

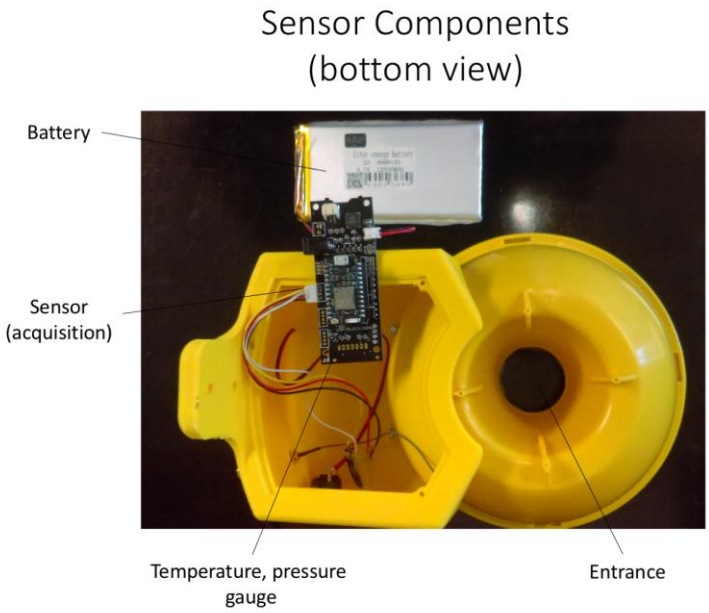

Figure 2: Bottom view of the modified Mcphail trap showing the battery unit, the data acquisition unit, the environmental measurement unit, and the entrance of the trap.

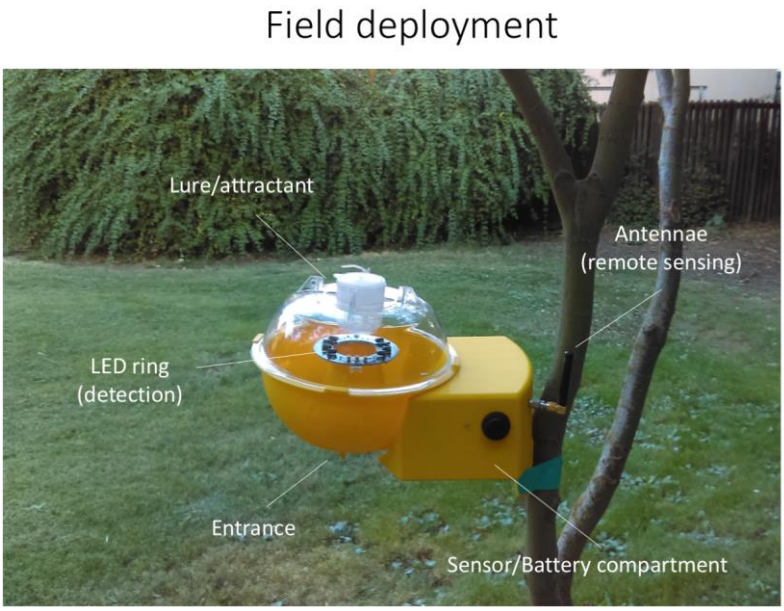

Figure 3: The modified Mcphail trap holding the sensor unit in a field setting. The antennae for transmitting remote sensing data is shown.

Temperature:		20C	25C	30C
D. sim	Male	x	x	x
	Female	x	x	x
D. tris	Male	x	x	x
	Female	x	x	x
D. suz	Male	x	x	x
	Female	x	x	x
D. biar	Male	x	x	x
	Female	x	x	x
D. mel	Male	x	x	x
	Female	x	x	x

Photoperiod		12:12
D. sim	Male	x
	Female	x
D. tris	Male	x
	Female	x
D. suz	Male	x
	Female	x
D. biar	Male	x
	Female	x
D. mel	Male	x
	Female	x

Figure 4: Temperature and photoperiod conditions for wing beat frequency recordings. Conditions marked with pink have been completed. *D. simulans (D. sim); D. tristis (D. tris); D. suzukii (D. suz); D. biarmipes (D. biar); D. melanogaster (D. mel).*

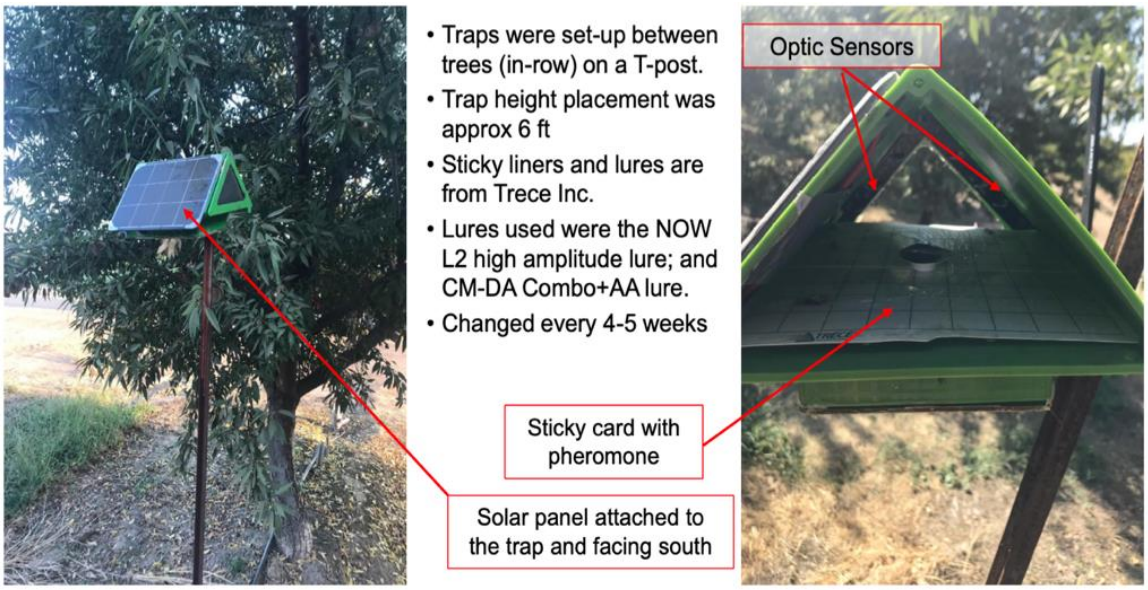

Figure 5: Experimental setup for field testing on NOW

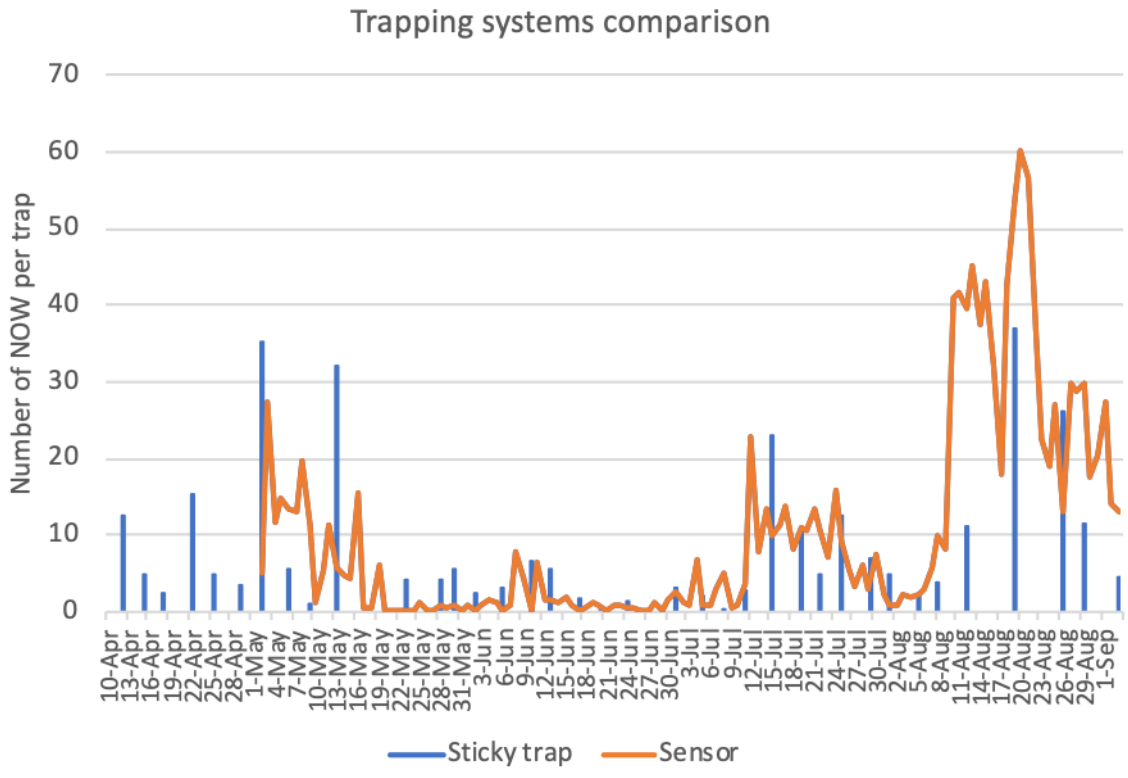

Figure 6: Insect count comparison between Sensor vs Sticky trap.

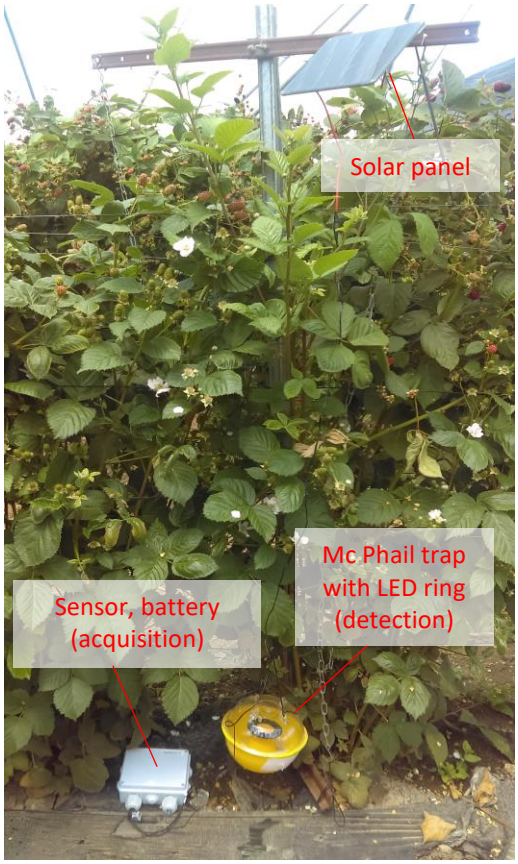

Figure 7: Field deployment of the sensors monitoring for SWD