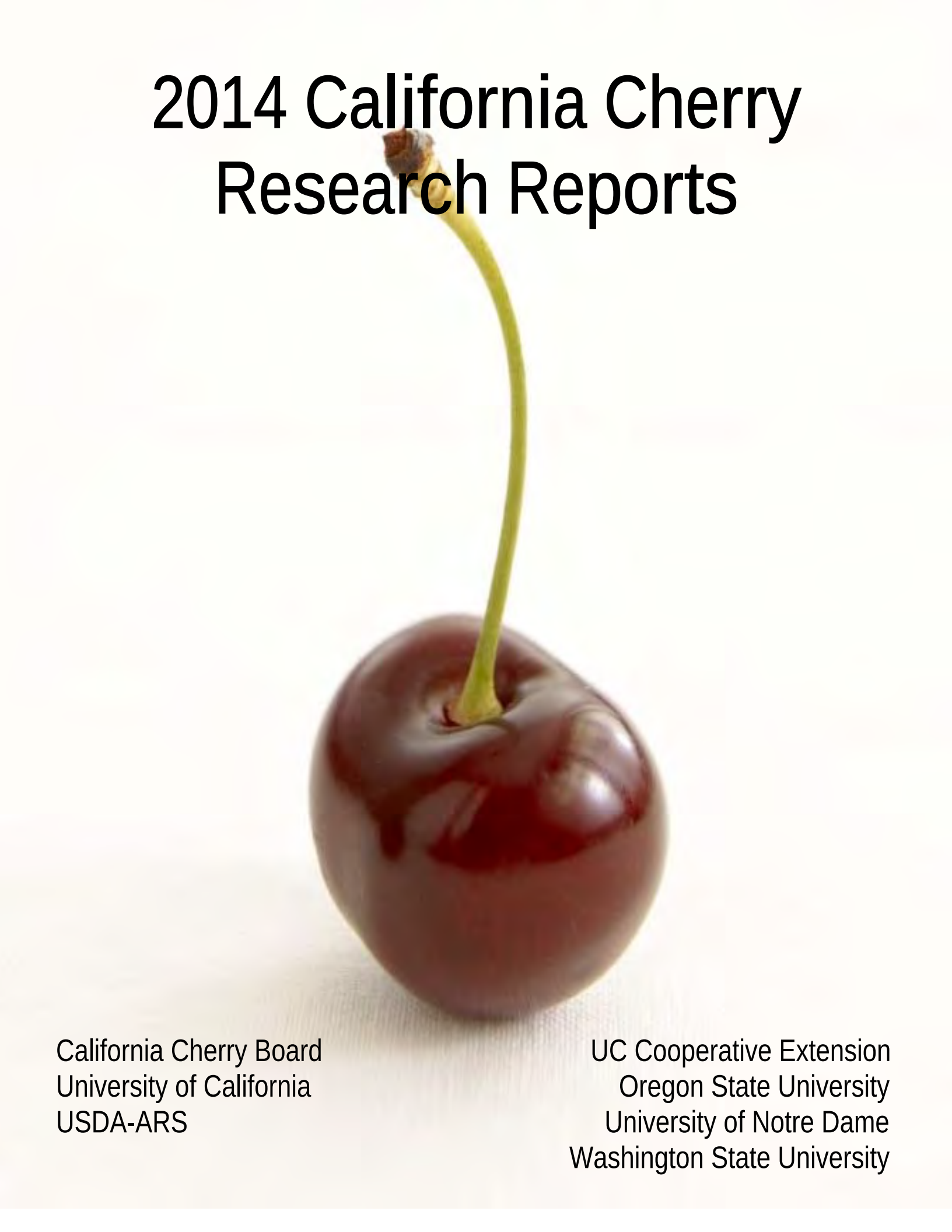
2014 California Cherry Research Reports



California Cherry Board
University of California
USDA-ARS

UC Cooperative Extension
Oregon State University
University of Notre Dame
Washington State University

CALIFORNIA CHERRY RESEARCH REVIEW
January 27, 2015

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

10:00 am	**Welcome** Joe Grant, UC Cooperative Extension, San Joaquin County
10:15	**Control of cherry fungal canker diseases** Dr. Doug Gubler, Dept. of Plant Pathology, UC Davis
10:45	**Evaluation of Spirotetramat as a post-plant nematicide in cherries** David Haviland, UC Cooperative Extension, Kern County
11:15	**SWD SCRI- Summary of key information and promising management techniques** Dr. Vaughn Walton, Oregon State University
11:55	**California Cherry Board Ongoing projects Update** CCB Research Committee
12:10	**Lunch** (courtesy of California Cherry Board) **Announcing the California Cherry Board's self-assessment workbook program for sustainable cherry production** Cliff Ohmart, SureHarvest
1:10	**Investigating biological controls to suppress spotted wing drosophila** Dr. Kent Daane, Dept. of ESPM, UC Berkeley and UC Kearney Agricultural Research & Extension Center, Parlier, CA
1:40	**Management and epidemiology of pre- and postharvest foliar and fruit diseases of sweet cherry** Dr. Jim Adaskaveg, Dept. of Plant Pathology, UC Riverside
2:10	**Optimizing postharvest methyl bromide treatments to control spotted wing drosophila in sweet cherries** Dr. Spencer Walse, USDA-ARS, Parlier, CA
2:40	**Phytosanitary irradiation using a cabinet X-ray tube machine** Dr. Peter Follett, USDA-ARS, Hilo, HI
3:10	**Adjourn**

3.5 hours of PCA Continuing Education Credit pending

CALIFORNIA CHERRY BOARD

2014-2015 RESEARCH COMMITTEE

MEMBER	CCB	PRIMARY REPRESENTATION AREA	INDUSTRY ACTIVITIES
NICK MATTEIS **(Staff)**	S	ALL	CCB (RESEARCH CO-OR)
RICH HANDEL	N	ALL	PACKER, GROWER, SHIPPER
JOE GRANT **(ex officio)**	N	SAN JOAQUIN CO.	U.C.EXTENSION CHERRY LIAISON
ARNIE TOSO	A	SAN JOAQUIN CO.	CCB, GROWER
ANDREW DASSO, JR.	A	ALL	CCB, PACKER, GROWER, SHIPPER
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BLAKE UEKI	S	TULARE CO.	CCB, GROWER, PACKER, SHIPPER
ROSS VAN VLACK	N	FRESNO CO.	GROWER, PACKER, SHIPPER

CALIFORNIA CHERRY BOARD -- Research Projects Approved April 2014
2014 Research Projects

	Project Leader	Project Title	Requested Amount	Total Funded Amount		
1	Syed	Identifying Drosophila Suzukii Attractants from Preferred Fruits and Yeast for Improved Monitoring and Management	$ 57,172.00	$57,172.00		
2	Adaskaveg	Management & Epidemiology of Pre- & Postharvest Foliar & Fruit Diseases of Sweet Cherry	$ 41,500.00	$ 41,500.00		
3	Gubler	Control of Canker Diseases in Sweet Cherry	$ 36,211.05	$ 36,211.05		
4	Chiu	Developing a Biopesticide to Combat Spotted Wing Drosophila	$ 21,145.00	$ 21,145.00		
5	Haviland	Evaluation of Spirotetramat as a Post-Plant Nematicide in Cherries	$ 16,024.00	$ 16,024.00		
6	Walse	The Post-Harvest Treatment of U.S. Cherries with Methyl Bromide/Phosphine-Oxygen Mixtures to Eliminate Key Insect Pests	$ 11,000.00	$ 11,000.00		
7	Beers/Eastwell	Developing a Management Strategy for Little Cherry Disease	$ 10,000.00	$ 10,000.00	OR Funding: $10,000 WA Funding: $43,479	NW Total Requested: $63,479
8	Wang	Extending Storage/Shipping Life and Assuring Good Arrival of Cherry	$ 8,000.00	$ 8,000.00	OR Funding: $16,466	NW Total Requested: $24,466
9	Walse	Postharvest Systems-Based Treatment of California Sweet Cherries for Brown Marmorated Stink Bug	$ 7,198.00	$ 7,198.00		
10	Einhorn	Early Season Estimation of Fruit Set and Size Potential	$ 5,000.00	$ 5,000.00	OR Funding: $50,368 WA Funding: $5,596	NW Total Requested: $60,964
11	Van Steenwyk	Ovicidal/Larvcidal Efficacy of Danitol and Malathion for the Control of Spotted Wing Drosophila	$ 2,000.00	$2,000.00		
12	Follett	Phytosanitary Irradiation Using a Cabinet X-ray Tube Machine	$ 11,100.00	$11,100.00		
13	Akbari/Hay	Engineered Transgenic Drosophila Suzukii for Wild Population Suppression and Eradication: Production, Performance Assessment and Effective Wild Releases	$ 61,000.00	$70,150.00		
14	Daane	Investigating Biological Controls to Suppress Spotted Wing Drosophila Populations	$ 37,431.00	$37,431.00		
			$ 324,781.05	**$333,931.05**		

CALIFORNIA CHERRY BOARD
2014 FINAL RESEARCH REPORTS

CALIFORNIA CHERRY BOARD
2014 FINAL RESEARCH REPORTS

Project year: 2014

Project leader: W. Douglas Gubler, C.E. Specialist
(530) 752-0304, wdgubler@ucdavis.edu
Location: University of California, Davis, CA 95616

Cooperating personnel:
Ara Avadisi Abramians, Graduate Student, Department of Plant Pathology, UC Davis, CA 95616; (530) 752-4982,aavadisiabramians@ucdavis.edu
Janet Caprile, Farm Advisor and County Director, Contra Costa and Alameda County, (925) 646-6129; jlcaprile@ucanr.edu
Joe Grant, Farm Advisor and CE Specialist, Cooperative Extension, San Joaquin County, (209) 468-2085; jagrant@ucdavis.edu

Project title: CONTROL OF CANKER DISEASES IN SWEET CHERRY
Keywords: Sweet cherry, canker diseases, Calosphaeria, Cytospora, Leucostoma, Eutypa dieback

Commodity: Sweet Cherry **Relevant AES/CE Project No.**

Project Summary of Accomplishments

Objective 1. Identify fungi and bacteria associated with cherry cankers in CA.

Isolations from diseased wood in 2014 continued to yield Calosphaeria, Cytospora, and Eutypa from all production areas. From two orchards in Stockton, we continue to recover Alternaria at a high rate and isolates do cause cankers in cherry branches. However, we consider Alternaria to be secondary and we are not doing anything with it currently. We recovered *Botryrosphaeria sp* from one orchard and *Phomopsis viticola* from another. Both are demonstrated pathogens but not prevalent.

Isolations for *Pseudomonas syringae* were negative nearly all cankers leading to the conclusion that fungal cherry cankers are not associated with bacterial canker.

Objective 2. Implement cultural practices to reduce risk of infection with Calosphaeria canker, Eutypa dieback, and Leucostoma (Cytospora) canker.

In order to have successful control of canker problem in sweet cherries, implementation of an integrated approach using cultural practices and chemical control measures is advisable. Knowing the biology of the pathogen is absolutely necessary for implementing effective and integrated control measures. To this end, in our current studies we studied the effect of temperature on lesion length development for three main canker causing fungi *Eutypa lata*, *Leucostoma persoonii* (Cytospora) and *Calosphaeria pulchella.* Accordingly, our results, showed fungal growth and canker formation occurs over a wide range of temperatures ranging from 60-80 F; however, temperatures between 70-75 °F were ideal for *Eutypa lata* and *Leucostoma persoonii. Calosphaeria pulchella* had a more irregular growth pattern and was more active at higher temperatures (80°F). These results indicate that pruning wounds that are made when the

average daily temperatures are less than 50°F will have less of a chance of becoming infected due to the negative action of cold temperatures on spore release and infection. Therefore, it makes sense that with summer pruning when temperatures are moderate, followed by sprinkler irrigation which releases spores of the fungal pathogens, there is going to be the potential of higher disease levels.

Which is most important, temperature or water? Right now I would have to say water because without it, the fungi can do nothing i.e. release spores or infect wood. Also temperatures are not so cut and dried since infection can occur over a pretty wide temperature range.

In addition to temperature, abundance of free water was shown to be the important means of fungal spore dispersal, as well as a favorable condition for fungal infection. Based on our studies, solid sprinkler irrigated orchards, on average, had almost twice as many cankered branches compared to those with drip and microsprinkler irrigation. Even though the average number of cankers in microsprinkler orchards were lower than solid set sprinklers, the difference between the two types was not significant. The variability we observed could be due to the variability in the angle of the water that was discharged from the nozzles and the proximity of the sprinklers from the tree trunks. Further investigation on this matter is necessary. In addition, since the pruning wounds are susceptible for such a long duration it might be more prudent to make sure water in absolutely not getting above the lower trunk or use of splitters might be used to keep water off the main tree.

Pruning diseased wood out of cherry trees is key to getting overall long term control. Due to lack of systemic fungicides that can cure the cankered branches, elimination of the lesions is the only way to stop the spread of the fungus inside the tree. Our study showed that most of the pruning that is done in commercial orchards is apparently not aimed at complete elimination of the canker problem. In one site we found that the same number of cankers in trees two weeks after pruning as it was before pruning. In another site pruning reduced the number of cankers from an average of 22/tree before pruning to 7/tree after pruning. Though this latter pruning was better, there are still too many cankers left in the trees after pruning. This latter branch disease will move into scaffold branches. In the case of Calosphaeria this movement happens quite rapidly.

This lack of disease elimination also effects fungicide trials. We know we cannot kill the pathogens once they get in the wood, thus if treatments are applied to branches with disease it is going to make the fungicides appear useless. This might explain the variation we have seen in our fungicide work.

Equally as important as pathogen biology is the importance of the host biology and the interaction between host and pathogen. Knowing the duration of wound susceptibility to *Eutypa lata, Leucostoma persoonii* (Cytospora) and *Calosphaeria pulchella* is instrumental in terms of knowing the biology of the host wound healing process and the interaction of the wounded branches of the host with any of the three pathogens. Our results indicate that pruning wounds of sweet cherry trees remain susceptible to *Eutypa lata, Leucostoma persoonii* (Cytospora) and *Calosphaeria pulchella* over the course of twelve weeks and longer. It is worthy to note that disease incidence for all three fungi is reduced significantly as wounds age.

Implementing measures such as using drip irrigation, scheduling the pruning based on the time of year, weather forecast to assure the absence of precipitation, using pre-pruning sprinkler irrigation during the summer, and favorable temperatures immediately before and after pruning, as well as preventing the exposure of the recent pruning wounds with the aforementioned disease-conducive weather conditions are integrative management measures.

Objective 3. Implement chemical control methods against Calosphaeria canker, Eutypa dieback, and Leucostoma (Cytospora) canker.

Based on our results, treating pruning wounds with fungicides will further protect the wounds and will lower disease incidence.

Our fungicide trials showed that almost all tested fungicides reduce disease incidence caused by *Eutypa lata*, *Leucostoma persoonii* (Cytospora) and *Calosphaeria pulchella.* Some of the fungicides had 100% control (0% disease incidence) when applied to pruning wounds that were inoculated by natural inoculum. Rally at 6.0 oz + Topsin at 1.5# per acre (one and two applications), Rally at 0.45g/500mL (only when applied twice in 14 days intervals) and Topsin at 1.99g/500mL were fungicidal treatments that had 0% disease incidence. Using paints and sealants, such as Farwell's Grafting Seal, was shown to be ineffective if used without amending with fungicides. Mixing Farwell's Grafting Sealant with Rally at 0.45g/500 ml + Topsin at 1.99g/500 ml and Topsin at 1.99g/500 ml gave 100% disease control *in Vitro* trials. More field trials are needed to fine tune the fungicide rates to increase their efficacy.

2014 Report

Objective 1. Identify fungi and bacteria associated with cherry cankers in CA.

Objective 2. Implement cultural practices to reduce risk of infection with Calosphaeria canker, Eutypa dieback, and Leucostoma (Cytospora) canker.

Objective 3. Implement chemical control methods against Calosphaeria canker, Eutypa dieback, and Leucostoma (Cytospora) canker.

Problem and Significance:

California is the second largest sweet cherry producer in the US with approximately 10,800 ha and an average annual crop value of about $200 million. Perennial canker diseases constitute major threats to the cherry industry productivity by reducing tree health, orchard longevity and yields. Recently, we described Calosphaeria canker caused by *Calosphaeria pulchella* as a new and widespread canker disease of sweet cherry (*Prunus avium* L.) in California (Trouillas et al., 2010). Additional pathogens reported to occur in cankers in sweet cherry in California have included *Eutypa lata* and *Leucostoma persoonii* (*Cytospora*). The epidemiology of these pathogens has been studied and there is evidence that spores are released in response to wetting caused by rain or irrigation, thus dispersing by wind or rain splashing. Infection normally occurs during the pruning season when fresh pruning wounds become exposed to spores. In California, release and dispersal of spores of *L. persoonii* occur during rain in all seasons (Bertrand and English, 1976). *Eutypa lata* spreads to new pruning wounds by wind-driven ascospores released during fall and winter rains (Ramos et al., 1975). Similarly, high spore concentrations of *C. pulchella* are found in California cherry orchards throughout the rainy season and during sprinkler irrigation events in the spring and summer months (Trouillas et al., 2012). Systematic pruning in summer and winter is widely implemented in sweet cherry orchards in California to keep trees to a suitable size, promote branching and early maturing of sweet cherries. Sprinkler irrigation also is broadly utilized. Based on previous studies we postulated that the implementation of tree pruning and generalized use of sprinkler irrigation in sweet cherry orchards in California have favored an outbreak of canker diseases.

Protection of pruning wounds with fungicides may reduce infection by fungal pathogens. However, this can be problematic because of the limited number of effective registered products and the limited duration of protection.

The objectives of this study are to (i) Identify fungi associated with cherry cankers in CA. (ii) Determine the role of other fungi in cherry cankers. (iii) Implement chemical control methods against Calosphaeria canker, Eutypa dieback, and Leucostoma (Cytospora) canker.

Growth Chamber Experiment

Procedure

In order to evaluate the effect of temperature on disease severity and lesion expansion growth chamber trials were established. Three main causal agents, namely *Eutypa lata*, *Leucostoma persoonii* (Cytospora) and *Calosphaeria pulchella* were used in this experiment. Two-three year

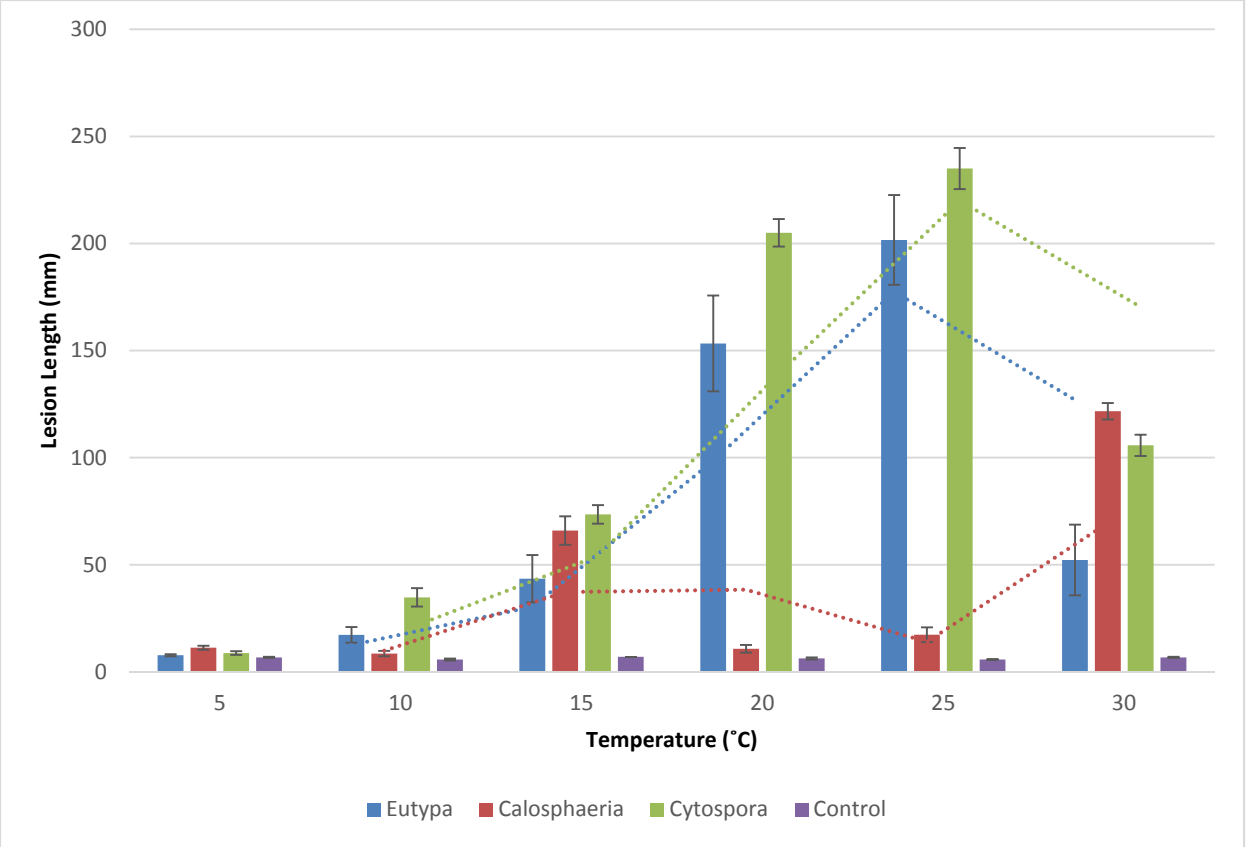
old sweet cherry branches were cut into 12-inch segments and all leaves were removed. The branches were soaked in a 10% bleach solution for 15 minutes and then rinsed with sterile distilled water. After air-drying, both ends of the woods were dipped in paraffin wax to prevent desiccation. The wood was inoculated by mycelial agar plugs that were placed in 4mm wounds made near the middle of each branch. The inoculated wounds were wrapped with parafilm and wood was placed in 12 X 9 inch plastic boxes with lids in place (crispers). Four wood segments were inoculated by each fungal isolate per given temperature. The crispers were incubated in growth chambers at 5, 10, 15, 20, 25 and 30°C.

Results

Temperatures of 20-25 °C were ideal for fungal growth (Figure 1). Using ANOVA, these differences were significantly different at $p<.0001$. Overall fungal development was highest at 25°C and the lesion length difference at this temperature was significantly different than those of 15, 20 and 30°C.

Eutypa and Cytospora had the most growth at 25°C and their lesion lengths were significantly different ($p<.0001$) than Calosphaeria. Similarly at 20°C Eutypa and Cytospora had significantly more growth than Calosphaeria ($p<.0001$). At 30°C, Cytospora and Eutypa had slower growth than Calosphaeria. At 30°C, the lesion length differences between Eutypa and Calosphaeria was statistically significant ($p<0.0004$). The trend line on the graph (Fig.1) indicates the development of lesion length pattern as the temperature increases in 5°C increments.

Figure 1. Lesion length development of *Eutypa lata, Calosphaeria puchella and Leucostoma personii* (Cytospora) at different temperatures. The best fit line (perforated line) indicates the trend of lesion length development by Cytospora (green), Eutypa (blue) and Calosphaeria (red).



Drip vs Sprinkler Trial- Statewide Survey

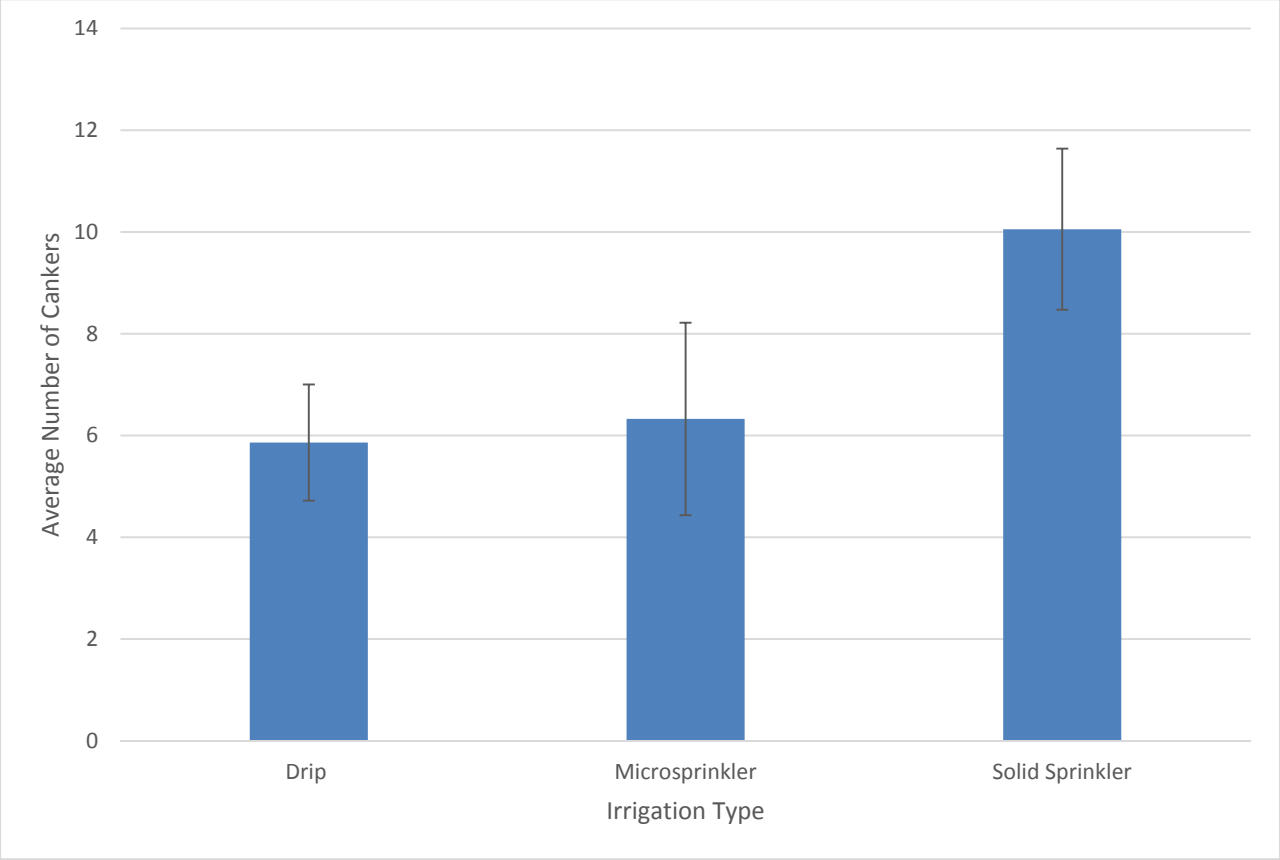
Procedure

In July and August 2014; Drip, micro-sprinkler and solid sprinkler irrigated orchards were surveyed in Contra Costa, San Joaquin, Sacramento and Fresno counties to assess disease incidence in orchards. In each orchard with their corresponding irrigation type, forty trees were surveyed for number of visible cankers. The trees were chosen at random. Approximately twenty five samples of cankered branches were collected and returned to the laboratory for assessment of the type of fungus that caused the canker. Wood samples were surface sterilized using ethanol and flaming. Wood chips from necrotic lesions were plated onto PDA-tetracycline plates. The recovered fungi were identified using physical properties and/or by molecular techniques. Some of the isolates were used in fungicide efficacy experiment (Automated Spiral Plater).

Results

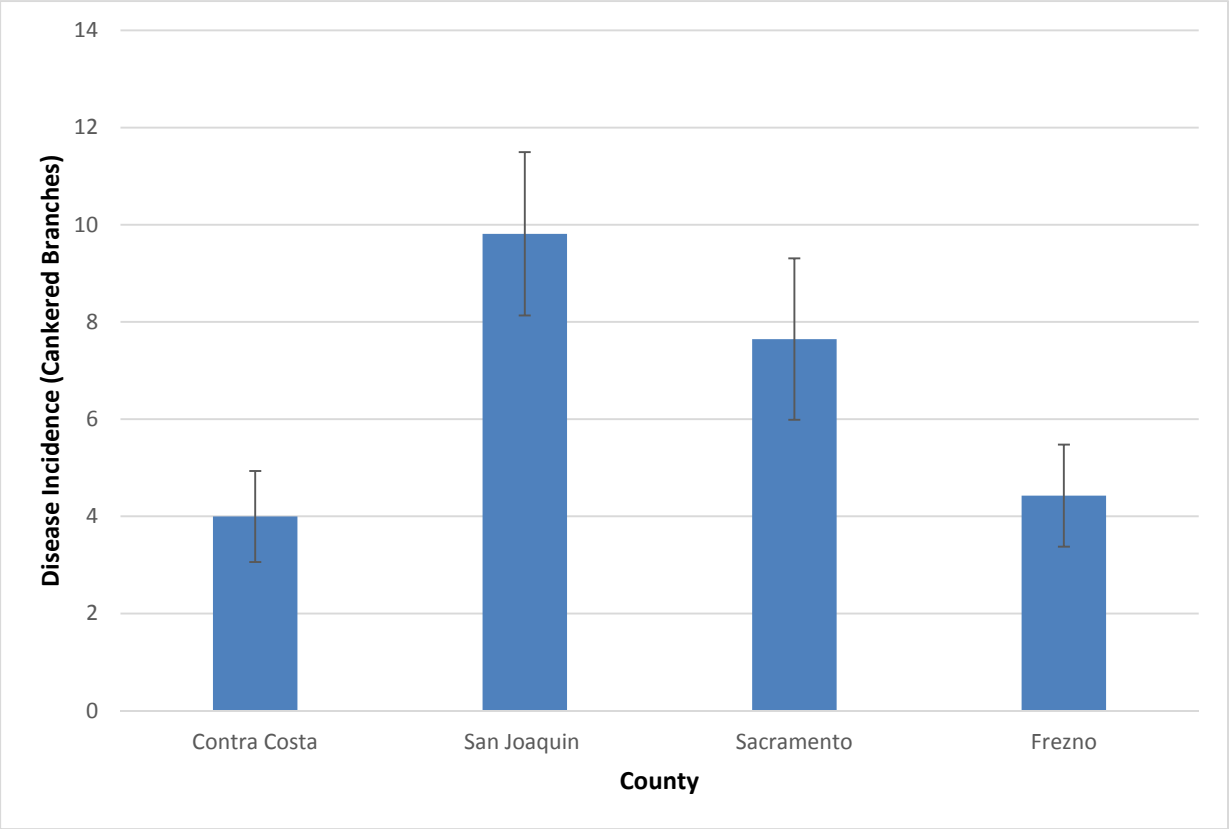
Drip orchards had significantly fewer cankers than sprinkler orchards, ($P<0.0500$) (Figure 2). Even though microsprinkler irrigated orchards on average had fewer cankers than solid sprinkler irrigated orchards, the differences were not statistically significant. Similarly the differences between Drip and micro-sprinkler irrigated orchards were not significant.

Figure 2. Average number of cankers per tree in drip, microsprinkler and sprinkler irrigated orchards.



Overall there was some difference in average number of cankers in orchards located in different counties (Fig. 3); however these differences were not statistically significant. The only significant difference was between Contra Costa and San Joaquin counties. Eight out of ten orchards that were surveyed in Contra Costa County were microsprinkler and one was drip irrigated. In San Joaquin County, almost fifty percent of orchards that were visited were sprinkler irrigated. The difference in irrigation type can be a major factor in disease incidence.

Figure 3. Average number of cankers per tree in Contra Costa, San Joaquin, Sacramento and Fresno Counies.



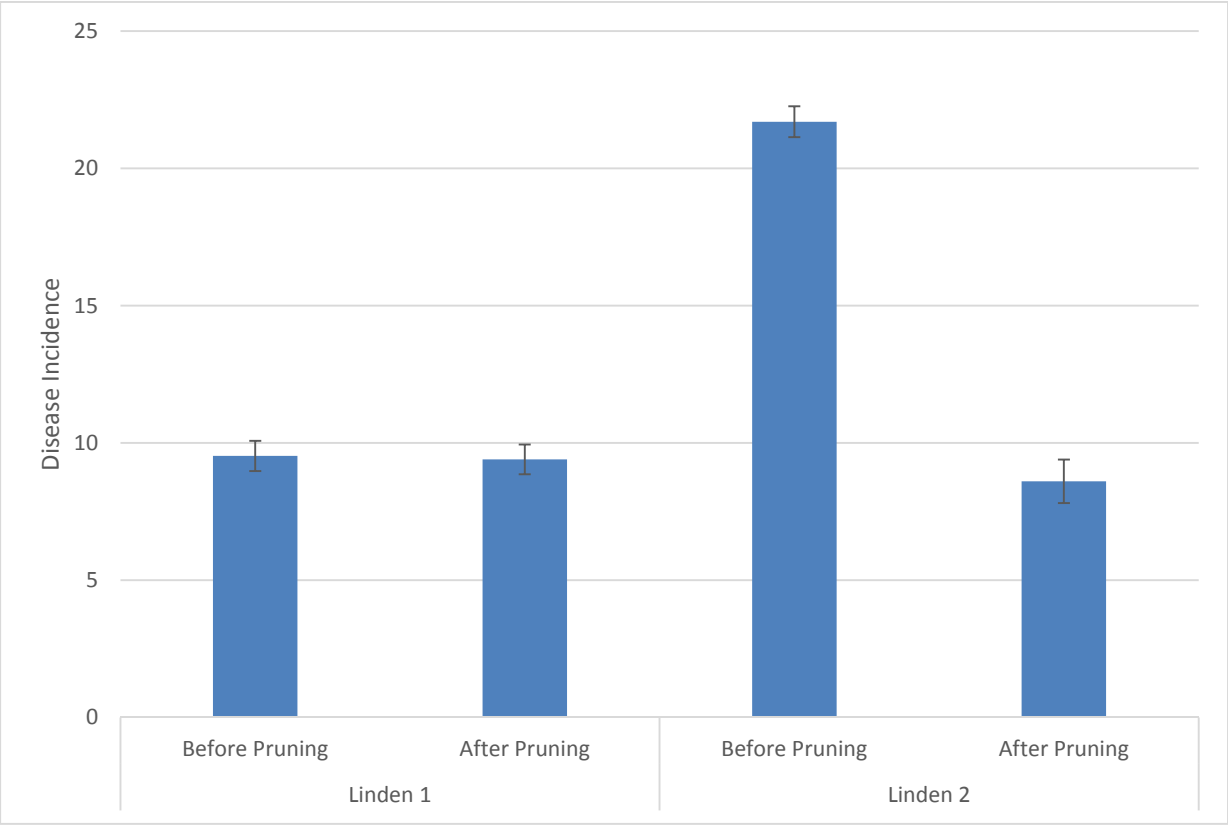
After Pruning Canker Assessment

Two orchards located in Linden that were previously surveyed were randomly chosen and revisited after the winter pruning was completed in these orchards. The procedure for surveying these two orchards after pruning was similar to our summer surveying procedure.

Results

One of the two orchards (Linden 1 in Figure 4) had an average of the same number of cankers after pruning as it had before pruning. The second orchard (Linden 2 in Figure 3) had approximately 60% less cankers after pruning. This difference was statistically significant ($p<.0001$. However, what this says about the pruning is that not all infections are being pruned out of the trees and this probably accounts for what we are seeing in terms of the amount of disease and how fast it seems to be moving in a branch. In other words what we have been seeing is not new infections as we had previously thought but one year old or older wounds that simply are continuing to progress.

Figure 4. Average number of cankers per tree in two orchards in Linden before and after pruning.



Pruning Wound Protection Trials

Several field trials have been conducted evaluating fungicide efficacy against canker pathogens. Field trials were established in Davis, Brentwood and Linden, CA in sweet cherry orchards (*Prunus avium* cv. Bing). For all fungicide trials, fresh stub cuts were made on two to three year-old wood in cherry orchards. Liquid formulations of fungicides were sprayed in a single or with repeating (shown as 2x in following sections) applications with 14-day intervals with 500 ml spray bottles immediately after pruning. After several months, treated branches were collected and returned to the laboratory for assessment of fungal colonization and wound protection. Wood samples were surface sterilized using ethanol and flaming. Wood chips from necrotic lesions were plated onto PDA-tetracycline plates. Fungicide efficacy was estimated by the number of fungal colonies of the various pathogens developing from plated tissues.

Natural Inoculum Fungicide Trial; Davis – March 2014

Procedure

Fresh pruning wounds (stub cuts) were made on two to three year old wood in Davis in March 2014. For each treatment, 20 stub cuts were treated. Liquid formulations of Rally at 0.45g/500m + Topsin at 1.99g/500mL, Rally at 0.45g/500mL, Topsin at 1.99g/500mL, Orbit at 0.63g/500mL, Luna Sensation at 0.63g/500mL and Cannonball at 0.53g/500mL were sprayed with 500 ml spray bottles immediately after pruning. Two weeks later, half of the branches were treated with second application of the same fungicides with the same rates. After six months treated branches were collected and returned to the laboratory for assessment of fungal colonization and wound protection. Wood samples were surface sterilized using ethanol and flaming. Wood chips from necrotic lesions or vascular discoloration just below the pruning wounds were plated onto PDA-tetracycline plates. Fungicide efficacy was estimated by the number of fungal colonies of the various pathogens developing from plated tissues.

Results

As shown in Figure 5, Eutypa was the most prevalent pathogen recovered from cankers in this trial. The incidence of Calosphaeria and Eutypa infection were reduced by fungicide treatment. (Figures 6 and 7). As shown in Figure 5, stub cuts treated with Rally + Topsin , Rally, Topsin, Orbit, Luna Sensation and Cannonball and treatments that received a second spray (except for Luna Sensation) were not infected by Calosphaeria. The differences in effect of fungicide on Calosphaeria was significant ($p<0.0087$). For Eutypa, Rally+Topsin, Rally+Topsin (2x), Rally (2x), Topsin, and Luna Sensation (2x) protected the stub cuts from this pathogen. The differences in fungicide effect on Eutypa was significant ($p<0.0011$). Rally+Topsin (2x), Luna Sensation and Cannonball treated stub cuts were not infected by Cytospora (Figure 8). There was no significant difference in fungicide treatments for Cytospora. When data for all 3 pathogens were combined, treatment means were significantly different ($p<.0.0005$).

Figure 5. Percent of stub cuts forming cankers using natural inoculum-Davis, March, 2014

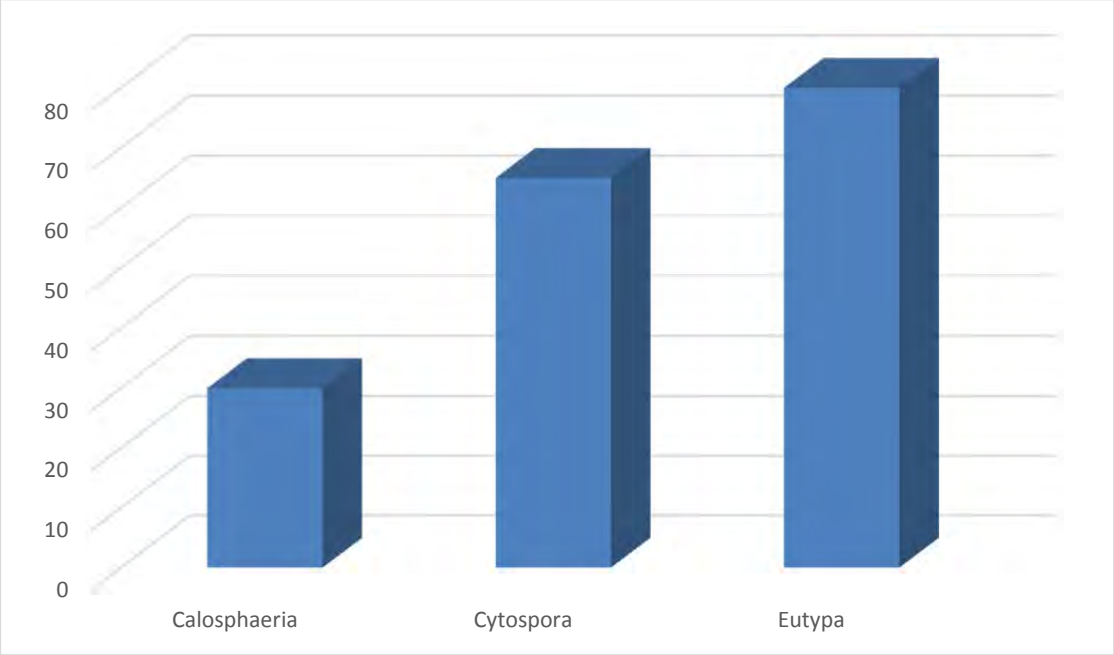


Figure 6. Percent of stub cuts developing cankers caused by *Calosphaeria puchella* in fungicide trial using natural inoculum in Davis fungicide trial, March 2014.

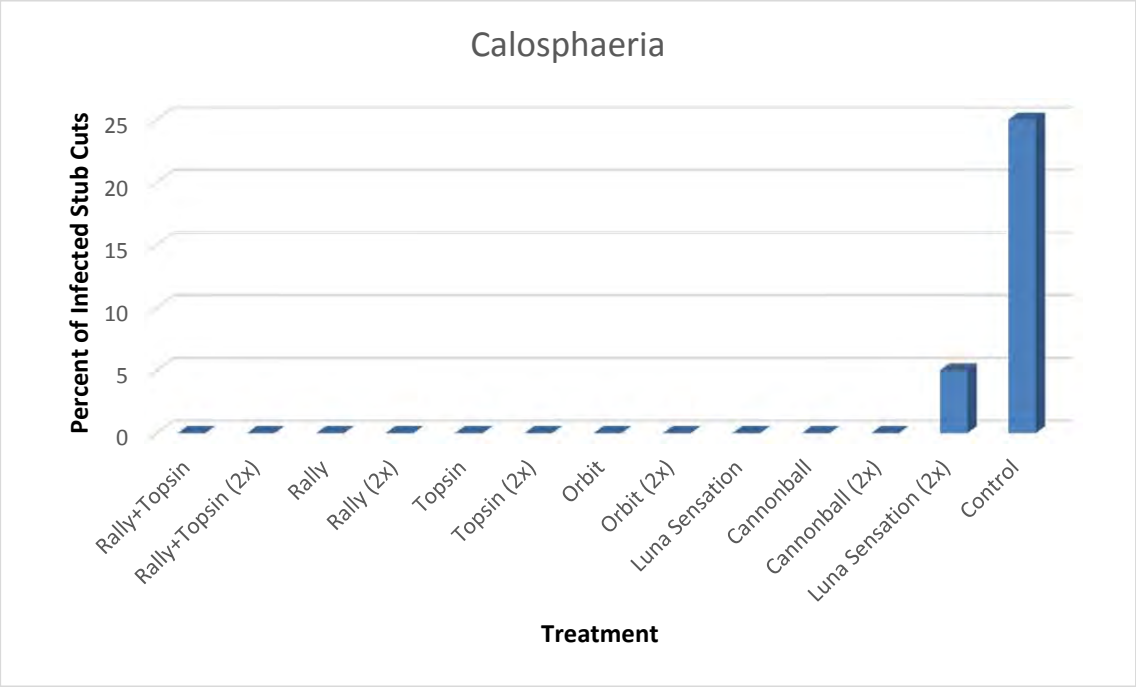


Figure 7. Percent of stub cuts developing cankers caused by *Eutypa lata* in a fungicide trial using natural inoculum in Davis fungicide trial, March 2014.

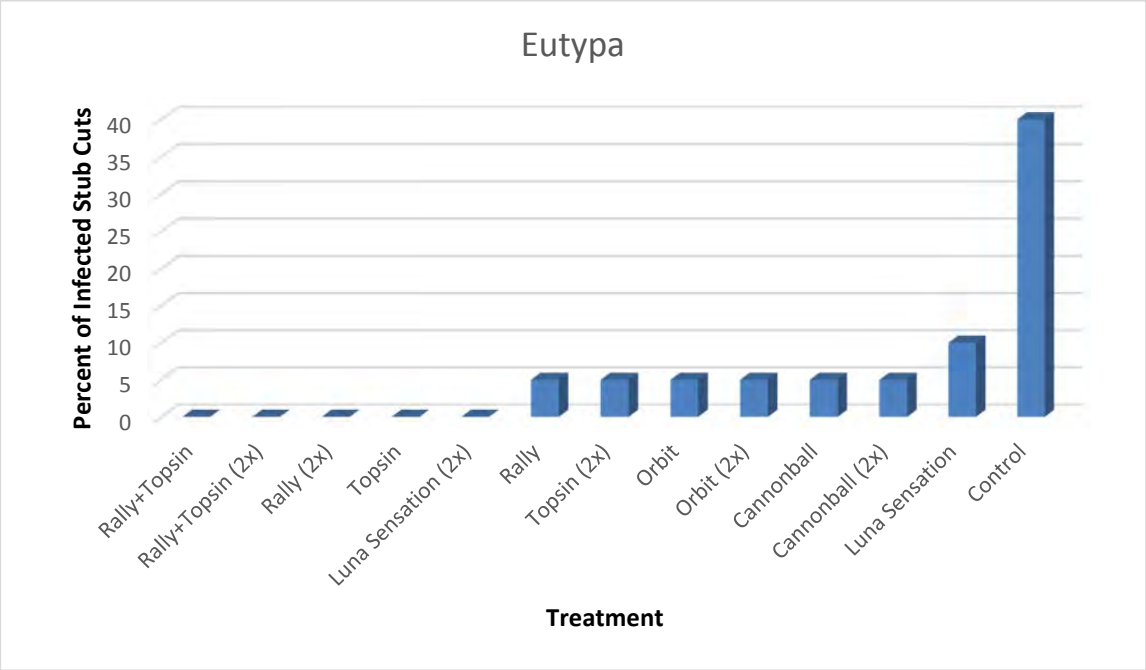
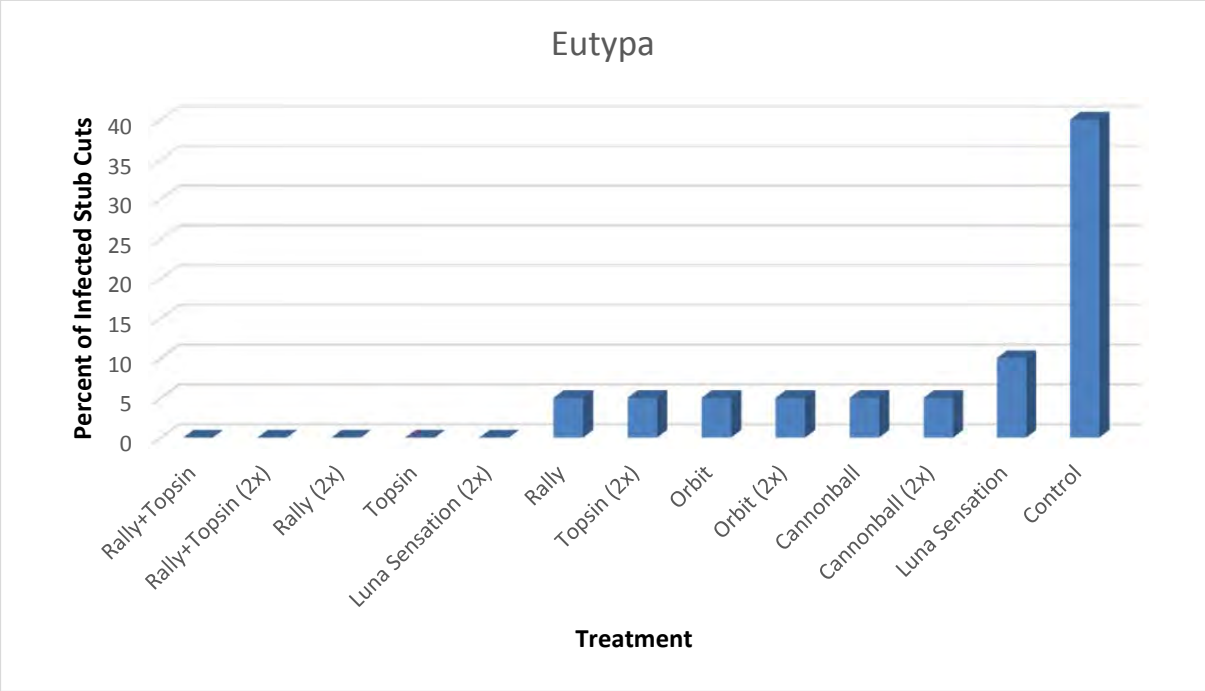


Figure 8. Percent of stub cuts developing cankers caused by *Leucostoma persoonii* (Cytospora) in a fungicide trial using natural inoculum in Davis fungicide trial, March 2014.



Natural inoculum Trial Linden – March 2014

Procedure

In March 2014, 240 stub cuts were made in Linden, CA. Eighty stub cuts each were sprayed with the following treatments: Rally at 0.45g/500 ml + Topsin at 1.99g/500 ml, Rally at 0.45g/500 ml + Topsin at 1.99g/500 ml and a control (no fungicide). Second application of the same treatments were done 14 days later. After several months, treated branches were collected and returned to the laboratory for assessment of fungal colonization and wound protection. Wood samples were surface sterilized using ethanol and flaming. Wood chips from necrotic lesions were plated onto PDA-tetracycline plates. Fungicide efficacy was estimated by the number of fungal colonies of the various pathogens developing from plated tissues.

Results

Cytospora was the most common pathogen isolated from lesions . All three fungi were reduced by fungicide treatments (Figures 9-11). As shown in Fig. 9, stub cuts that received one time and two times applications of Rally+Topsin treatment were not infected by Calosphaeria. The differences between two treatments was not significant. In Cytospora (Fig.11), two applications of Rally+Topsin in 14-days interval significantly reduced (53%) the percent of infected stub cuts compared to the single treatment. The differences in percent of infected stub cuts were significant ($p<0.0202$) between treatments and the control. In Eutypa, stub cuts that received two treatments of the fungicide in 14-days interval were not infected. Stub cuts that received one time treatment of Rally+Topsin had 48% less infection than control (Fig. 10). The differences between treatments and the control was significant ($p<0.0159$).

Figure 9. Percent of stub cuts developing cankers caused by *Calosphaeria puchella* in fungicide trial using natural inoculum in Linden fungicide trial, March 2014.

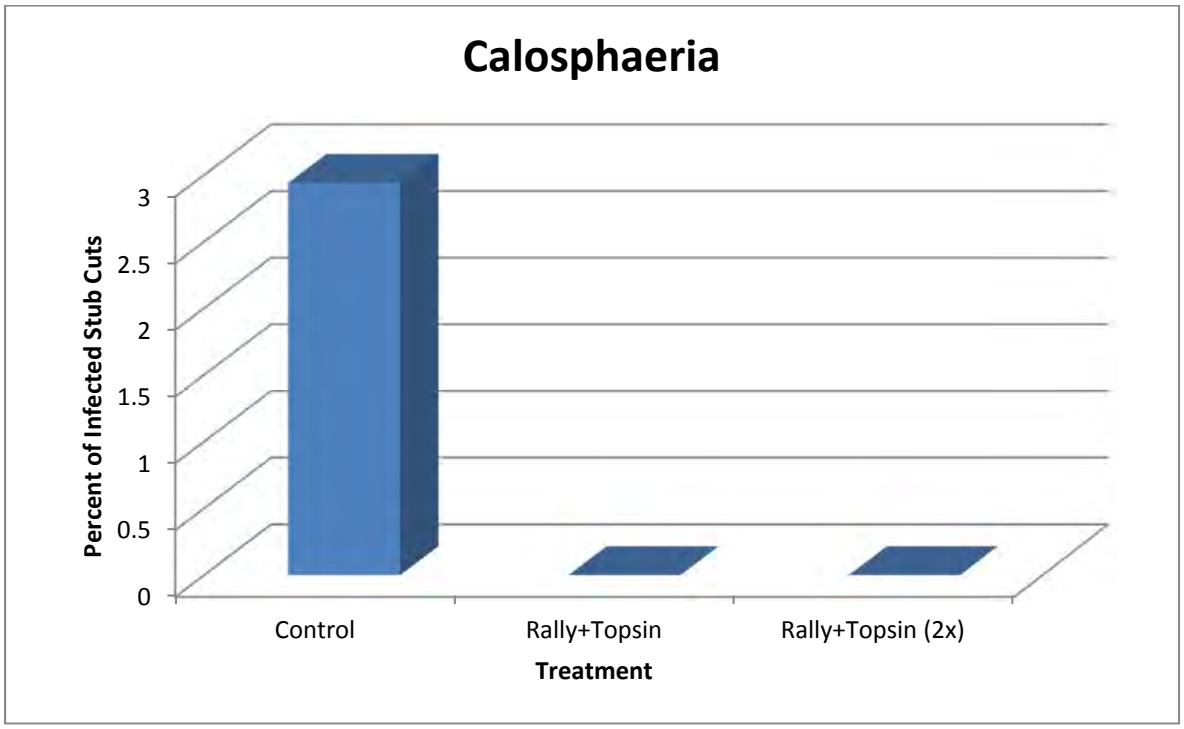


Figure 10. Percent of stub cuts developing cankers caused by *Eutypa lata* in fungicide trial using natural inoculum in Linden fungicide trial, March 2014.

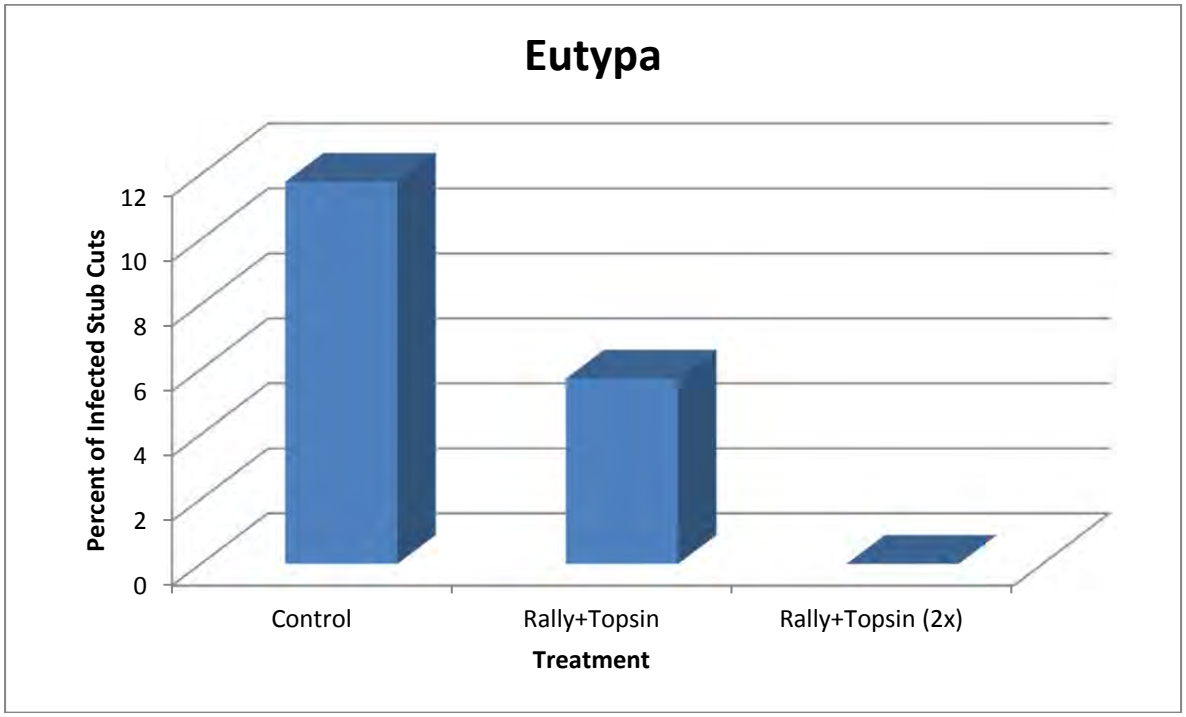
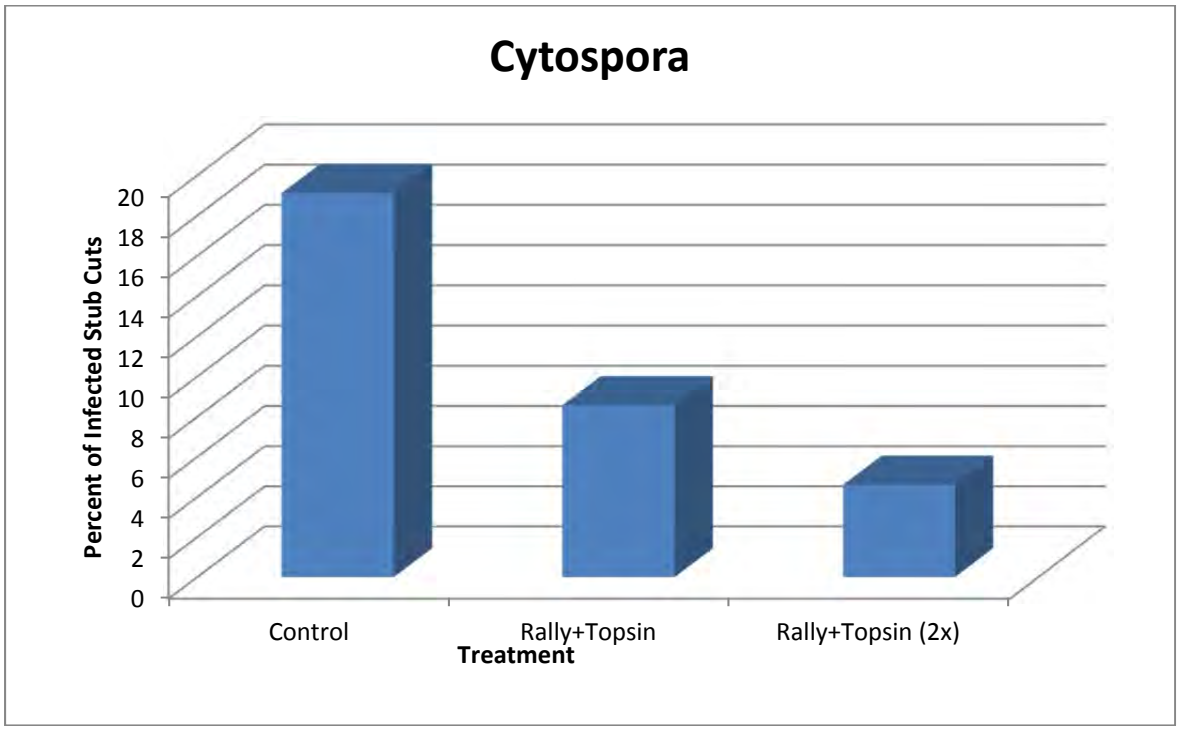


Figure 11. Percent of stub cuts developing cankers caused by *Leucostoma persoonii* (Cytospora) in fungicide trial using natural inoculum in Linden fungicide trial, March 2014.



Davis Pruning Wound Susceptibility Trial – February 2014

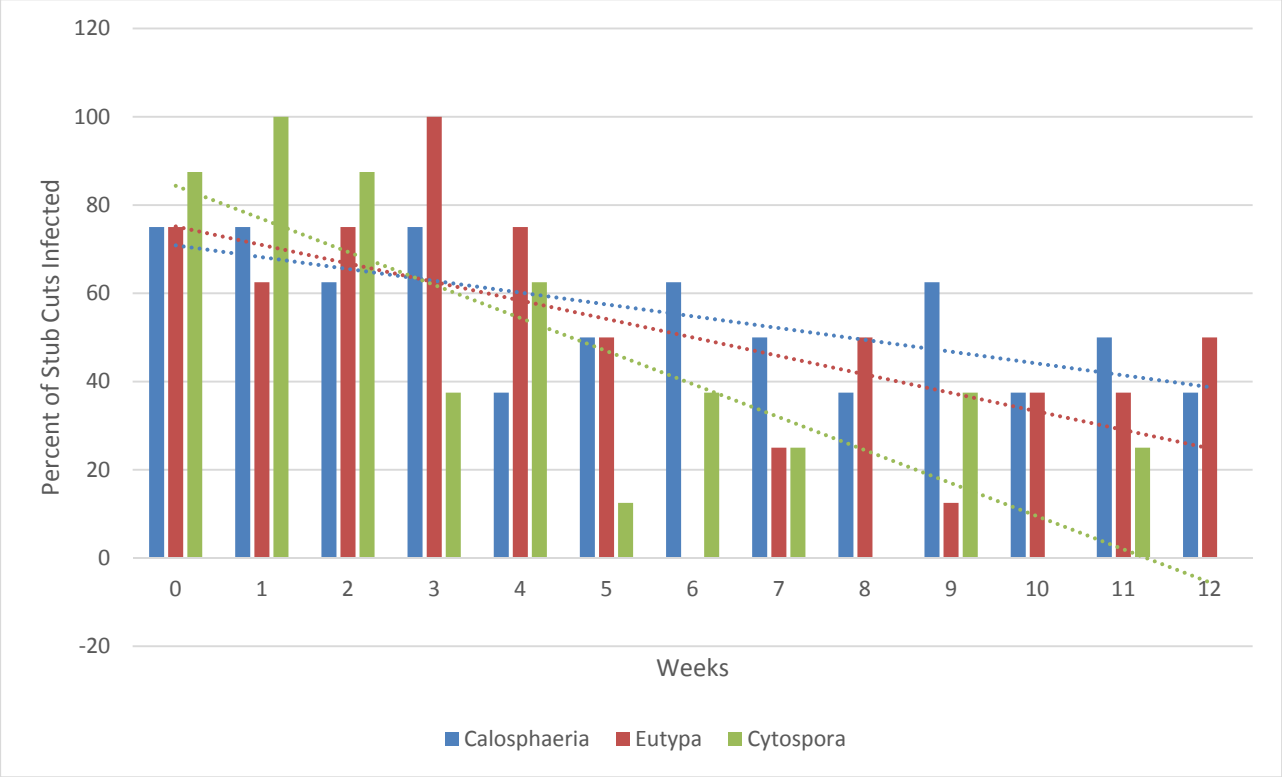
Procedure

Two hundred eighty eight pruning wounds were made on February 14, 2014 in Davis. Every week, a subset of eight branches per pathogen was inoculated with Eutypa, Cytospora, or Calosphaeria for a total of 24 branches. Mycelial plugs were placed on the pruning wounds and covered with parafilm. This process was repeated every week for twelve weeks. Four months after the last pruning wounds were inoculated, the treated branches were removed. Wood samples were surface sterilized using ethanol and flaming. Wood chips from necrotic lesions or vascular discoloration just below the pruning wounds were plated onto PDA-tetracycline plates to look for fungal growth.

Results

Results show Calosphaeria and Eutypa can infect twelve week old pruning wounds. However, as it is shown by the descending trend line in Fig. 19, susceptibility declined over the twelve week period for both pathogens. Cytospora was able to infect stub cuts up to eleven week. The steeper declining trend line (green line) indicates more reduction in infectivity by Cytospora (Figure 12). The differences in disease incidence caused by Eutypa and Cytospora were significant ($p<0.0001$ for Cytospora and 0.0007 for Eutypa) but not significant for Calosphaeria.

Figure 12. Stub cut susceptibility to Calosphaeria, Cytospora and Eutypa through twelve weeks. The best fit line (perforated line) indicates the descending trend in percent of stub cuts infected by Calosphaeria (blue line), Eutypa (red line) and Cytospora (green line).



In Vitro (Bottle) Fungicide Trials

Farwell's Grafting Seal

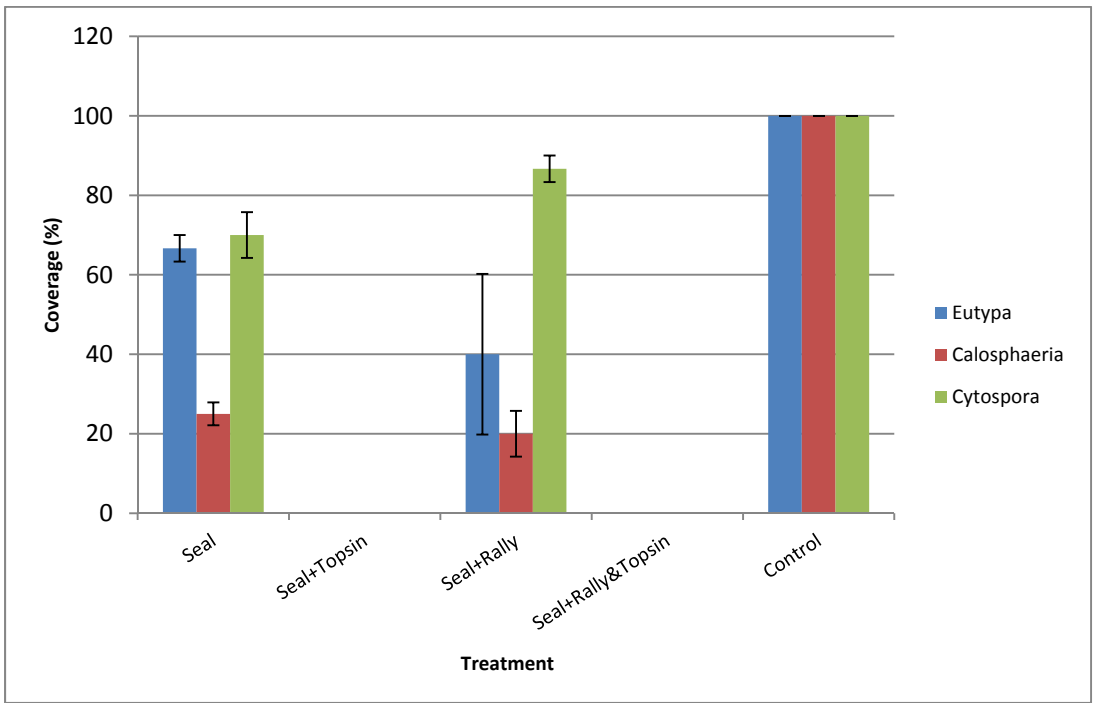
Procedure

In order to assess the efficacy of Farwell's Grafting Seal, a sealing product that is commonly used for sealing pruning wounds by cherry growers, a bottle trial was established. Two and three year old cherry branches were cut in about one inch pieces and autoclaved twice. Farwell's Grafting Seal, Sealant+ Rally at 0.45g/500mL, Sealant + Topsin at 1.99g/500mL, Sealant + Rally at 0.45g/500mL+ Topsin at 1.99g/500mL and a control (no sealant/fungicide) were the five treatments tested in this trial. *Eutypa lata*, *Leucostoma persoonii* (Cytospora), *Calosphaeria pulchella,* were cultured in bottles containing PDA tetracycline medium. Three replications of each treatment were used in this trial. After one week of incubation period and fungal colony growth, cut cherry wood was submerged in fungicide solutions and placed in the bottles. The fungal growth on the wood was estimated on a weekly basis.

Results

The sealant treated wood had less mycelial coverage compared to the control. However, Compared to Sealant + Topsin at 1.99g/500mL and Sealant + Rally at 0.45g/500mL+ Topsin at 1.99g/500mL that had no mycelial coverage the sealant treatment had significantly more ($p<0.0001$) myucelial coverage (Fig. 13). Sealant+ Rally also reduced the mycelial coverage, but this treatment was not significantly different than the sealant alone.

Figure 13: Percent coverage with fungal mycelium of *Eutypa lata*, *Leucostoma persoonii* (Cytospora), *Calosphaeria pulchella* on woods treated with Sealant with and/or without fungicide three weeks after wood exposure to the fungi.



Vapor Gard

One of our objectives of past year's research was to screen different products that can be used by both organic and conventional growers for controlling canker problem. After testing number of natural products including different essential oils, fertilizer formulations and mineral oils (the results are not shown) Vapor Guard was found to be effective against canker causing fungi when applied as concentrated (stock) solution in our preliminary in vitro trials. Vapor Gard is an organic (pine derivative) product that is used as an anti-transpirant agent in agricultural and horticultural production. In order to assess the efficacy of diluted form of this product an in vitro trial was established.

Procedure

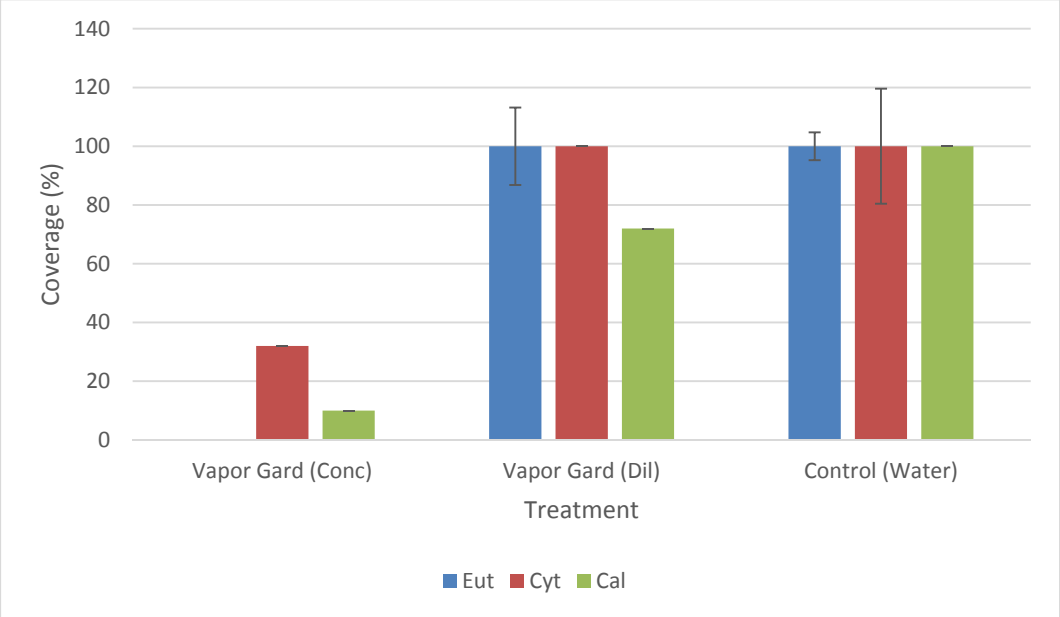
Two and three year old cherry branches were cut in about one inch pieces and autoclaved twice. Concentrated Vapor Gard, diluted Vapor Gard (1:10) and a control were the three treatments tested in this trial. *Eutypa lata*, *Leucostoma persoonii* (Cytospora), *Calosphaeria pulchella*, were cultured in bottles containing PDA tetracycline medium. Five replications of each treatment were used in this trial. After one week of incubation period and fungal colony growth, cut cherry wood

was submerged in Vapor Gard solutions and placed in the bottles. The fungal growth on the wood was estimated on a weekly basis.

Results

Woods treated with concentrated (stock) form of Vapor Gard had no Eutypa mycelial coverage (Figure 14). Calosphaeria and Cytospora mycelial coverage was also significantly reduced (90 and 70%) compared to control and the diluted formula ($p<0001$). The Diluted Vapor Gard Reduced the Calosphaeria mycelial coverage by 28%, but did not have significant difference by control.

Figure 14. Percent coverage with fungal mycelium of *Eutypa lata*, *Leucostoma persoonii* (Cytospora), *Calosphaeria pulchella* on woods treated with Vapor Gard three weeks after wood exposure to the fungi.



Brentwood Vitiseal Trial- August 2014

A Vitiseal fungicide trial is still in progress in Contra Costa. The results of this trial will be submitted upon completion.

Discussion

Cherry canker diseases have been highly problematic in Central California. Fungicide trials using artificial inoculum show fungicides are effective in controlling canker formation. Fungicides in natural inoculum trials were shown effective against Eutypa. Future research will focus on bacterial isolations. To date, the three primary cherry canker pathogens are *Eutypa lata, Leucosoma persoonii* and *Calosphaeria puchella*.

Many factors influence canker formations. Sprinkler irrigation, pruning, debris piles near orchards, and spreading woodchips on orchard floors all potentially contribute to canker formation. We recommend using drip irrigation, following pruning with a fungicide treatment, and removing debris piles from orchards.

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Annual Report - 2014

Prepared for the California Cherry Advisory Board

Project Title:	Management and Epidemiology of Pre- and Postharvest Foliar and Fruit Diseases of Sweet Cherry
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Cooperators:	Dr. H. Förster, D. Thompson, L. Wade, and J. Grant (Farm Advisor)

Summary

We continued our research on dormant, blossom, preharvest, and postharvest treatments for the management of major foliar and fruit diseases of sweet cherry in California. We focused on bacterial blast and canker, powdery mildew, brown rot blossom blight and fruit decays, and postharvest decays including brown rot, gray mold, and Rhizopus rot.

1) In studies on bacterial canker and blast (flower infections) caused by *Pseudomonas syringae* pv. *syringae*, the efficacy of copper treatments was compared to the antibiotics kasugamycin (Kasumin), streptomycin and oxytetracycline, the biocontrols Actinovate and Botector, the plant extract Regalia, and the new copper formulation MagnaBon.

   a. In two small-scale field studies on cv. Coral, branch wounds were treated and inoculated. Kasumin was the most effective treatment of the single-treatments. Kasumin mixed with streptomycin, Actinovate, or Botector also significantly reduced the incidence of canker development and canker length

   b. The incidence of blossom blast was very low in the spring of 2014. Data were obtained from evaluation of developing fruit. Kasumin, oxytetracycline (Mycoshield), and Actinovate were identified as effective treatments, whereas Kocide 3000 did not reduce the incidence of fruit with blast symptoms. Oxytetracycline is currently in the IR-4 program for registration on cherry with support from the registrants, the California Cherry Board, and other researchers in the North Central and North Eastern regions of the US.

2) In powdery mildew studies, the disease developed at high incidence on leaves of water sprouts and then on new shoots on terminal branches. The incidence of fruit infections was relatively low in 2014.

   In a trial San Joaquin Co., the most effective treatments included the SDHI (FG 7)-containing fungicides Fontelis, Luna Sensation, and Merivon, as well as selected DMI (FG 3)-containing fungicides such as Rhyme, Procure, and Quadris Top (Fig. 4). Rotation programs of Merivon and Vivando (FG U8) or of Luna Sensation and Serenade Optimum were also very effective; whereas the Rovral-Quintec rotation showed reduced performance. Because of the potential of resistance to single-site mode of action fungicides, FG 7 materials should be tank-mixed with FG 3 or FG 11 compounds. Pre-mixtures and tank mixtures should be used in rotation with other fungicides with different modes of action. Vivando (FG U8) is potentially an excellent mix partner because of its unique mode of action and specificity against powdery mildew fungi.

3) For brown rot and gray mold blossom blight, highly effective fungicides with excellent pre- and post-infection activity included FG 7/11 fungicides (e.g., Luna Sensation, Luna Experience, Merivon) and the FG 7 Fontelis. The FG 3 (DMI) fungicides Quash and Rhyme were very effective against brown rot but also showed good efficacy against gray mold, whereas Ph-D was more effective against gray mold. The natural product Fracture and the biocontrol yeast Botector showed intermediate efficacy and overall performed quite well under these stringent experimental conditions.

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

a. Applications at 6-day PHI with a Ph-D/Elevate mixture, Quadris Top, Luna Experience, and the new experimental EXP-1 by itself and mixed with Headline or EXP-2 provided excellent protection against brown rot in wound inoculation studies using non-washed fruit. For gray mold, only the Ph-D/Elevate mixture showed high efficacy in one of the trials. When non-washed fruit were non-wound drop inoculated with *M. fructicola*, all fungicides evaluated demonstrated high efficacy indicating that many of the newer fungicides have little locally systemic activity.
b. When harvested fruit were washed for 5 min and then inoculated, Procure, Fontelis, Quadris Top, the Ph-D/Tebucon mixture, and treatments that included the new experimental EXP-1 were still highly effective in both wound- and non-wound inoculations with *M. fructicola*. None of the fungicides was highly effective after fruit washing and wound-inoculation with *B. cinerea*.

These studies demonstrate that preharvest treatments can protect fruit from disease-causing organisms before and during harvest. Postharvest decays, however, can still develop due to minor injuries that occur during the bulk handling of fruit and the lack of local systemic action of some fungicides.

5) In laboratory studies on the evaluation of postharvest fungicides, we focused on two natural fermentation products, polyoxin-D (Oso, Tevano, or CX-10440) and the experimental EXP-13. Both compounds showed consistent high efficacy in reducing brown rot and gray mold when inoculated fruit were treated 13 to 20 h after inoculation. Efficacy against Rhizopus rot, however, was very inconsistent. With increasing emphasis on food safety and consumer concerns, treatments like these with 'exempt from tolerance status' these may become important in the future. A generic tebuconazole (Tebucon) was also evaluated and was comparable in efficacy to the previously registered Elite.

INTRODUCTION

Overview. The goals of this project focus on improved pre- and postharvest management of fungal and bacterial pathogens causing flower, foliar, fruit, and branch diseases of sweet cherry. For this, we evaluated new fungicides, bactericides, natural products, and biologicals. Compounds used in our 2014 studies, including their trade names, active ingredients, and FRAC groups (FG) are summarized in Table 1. Except for the pre-mixtures, all of the newer fungicides have a single-site mode of action. This emphasizes the implementation of resistance management strategies to avoid the development of resistant pathogen populations regardless of the effectiveness of the fungicides. The use of pre-mixtures with at least two ingredients of different mode of action that are both active against the pathogen(s) reduces the risk of resistance development. Merivon (fluxapyroxad + pyraclostrobin) and Luna Sensation (fluopyram + trifloxystrobin), are the newest of the pre-mixtures for sweet cherry after Pristine (boscalid + pyraclostrobin), Quilt Xcel (azoxystrobin + propiconazole), and Quadris Top (azoxystrobin + difenoconazole). All these were continued to be evaluated in 2014 because different environmental conditions occur each year. Goals are to identify and develop treatments to prevent overreliance on any one fungicide class and develop treatments that allow for rotations and high levels of control of brown rot and gray mold blossom blight and fruit rot, as well as powdery mildew. Because most fungicides have different efficacies against different diseases, we are helping to design rotation programs where each fungicide is applied at a timing when its efficacy is optimal. Natural products/biocontrols are also evaluated to possibly provide organic growers with alternative treatments for managing major diseases of sweet cherry.

In an additional objective, we are evaluating new treatments for the management of bacterial blossom blast and canker caused by *Pseudomonas syringae* pv. *syringae.* Previously, only copper was available, however, widespread copper resistance in the pathogen has been documented in California. The antibiotics oxytetracycline (Mycoshield, Fireline), streptomycin (Firewall), and kasugamycin (Kasumin) that are currently registered in the United States for management of other bacterial diseases of agricultural

crops were evaluated, as well as the new copper product Magnabon and the biocontrols Actinovate and Botector.

For postharvest management, fungicides with mostly unique modes of action registered on sweet cherry include: tebuconazole (Tebucon), fludioxonil (Scholar), fenhexamid (Judge), pyrimethanil (Penbotec), and propiconazole (Mentor). These products can be used alone or in mixtures. Our studies in 2014 focused on the evaluation of polyoxin-D that previously showed high efficacy against gray mold and brown rot and on the new experimental EXP-13 that represents another natural fermentation product. We also did comparative studies with two formulations of tebuconazole, Elite and Tebucon.

With the establishment of MRLs in many export countries in the last five years and with the establishment of a food additive tolerance (FAT) for fludioxonil in Japan in 2011, Scholar is the first postharvest fungicide that the North American cherry industry can use for domestic and international markets including Japan. The FAT for pyrimethanil was obtained in Japan in 2013. Scholar, but not Penbotec (pyrimethanil), is very stable in the presence of chlorine in re-circulating drench or flooder treatments and in combination with other postharvest fungicides, and can be used at reduced rates, making it cost-effective. The availability of several fungicides belonging to different chemical classes and of different sanitizers for wash treatments is essential for managing the major diseases occurring on sweet cherry after harvest in California. The development of new products that are considered so safe that they will be registered as "exempt from tolerance" will also be critical for preserving the efficacy of these fungicides against postharvest fruit decays and for the successful marketing of sweet cherry in global markets where maximum residue limits (MRLs) will be important factors in the future.

Objectives

1. Evaluate new products against bacterial blast in flower inoculation studies and/or canker in stem inoculation studies. (Cooperate with J. Grant/C. Ingels).
   a. Biologicals/natural products (e.g., Actinovate, polyoxin-D, Double Nickel 55, Blossom Protect).
   b. Antibiotics – Kasugamycin – large-scale trials once federally registered.
   c. Systemic acquired resistance (SAR) compounds – Actigard, PM-1, and possibly others.
2. Evaluate, under field conditions, bloom and preharvest applications of new compounds (e.g., Fontelis), premixtures (e.g., Luna Sensation, Merivon, Quadris Top, Custodia), as well as Scholar, polyoxin-D, 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 (e.g., Vivando), polyoxin-D, SDHI compounds (fluopyram, fluxapyroxad, penthiopyrad, and premixtures using these fungicides) and biologicals such as Fracture 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 (fluopyram, fluxapyroxad, penthiopyrad), HAs (Protexio), and premixtures, and polyoxin-D. We will also evaluate biologicals and OMRI approved organic treatments such as polyoxin-D (Ph-D, Oso) and Fracture.
   c. Test the efficacy of fludioxonil as a preharvest fruit treatment to control postharvest decays for fruit going to international markets (e.g., Japan).
3. Evaluate new fungicides as postharvest treatments and develop cost-effective application methods:
   a. Continue to evaluate Scholar, Penbotec, Mentor, as well as Scholar-Mentor, and Tebucon-Elevate and polyoxin-D-Scholar mixtures with an emphasis on Scholar and Penbotec due to their recent approved 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. Evaluate biologicals, 'exempt from tolerance' materials (EXP-13, polyoxin-D) and possibly OMRI approved organic treatments.

Table 1: Fungicides, bactericides, and biologicals used in 2014 studies*.

Pesticide	FRAC group	Trade name	Active ingredient
Fungicides	***Single***		
	2	Rovral, Iprodione	iprodione
	3	Elite, Tebucon, Tebuzol	tebuconazole
	3	Procure	triflumizole
	3	Quash	metconazole
	3	Rhyme	flutriafol
	7	Fontelis	penthiopyrad
	11	Headline	pyraclostrobin
	12	Scholar	fludioxonil
	13	Quintec	quinoxyfen
	17	Elevate	fenhexamid
	19	Ph-D, Oso, CX-10440	polyoxin-D
	U8	Vivando	metrafenone
	Experimentals	EXP-1	not disclosed
		EXP-2	not disclosed
		EXP-13	not disclosed
	Double (Premixtures)		
	7 + 11	Luna Sensation	fluopyram + trifloxystrobin
	7 + 3	Luna Experience	fluopyram + tebuconazole
	7 + 11	Merivon	fluxapyroxad + pyraclostobin
	7 + 11	Pristine	boscalid + pyraclostrobin
	3 + 11	Quadris Top	difenoconazole + azoxystrobin
	Multiple		
	M1	Kocide 3000, MagnaBon	copper hydroxide, copper sulfate pentahydrate
	M3	Captan	captan
Bactericides	Aminoglycoside	Kasumin	kasugamycin
		Firewall	streptomycin
	Tetracyclines	Mycoshield	oxytetracycline
Biologicals	Bacterium	Actinovate	*Streptomyces lydicus* WYEC108
	Bacterium	Serenade Optimum, Taegro	*Bacillus subtilis* QST713, *B.s. amyloliquefaciens* strain FZB24
	Plant extracts	Fracture, Regalia	protein from *Lupinus. Reynoutria* spp.
	Yeast	Botector	*Aureobasidium pullalans* DSM14940/14941

* - Alphabetical by trade name for each Fungicide Resistance Action Committee (FRAC) group or mode of action. Some fungicides were used with adjuvants such as Silwet or Dyne-Amic.

MATERIALS AND METHODS

Evaluation of treatments for control of bacterial blossom blast and canker. In two trials in mid-December of 2013, the bark of 2-year-old twigs was puncture-wounded using a 12-gauge needle (3 wounds per twig). Wounds were sprayed to run-off using a hand sprayer, allowed to air-dry, and spray-inoculated with *Pseudomonas syringae* pv. *syringae* (2 x 10^8 cfu/ml). Treatments included Kocide 3000, Captan, Fireline, Kasumin, Actinovate, Botector, Kasumin-oil, Kasumin-Captan, Kasumin-Fireline, Kasumin-Botector, and Kasumin-Actinovate. In late April 2014, inoculated branches were sampled and evaluated for the incidence. Severity (canker length) was determined by measuring canker length.

Several trials on bacterial blossom blast were done on cv. Coral cherry in San Joaquin Co. In hand-spray/inoculation studies, blossoms of flower 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. Bactericide applications were made using a hand sprayer. After air-drying for 2 h,

blossoms were inoculated with *P. syringae* (2 x 10^8 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 blossoms per total number of blossoms) was evaluated after approximately 2 weeks.

For evaluation of commercial treatments to control the natural incidence of blossom blast, applications to blocks of trees were done on Feb. 25, 2014 using an air-blast sprayer at 100 gal/A. In mid-April, fruit and leaves were evaluated for the presence of blast symptoms. Leaves were evaluated using a rating scale from 0 to 5. Data were analyzed using analysis of variance and LSD mean separation procedures of SAS 9.4.

Evaluation of new fungicides for control of powdery mildew of sweet cherry. A field trial in San Joaquin Co. was conducted to evaluate fungicides for powdery mildew control. Treatments were done at full bloom on 3-21-14 for protection from primary inoculum (ascospores from overwintering chasmothecia), and were followed by two additional treatments on 4-11 (petal fall) and 5-1-14 (early fruit development) for protection from secondary infection from conidia. Single fungicides, pre-mixtures, and four rotation programs were evaluated (Fig. 4). The incidence of powdery mildew was evaluated on 20 leaves from five random shoots each from inside the tree and from the outer tree perimeter for each of the four single-tree replications on May 21, 2014. Data were analyzed using analysis of variance and LSD 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. For pre-infection activity (protection), blossoms were collected at white bud, allowed to open in the laboratory, and treated using a hand sprayer. After 12 h, blossoms were inoculated with a spore suspension of *M. fructicola* or *B. cinerea* (30,000 conidia/ml) until water droplets formed on anther filaments. To evaluate the post-infection ("kick-back') activity, blossoms were collected, inoculated, and treated after 16 h with a hand-sprayer. Blossoms were evaluated for stamen infection after 4-5 days of incubation at 20 C, >95% relative humidity. Disease incidence was evaluated as the number of stamens infected divided by the total number of stamens per blossom. Three replications of 8 blossoms were used for each treatment and data were analyzed using analysis of variance and LSD mean separation procedures (SAS 9.4).

To evaluate preharvest fungicide applications for control of fruit decay, orchards were used in San Joaquin Co. (commercial orchard) and at UC Davis (experimental orchard). In the San Joaquin trial, fungicides were applied to trees 6 days before harvest using a back-pack sprayer calibrated to deliver 100 gal/A. Fruit were harvested, 8 fruit from each of four single-tree replications were wounded with a glass rod (1 x 1 x 0.5 mm; 8 fruit from each of four single-tree replications), and inoculated with 20 µl of a conidial suspension of *M. fructicola* or *B. cinerea* (30,000 conidia/ml). In non-wound inoculations, approximately 50 to 60 fruit from each replication were sprayed with conidia of *M. fructicola* and incubated at 20C. In the UC Davis trial, treatments were also applied 6 days PHI using a back-pack sprayer. Fruit (8 fruit from each of three single-tree replications) were harvested and wound-inoculated with *M. fructicola* or *B. cinerea* as described above or non-wound, drop-inoculated with a spore suspension of *M. fructicola* (50,000 spores/ml). All fruit were incubated for 3-7 days at 20 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.

To evaluate preharvest fruit treatments for postharvest decay management and the persistence of the fungicides on the fruit that were treated in San Joaquin orchard, fruit were washed by spraying with high-volumes of water for 5 minutes prior to wound- and non-wound inoculations of harvested fruit. Fruit were inoculated with *M. fructicola* or *B. cinerea* and decay was evaluated as described above.

Efficacy of new and registered postharvest treatments for managing brown rot, gray mold, and Rhizopus rot fruit rots of sweet cherry. Four laboratory studies focused on the efficacy of the SC formulation of polyoxin-D and the new experimental compound EXP-13 against brown rot, gray mold, and Rhizopus rot. These compounds were evaluated by themselves, in mixtures, and in mixtures with Scholar or Tebucon. We

also compared the efficacy of two formulations of tebuconazole, Elite and Tebucon (the Elite replacement). Fungicides were applied as aqueous solutions using an air-nozzle sprayer either 13-20 h after (Inoculated-Treated) or before (Treated-Inoculated) inoculation with the respective fungal pathogens. Fruit were wound-inoculated with 20 µl of a spore suspension of *M. fructicola, B. cinerea, R. stolonifer* (30,000 spores/ml each). Fruit were incubated for 4-7 days at 20 C, >95% RH. Incidence of decay was determined as the number of fruit infected of the total fruit evaluated. Data were analyzed using analysis of variance procedures of SAS 9.4.

RESULTS AND DISCUSSION

Evaluation of treatments for control of bacterial canker and blossom blast. Research on these diseases is important because no effective treatments are currently available. We focused on the bacterial blast phase of the disease because there is a known period of high susceptibility for infection. Additionally, management of blast is becoming increasingly important because rest-breaking treatments are being used widely by the cherry industry to achieve an early harvest. This shifts the bloom period to an earlier date when disease-predisposing cold, rainy weather conditions are more likely to occur. Additionally, the highly susceptible cultivar Coral Champaign is increasingly being planted due to resistance of the fruit to rain cracking.

In two studies on cv. Coral cherry for the control of bacterial canker where freshly wounded branches were treated and then inoculated with a copper-resistant strain of the pathogen, treatments that included Kasumin reduced incidence and severity of cankers the most, whereas Kocide 3000 was not effective (Fig. 1). In the first experiment, Kasumin-Fireline reduced the incidence of canker formation from 100% in the control to 30%. Kasumin by itself was less effective but canker length for this treatment was reduced from 33 mm in the control to 11 mm. In addition to Kocide 3000, Captan and Fireline (oxytetracycline) were also not effective, and the addition of oil or Captan to Kasumin reduced the effectiveness of Kasumin.

In the second experiment, Kasumin by itself resulted in the lowest disease ratings, followed by Kasumin-Actinovate (kasugamycin has a minor direct impact on the bacterial-based biocontrol product), and Kasumin-Botector (kasugamycin does not directly affect the yeast-based product); but there were no statistically significant differences between these three treatments. These two biocontrols by themselves resulted in similar canker incidence and severity as the control treatment and thus, were not effective.

Unfavorable environmental conditions in the spring of 2014 resulted in a very low incidence of bacterial blossom blast, also on the most susceptible cherry cultivar Coral. Even in our inoculation studies, disease only developed on developing fruit. In one trial, 10.7% of the fruit showed typical spotting symptoms in the untreated control. All treatments evaluated significantly reduced the incidence of blossom blast from that of the control (Fig. 2). Mycoshield (oxytetracycline) resulted in the lowest incidence of disease (1.3%). Other effective treatments were Kasumin, MagnaBon, Actinovate, and several mixture treatments. The effectiveness of MagnaBon is interesting because a copper-resistant strain was used for blossom inoculation and in previous years' experiments, copper (e.g., Kocide 3000, Badge X2) only slightly reduced the incidence of blossom blast. A possible explanation is that blast symptoms on the fruit were not caused by the copper-resistant inoculation strain of *P. syringae* used during bloom but by a naturally occurring copper-sensitive pathogen population that displaced the inoculation strain during early fruit development (copper resistance is widespread but not present at all locations).

In a large-scale commercial trial, again symptoms of blossom blight occurred at a very low incidence and leaves and fruit were evaluated for blast symptoms. Kasumin was the most effective treatment in this trial and Actinovate mixed with table sugar also significantly reduced the disease from the control (Fig. 3). Actinovate by itself was not effective, and Regalia only slightly reduced the incidence of fruit symptoms, but not of leaf symptoms.

In summary, in five years of research on the management of bacterial canker and blossom blast, we identified Kasumin as the most effective treatment. Oxytetracycline was only evaluated in some of the trials but was identified as a very promising bactericide against *P. syringae*. Registrants of both of these

antibiotics are supportive of a registration on sweet cherry and this is currently pursued. Oxytetracycline was accepted into the IR-4 program in Sept. 2013 and residue studies were performed in 2014. Kasumin was fully registered on pome fruit in 2014 for management of fire blight, and oxytetracycline has been available for this disease for numerous years. Over the years of our evaluations, Actinovate also showed good efficacy in reducing blossom blast (but was less effective against canker), and Blossom Protect/Botector also reduced the disease. The performance of these two biocontrol treatments possibly can be improved. Thus the addition of table sugar increased the efficacy of Actinovate in the commercial trial in 2014, although a citrate-sugar mixture did not increase effectiveness in the small-scale study.

Our work has focused on bacterial blast because this phase of the disease mostly occurs during bloom and thus, this is a defined period of susceptibility. Progress is also being made on the management of bacterial canker. Our trials, however, are based on inoculations at a specific time after treatment. Epidemiological studies are needed to determine conditions that are most favorable for canker development. In general terms, cold and wet winter weather is presumed to be an optimal period for infections. Due to the long infection period for woody tissues, application timings are difficult to determine and most likely will focus on the most favorable infection periods (e.g., after pruning). The use of a biocontrol agent may provide a longer residual efficacy as compared to organo-chemical treatments such as oxytetracycline and kasugamycin that are metabolized and have a short persistence period.

Evaluation of new fungicides for control of powdery mildew of sweet cherry. The efficacy of new fungicides and new pre-mixtures was evaluated in a trial in San Joaquin Co. Three applications were done over a 6-week period starting at full bloom with fungicide applications for brown rot blossom blight. At evaluation time, all sampled leaves on trunk shoots (water sprouts) and over 80% of leaves of the outside canopy showed symptoms of powdery mildew in the untreated control. As in previous years, the most effective treatments included the SDHI (FG 7)-containing fungicides Fontelis (used at 20 fl oz/A), Luna Sensation, and Merivon, as well as selected DMI (FG 3)-containing fungicides such as Rhyme, Procure, and Quadris Top (Fig. 4). Rotation programs of Merivon and Vivando (FG U8) or of Luna Sensation and Serenade Optimum were also very effective. Serenade Optimum by itself, Pristine, as well as mixtures of Ph-D and Elevate, or of Ph-D and Tebucon were less or not effective. Quadris Top by itself showed good efficacy, but the rotation of Quadris Top with the biocontrol Taegro had little effect on reducing the incidence of powdery mildew. Luna Experience also showed little to no efficacy, although this premixture, like Luna Sensation, contains the SDHI fluopyram. Quintec (FG 13), that was highly effective in the first years after its registration on cherry only showed moderate efficacy. The possibility that reduced sensitivity or resistance has developed against this fungicide needs to be evaluated.

Thus, this research has demonstrated excellent activity of several new fungicides against powdery mildew and we show that the disease can be reduced to acceptable levels by properly timed applications. The FG 7/11 fungicides Luna Sensation and Merivon, as well as the FG Group 7 Fontelis are excellent powdery mildew fungicides. Because of the potential of resistance to single-site mode of action fungicides, FG 7 materials should be tank-mixed with FG 3 or FG 11 compounds. Pre-mixtures and tank mixtures should be used in rotation with other fungicides with different modes of action. Similarly, Vivando (FG U8) is potentially an excellent mix partner because of its unique mode of action and specificity against powdery mildew fungi. Mildew fungicides should be applied during bloom and again during petal fall periods. Materials could be selected that are very effective against blossom blight and powdery mildew diseases. Rotation of these different mode-of-action fungicides potentially may off-set resistance selection by limiting the use of any single-site mode of action fungicide (i.e., single FG number) and thus, this reduces the selection pressure. Limiting any one fungicide product will also reduce the residue and ensure that MRLs are not exceeded with any of the trade partners of the cherry industry.

Our epidemiological studies to date have shown that mildew develops on leaves of inside shoots (water sprouts) followed by leaves of outer shoots, stems of fruit, and then on ripening fruit. Young leaves are more susceptible than old leaves. Signs of the pathogen were not found on green fruit but were observed on mature fruit. Additional studies are needed to determine when fruit become susceptible.

Efficacy of new fungicides for control of brown rot and Botrytis blossom blight. Fungicide treatments were evaluated on detached opened blossoms in comparative laboratory studies. In pre- and post-infection studies, new and registered fungicides were very effective against brown rot and Botrytis blossom blights (Fig. 5). Highly effective fungicides with excellent pre- and post-infection activity against both blossom diseases included FG 7/11 fungicides (e.g., Luna Sensation, Luna Experience, Merivon) and the FG 7 Fontelis. The FG 3 (DMI) fungicides Quash and Rhyme were very effective against brown rot but also showed good efficacy against gray mold, whereas Ph-D was more effective against gray mold. The natural product Fracture and the biocontrol Botector (a yeast-based product) showed intermediate efficacy and overall performed quite well under these stringent experimental conditions where inoculated blossoms were incubated at highly favorable conditions. These products potentially may provide alternatives to conventional fungicides. Due to the good pre- and post-infection activity of most of the conventional fungicides, the practice of a single delayed-bloom application when environmental conditions are not favorable for disease development is an excellent strategy for obtaining highly effective blossom disease management and result in a minimal number of blossom treatments on sweet cherry.

Evaluation of preharvest treatments for fruit decay control without postharvest washes and for postharvest decay control after postharvest washes. Two preharvest efficacy trials with 6-day PHI applications were done in 2014. In wound inoculation studies using non-washed fruit, several fungicides provided excellent protection against brown rot and these included Ph-D mixed with Elevate, Quadris Top, Luna Experience, and the new experimental EXP-1 by itself or mixed with Headline or EXP-2 (Figs. 6A,7). For gray mold, only the Ph-D/Elevate mixture showed high efficacy in one of the trials (Fig. 6B). In contrast, when non-washed fruit were non-wound drop inoculated with *M. fructicola*, all fungicides evaluated demonstrated high efficacy indicating that many of the newer fungicides have little locally systemic activity.

When harvested fruit were washed for 5 min and then inoculated, several treatments were still highly effective in both wound- and non-wound inoculations with *M. fructicola*. These included Procure, Fontelis, Quadris Top, the Ph-D/Tebucon mixture, and treatments that included the new experimental EXP-1 (Fig. 6A). Pristine was only very effective on non-wound-inoculated fruit. None of the fungicides was highly effective after fruit washing and wound-inoculation with *B. cinerea*, but significant reductions in gray mold from the control were obtained using Fontelis, Ph-D/Elevate, Ph-D/Tebucon, and Pristine.

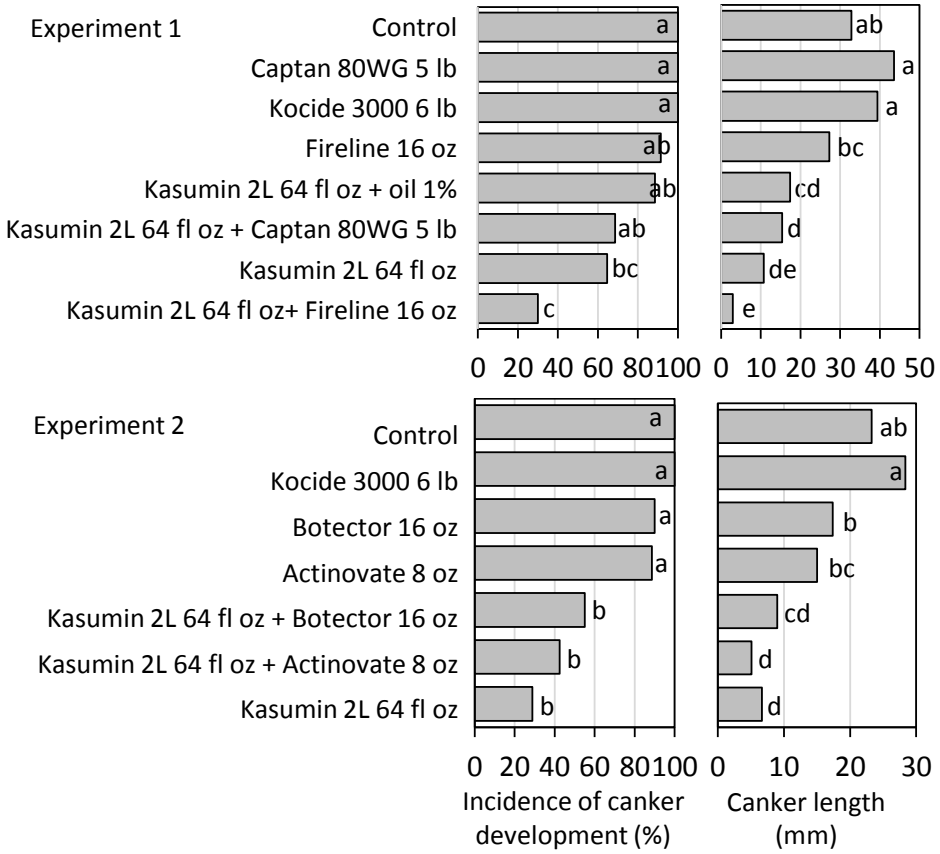
Efficacy of new and registered postharvest treatments for managing brown rot, gray mold, and Rhizopus rot of sweet cherry. In postharvest decay management in 2014, we focused on evaluating two fermentation products, polyoxin-D (Oso, Tevano, or CX-10440) and the experimental EXP-13. Polyoxin-D is currently exempt from tolerance and efficacy data will allow registration on sweet cherry. Previously we also evaluated the WG formulation of this chemical, but the SC formulation is the one that is pursued for postharvest registration. The 13-fl oz-rate of CX-10440 showed consistent good efficacy in reducing brown rot and gray mold when inoculated fruit were treated 13 to 20 h after inoculation (Figs. 8A, 9A,B,C). Efficacy against Rhizopus rot, however, varied from no control (Figs. 8A, 9A,C) to very good control (Fig. 9B). This inconsistency against Rhizopus rot was also observed in previous years. Polyoxin-D also was effective when treated fruit were wound-inoculated with *M. fructicola* or *B. cinerea*, indicating some penetration into the fruit. When polyoxin-D was applied in a mixture with Tebucon or Elite, this treatment was also effective against Rhizopus rot in two of the three studies (Fig. 9A,B).

The new experimental EXP-13, also with potential exempt registration status, was evaluated in several studies. In all four studies, this treatment was highly effective when treating fruit inoculated with *M. fructicola* or *B. cinerea* (Figs. 8A, 9A,B,C) or when treated fruit were inoculated with these two pathogens (Fig. 8B). As with polyoxin-D, inconsistency was observed with Rhizopus rot, ranging from very high (Fig. 9A,B) to intermediate (Fig. 8A) to no efficacy (Fig. 8C). The reasons for this are not known. Mixtures of EXP-13 with Tebucon or Elite (Fig 8A, 9A), with CX-10440 (Fig. 8A, 9A,B), or with Scholar at a low rate of 150 ppm (Fig. 9C) were highly effective against all three decays.

Thus, in our postharvest studies, two fermentation products with possibility for organic registration demonstrated high potential as new postharvest treatments for sweet cherry. One US company is supporting registration for polyoxin-D, whereas currently two companies show strong interest in EXP-13. We will continue our evaluations of these treatments in 2015. With regulatory changes occurring with major trade partners to harmonize maximum residue limits (MRLs) for postharvest fungicides, establish common food additive tolerances, and to move toward accepting these treatments as food preservatives rather than pesticides based on their levels of safety, the future will put greater importance on fludioxonil and 'exempt from tolerance' materials such as polyoxin-D and possibly EXP-13 for preventing decays of sweet cherry in international markets.

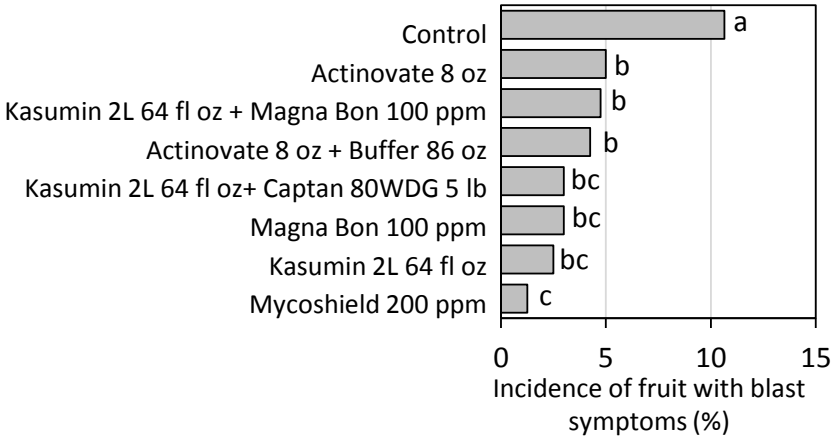
In trials comparing Elite that is no longer registered for postharvest use and a new generic formulation (Tebucon), both products showed similar performance against brown rot, gray mold, and Rhizopus rot (Fig. 9B). Thus, Tebucon can be used effectively as a replacement for Elite. The DF formulation of Tebucon was more difficult to directly mix with the fungicide solution. Thus, the fungicide should be prepared separately in water as a stock solution and then added to the existing fungicide solution under constant agitation.

Fig. 1. Evaluation of antibacterial treatments for protection of cv. Coral cherry trees from bacterial canker in 2013/14



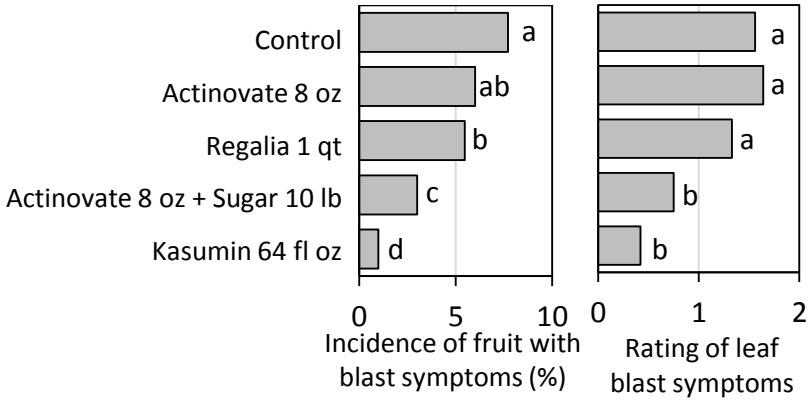
Branches were puncture-wounded, treated using a hand sprayer, allowed to air-dry, and then inoculated with *Pseudomonas syringae* on 12-18-13. Evaluations were done in late April 2014.

Fig. 2. Evaluation of antibacterial treatments for protection of cv. Coral cherry fruit from bacterial blast in 2014



Applications were done on 3-11-14 at 50% bloom using an airblast backpack sprayer at a rate of 100 gal/A. Fruit were evaluated for the presence of blast symptoms in mid-April.

Fig. 3. Evaluation of antibacterial treatments for protection of cv. Coral cherry fruit and leaves from bacterial blast in a commercial trial in 2014



Applications were done on 2-25-14 using a commercial airblast sprayer at a rate of 100 gal/A. Fruit and leaves were evaluated for the presence of blast symptoms in mid-April. Leaves were evaluated using a rating scale from 0 to 5.

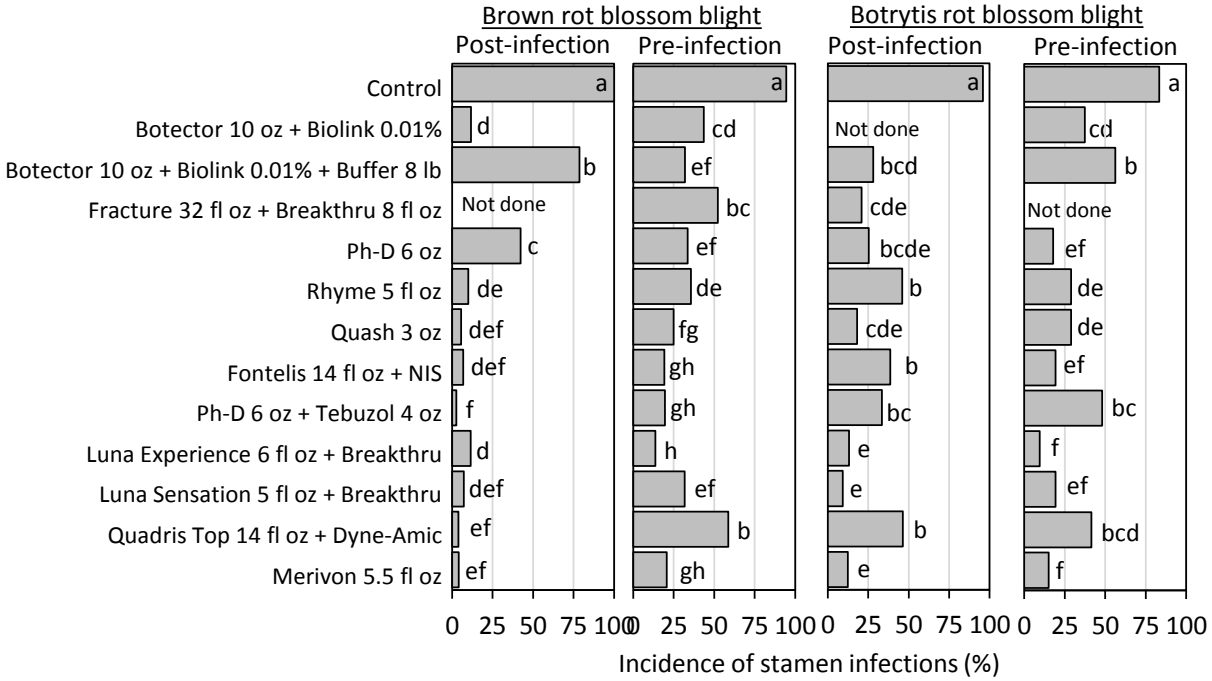
Fig. 4. Evaluation of fungicide treatments for management of powdery mildew of Bing cherries in San Joaquin Co. 2014

	Treatment	3/21	4/11	5/1	Inside shoots	Outside shoots
	Control	---	---	---	a	a
Single	Serenade Optiva 16 fl oz	@	@	@	abc	ab
	Rhyme 5 fl oz	@	@	@	fg	fg
	Rhyme 7 fl oz	@	@	@	defg	cdefg
	Procure 12 fl oz	@	@	@	defg	bcde
	Fontelis 14 fl oz/NIS	@	@	@	bcde	cdefg
	Fontelis 20 fl oz/NIS	@	@	@	fg	efg
Mixtures	Ph-D 6.2 oz + Elevate 24 oz	@	@	@	abcd	defg
	Ph-D 6.2 oz + Tebucon 45DF 4 oz	@	@	@	abc	cdef
	Quadris Top 14 fl oz	@	@	@	defg	g
Pre-mixtures	Pristine 14.5 oz	@	@	@	abc	cdefg
	Merivon 5.5 fl oz	@	@	@	efg	efg
	Luna Experience 5.5 fl oz	@	@	@	abc	abc
	Luna Sensation 5 fl oz	@	@	@	g	g
Rotations	Merivon 5.5 fl oz	@	---	---	efg	defg
	Vivando 15.4 fl oz + Breakthru	---	@	@		
	Luna Sensation 5 fl oz	@	---	@	fg	defg
	Serenade Optiva 16 fl oz	---	@	---		
	Iprodione 32 fl oz	@	---	---	cdef	defg
	Quintec 7 fl oz	@	@	@		
	Iprodione 32 fl oz	@	---	---	abc	bcd
	Fracture 32 fl oz	@	@	@		
	Quadris Top 14 fl oz	@	---	@	ab	bcd
	Taegro 5 fl oz	---	@	---		

0 50 100 0 50 100

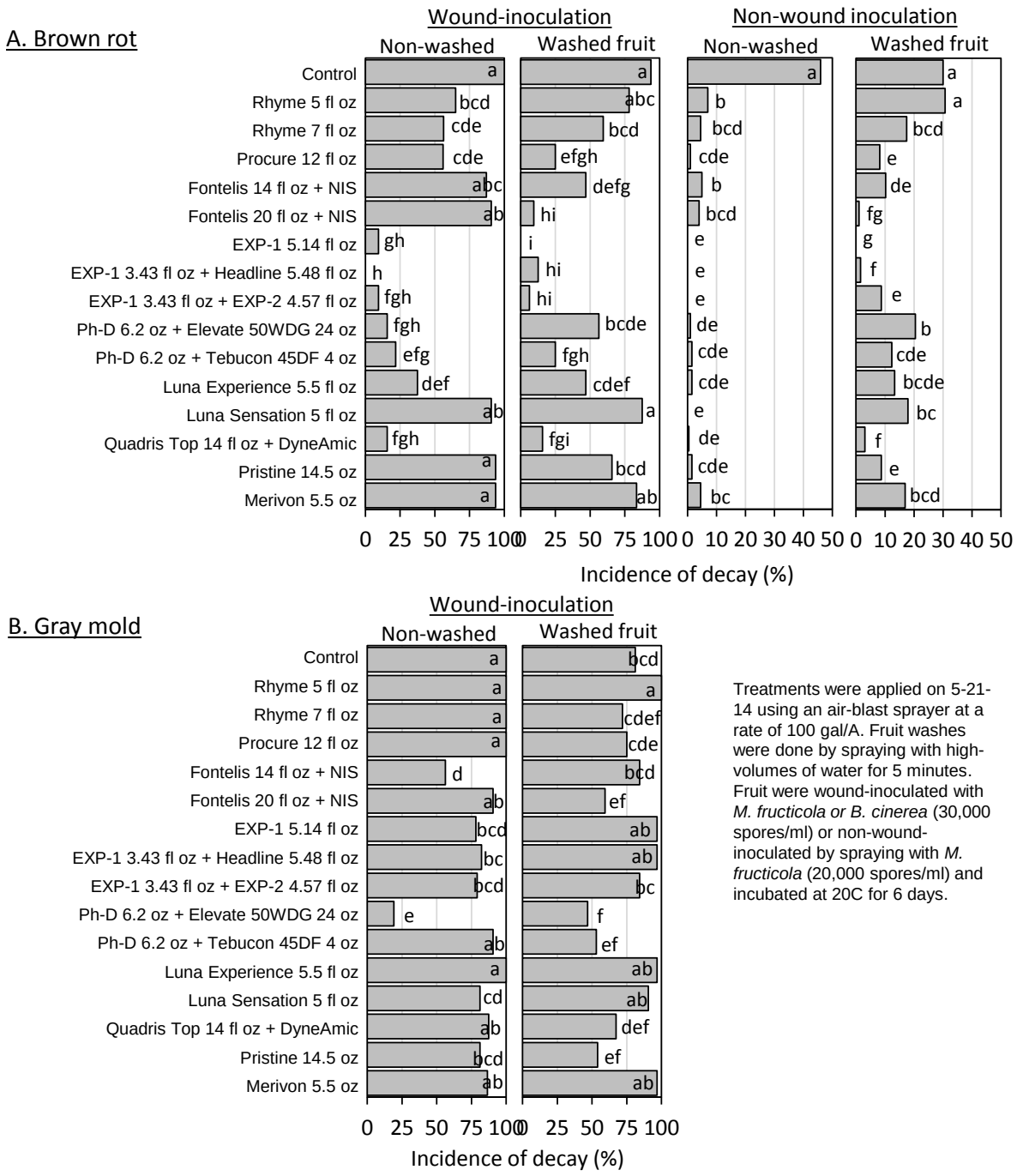
Applications were done using an airblast sprayer at a rate of 100 gal/A. Evaluation was done on 5-21-14. For this, 20 leaves from 5 random shoots from the inside or outside of the tree were rated using the following scale: 0 = healthy, 1 = 1-3 lesions, 2 = <25%, 3 = up to 50%, 4 = >50% of leaf area affected.
*treatments were applied in combination with 14 fl oz DyneAmic

Fig. 5. Efficacy of pre- and post-infection treatments for management of brown rot and Botrytis blossom blight of Bing cherry 2014



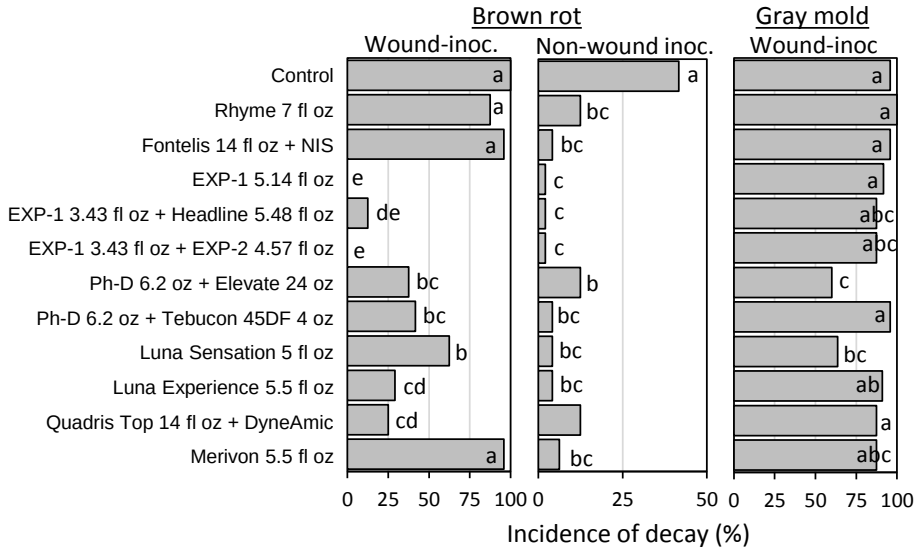
For evaluation of the pre-infection activity, closed blossoms were collected in the field, allowed to open, and treated in the laboratory using a hand sprayer. After 12 h blossoms were inoculated with a spore suspension of *M. fructicola* or *B. cinerea* (30K/ml). For post-infection activity, blossoms were inoculated, incubated at 22 C, and treated after 16 h. Blossoms were evaluated for stamen infections after 4-5 days of incubation at 20 C.

Fig. 6. Efficacy of 6-day preharvest fungicide treatments for management of postharvest brown rot and gray mold of Bing cherries - Orchard 1 - 2014



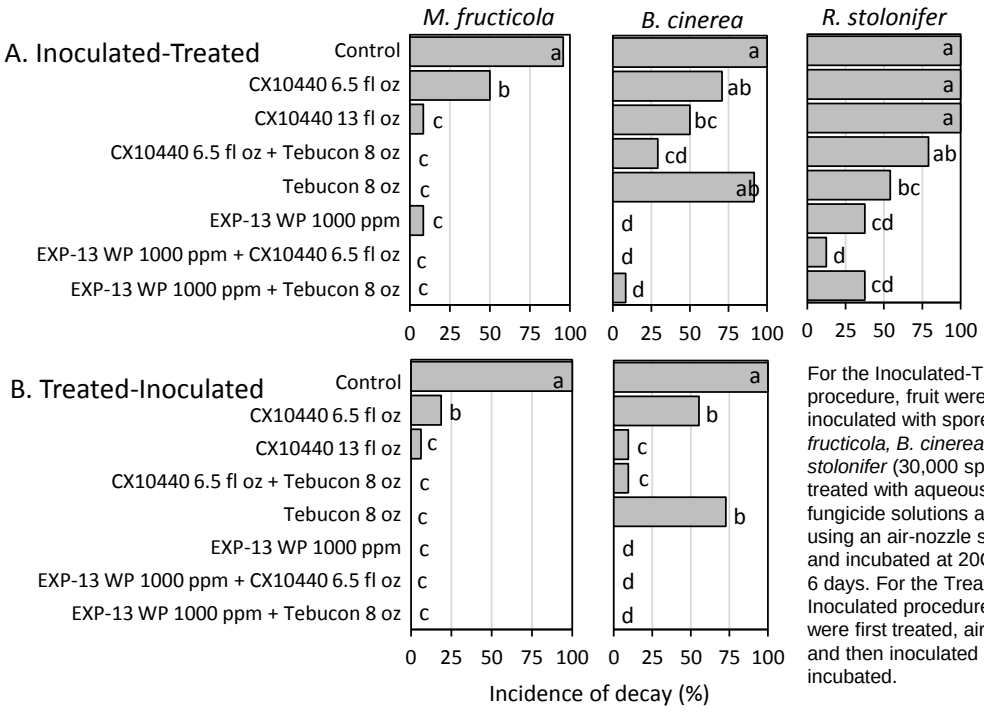
Treatments were applied on 5-21-14 using an air-blast sprayer at a rate of 100 gal/A. Fruit washes were done by spraying with high-volumes of water for 5 minutes. Fruit were wound-inoculated with *M. fructicola or B. cinerea* (30,000 spores/ml) or non-wound-inoculated by spraying with *M. fructicola* (20,000 spores/ml) and incubated at 20C for 6 days.

Fig. 7. Efficacy of 6-day preharvest fungicide treatments for management of postharvest brown rot and gray mold of Bing cherries - Orchard 2 - 2014



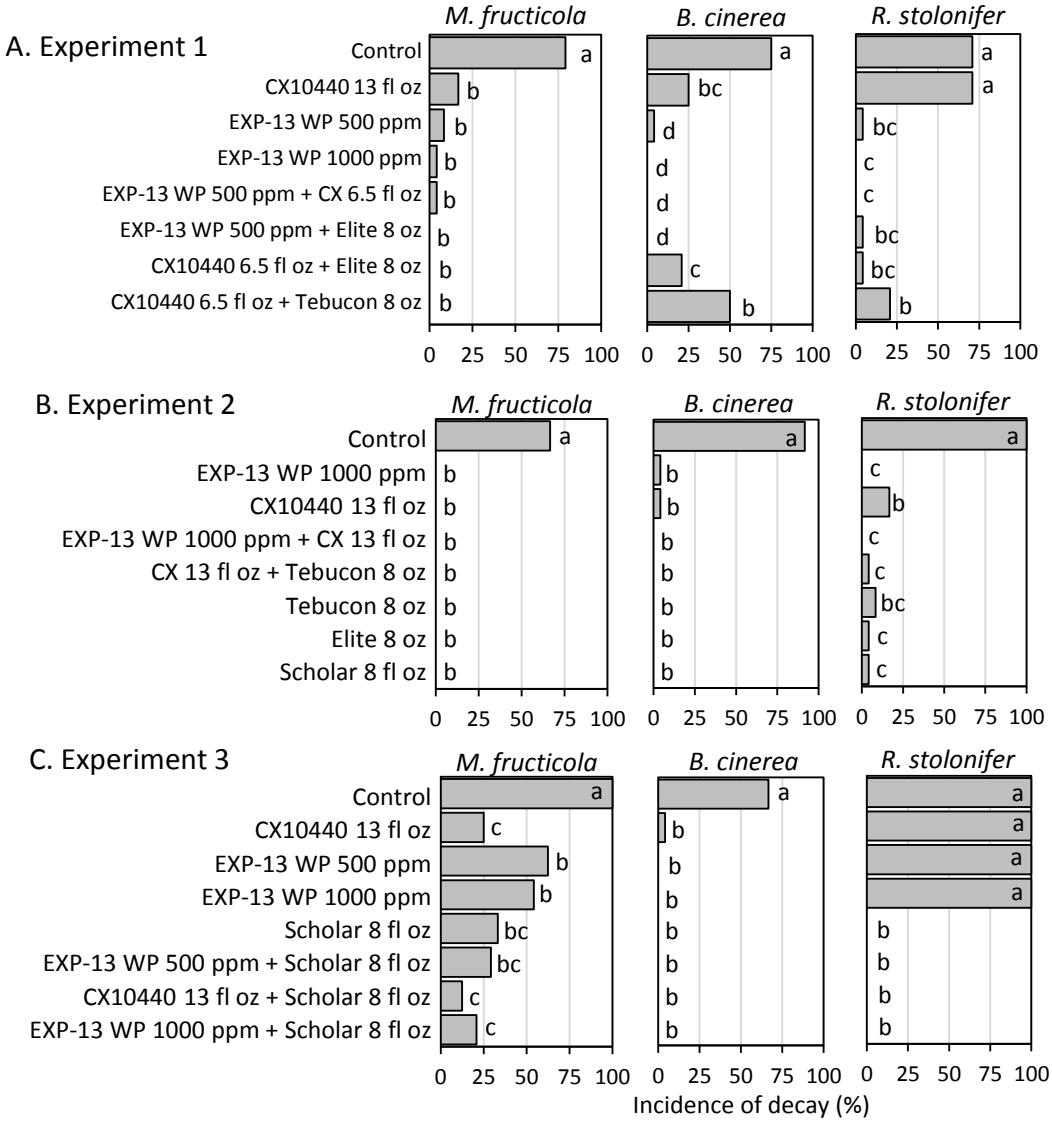
Treatments were applied on 5-21-14 using an air-blast sprayer at a rate of 100 gal/A. Fruit were wound-inoculated with *M. fructicola or B. cinerea* (30,000 spores/ml) or non-wound-inoculated with *M. fructicola* (50,000 spores/ml) and incubated at 20C for 6 days.

Fig. 8. Postharvest treatments with registered and new fungicides for decay control of sweet cherry fruit in laboratory studies - 2014



For the Inoculated-Treated procedure, fruit were wound-inoculated with spores of *M. fructicola, B. cinerea*, or *R. stolonifer* (30,000 spores/ml), treated with aqueous fungicide solutions after 13 h using an air-nozzle sprayer, and incubated at 20C for 4 to 6 days. For the Treated-Inoculated procedure, fruit were first treated, air-dried, and then inoculated and incubated.

Fig. 9. Postharvest treatments with registered and new fungicides for decay control of inoculated sweet cherry fruit in laboratory studies - 2014



Fruit were wound-inoculated with spores of *M. fructicola, B. cinerea*, or *R. stolonifer* (30,000 spores/ml), treated with aqueous fungicide solutions after 13 h (experiments 1 and 2) or 20 h (experiment 3) using an air-nozzle sprayer, and incubated at 20C for 4 to 6 days.

Project Title: Evaluation of Spirotetramat as a Post-Plant Nematicide in Cherries (Year 2 of 2)

Principal Investigators

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Objectives of Proposed Research (Year 1 of 2)

Evaluate the use of Movento as a nematicide in cherries by

1) determining the effects of foliar-applied Movento on the concentration of spirotetramat in leaf and root tissues
2) determining the effects of spirotetramat on the density of plant-feeding and predatory nematodes in the soil
3) determining if there is a positive plant response following application of spirotetramat

Introduction

Damage from nematodes can play an important role in the vigor of cherry trees. Left untreated, feeding by nematodes can impair root functions such as the uptake of nutrients and water and cause reductions in tree vigor and productivity. Historically, nematode management has been accomplished through the use of pre-plant fumigation and rootstock selection. However, pre-plant fumigants have been under intense regulatory scrutiny that has made applications more difficult to make, or in some cases not even possible. For that reason there is great interest among cherry and other *Prunus* sp. growers to identify post-plant options for nematode control.

Over the past few years some cherry growers have started to use Movento as a post-plant nematicide. However, the effects of Movento applications on nematode populations in cherries have not been document scientifically. The only work that has been done is a series of studies by Dr. Mike McKenry prior to his retirement in other crops like grapes and walnuts. However, reports from that work only show sparse details about how the trials were conducted, only show a portion of the results, lack the statistical analyses needed to determine if statistical differences occurred, and none of that work has been made available for peer review by other scientists. As a result, these data need to be considered inconclusive due to their inability to either prove or disprove spirotetramat's effectiveness.

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The current status in California is that a supplemental 2(ee) label now exists for stone fruits (including cherries) that allows for the use of Movento as a post-plant nematicide. Many growers have started using the product based on the hope that it is effective. Other more skeptical growers are waiting until there are data to support the use of Movento before they start

writing recommendations. The purpose of this project is to collect data that will help cherry growers determine if, and when, the use of Movento might be justified for post-plant nematode control.

Materials and Methods

During 2013 and 2014 we conducted a multi-year study to evaluate the uptake and distribution of spirotetramat in two mature commercial cherry orchards in the lower San Joaquin Valley. The sites were located near Wasco and Arvin and each was organized as a randomized complete block design with four blocks of two treatments. Plot size was 15 and 20 trees, respectively. Half of the plots did not receive spirotetramat treatments and served as the untreated check. The other half of the plots were treated with Movento at 9 fl oz/acre in 100 gal/acre of water on 11 July 2013 and then again on 22 May 2014 using an Air-blast sprayer at 2 mph with 4 fl oz of Dyne-Amic per 100 gal of water as a surfactant.

Objective 1. Concentrations of spirotetramat in plant tissues

During 2013 leaf and root tissue samples were collected just prior to treatment on 11 Jul and then 2, 4, 6, 8, 10, 12, 14, 16, and 18 weeks after treatment on 26 Jul, 8 Aug, 22 Aug, 5 Sep, 19 Sep, 3 Oct, 17 Oct, 31 Oct and 14 Nov, respectively. On each sample date, 15 leaves were collected from each plot and brought back to the lab. A 'punch' was used to excise a 15.9 mm diameter circle from a region approximately half-way between the midvein and edge of each leaf. The 15 leaf discs from each plot were placed into one well of a 12-well TC 6.9 ml plate (Fisher scientific) and frozen until processing. Root samples were collected with a shovel. On each evaluation date we collected one shovel full of soil from the area approximately 1 to 1.5 ft from the base of the trunk of each of three trees per plot. This was the region where the drip emitters were located. Within each plot the three soil samples were mixed and roots were pulled out by hand. Approximately 2.0 g of roots from each plot were collected and returned to the lab where a subsample of 0.5 grams of roots between 1/8 and 1/4 diameter were collected and placed into a well of the same 12-well TC plate previously described. In total, each sampling date resulted in 32 wells for tissue analysis (2 sites x 2 treatments x 4 replications x 2 types of tissues (leaves and roots)). After collection all samples were frozen until they could be analyzed.

Samples were processed during the winter of 2013-4 using a multi-step process whereby tissues are placed into plastic wells, the wells were filled with a solvent, and then were shaken for a period of time for spirotetramat and other molecules to leave the plant tissues and move into the solvent. The solvent was then removed from the wells, placed into vials, and then put through a HPLC analysis machine at the USDA-ARS lab in Maricopa, AZ. The HPLC analysis was used to produce a chart that shows peaks for each organic molecule present in the solvent. Previous tests to calibrate the machine in 2013 were used to identify which peaks were associated with spirotetramat and its principal breakdown product spirotetramat-enol. The machine then measured the heights of each peak compared to standards to determine the parts per billion (ppb) of spirotetramat and spirotetramat-enol in the solvent. Data were analyzed graphically to evaluate trends in spirotetramat in leaf and root tissues over time.

Objective 2- Evaluate effects of spirotetramat on nematode density

In 2013 nematode samples were collected prior to treatment on 11 Jul and then monthly through five months after treatment (MAT) on 8 Aug, 5 Sep, 3 Oct, 31 Oct, and 26 Nov. In 2014 samples were collected on 19 May and 18 Sep. Samples were made by collecting one shovel full of soil from a moist soil zone containing feeder roots from each of three trees per plot. Soil from the three subsamples in each plot was combined into a bucket, mixed, and then approximately 1892 cm^3 of soil was placed into a gallon plastic bag that was labeled and refrigerated. Samples were delivered to a commercial nematode evaluation laboratory (ID Services) in Wasco, CA within one day of collection. Once at the lab they were processed within one week according to industry standard procedures for sugar flotation and counting of plant parasitic nematodes. Data were summarized and analyzed by ANOVA with means separated by Fisher's Protected LSD at P=0.05.

Objective 3- Effects of spirotetramat on tree vigor and yields

During 2014 we planned on collecting data on crop yield and tree vigor. The goal was to collect crop yields from a subset of trees from each plot and then measure the relative lengths of vegetative regrowth. However, due to a severe lack of winter chill hours, both orchards had minimal crops with anticipated yields less than 10% of normal. After discussions with the grower cooperators at each location it was determined that it was not worth trying to collect yield data from orchards where growers were questioning whether or not harvest efforts should be completely abandoned during 2014. It was determined to forego harvest in 2014 and watch data to determine if significant differences were seen in nematode counts during 2014. Based on those efforts we currently have no plans to harvest research plots in 2015.

Results and Conclusions

Objective 1. Concentrations of spirotetramat in plant tissues

The effects of a foliar application of Movento on spirotetramat and spirotetramat-enol concentrations in leaf and root tissues are shown in figures 1 and 2, respectively. In both figures the top chart reports spirotetramat concentrations whereas the bottom chart displays data for the spirotetramat-enol derivative. Each chart also shows data from each of the two sites with the cherry orchard near Arvin shown on the left side and data from the Wasco orchard shown on the right.

At both research sites concentrations of spirotetramat and spirotetramat-enol were negligible prior to treatment (Fig. 1). Following treatment spirotetramat was immediately found in the extract from leaf tissue samples at a level of approximately 400 ppb for weeks 2 to 8 WAT at Arvin and from approximately 800 to 1600 ppb for weeks 1 to 4 WAT in Wasco. These levels are relatively high because they include any spirotetramat residues still present on the surface of the leaf as well as any spirotetramat that might be present inside of the leaf. Beginning at 10 WAT the amount of spirotetramat extracted from tissues began to decline through 18 WAT to levels close to zero in Arvin and down to approximately 400 ppb in Arvin.

Concentrations of spirotetramat-enol in leaf tissues followed a similar pattern as the parent compound with the greatest amount of active ingredient extracted 2 to 8 WAT that led to decreased amounts beginning 10 WAT. However, concentration levels of the -enol derivative were much lower than those of the parent compound in the leaf tissue. Peak concentrations during first two months after application typically ranged from 10 to 25 ppb compared to 400 to 1600 ppb for the parent compound from the same sample. One explanation for the difference is that the parent compound extracted includes product on the surface of the leaf as well as any parent compound that may have passed through the leaf whereas it is expected that spirotetramat-enol would not be present on the surface on the leaf and is likely only found within plant tissues.

Concentrations of spirotetramat and spirotetramat-enol in root tissues were much lower than in leaf tissues and followed a different pattern (Fig. 2). There was a delay of approximately 4 weeks in Arvin and 6 weeks in Wasco before any significant increases in spirotetramat concentrations were found after treatment. Once the increased occurred, this compound continued to be found at levels of approximately 2 to 10 ppb through 14 WAT in Arvin and at levels of approximately 15 to 25 ppb from weeks 6 through the end of the study (18 WAT) in Wasco. The patterns in spirotetramat-enol in the roots were similar to the patterns of the parent compound, except that the concentrations of the -enol derivative were much lower and never exceeded 2 ppb in Arvin and 4 ppb in Wasco.

Objective 2- Evaluate effects of spirotetramat on nematode density

The effects of Movento applications on nematode density are shown in Table 1 and are represented graphically in Figure 3. At the Wasco site the predominant nematodes present were dagger nematode (*Xiphenema americanum*) and pin nematode (*Paratylenchus* sp.). At the Arvin site the predominant nematode species was lesion nematode (*Pratylenchus vulnus*).

Densities of dagger nematode in untreated plots ranged from 6 to 114 nematodes per 500 cc of soil with an average of 36.3 ± 15.7 across all dates. Nematode densities in plots treated with Movento ranged from 7 to 141 nematodes per 500 cc of soil with an average of 59.9 ± 23.6. There were no significant differences in nematode density between the treated and untreated plots prior to treatment or during any of the evaluation dates in 2013 or 2014 ($P > 0.33$).

Densities of pin nematode in untreated plots ranged from 410 to 1,861 nematodes per 500 cc of soil with an average of 830 ± 177 across all dates. Nematode densities in plots treated with Movento ranged from 160 to 1,412 nematodes per 500 cc of soil with an average of 598 ± 97. There were no significant differences in nematode density between the treated and untreated plots prior to treatment or during any of the evaluation dates in 2013 or 2014 ($P > 0.20$).

Densities of lesion nematode in untreated plots ranged from 41 to 300 nematodes per 500 cc of soil with an average of 164.8 ± 12 across all dates. Nematode densities in plots treated with Movento ranged from 55 to 765 nematodes per 500 cc of soil with an average of 277 ± 77. There were no significant differences in nematode density between the treated and untreated plots prior to treatment or during any of the evaluation dates in 2013 or 2014 ($P > 0.26$).

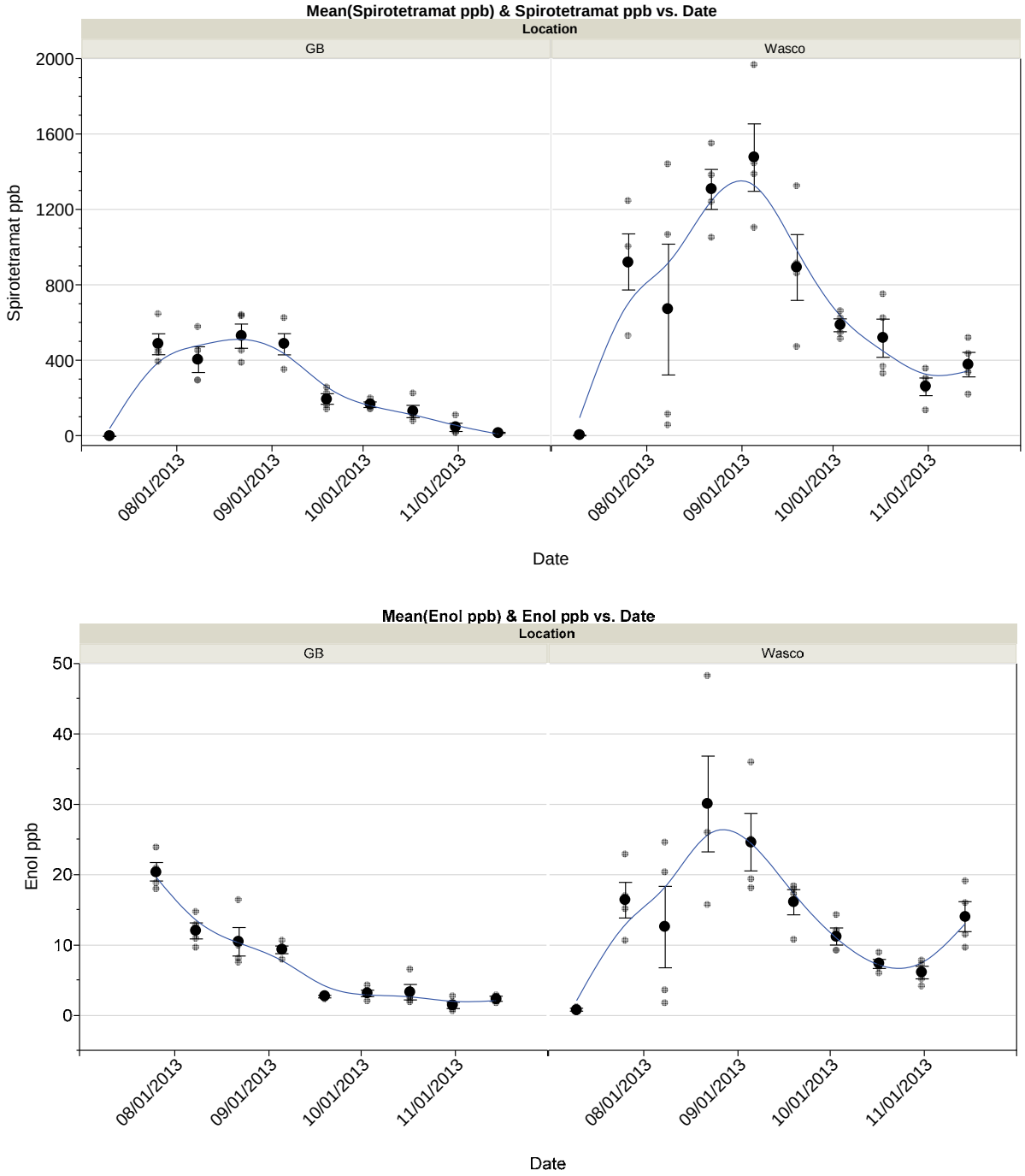

Figure 1- leaves. Concentrations of spirotetramat (top) and spirotetramat-enol (bottom) in cherry leaves at two field sites in Kern County, 2013.

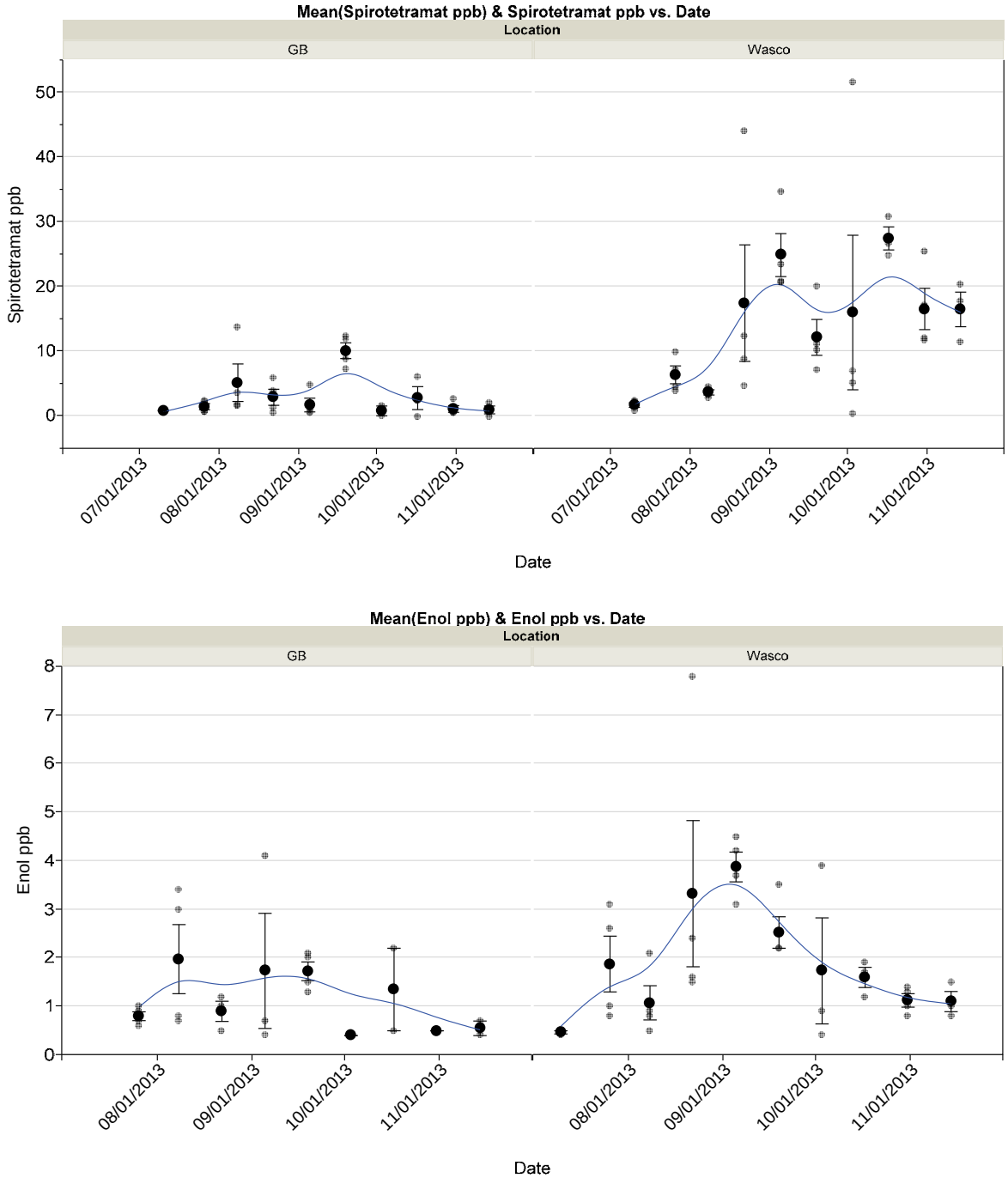

Figure 2- roots. Concentrations of spirotetramat (top) and spirotetramat-enol (bottom) in cherry roots at two field sites in Kern County, 2013.

Table 1. Effects of applications of Movento (spirotetramat) on 11 Jul 2013 and 22 May 2014 on the principal nematode species in two commercial cherry orchards in Kern Co, CA.

Dagger nematode (*Xiphenema americanum*) per 500 cc of soil, Wasco

	Precounts 7/16/13	8/8/13	9/5/13	10/3/13	10/31/13	11/26/13	5/19/14	9/18/14	Average
Movento	10	24	141	14	102	58	75	7	60
Untreated	9	94	7	21	58	114	39	6	36
F	0.04	0.60	1.02	0.23	0.53	0.29	0.46	0.01	0.40
P	0.8418	0.4513	0.3286	0.6388	0.4796	0.5994	0.5103	0.9049	0.5391

Pin nematode (*Paratylenchus* sp.) per 500 cc of soil, Wasco

	Precounts 7/16/2013	8/8/13	9/5/13	10/3/13	10/31/13	11/26/13	5/19/14	9/18/14	Average
Movento	1,412	1000	707	320	453	700	844	159	598
Untreated	1,861	1224	973	410	1206	959	574	450	828
F	0.15	0.10	0.26	0.15	0.65	0.23	0.33	1.79	0.29
P	0.7025	0.7533	0.6210	0.7023	0.4326	0.6388	0.5760	0.2025	0.5994

Lesion nematode (*Pratylenchus vulnus*) per 500 cc of soil, Arvin

	Precounts 7/16/13	8/8/13	9/5/13	10/3/13	10/31/13	11/26/13	5/19/14	9/18/14	Average
Movento	77	765	218	55	285	285	146	185	277
Untreated	248	300	268	41	91	272	72	110	165
F	1.18	1.03	0.04	0.08	1.36	0.00	0.15	0.23	0.59
P	0.2964	0.3274	0.8426	0.770	0.2622	0.9504	0.7004	0.6387	0.4565

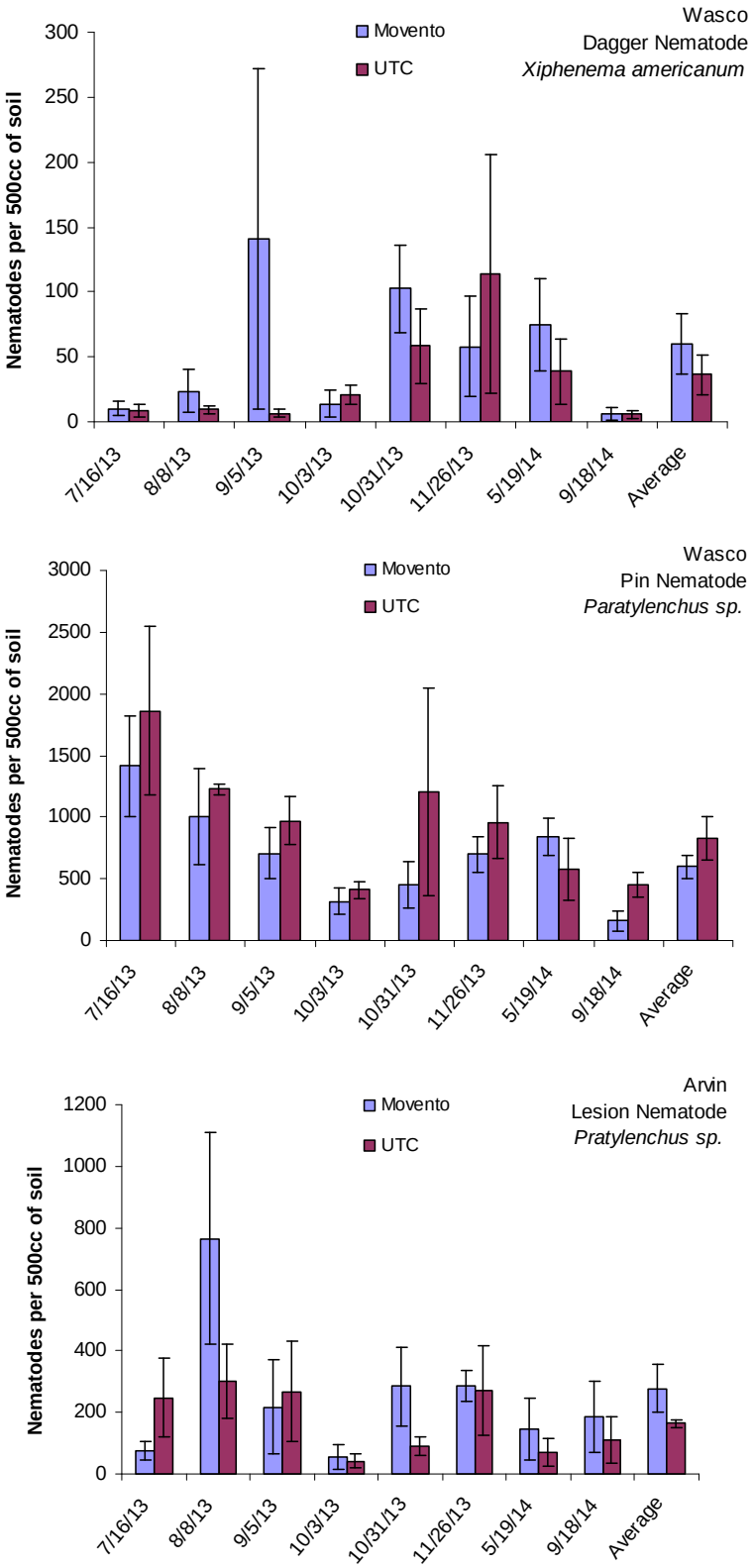

Figure 3. Effects of applications of Movento (spirotetramat) on 11 Jul 2013 and 22 May 2014 on the principal nematode species in two commercial cherry orchards in Kern Co, CA.

Conclusions

During the first year of the study we were able to develop data to help describe how spirotetramat moves in cherry trees. First, we determined that spirotetramat has a long persistence on the leaves. Compared to many other products that break down within a period of hours, days or a few weeks, spirotetramat and its derivative were routinely found in leaf tissues through the end of the study 18 weeks after treatment. Second, spirotetramat can successfully move across the leaf surface and become systemic within cherry trees and can be found as both the parent compound and the -enol derivative. In this study spirotetramat and its derivative moved systemically to the roots following a foliar application. Third, concentrations of spirotetramat remain highest in leaf tissues during the first two months after application and decline thereafter whereas spirotetramat didn't reach root tissues until approximately 4 to 6 weeks after application. Lastly, concentrations of spirotetramat are highest in leaf tissues that were sprayed directly with Movento. However, concentrations of the -enol derivative in the same leaves and concentrations of either compound in the roots were orders of magnitude lower.

These data suggest that applications of spirotetramat against foliar pests that are susceptible to the active ingredient are likely to be effective. However, the data show that concentrations of spirotetramat in the roots are very low, which brings into question whether or not sufficient concentrations exist to be able to provide efficacy against root-feeders such as nematodes. This is complicated by the fact that we do not know the concentrations and exposure times of spirotetramat that are required to kill different species of nematodes. Data also suggest that if nematode control is going to occur, that the effects are not likely to be seen during the first month after application and are more likely to be seen six to twelve weeks after application when concentrations are at their highest. This rule, however, may not hold true for other times of the year as movement of spirotetramat in the plant is influence by xylem and phloem chemistry. For example, in a separate study in grapes, data showed that spirotetramat movement in grapevines following a spring application was very similar to the results reported in this study, whereas applications in the fall led to a much quicker, but short-lived, movement to the roots.

Regarding efficacy against nematodes, during this 2-year study we were unable to detect any significant differences in population density of any of the predominant nematode species evaluated in the two cherry orchards. These data, coupled with data from the first objective showing extremely low titers of spirotetramat in the roots, do not support the recommendation of Movento applications to cherries for control of nematodes. However, it is important to note that while we were not able to detect any benefit to nematode control in our research plots, this does not mean that a suppressive effect of spirotetramat cannot occur in certain circumstances. In other words, just because this project was unable to prove that it works, this project likewise does not prove that it can't ever work. Cherry growers who feel that they have unique situations are encouraged to experiment on their own to determine if they can identify a way to generate more positive results than the ones presented in this report.

FINAL REPORT **YEAR: 2 of 2**

Project Title: Early season estimation of fruit set and size potential

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Cooperators: Matthew Whiting

Total project Funding: $120,874

Other funding sources: None

Budget 1-Einhorn
Organization Name: OSU-MCAREC **Contract Administrator: L.J. Koong**
Telephone: 541 737-4866 **Email address: l.j.koong@oregonstate.edu**

Item	2013	2014
Salaries	28,784	29,648
Benefits	18,064	18,604
Wages	3520	3520
Benefits	352	352
Equipment		
Supplies	2310	1960
Travel	1000	1000
Miscellaneous		
Plot Fees		
Total	54,030	55,084

Footnotes: Salaries for 0.75 FTE postdoc (3% is added to year 2); benefits were calculated based on Actuals; wages are for 300 hours part-time summer employee for image analysis of cherry fruit ($11/hr); benefits for part-time (10%); supplies include fixative, PGRs, tubes for storage of fruit in fixative, bee exclusion netting (only factored into year 1), Ziploc plastic bags, flagging and lab tape for limb and fruit selection; travel includes 1,700 miles estimated for all sample collections and growth rate analyses at $0.55 per mile.

Budget 2- Long
Organization Name: OSU-MCAREC **Contract Administrator: L.J. Koong**
Telephone: 541 737-4866 **Email address: l.j.koong@oregonstate.edu**

Item	2013	2014
Salaries		
Benefits		
Wages	4800	4800
Benefits	480	480
Equipment		
Supplies	200	200
Travel	400	400
Plot Fees		
Miscellaneous		
Total	5880	5880

Footnotes: Wages are for 2.5 months of part-time summer employee for fruit sample collection ($12/hr); benefits for part-time (10%); supplies include Ziploc bags, flagging, and lab tape and dry ice for transport; travel includes 740 miles estimated for all sample collections for fruit set estimates and growth rate analyses at $0.55 per mile.

Objectives:

1) Develop sampling and measurement protocols at the tree, row and orchard scale for Rainier, Bing, Chelan, and Sweetheart. Define the number of fruitlets required for precise crop estimates
2) Analyze growth rates of unfertilized and fertilized fruit of Rainier, Bing, Chelan, and Sweetheart to strengthen our model
3) Develop models of fruit growth that incorporate calendar date and growing degree units so they may be broadly applicable to the cherry growing regions of the PNW
4) Time whole-tree PGR applications with early-season growth of cherry and determine their effect on fruit set, yield, harvestable fruit size, and fruit quality

Significant Findings:

1) The dry weight of 2000 to 3000 ovaries sampled randomly was sufficient for crop estimates by 18 days after bloom
2) Ovary length to width ratios improved detection of potential fruit versus developmentally failed fruit
3) Crop estimates based on fruit from 30 spurs per sampling date, when combined with ovary shape, provided estimates of fruit set by 20 days after bloom
4) Sweetheart grown in three locations with differing seasonal temperature indicated the Base Temperature for accumulation of Degree Days (43°F) is inappropriate and should be lowered
5) Pre-bloom ovary growth was significantly and positively related to temperature
6) The calendar day order for beginning of the Sweetheart season at five locations was The Dalles (BA, SK, JH), Hood River and Parkdale
7) 40°F was sufficient, and 50°F was near the upper limit of a growth response in the green tip phase
8) 70°F produced a large growth effect during the open cluster and first white phases
9) Flowers that bloom early, with respect to average bloom date, produce larger fruit at harvest
10) Pre-bloom (~first white) application of Promalin or cytokinin alone (CPPU) increased fruit size between 7% and 14% when sampled around pit hardening. Promalin significantly increased stem length and leaf area indicating absorption

Results:

Fruit Growth. Our first goal was to complete a growth analysis from dormancy to bloom. An essential component of these growth analyses was the segregation of fertilized fruit from non-fertilized fruit, *prior to their abscission*. These two populations cannot be statistically differentiated within the first 18-20 days from bloom based on their growth rates (Fig. 1).

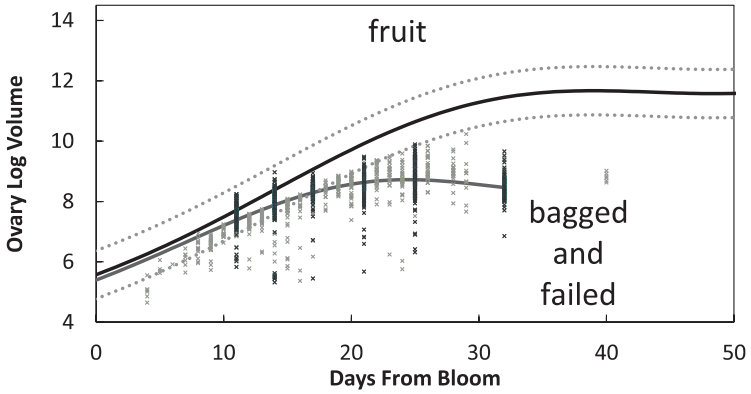

Figure 1. Ovary growth from bloom of a population of fruit comprising both fertilized fruits and fruits destined to abscise compared to non-fertilized fruits developing in bee-exclusion bags.

We then eliminated all fruit that were destined to drop through statistical procedures, of cluster and discriminate analysis, in order to only describe the growth of harvestable fruit of Chelan, Bing, and Sweetheart. Surprisingly, relative growth rates (and timing) defining growth of early developmental stages (First swelling through Stage II) did not differ among these three cultivars (Table 1).

Table 1. Days from bloom of growth phase transitions determined from the minima, maxima and up or down inflexion points of relative growth rate (RGR) curves (not shown).

Variety	**Growth phase**							
	FS,SG	SG,GT	GT,OC	I	I,II	II,III	III	Maturation
	Direction of relative growth rate curve							
	minimum	down	up	maximum	up	minimum	maximum	asymptote*
	Days from bloom							
'Sweetheart'	-39	-31	-17	11	29	44	60	75,79,88
'Bing'	-37	-29	-14	12	30	45	64	70,72,77
'Chelan'	-38	-29	-14	15	30	43	56	59,61,65

First swelling (FS), side green (SG), green tip (GT), open cluster (OC).
**Days from bloom of the additional 90, 95 and 99% increase in phase III volume as determined by logistic functions.*

Based on these similarities, we then developed sampling protocols that provide a good representation of fruit set and variability in fruit size. We attempted moderate (300) and large (3,000) fruit sampling protocols.

Fruit set. Set was determined in two ways. Recounting fruits per flower on flagged limbs at weekly intervals during the season yielded good results but was difficult (see last year's continuing report). A more random sampling proved to be more informative. Sampling at random for dry weight measurements was good but required a lot of sample (>>1000; Fig. 2). A convenient unit to base fruit set on is the spur. Spurs can be sampled as random units throughout the orchard and based on pre-determined average bud and flower numbers per spur (Table 2), the fruit remaining on a spur represents the percentage of fruit set (Table 3). In comparison to limb sampling, sampling entire spurs captured much of the variability and was possibly more accurate; this is attributed to each spur representing flowers at various stages of development so sampling by single spurs from many trees is more likely to represent the orchard as a whole.

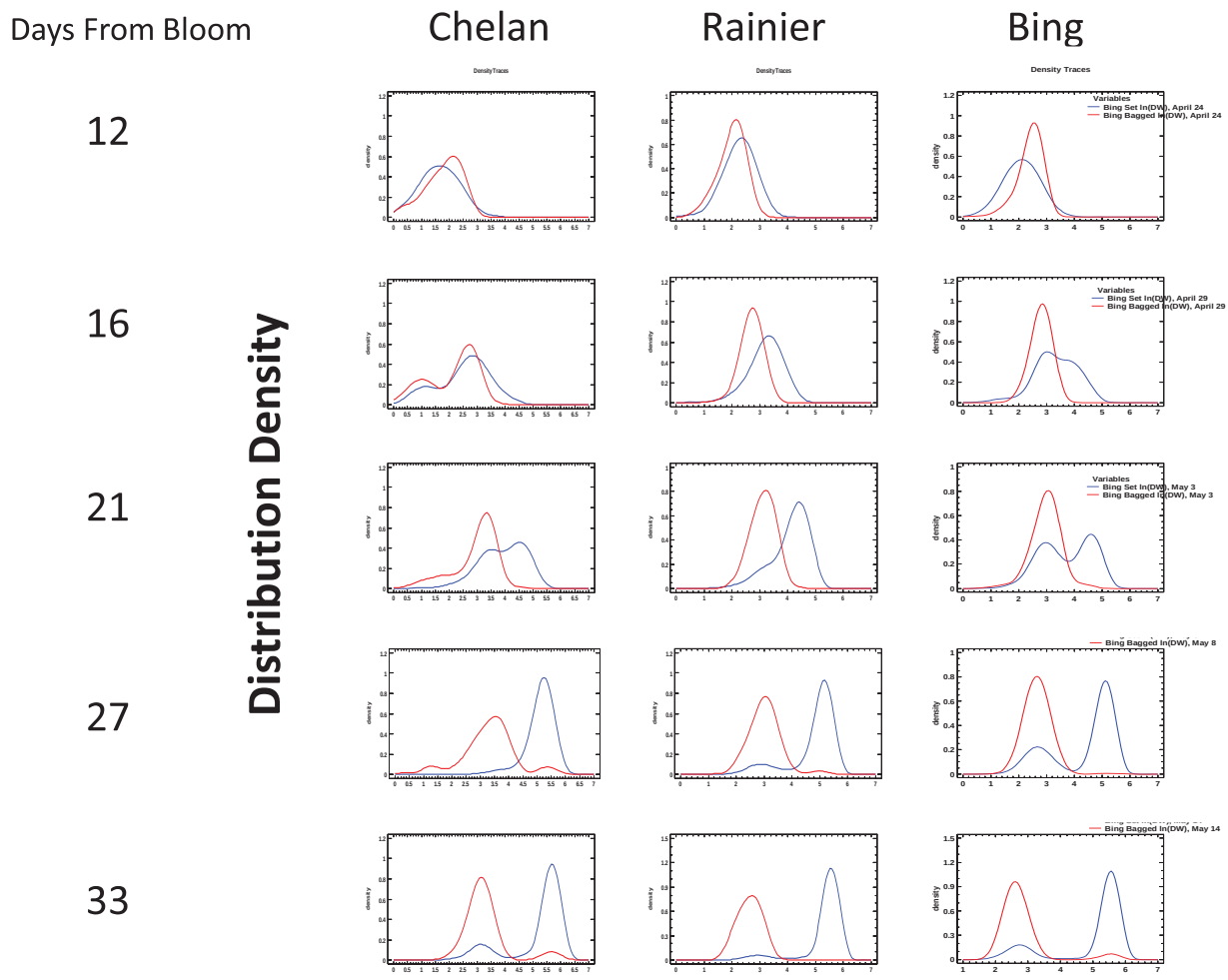

Figure 2. Dry weight gave a sufficient early estimate of fruit set, 16 to 21 DFB, but only if >1000 ovaries were measured. Populations of fruit form two distinct curves- the curve to the left of each graph is for bagged, non-fertilized fruit. The curve to the right is from a random sampling (it is comprised of both fertilized and unfertilized fruit, as can be seen by the bi-modal distribution beginning ~16 to 20 DFB).

Table 2. Spur data used for the fruit set and growth analysis of Sweetheart across multiple sites. This baseline data were best taken before bud break.

	The Dalles			Hood River	Parkdale
	BA	SK	JH	HR	PD
	Average of 30 spurs				
Flower per bud	2.87	3.06	3.48	3.06	3.69
Bud per spur	4.42	4.37	4.44	5.12	3.61
Potential Flowers per spur	12.6	13.3	15.4	15.5	12.8

Location, which includes biological variability attributed to tree age, rootstock, etc., affected flower and bud number.

To reduce sample size we developed a better sampling protocol. In addition, a more sensitive, discriminant measure of ovaries was conducted by integrating shape and volume estimates from

digital images (data not shown). A fruit set estimate was reliably detected about 15 DFB from 200-300 fruit collected from sampling 30 spurs on separate trees (Table 3).

Table 3. Fruit set of Sweetheart determined from spur sampling and photographic analysis.

	The Dalles			Hood River	Parkdale
	BA	SK	JH	HR	PD
			% fruit set		
10 to 19 DFB	47	52	68	37	35
Harvest	41	46	56	37	42

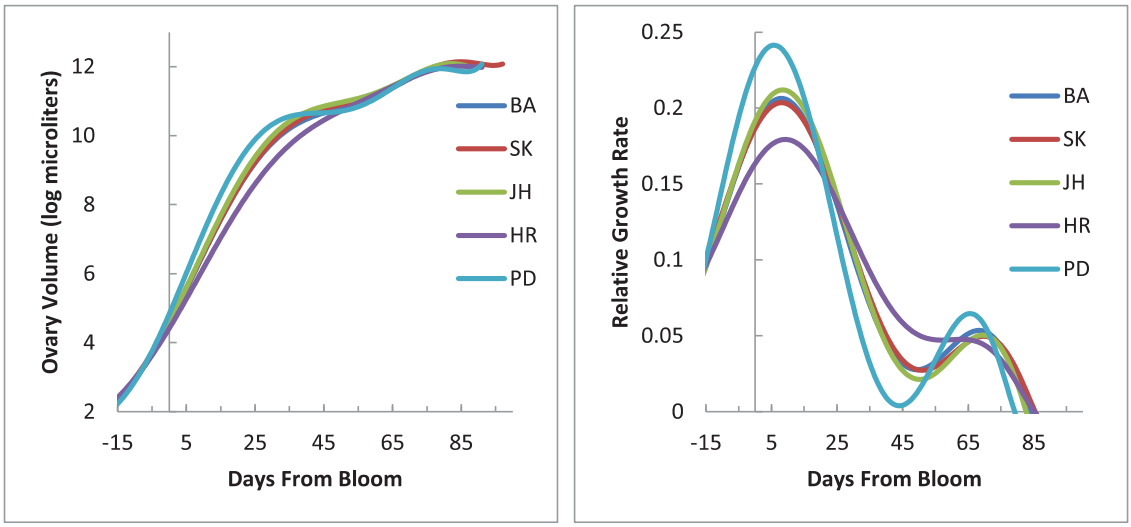

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Figure 3. Growth curves of Sweetheart were derived from spur sampling twice weekly at five locations. Left: growth in volume expressed in logarithmic form. Right: Relative growth rates. The similarity of the minima, maxima and inflection points (data not shown but see Table 1) on relative growth rate curves from 2014 (Fig. 3, right panel), and those of the previous year (provided in 2013 continuing report) show synchrony in development despite varied environments.

Sources of variability in growth and fruit size. Bloom dates have always presented a question mark with no uniformly agreed upon protocol for its determination. And this is surprising considering how important bloom date can be in determining fruit size. Given that cherries are typically harvested in one pass, bloom that is significantly behind the curve (as we have previously demonstrated) do not catch up and will be smaller at harvest. The most straight forward way to approach this question is to count blooms as they open (Fig. 4). As expected, a range in bloom progression and timing was observed at different sites. A consistent ranking of size on given dates was not found between sites; however, after pit hardening (45 DFB) fruit from sites in The Dalles were larger than Hood River and Parkdale where protracted bloom periods were observed (Fig. 4).

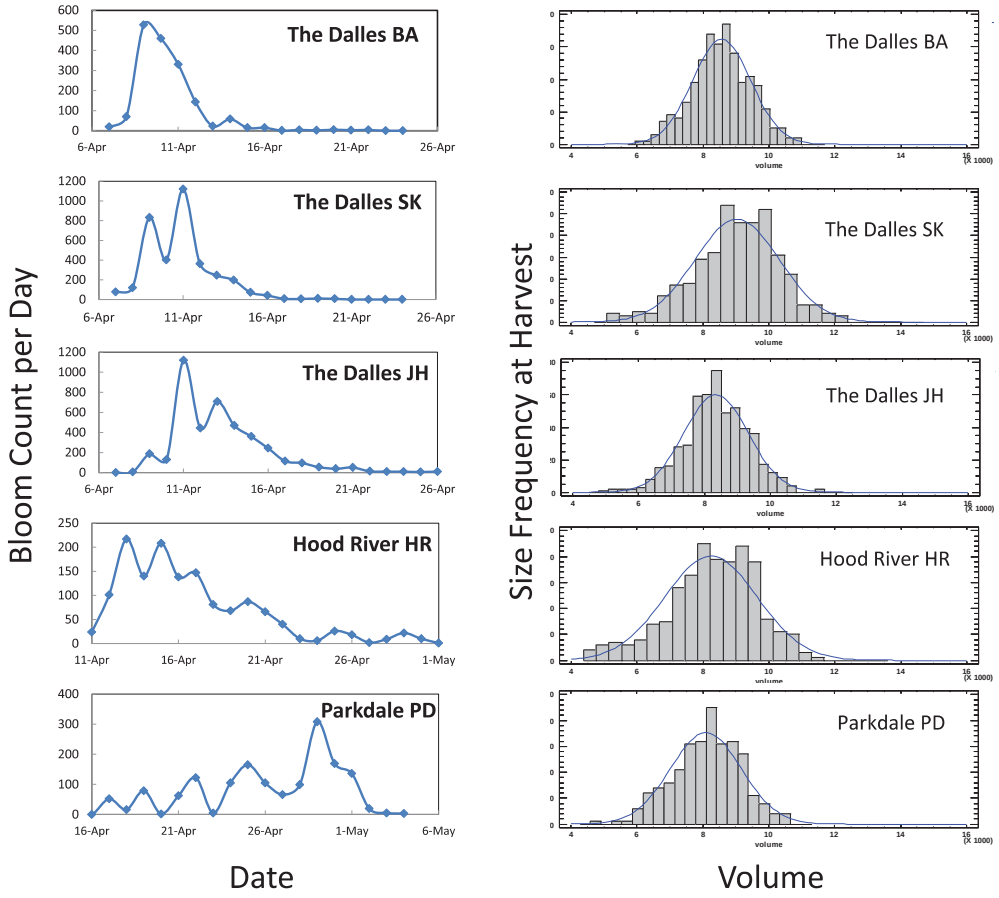

Figure 4. Bloom progression of Sweetheart at 5 sites may offer insights into fruit volume differences at harvest. Left panel: Blooms were removed and counted on the day they opened from portions of 15 limbs (of separate trees). Right panel: The narrowest distribution in fruit size was from site BA (10% variation) and the broadest was HR (17% variation) mirroring the bloom progression. PD had fewer large fruit than may be expected (poor pollination of early bloom) explaining the smaller size, but narrow distribution.

We've settled on an approximate 50% bloom to begin our fruit growth and set calculations, but this choice is debatable (HR) or delayed blooming (PD) could have a large effect on the variation of fruit size, and possibly detrimental to overall size if the early bloom was left unfertilized. Additionally, the prolonged bloom would have affected the fruit vs. failure determination adding to the variation. HR and PD were smaller and had long duration of bloom.

An experiment with Regina also tested the importance of bloom date and its relationship to final fruit size. In 2014, 250 flowers were tagged each day as they opened from the beginning to the end of the bloom period. At harvest the fruit were recovered to record the fruit size. As we have previously shown, early flowers yield the largest fruit (Fig. 5). Interestingly, fruit set of this orchard was quite low indicating that even under ample carbon supply, potential fruit size (of later blooming ovaries) cannot be made up.

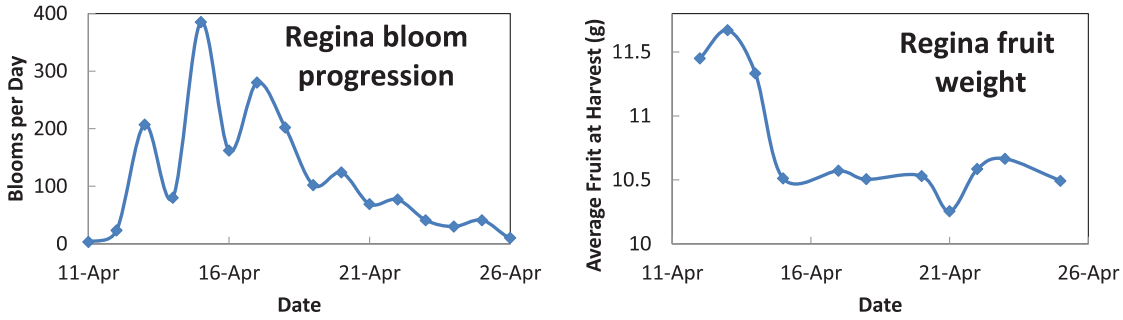

Figure 5. Relationship of bloom date to fruit size. The first three dates of bloom resulted in significantly larger fruit.

Growth models.

Temperature affects the progression of bloom and the growth of ovaries. We experimentally manipulated temperature prior to bloom in order to determine temperature optima for ovary growth. This is a necessary step toward model development. For these experiments, Bing and Regina whole limbs were harvested and placed in temperature controlled growth chambers. These two cultivars were selected based on their different developmental timelines in early spring. As low as 40°F was sufficient for growth effects approximately 22 DFB (i.e., in the green tip phase; Fig. 6). Near 50°F was probably the upper limit for growth but did appear to have a marked influence on Regina ovary growth.

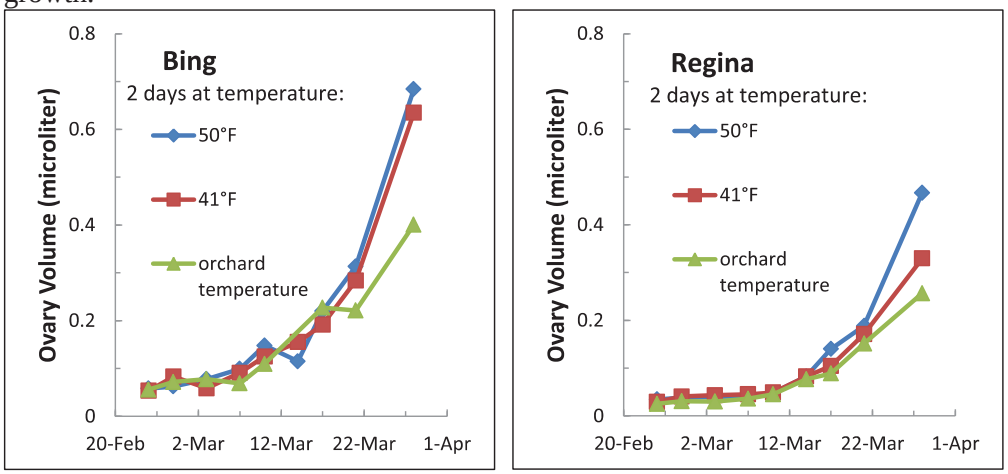

Figure 6. Growth of Bing and Regina ovaries between dormancy and green tip as affected by temperature.

However, for advanced stages of bud break, temperatures of 70°F produced a significant growth effect approx. 9 DFB when buds were in the open cluster to first white phases. These responses need to be expanded upon (see Einhorn New Proposal) in a systematic manner to determine how temperature optima for growth change with development. This is absolutely essential to the development of an accurate growth model.

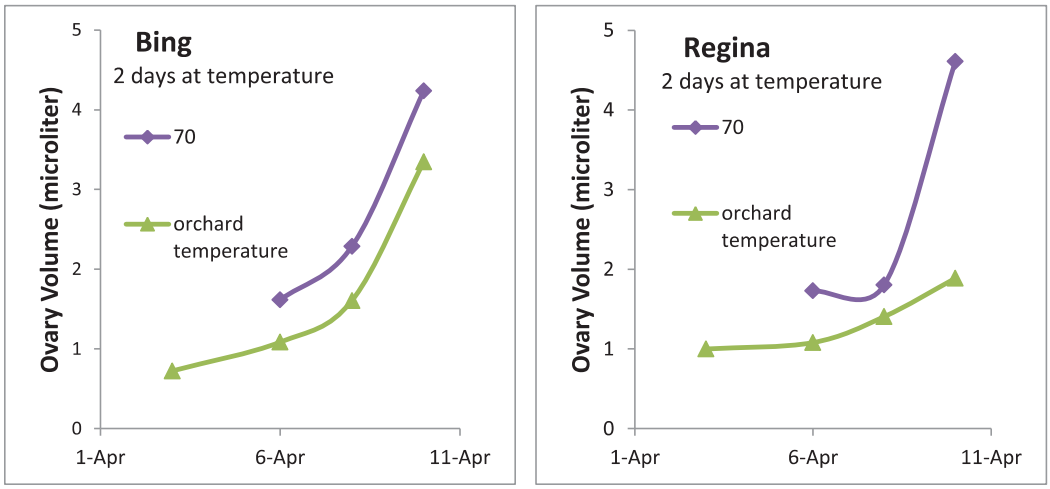

Figure 7. Growth of Bing and Regina ovaries in advanced stages of bud-break.

Adjusting model indices in step with the season

In addition to experimentally determining the optima of the growth response, temperature indices can be manipulated to explain the seasonality of growth, especially post-bloom. We created a spreadsheet with inputs for temperature data from the IFPnet, sunrise and sunset data from the Naval Observatory, and of course growth measurements. The spread sheet uses easily adjusted temperature indices for asymmetric curves of the growth response to temperature. The temperature response we observed in the pre-bloom phases (Fig 6 and 7) matches well with the empirical choice of temperature indices we used in our new model (Table 4).

Table 4. Adjusting temperature indices can result in a more accurate model.

Location, Year	Calendar Days From Bloom	Linear Degree Hour Model	Our NEW Adjusted Degree Model
The Dalles, 2013	91 (April 14)	20820	6129
Hood River, 2013	**92 (April 21)**	**27085**	**6077**
Parkdale, 2013	97 (April 27)	25252	6119
The Dalles2, 2011	**95 (May 2)**	**27026**	**6150**
Average Coefficient of Variation	94 3% (+/- 3 days)	25046 12% (+/- 11 days)	6119 0.5% (<1 day)

This model changes indices for day/night, and seasonal progression. Day and night indices are changed to account for photosynthesis and respiration, while indices are also adjusted seasonally to account for phenology and year to year variation.

PGRs

Stem growth is complete by pit-hardening; in nearly every case pre-bloom applications of solutions containing GA were highly effective in elongating stems (comparable results were observed, but not quantified, for leaf area- a process similarly completed in a relatively short time span). These data provided evidence of uptake and translocation when applied at first white; a possibly prohibitive time given the relatively limited supply of absorptive green tissue present. Fruit growth, however, appeared to be more greatly affected by cytokinins. Packout data (~2,000 fruit per treatment) of Sweetheart revealed a significant size improvement for the prebloom (-7 dfb) CPPU application producing 72% 9.5 row and larger fruit compared to 59% for the control.

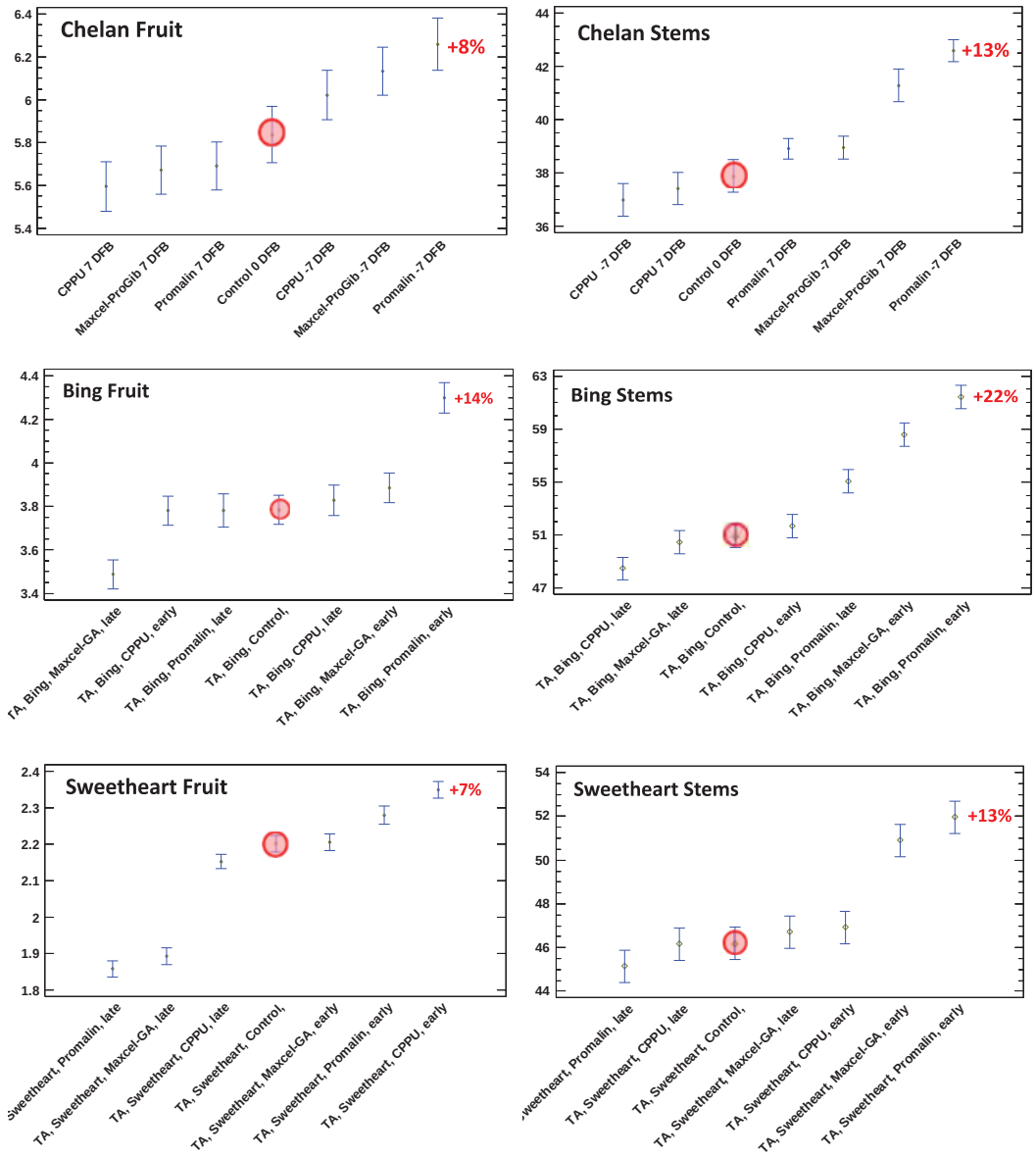

Figure 8. Pre-harvest sampling of Chelan, Bing, and Sweetheart fruit from a commercial orchard in WA. Fruit size data are grams (n=250); stem length data are mm (n=250). Treatments on the x-axis are ascending with respect to the data and are therefore not consistently ordered across graphs. On each graph, controls are circled for comparisons and treatments showing the greatest percent increase relative to the control are indicated. Late applications were performed at ~7 days after bloom; early applications were made between open cluster and first white (-5 to -7 days from bloom).

These data aligned with our pre-season measurements, which were taken prior to pit hardening (in the case of Sweetheart). For Bing and Chelan, however, no significant differences at harvest were quantifiable- a perplexing outcome given a visibly noticeable size improvement in rows treated with early Promalin. Our pre-harvest sampling of individual fruit of Chelan, for example, was taken ~2.5 weeks prior to harvest. Chelan cropload (and yield) was exceptionally high, and could have increased the demand for carbon during the last few weeks of stage III growth, thus limiting the growth potential established early by CPPU and Promalin, relative to controls. The greater leaf area, produced by Promalin in particular, would have likely augmented carbon available to supply fruit. More work is needed on early-season PGRs before programs can be recommended.

Executive summary

Growers can use these guidelines for assessing their orchard:

- Sample one spur from at least 30 trees for a good size and set estimate
- Count bloom progression from one limb portion from 15 trees to set bloom date accurately
- 40°F is sufficient to enhance ovary growth at green tip phase
- 70°F at open cluster to first white advances growth considerably
- Good crop estimates can be made 20 days after bloom
- Pre-bloom PGR applications increased fruit size, stem length and/or leaf area
- Effort to set early bloom should be made; these flowers produce big fruit

Further work is proposed because:

- Maturation could be better qualified with photographic analysis of color
- A more descriptive model of growing degree units can, and needs, to be done
- Early season cytokinin sprays to enhance fruit size appear promising

FINAL PROJECT REPORT **YEAR**: 3 of 3 years

Project Title: Extending storage/shipping life and assuring good arrival of sweet cherry

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Total Project Request: **Year 1:** $26,375 **Year 2:** $26,913 **Year 3:** $24,466

Other funding sources: None

WTFRC Collaborative expenses: None

Budget 1: Yan Wang
Organization Name: OSU-MCAREC **Contract Administrator:** L.J. Koong
Telephone: 541-737-4066 **Email address:** l.j.koong@oregonstate.edu

Item	2012	2013	2014
Salaries		10,384[1]	10,696[7]
Benefits		1,848[2]	1,903[7]
Wages	9,600	5,312[3]	5,471[7]
Benefits	8,275	1,222[4]	1,259[7]
Equipment			
Supplies	8,000	7,647[5]	4,637
Travel	500	500[6]	500
Miscellaneous			
Total	26,375	26,913	24,466

Footnotes:
[1]Postdoctoral Research Associate (Dr. Xingbin Xie): 550hr at $18.88/hr.
[2]OPE: $3.36/hr.
[3]Wages: 390hr for a Biological Science Tech. at $13.62/hr.
[4]OPE: 23% of the wage.
[5]Supplies: fruit, Ca and Cl analysis, GC-MS volatile analysis, gases (helium, nitrogen, hydrogen, standard gases), gas tank rental, chemicals, and MCAREC cold room use fee.
[6]Travel to grower's fields
[7]3% increase

OBJECTIVES

The goal of this project was to minimize pitting, postharvest splitting, acid loss, dull color, and stem browning, therefore improve shipping quality of the PNW and California sweet cherries.

The key objectives were to:

1. ***Modified atmosphere packaging (MAP):*** Determine the optimum MAP parameters (O_2, CO_2) and efficacy of the major commercial MAP liners and consumer packaging for improving shipping quality of the PNW and California cultivars.

2. ***Calcium (Ca):*** Study the mechanism and practical postharvest Ca treatment to minimize pitting and splitting of PNW sweet cherries.

3. Evaluate ***edible coatings and GRAS compounds*** on shipping quality of PNW sweet cherries.

SIGNIFICANT FINDINGS

1. Respiration physiology influenced by $O_2$and CO_2, temperature, and cultivars

- At shipping temperatures, respiration rates of the major PNW and California cultivars were affected very little by reduced O_2 from 21 to 10%, but declined significantly from 10 to 5%.
- Estimated fermentation induction points were about1-4% O_2 for the major cultivars depending on temperatures.
- CO_2 at 0-15% did not affect respiration rates of 'Bing', 'Sweetheart', and 'Coral'.
- 'Skeena' had a higher RQ (respiration quotient) and respiration Q_{10} than other cultivars. Therefore, 'Skeena' is more susceptible to anaerobic injury.
- 'Skeena' fruit stressed by heat had a higher respiration rate and are more susceptible to anaerobic injury.

2. MAP Technologies

- It was found that the major commercial MAP liners (7) had extremely varied equilibrium O_2 (i.e., 1-15%) and CO_2 (i.e., 5-13%) concentrations for the major PNW and California cultivars at simulated commercial shipping conditions.
- ***O_2 concentration affected flavor.*** MAP liners with equilibrium O_2 5-8% at 32 °F reduced respiration rate and therefore maintained titratable acidity (TA) and flavor of the major cultivars after 4-6 weeks of cold storage. MAP liners with O_2 > 10% did not maintain flavor. MAP liners with O_2 < 5% may cause anaerobic fermentation during commercial storage/shipping.
- ***CO_2 concentration affected fruit color darkening.*** MAP liners with equilibrium CO_2 10-15% maintained the shiny fruit color at simulated storage/shipping conditions. MAP liners with CO_2 < 8% had little beneficial effect on maintaining fruit shiny color.
- 'Regina', 'Skeena', and 'Lapins' produced a bitter taste after 3-6 weeks storage/shipping. MAP liners with O_2 at 5-8% prevented or reduced bitter taste development.
- 'Skeena' is more susceptible to anaerobic fermentation at fluctuated temperatures, therefore, needs MAP liners with relatively higher gas permeability (i.e., O_2 8-10%) to avoid anaerobic injury in commercial storage/shipping.
- ***Consumer packaging.*** Zipper-lock bags and clamshells with perforation ratio of 0.5% (3mm diameter) maintained cherry pedicel healthier than the commercial ones (perforation at 2-5%, 8mm diameter), without generating extra condensation or fermentation after a simulated storage/shipping/marketing period.

3. Postharvest Ca application in hydro-cooling water

- Pitting susceptibility was found to be correlated negatively with fruit tissue Ca content.
- Splitting potential was correlated with fruit tissue Ca content and pectin chemistry.
- Adding Ca (0.2-0.5%) in hydro-cooling water (32 °F) efficiently increased fruit tissue Ca content in 5 min.
- The enhanced Ca concentration increased fruit firmness (FF) and retarded fruit senescence, therefore, reduced pitting susceptibility, maintained TA and Vc, and reduced postharvest splitting and decay of 'Bing', 'Skeena', 'Lapins' and 'Sweetheart'.
- EDTA (a chelator of divalent cation) or low pH (i.e., <4) depleted Ca from fruit and increase splitting of cherry fruit.
- Ca application rate and temperature gradient between fruit and solution were the key factors determining efficacy of the Ca treatments.
- Higher Ca rates (1.0-2.0%) damaged cherry stems.
- Cherry fruit didn't take up Cl.

4. Edible coatings and GRAS compounds

- Semperfresh™ at appropriate rates (i.e., 0.5% a.i.) reduced moisture loss, maintained stem quality, and reduced pitting expression of cherries packed in clamshells. Semperfresh™ at its label rate of 1.0% a.i. increased pitting expression of 'Sweetheart'.
- Postharvest applications of salicylic acid (SA) and oxalic acid (OA) tended to reduce respiration rate and maintain higher TA during storage.
- There may be little benefit at commercial level from postharvest applications of Chitosan, Sodium alginate, Jasmonic acid (JA), Methyl Jasmonate (MeJA), ethanol, GA_3, and Homobrassinolide (HBR) on PNW sweet cherries.

METHODS

1. Respiration physiology

Cherry samples of ~500g of 'Bing', 'Skeena', 'Regina', 'Lapins', 'Sweetheart', and 'Coral' were placed in hermetically sealed glass containers (960mL) equipped with 2 rubber sampling ports at 32 and 68°F. Headspace O_2 and CO_2 concentrations were periodically monitored by an O_2/CO_2 analyzer. Respiration rates based on O_2 consumption and CO_2 production, fermentation induction point, and respiration quotient (RQ) were plotted with O_2 and CO_2 concentrations.

2. MAP Trials

The major commercial MAP liners (ViewFresh, Xtend, LifeSpan, Breatheway, Primpro, PEAKfresh, FreshLOK) with distinct technologies were obtained from the manufactures. Fruit of different cultivars were either obtained from packinghouses shortly after packing or harvested directly from the field and then packed into different MAP liners after pre-cooling. The concentrations of O_2 and CO_2 in MAP liners were determined every day in the first week then every 3-5 days until at the end of the tests. At 2, 4, and 6 weeks, 50 fruit were randomly selected from each box for determinations of respiration, FF, color, anthocyanin, SSC, TA, Vc, ethanol, and volatile-aroma compounds (GC-MS) immediately after cold storage and plus 2 days at 68°F. Fifty fruit were randomly selected for evaluations of pitting, splitting, stem quality, decay, and sensory evaluation. Experimental units were boxes and there were three replications per treatment at each evaluation period. The experimental design was completely randomized.

3. Postharvest Ca Application in hydro-cooling water

Ca solutions at 0, 0.2, 0.5, 1.0, and 2.0% were cooled to 32 °F before treatments. Fruit harvested at commercial maturity from MCAREC with fruit pulp temperature 70-80 °F were immediately hydro-cooled in the cold Ca solutions for 5 min to simulate the commercial hydro-cooling procedures. Fruit tissue Ca and Cl content (ICP-AES and Lachat Quikchem autoanalyzer methods, respectively),

shipping quality (pitting, splitting), eating quality, nutracuetical values, and biochemical changes were evaluated after 2, 4, and 6 weeks of cold storage.

4. Postharvest Applications of edible coatings and GRAS Compounds

Semperfresh™, Chitosan, Sodium alginate, Salicylic acid (SA), Oxalic acid (OA), Jasmonic acid (JA), Methyl Jasmonate (MeJA), ethanol, GA_3, Homobrassinolide (HBR, a brassinosteriod) are applied postharvest on certain PNW cultivars.

RESULTS AND DISCUSSION

1. Respiration Dynamic

While respiration rate of cherry fruit was inhibited linearly by reduced O_2 concentration from 21% to 3-4% at 68 °F, at 32 °F it was affected very little from 21% to ~10% but declined significantly from ~10% to ~1% for 'Bing', 'Sweetheart', and 'Coral' (Fig. 1). Estimated fermentation induction points determined by a specific increased RQ were ~1% and 3-4% O_2 for all cultivars at 32 and 68 °F, respectively. As a consequence, the gas permeability of MAP has to be modified to reduce O_2 between 10-5% at 32 °F within the package to inhibit cherry fruit respiration activity to maintain fruit quality (flavor) without anaerobic fermentation during commercial storage/shipping.

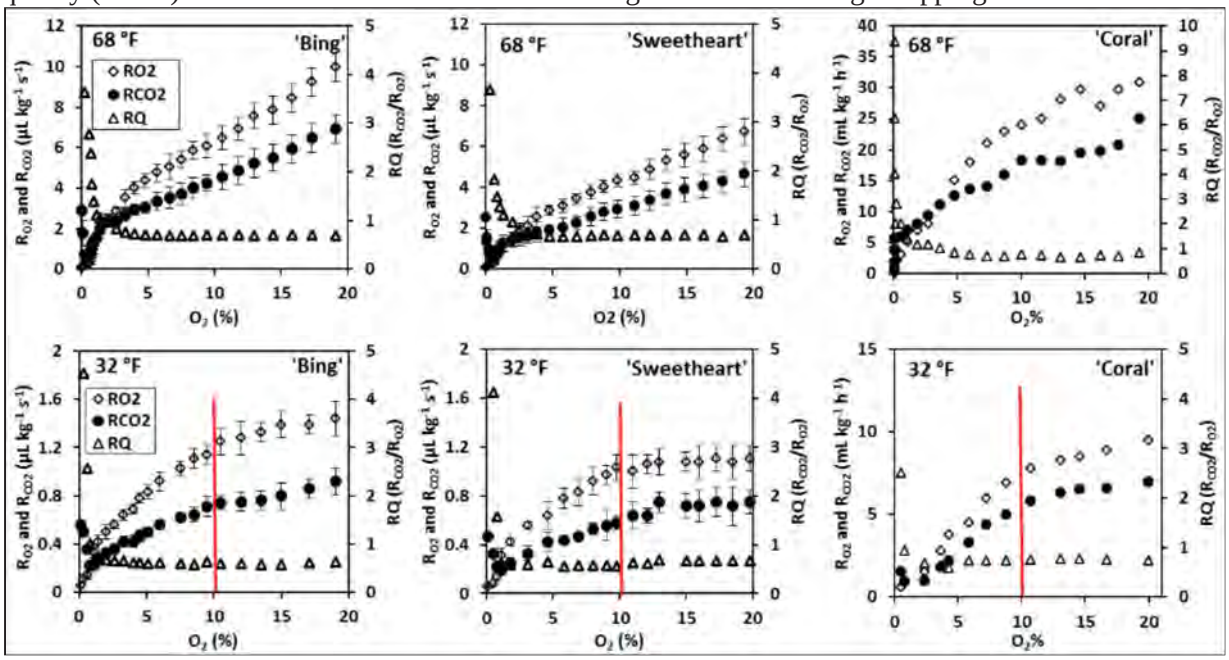

Fig. 1. Respiration dynamics of sweet cherries affected by O_2, temperature, and cultivars.

'Skeena' has a higher RQ at elevated temperatures and therefore is more sensitive to anaerobic injury due to temperature fluctuations during shipping (Fig. 2). MAP liners with equilibrium 8-10% O_2 at 32 °F may be suitable for 'Skeena' at commercial shipping. Q_{10} was determined to be 3.5, 3.3, 3.1, and 3.0 at temperatures from 32 to 50 °F for 'Skeena', 'Lapins', 'Regina' and ''Sweetheart', respectively. 'Skeena' fruit stressed by heat in the field had higher respiration rates, a shorter shelf-life, and were more susceptible to anaerobic injury (Data not shown). Heat stressed Skeena could show pitting on the trees.

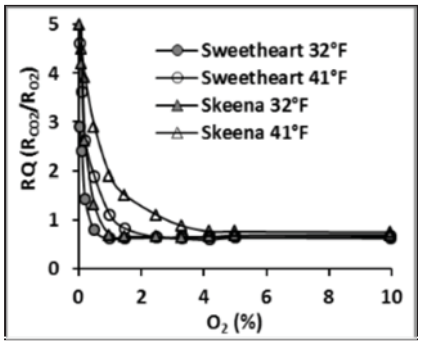

Fig. 2. RQ of Sweetheart and Skeena.

2. MAP Technologies

1) Gas permeability of different MAP liners.

The seven commercial MAP liners used in sweet cherry industry generated extremely varied equilibrium O_2 and CO_2 concentrations for different cultivars at recommended shipping temperatures (Fig. 3). O_2 ranged from 1-15% and CO_2 ranged from 5 to 15% for 'Bing', 'Lapins', 'Skeena', 'Regina', 'Sweetheart', and 'Coral'.

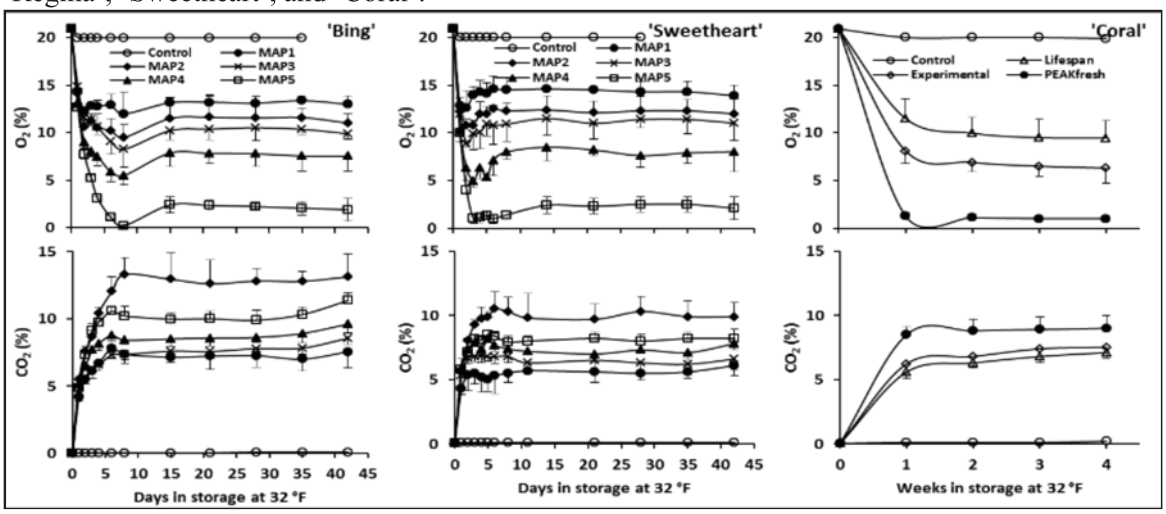

Fig. 3. O_2 and CO_2 contents in different MAP liners for 'Bing', 'Sweetheart', and 'Coral' at 32°F.

2) Effect of elevated temperatures on O_2 and CO_2 in MAP liners and anaerobic fermentation.

Elevated transit temperatures from 32 to 41 °F reduced O_2 significantly (Fig. 4) but did not change CO_2 much in MAP liners. The equilibrium O_2 in MAP4 and MAP5 were reduced from ~6% and 2% at 32 °F to ~3.5% and 0.5% at 41 °F for Sweetheart and Skeena, respectively (Fig. 4). At 36 °F, the equilibrium O_2 was 4.5% and 1% in MAP4 and MAP5 during 2 weeks of cold storage and there was no significant accumulation of ethanol in 'Sweetheart' and Skeena after 2 weeks of cold storage (data not shown). At 41 °F, ethanol was accumulated significantly in 'Sweetheart' packed in MAP5 and Skeena packed in MAP4 and MAP5 (Fig. 4). Fermentation flavor was detected in the fruit with significant ethanol accumulation. In conclusion, MAP with appropriate gas permeability (i.e., 5-8% O_2 for most of the cultivars and 8-10% O_2 for Skeena) are suitable for commercial application to maintain flavor without damaging the fruit through fermentation, even if temperature fluctuations, common in commercial storage/shipping, do occur.

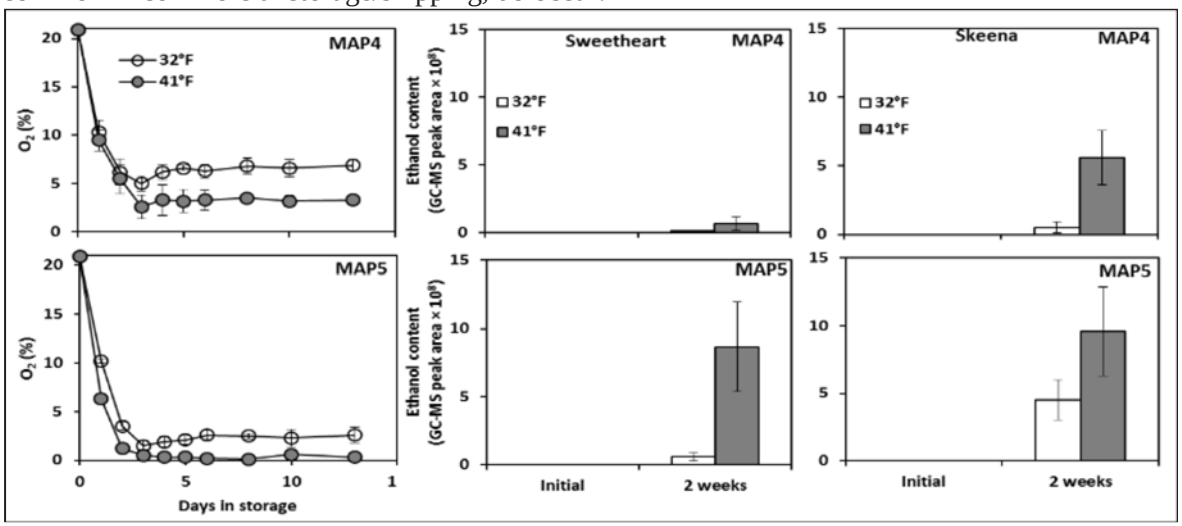

Fig. 4. Effect of elevated temperature on O_2 in MAP and ethanol accumulation in cherry fruit.

3) Efficacies of different MAP liners on maintaining fruit shipping quality. While all the MAP liners maintained higher FF and reduced decay, only the MAP liners with lower O_2 permeability (i.e., equilibrated at 5-8% O_2) reduced fruit respiration rate and maintained TA and flavor compared to the standard macro-perforated PE liners after 4-6 weeks of cold storage. In contrast, MAP liners that equilibrated with atmospheres of 10-15% O_2 had little effect on inhibiting respiration rate and TA loss, MAP with 1-2% O_2 enhanced ethanol accumulation and fermentation flavor during cold storage (Fig. 5).

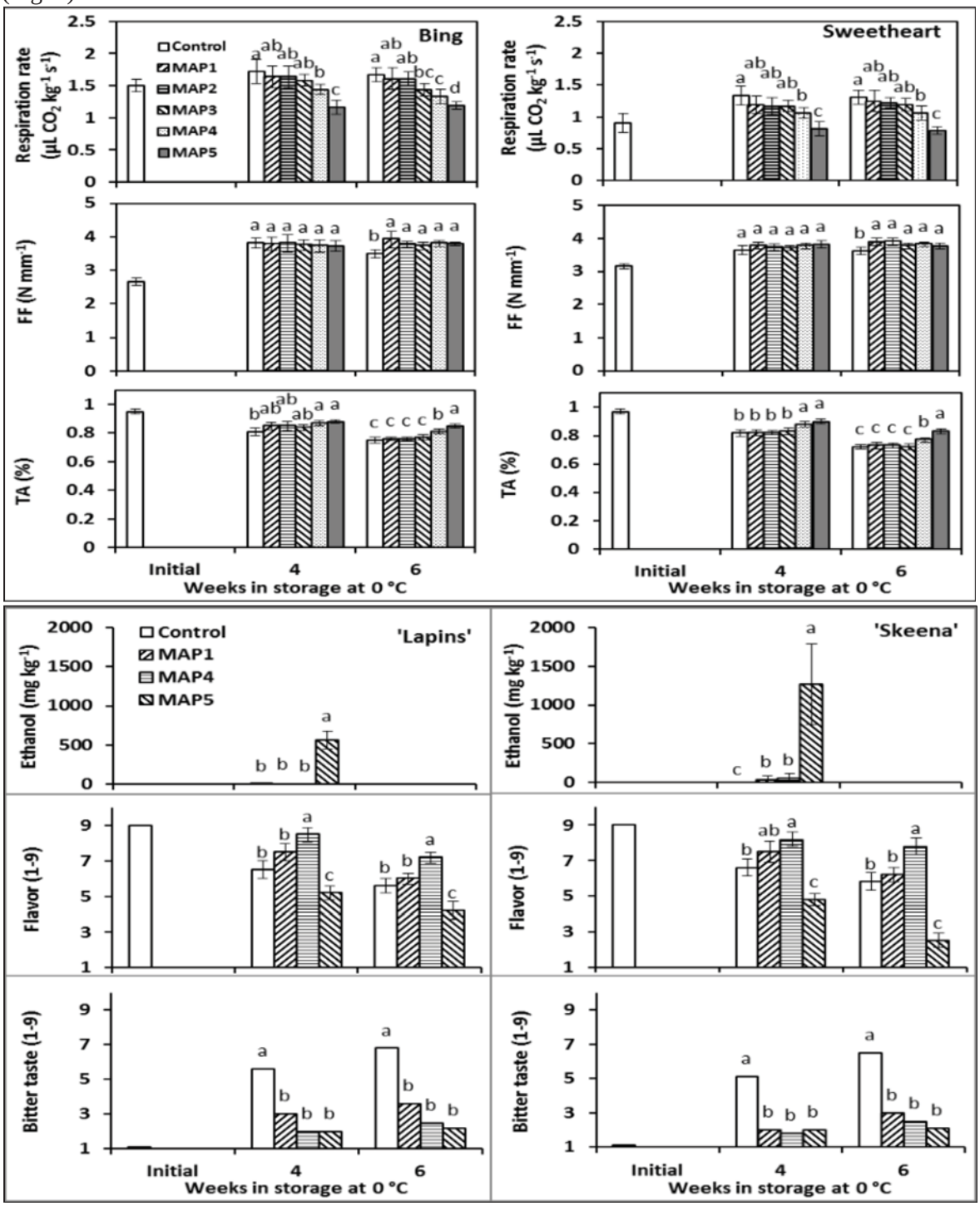

Fig. 5. Effect of MAP on cherry fruit quality during storage.
Cherry fruit skin darkening during storage gave the fruit a dull and over-ripe appearance that affected consumer preference. Fruit skin darkening during storage was reflected by reduced L* and increased anthocyanin accumulation. Higher CO_2 concentrations (10-15%) in MAP retarded anthocyanin accumulation and fruit skin color darkening significantly. In contrast, CO_2 < 8% had much less effect on retarding anthocyanin synthesis and maintaining the luster skin color of cherry fruit after cold storage/shipping (Fig. 6).

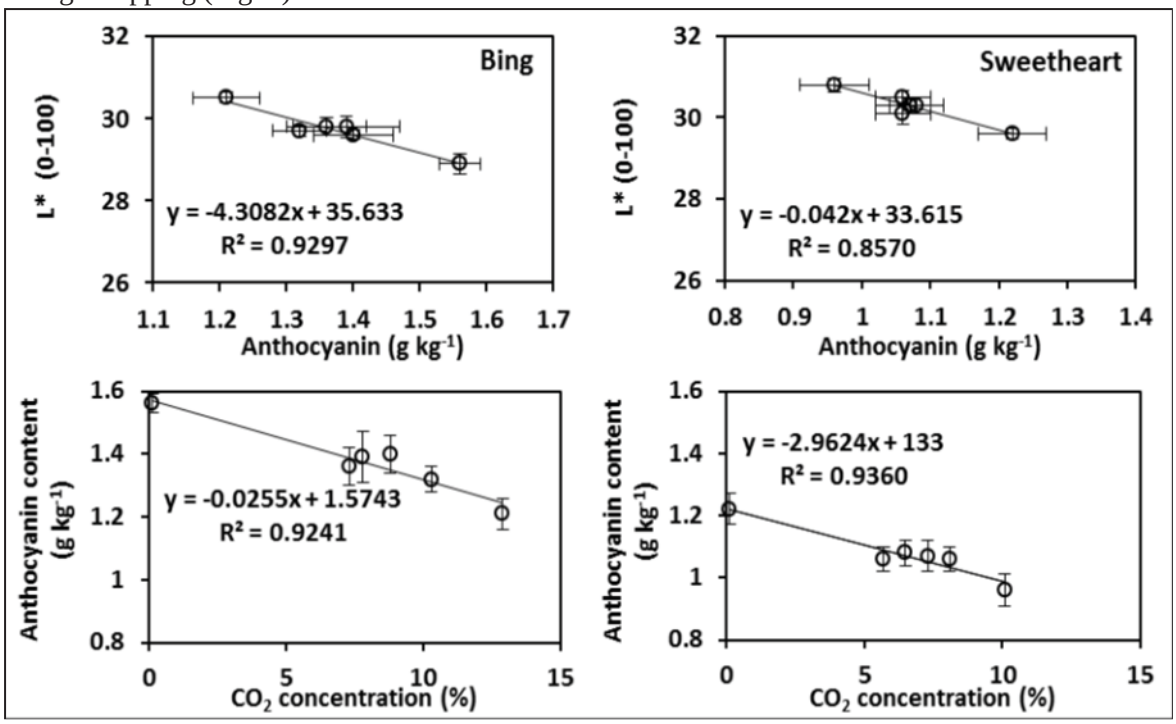

Fig. 6. The relationship of cherry fruit skin darkening with anthocyanin and CO_2 concentration in MAP during storage at 32 °F.

4) Consumer packaging. The perforation ratios of commercial zipper-lock bags or clamshells were ranged from 2-5%. The RH within zipper-lock bags with perforation of 2% were 96%, 93%, and 91% at environment temperatures of 32°F (RH 88%), 50°F (RH 75%), and 68°F (RH 65%), respectively. The RH within zipper-lock bags with perforation of 0.5% were 99%, 98%, and 96% at 32°F, 50°F, and 68°F, respectively. RH within the bags with perforation of 0.05% was close to 100% at each of the temperatures tested (Fig. 7). Stem moisture losses of Chelan and Lapins were higher in bags with 2% perforation than 0.5% and 0.05% at each of the simulated marketing stages. Stem visual quality was higher in bags with perforation at 0.5% than at 2% after 1 week at 32°F + 2 days at 50°F + 2 days at 68°F. Bags with perforation at 0.05% had higher condensation and higher decay incidence (data not shown).

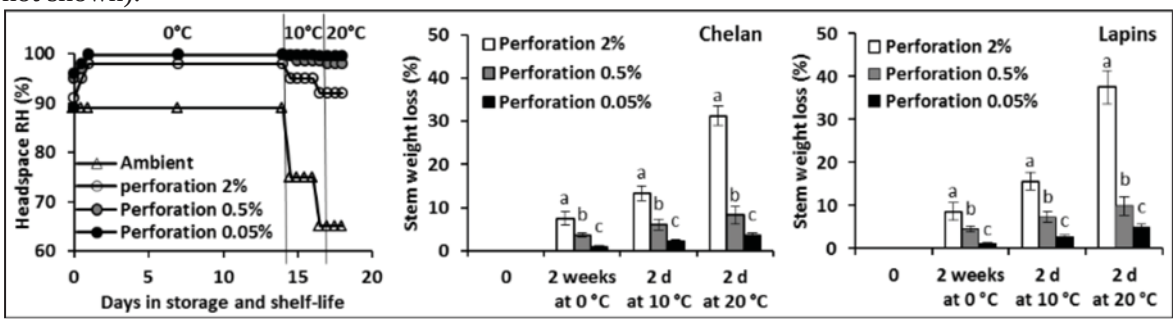

Fig. 7. Effect of perforation ratio in zip-lock bag or clamshell on RH and cherry stem weight loss.

3. Postharvest Ca Application in Hydro-Cooling Water

1) Increasing fruit tissue Ca content. Cherry fruit absorbed Ca with increasing Ca concentration from 0.2 to 2.0% in cold water (0 °C) for 5 min (simulating commercial hydro-cooling), but did not take up Cl (Fig. 8). Extending the exposure time from 5 to 30 min increased tissue Ca content of both cultivars at each Ca rate numerically but not at a statistically significant level.

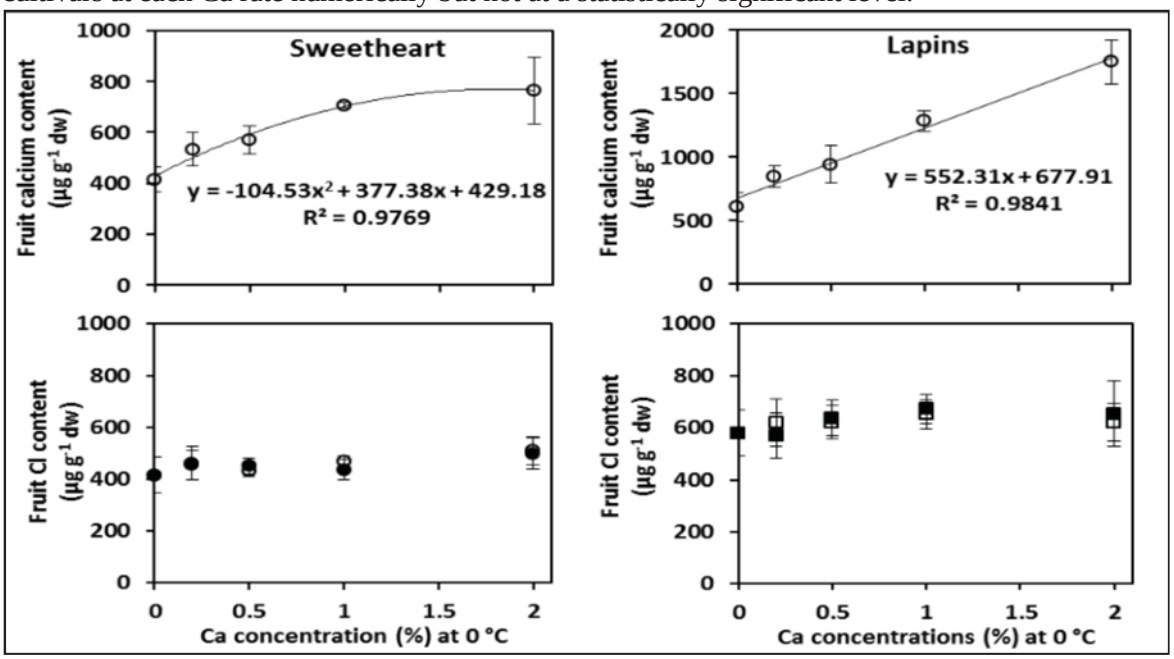

Fig. 8. Tissue Ca and Cl uptakes by cherry fruit **as** affected by $CaCl_2$ in cold water at 0 °C.

2) Retarding senescence, increasing firmness, and reducing pitting. The increase of fruit tissue Ca content was accompanied by reductions in respiration rate, ascorbic acid (AsA) degradation, and membrane lipid peroxidation (Fig. 9). The Ca treatments enhanced total phenolics content and total antioxidant capacity, and resulted in increases in fruit firmness and pitting resistance (Fig. 10) and decreases in TA loss and decay (data not shown) of both cultivars. Pedicel browning was inhibited by Ca at 0.2% and 0.5%, but increased by higher rates at 1.0% and 2.0% (Fig. 11), possibly via modifying membrane lipid peroxidation.

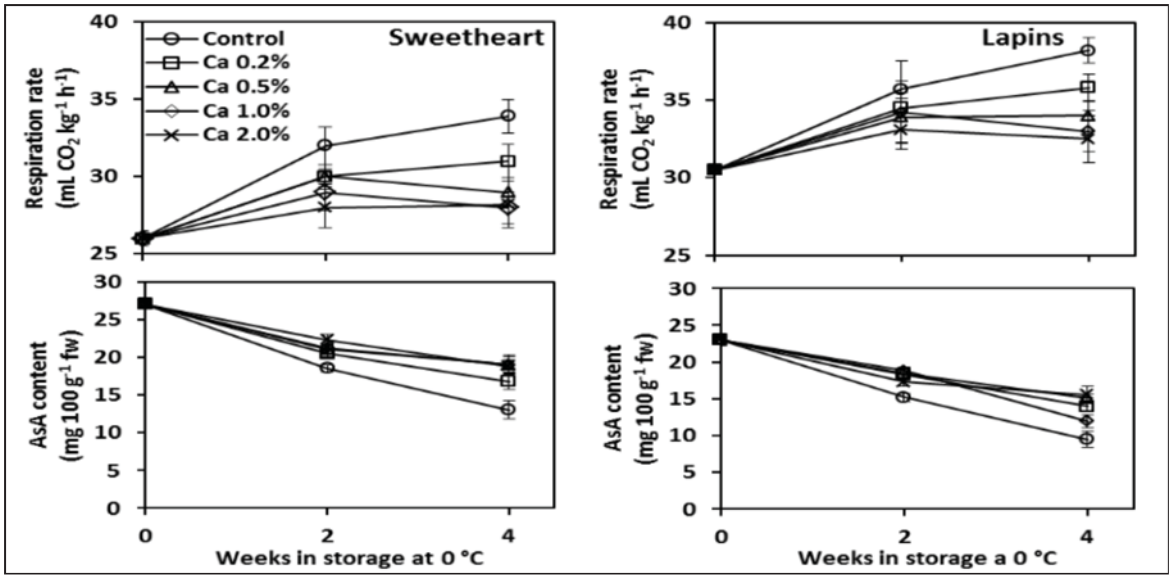

Fig. 9. Effect of Ca in hydro-cooling water on cherry respiration rate and AsA degradation.

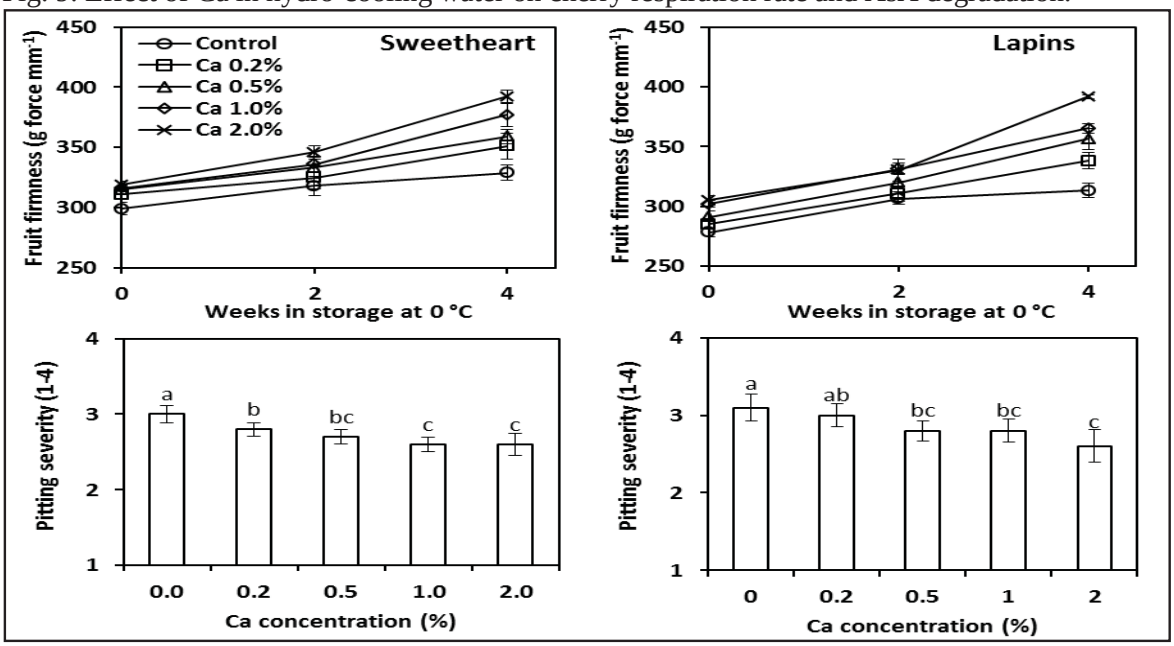

Fig. 10. Effect of Ca in hydro-cooling water on cherry fruit firmness and pitting susceptibility.

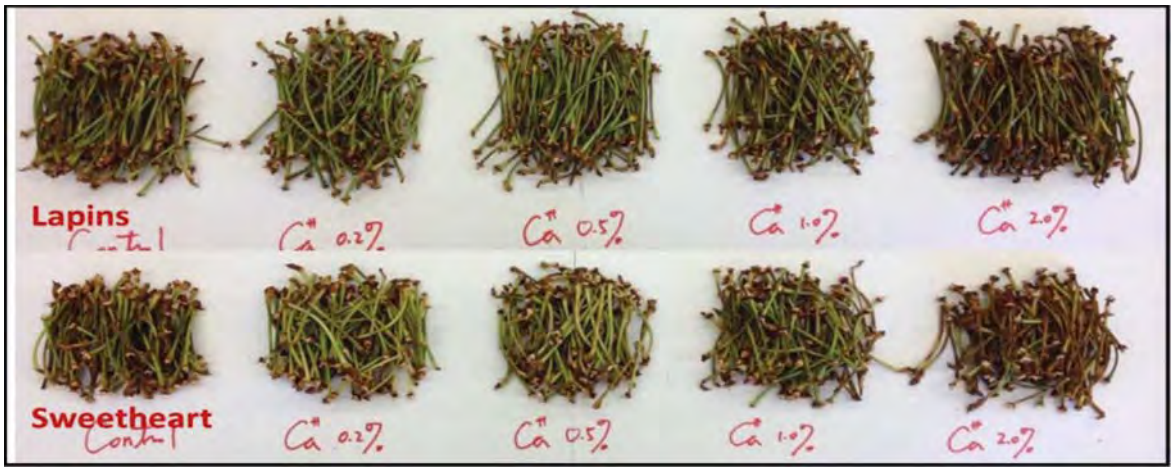

Fig. 11. Effect of Ca in hydro-cooling water on cherry stem quality after 2 weeks of storage.

3) Reducing splitting of Skeena and Bing. The enhanced tissue Ca content reduced splitting potential of the splitting-susceptible cultivars (i.e., Skeena) by decreasing fruit soluble pectin release and increasing the splitting threshold. In contrast, depleting Ca from fruit tissue by EDTA or low pH increased soluble pectin release and splitting potential (Fig. 12).

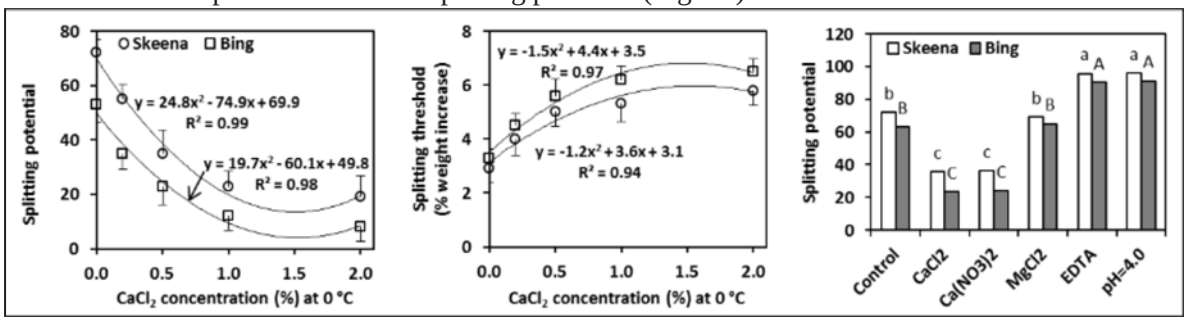

Fig. 12. Effect of Ca in hydro-cooling water on splitting potential of Skeena and Bing cherries.

4. Postharvest Treatments with GRAS Compounds and edible coatings

1) SA, OA, JA, MeJA, ethanol, HBR,

Postharvest applications of SA and OA tended to reduce respiration rate and maintain TA of PNW cultivars packed in clamshells during storage (Fig. 13). It was reported that both SA and OA enhanced total antioxidant capacity (TAC) in 'Cristilina' and 'Prime Giant' cultivars (Valero et al., 2011), however, they do not seem to affect TAC of PNW cultivars during cold storage (Fig. 13). Postharvest treatment with JA, MeJA, ethanol, and HBR had little effect on shipping quality of 'Lapins' and 'Skeena' at commercial level (data nor shown).

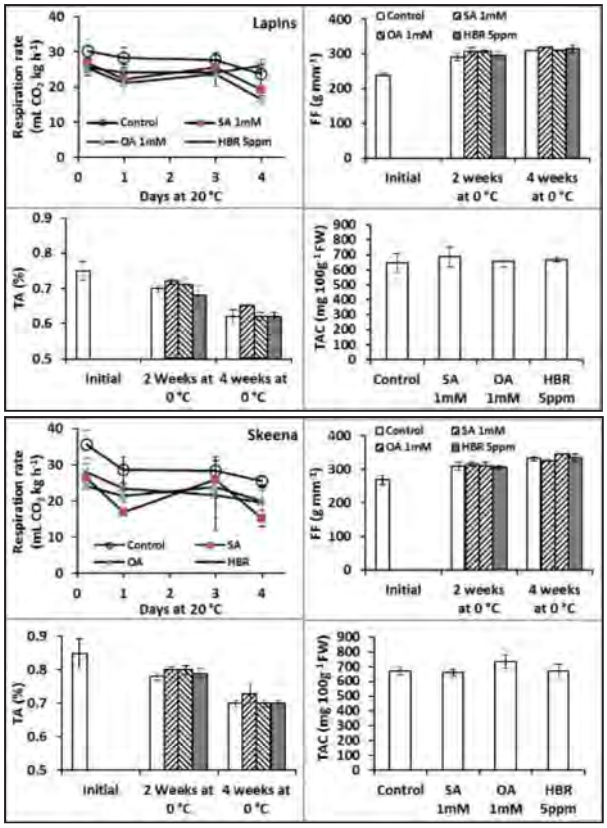

Fig. 13. Effect of SA, OA, and HBR on respiration rates, TA, FF, and total antioxidant capacity (TAC) of 'Lapins' and 'Skeena'.

2) Semperfresh^{TM}, GA_3, sodium alginate, chitosan

Semperfresh^{TM} at 0.5% a.i. reduced moisture loss and maintained green stem of 'Chelan' and 'Lapins' packed in clamshells at simulated marketing conditions (Fig. 14). GA_3 at 100ppm did not affect shipping quality of 'Chelan' and 'Lapins'. Semperfresh^{TM} reduced pitting of 'sweetheart' at application rate of 0.5% a.i., but increased pitting at its label rate of 1.0% a.i.(Fig. 15). Pitting formation seems to be associated with moisture loss and localized O_2 deficiency. Chitosan and alginate had little effect on shipping quality of 'Chelan' and 'Lapins' (data not shown).

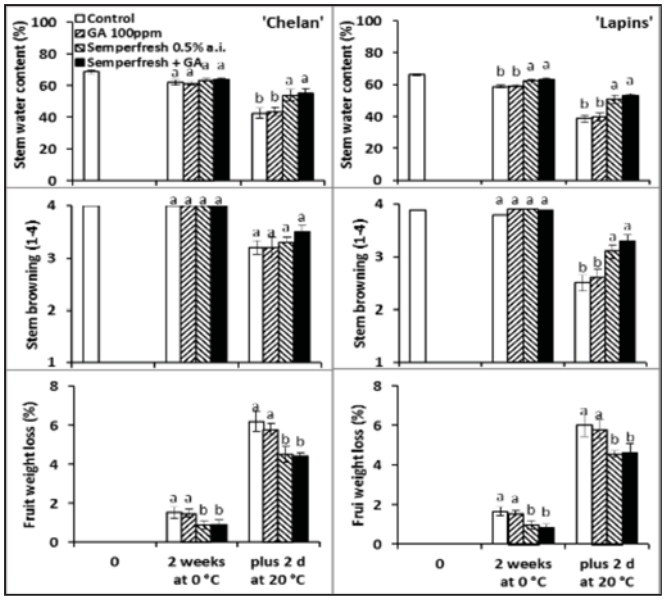

Fig. 14. Effect of Semperfresh™ and GA_3 on shipping quality of Chelan and Lapins at simulated marketing conditions.

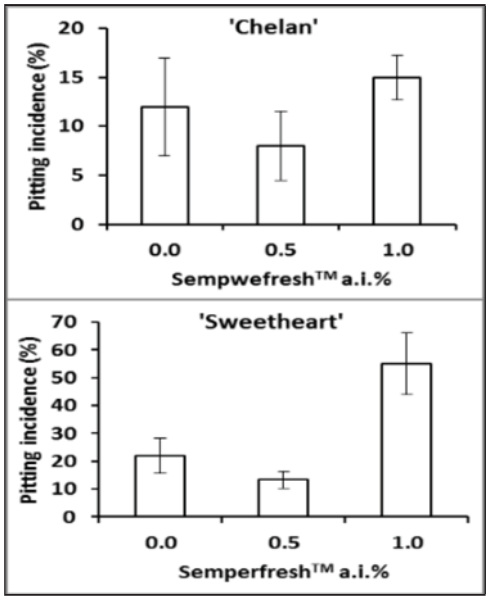

Fig. 15. Effect of Semperfresh™ on pitting incidences of Chelan and Sweetheart after 2 weeks of cold storage.

EXCUTIVE SUMMARY

Project title: Extending storage/shipping life and assuring good arrival of sweet cherry

Due to a high respiratory activity, minimal reserve carbohydrate, and high susceptibility to mechanical damage and water internalization injury, sweet cherries are highly perishable and have a shelf life of only about 2 weeks under cold chain management. Their shelf life is often shortened due to loss of flavor, darkening of fruit skin color, pitting, splitting, pedicel browning, and decay development. Choosing the MAP liners with right gas permeability and postharvest Ca treatment are found to improve shipping quality of sweet cherries.

Modified atmosphere packaging (MAP).

Understanding respiration dynamics influenced by $O_2$and CO_2, temperature, and cultivars is an essential knowledge for reducing respiration rate and extending storage/shipping life of cherries. We found that while respiration rate of PNW and California cultivars was inhibited linearly by reduced O_2 concentration from 21% to 3-4% at 20 °C, it was affected very little from 21% to ~10% but declined significantly from ~10% to ~1% at 0 °C. Estimated fermentation induction points were ~1 - ~4% O_2 for PNW and California cultivars depending on temperature. CO_2 between 0-15% did not affect respiration rate, but inhibited fruit skin darkening by retarding anthocyanin accumulation.

The commercially available MAP box liners for sweet cherries were found to have extremely varied gas permeability (i.e., 1-15% O_2 + 5-15% CO_2). While all the MAP liners maintained higher fruit firmness, greener stem, and reduced decay, only the MAP liner with 5-8% O_2 maintained higher TA and better flavor by reducing respiration rate. The MAP liners with 10-15% CO_2 maintained shiner skin color. The MAP liners with 1-2% O_2 increased fruit ethanol accumulation and therefore anaerobic flavor after storage/shipping. Most of the PNW and California cherry cultivars packed in the MAP liners with 5-8% O_2 did not accumulate ethanol at temperature fluctuation between 32-41 °F. Skeena is more susceptible to anaerobic injury and should be packed in MAP liners with 8-10% O_2.

Implementing Ca in hydro-cooling water (5 min)

Calcium (Ca^{2+}) plays an extremely important role in the fruit for cell wall structure and strength, plasma membrane structure and integrity, and cellular signaling responses. However, fruit are often deficient in Ca due to its low mobility in plants. Enhancing Ca content can be extremely beneficial in reducing disorders and maintaining quality of fruit during storage. We found that cherry fruit tissue Ca content increased significantly as Ca rate increased from 0.2% to 2.0% at 0 °C for 5 min. The increase of fruit tissue Ca content was accompanied by reductions in respiration rate, ascorbic acid degradation, and membrane lipid peroxidation, which enhanced total phenolics content and total antioxidant capacity, and resulted in increases in fruit firmness and pitting resistance and decreases in titratable acidity loss and decay. The enhanced tissue Ca content also reduced cherry fruit splitting potential by decreasing fruit soluble pectin release and increasing the splitting threshold. In contrast, depleting Ca from fruit tissue by EDTA or low pH increased soluble pectin release and splitting potential. Pedicel browning was inhibited by Ca at 0.2-0.5%, but increased by higher rates at 1.0-2.0%, possibly via modifying membrane lipid peroxidation.

Edible coatings and GRAS compounds

Some benefits on cherry fruit quality from applications of edible coatings and GRAS compounds are reported in literatures. We did not find significant improvement at commercial level on shipping quality of PNW cultivars by postharvest applications of SA, OA, JA, MeJA, ethanol, HBR, GA_3, sodium alginate, chitosan in our conditions. Semperfresh™ helps reducing stem browning, but the rate at 1.0% a.i. may increase pitting expression for certain cultivars.

CONTINUING PROJECT REPORT **YEAR**: 1 of 3
WTFRC Project Number: CH14-10

Project Title: Developing a management strategy for little cherry disease

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Cooperators: Tim Smith–WSU Regional Extension Specialist, Grower cooperators

Total Project Request: Year 1: $63,479 **Year 2**: **$65,020** **Year 3**: $62,743

Other funding sources

Agency Name: Stemilt Growers LLC
Amt. requested: $10,000
Notes: This funding is to support the development of field diagnostic kits for Little Cherry Virus 2.

Agency Name: WSDA Specialty Crop Block Grant – 'Managing Little Cherry Disease'
Amt. Funded: **$**199,820
Notes: WTFRC funding was used as match for this grant

Budget 1
Organization Name: WSU-TFREC **Contract Administrator:** C. Johnston/J. Cartwright
Telephone: 509-335-4564/ 509-663-8181 **Email address:**carriej@wsu.edu/joni.cartwright@wsu.edu

Item	2014	2015	2016
Salaries[1]	26,738	27,808	26,499
Benefits[2]	9,074	9,436	8,934
Wages[3]	6,240	6,490	6,750
Benefits[4]	605	630	655
Supplies[5]	15,756	15,590	14,580
Travel[6]	5,066	5,066	5,325
Plot Fees	0	0	0
Total	63,479	**65,020**	62,743

Footnotes: [1]Salaries are for post-doctoral scientists (Beers, Eastwell) and faculty salaries (Gallardo) and research associate (Gallardo). [2]Benefits range from 27.47 to 41.85%. [3]Wages are for summer help (Beers). [4]Benefits for wages are 9.7%. [5]Supplies are PCR supplies (Eastwell); diagnostic kits (Beers), and grafted cherry trees/potting supplies (Beers). [6]Travel is for Motor Pool rental and gas (Beers) for travel to plots, and travel for focus group meetings (Gallardo).

OBJECTIVES

Obj. 1: Determine mechanisms of LChV2 transmission via insect vectors (apple and grape mealybug [AMB and GMB]). Our first objective aims to better understand the vector/disease relationship. Little is known regarding mechanisms of LChV2 transmission within and between orchards via grape and apple mealybug. In 2014, we located LChV2 infected trees, and extracted mealybugs of different life stages to determine corresponding rates of infection. So far, we have detected LChV2 in field-collected crawlers, but the analysis of other stages is still underway (see results); the difficulty with the diagnostic kit delayed this work somewhat. In 2015-16, we plan to continue to develop basic information such as: which species and/or stage of mealybug is more efficient at passing virus to healthy trees or acquiring virus from an infected tree; how much time and how many vectors are necessary to successfully pass virus to an uninfected tree; and can mealybug eggs that hitch a ride on non-plant material (bins, tractors) and orchard prunings, transmit virus to healthy trees. Results from these studies will help us to better understand how LChV2 is spreading between trees and orchards, improve management efforts, and ultimately slow the spread of LChV2.

Obj. 2. Determine control methods for AMB and GMB in conventional and organic cherries. Our second objective is to explore the efficacy of various pesticides for the control of apple and/or grape mealybug. In 2014, we examined delayed dormant, foliar and soil-applied systemic, and contact-foliar sprays for control of apple mealybug (AMB). In 2015, we will look at repeated applications of dormant and neem oil on mealybug populations for organic control.

Obj. 3*:* Develop and deploy field diagnostic assays to detect LChV2 and differentiate it from other pathogens that induce similar symptoms (LChV1 and Western X phytoplasma). Our third objective is to validate a low cost test for LChV2 that can be performed with minimal specialized equipment. Results from 2014 revealed the presence of genetic variants of LChV2 in WA State; these genetic differences dramatically reduced sensitivity of the assay system under investigation. Using this information (see results), modifications are being made to the detection kit to improve detection of LChV2, including the newly revealed genotypes. Before the issue with the diagnostic kit/genetic variants was discovered, several user groups were trained on the use of the kit. The experience gained from using this technology in the 2014 season will be applied during the 2015 season. Modifications in protocol were delineated in an effort to increase sensitivity. The redesigned assay system and protocols will be validated during the 2015 growing season, and training will be available on request.

Another goal for this objective is to develop field diagnostic tests that will differentiate LChV2 from two other pathogens that occur in sweet cherries and cause little cherry disease (LCD), namely LChV1 and Western X phytoplasma (WX). The contributions of LChV1 and particularly WX to LCD in WA State became more evident during testing in the 2014 season. Sequence information was developed for isolates of LChV1 and WX detected in 2014. This information was used to design primers and probes for detection purposes. The primers and probes for detection of LChV1 and WX will be evaluated during the 2015 growing season.

An accurate assessment of the contribution of LChV1, LChV2 and WX to LCD is critical for the development of management plans by affected growers.

Obj. 4. Assess the economic impact of LChV2 given its effects on crop yield, crop quality, and tree death. The post-harvest period (fall) was targeted as the most convenient time for growers to participate in focus groups, and a list of names was developed. Despite numerous contacts, participants willing to share production cost information have not been identified. In 2015, we will assess the impacts of LChV2 by using a partial budgeting procedure and assessment of three yield-loss scenarios (see Methods).

SIGNIFICANT FINDINGS

- Control strategies for AMB, including a delayed dormant spray in combination with a spray targeting the crawler stage provided the best results.
- Loresban+oil sprayed at delayed dormant and Delegate sprayed to target AMB crawlers resulted in significantly lower crawler numbers when compare to control.
- A new genetic variant of LChV2 was discovered in Washington orchards. This genetic variability contributes to reduced sensitivity of the assay systems.
- WX has been found to be an important pathogen associated with LCD in Grant and Chelan counties. It was previously primarily associated with LCD in Yakima County.

METHODS

Obj. 1. *Vector transmission:* Mealybugs will be collected from virus-free trees in the field during 2015 growing season, and transferred to infected and uninfected, small, potted 'Bing' cherry trees grown in a greenhouse at WSU TFREC. Ten mealybugs will be removed from infected trees at 1, 3, 7, and 9 days, and will be subject to virus verification procedures. At least five mealybugs feeding on virus-free trees will be tested for virus as a check. These results will tell us the number of mealybug feeding days required, on an infected plant, to acquire virus. The remaining mealybugs will be transferred to 20 virus-free cherry trees and will be allowed to feed for 1, 3, 7, and 9 days, before removal (five trees per group). Trees will be verified for LChV2 after 30 days (when virus becomes detectable). These results will tell us the number of mealybug feeding days required to inoculate a healthy tree with virus. These experiments will be completed using crawlers and only other life stages verified to acquire virus, as determined in 2014 experiments. Experiments will occur as mealybugs become available in the field. In 2016 we will revise these methods to determine if mealybug density can affect the likelihood of uninfected healthy trees acquiring virus by examining the time to inoculation of a virus-free seedling when infected mealybugs are allowed to feed in high and low numbers.

Obj. 2. *Vector Control:* During the 2015 growing season we plan to examine the use of two organic control strategies for the management of apple mealybug in a block of organic apple trees at WSU Sunrise Research Orchard, which is currently infested with apple mealybug. Treatments will include dormant oil and Neemazad, which will be compared to an untreated check. Experimental setup, monitoring, and sampling will occur as described in the proposal.

Obj. 3. *Field Diagnostic Assay:* A new genotype of the LChV2 virus was discovered in WA orchards during 2014. The molecular assay has been redesigned to compensate for this genetic variation. Also, testing parameters were further refined during the 2014 season and this information will be incorporated into operating procedures during the 2015 season to validate the reliability of the assay system.

Sequence information obtained in 2014 from Washington State pathogen isolates, in conjunction with other available sequence information, was used to design reagents for detection of WX and LChV1; these components of the assay system are being synthesized. In 2015, these will be incorporated into detection assays for WX and LChV1, and evaluated in the laboratory setting using known reference isolates of the pathogens. Once basic operating protocols have been established, testing of grower samples will be implemented late in the 2015 growing season to further validate the assay system. During the course of the evaluation, results from the new molecular assay system will be compared to those from established polymerase chain reaction assay systems, in terms of both sensitivity and reliability.

Obj. 4. *Economic impact and decision-making tools:* We will develop a baseline enterprise budget for production of 'Bing' sweet cherries not infected with LChV2, and three partial budgets that take into account the three scenarios mentioned above for LChV2-infected trees (assume losses due to

lower yields per acre, assume losses due to diminished pack-outs and removal of infected trees). To develop the budgets, we will gather information on production costs by organizing focus group meetings with at least ten sweet cherry producers who have experience in dealing with LCD, to be held during the Spring-Summer of 2015. The purpose of the focus group will be to achieve a consensus among producers and to generate a representative cost of production study for "Bing" cherries. The group session will take place on a date and venue agreed upon by the participants. We will follow this group session with in-person visits to growers' sites.

RESULTS & DISCUSSION

Objective 1: Mechanisms of LChV2 transmission via insect vectors

During the 2014 growing season, we addressed LChV2 acquisition for various stages of apple and grape mealybug from infected trees in the field. Mealybug nymphs and eggs were collected from trees that tested positive for LChV2 as well as trees that tested negative, but are located in orchards with a history of LChV2 infection. Molecular analysis of plant material was used to determine/verify virus infection. Grape mealybug eggs, nymphs and adults were collected from four LChV2 positive trees in two orchards, and nine LChV2 negative trees in three orchards. Apple mealybug eggs and nymphs were collected from one tree testing positive for LChV2. Mealybugs/mealybug eggs were also collected from trees other than cherries to act as negative controls for molecular analysis. Mealybugs were extracted from plant material and placed in sterile micro-centrifuge tubes, and stored at -80º C until analysis could be performed. A small group of samples from a virus positive tree were analyzed. It included seven samples of newly hatched grape mealybug (GMB) crawlers (≈ 0.5mm), containing 10 crawlers/sample, and one sample containing two late instar crawlers (≈ 1.5mm). Molecular analysis (RT-PCR) of virus extract showed the virus to be present in all eight samples tested. However, when we tested those same extracts using the LChV2 diagnostic kits from Agdia (RT-RPA), 50% of the samples showed a negative result (Table 1). We plan to use molecular analysis for all the remaining samples, and because it is more time consuming than the diagnostic kits, we are still in the process of analysis (see objective 3).

Table 1: Detection of LChV2 in extracts from GMB nymphs, using RT-PCR and RT-RPA (diagnostic kit)

GMB sample #	RT-PCR	RT-RPA
1	+	-
2*	++	++
3	++	-
4	+	-
5	++	+
6	++	++
7	+	-
8	++	++

Reaction: ++, strong positive, +, weak positive, -, negative
* Sample # 2 contained 2 late instar crawlers. All other samples contained 10 newly hatched crawlers.

Objective 2: Control methods for apple mealybug

Apple mealybug (AMB), the documented vector of LChV2, was only recently recorded for the first time on sweet cherry in Washington; therefore, little is known about its phenology in this region. In 2014, AMB was monitored weekly at WSU's Sunrise Orchards, in a conventional apple orchard with a high density of AMB (Fig. 1). We found that second to third instar females are present and feeding on woody plant parts near buds as early as 21 March, while immature males were still in overwintering structures. Nymphs of both sexes overwinter in individual structures underneath bark. Females continued to feed under loose bark

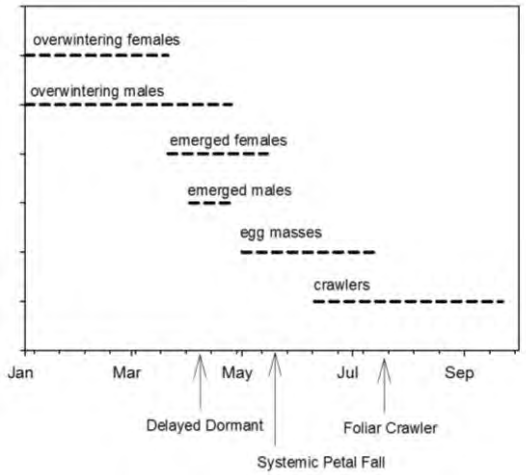

Fig. 1. Phenology of apple mealybug (AMB) at Sunrise Orchards (2014) and chemical timing of delayed dormant sprays; systemic treatments, applied 14 days after petal fall; and foliar sprays targeting crawlers on leaves.

and grow rapidly throughout the spring (21 March – 24 April), then became active, dispersing to more exposed parts of the tree (cracks in the bark, old pruning scars, and at the ends of twigs), before settling to lay eggs. Emerged males were observed as early as 2 April from bark removed from infested trees. Males emerged gradually over time and were observed mating starting on 9 April, however, some could still be found in overwintering structures a week later. Crawlers emerged gradually from egg masses staring around10 June through 16 July, and remain feeding on the underside of leaves though the fall.

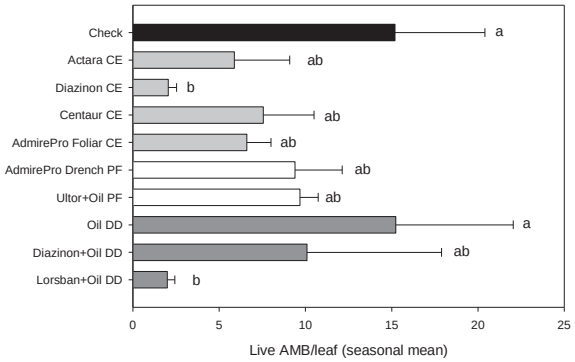

Fig. 2. Seasonal averages of AMB crawlers/leaf (17 June – 22 Sept.) from leaves collected from apple trees treated with delayed dormant (DD), petal fall (PF), and crawler emergence (CE) spray timings.

During the 2014 growing season, a field experiment was conducted in the aforementioned AMB infested orchard, to determine the effects of chemical insecticides on AMB populations. Treatments were applied using a speed sprayer to groups of three trees per replicate (4). Twenty leaves per tree were collected weekly, from 17 June – 5 Aug, and then every other week, from 5 Aug. – 22 Sept. The average number of crawlers/leaf was calculated for each treatment for each sampling date, and then those means were averaged again for the entire season. Delayed dormant treatments included Lorsban+oil, Diazinon+oil, and oil targeted overwintering females. Lorsban +oil (2.0 ± 0.4 crawlers/leaf) was the only compound that showed a significant lower crawler population compared to the check (15.2 ± 5.2 crawlers/leaf) (Fig. 2). Two systemic compounds, Ultor+oil (foliar) and Admire Pro (soil drench), were applied 14 days after petal fall, however season crawler averages were similar to the control. Finally, we applied Admire Pro (foliar), Centaur, Diazinon, and Actara to target active crawlers on leaves. Trees treated with Diazinon (2.1 ± 0.5 crawlers/leaf) had significantly lower crawler numbers than the control trees (15.2 ± 5.2 crawlers/leaf), while all other treatments targeting crawlers had similar seasonal crawler averages compared to the control.

Pinning down the phenology of the AMB in Washington is important for determining the timing, application method, and active ingredient of successful chemical control strategies. Mealybugs tend to live in protected areas of trees, such as bark cracks and crevices, and the undersides of leaves, and the eggs are protected by waxy filamentous secretions of the ovisac, making them extremely difficult to reach with insecticides. We know that newly hatched crawlers are the most susceptible stage, but delayed dormant sprays targeting females emerging from overwintering places can also be a successful management strategy. The timing of delayed dormant sprays allows control of females before they have a chance to lay eggs. A management plan that includes a delayed dormant spray in combination with a spray targeting the crawler stage is likely to provide the best results.

Objective 3: Validation of Little cherry virus 2 (LChV2) field kits

A diagnostic kit for LChV2, based on the reverse transcription recombinase-polymerase amplification (RT-RPA) technology, was made available during this year's growing season. During May to June of the 2014 growing season, samples from symptomatic trees showing little cherry disease symptoms (e.g., small fruits) were tested for the presence of LChV2 by RT-RPA assays. However, many samples from symptomatic trees did not give positive results in the RT-RPA assay format. Initially, these apparent discrepancies were investigated on 13 symptomatic samples. Reverse transcription polymerase chain reaction (RT-PCR) confirmed that each was infected with LChV2. When crude

plant sap extracts (the method being employed in the RT-RPA kit) from these samples were tested, only 7 of the 13 samples yielded positive results. When RT-RPA was repeated using purified RNA, 12 out of 13 samples yielded positive reactions for LChV2. The RNA purification process concentrates the RNA relative to crude extracts and removes many enzyme inhibitors from the sample. Although RNA purification greatly enhanced sensitivity of LChV2 detection by RT-RPA, this approach adds significantly to the cost of the assay and requires more specialized equipment that would not be amenable for use in field offices. These results suggested that the sensitivity of RT-RPA relative to RT-PCR is resulting in inaccurate test results.

Different parameters were investigated to increase sensitivity of the RT-RPA assay. Increasing the reaction incubation time from 15 minutes to 30 minutes increased signal strength and the results easier to interpret, but it did not increase the sensitivity of the assay in terms of the number of positive samples detected by RT-RPA. Increasing the incubation time to 45 minutes resulted in a marked decrease in sensitivity. Increasing the sample volume per reaction from 1 µl to 5 µl per reaction greatly inhibited RT-RPA reactions, and positive reactions were lost for all but the strongest positives.

Previous studies had suggested that detectable LChV2 might be increased by storing samples at 39°F and/or by collecting samples later in the growing season. Storing samples for 10 days did not increase sensitivity of the RT-RPA assay system. Testing symptomatic trees by RT-RPA using crude sap preparations during the latter part of the growing season (beginning early July) provided much more accurate virus detection without the need to purify RNA (Table 2). This improved detection during the latter part of the season is presumably due to decreased carbohydrate inhibitors present in crude sap and the increase in concentration of LChV2 in infected tissue.

Table 2. Detection of Little cherry virus 2 (LChV2) by RT-RPA using crude sap preparations at different times of the growing season

Sample	RT-PCR	RT-RPA	
		Jun-14	Jul-14
1-1	+	-	-
1-2	-	-	-
1-3	++	++	++
1-4	++	-	++
1-5	++	-	++
1-6	++	-	+
1-7	++	-	++
1-8	++	-	++
1-9	++	-	+
1-10	++	-	++
1-11	++	+	++
non LChV2 infected	-	-	-
		Jun-14	Aug-14
2-1	++	-	++
2-2	++	-	++
2-3	++	-	++
2-4	+	-	-
2-5	++	-	++
2-6	++	-	++
2-7	++	-	++
2-8	+	-	-
2-9	++	-	++
2-10	++	-	++
2-11	++	n/a	-
2-12	++	n/a	-
2-13	++	n/a	++
2-14	++	n/a	++
2-15	++	n/a	++
2-16	++	n/a	++
2-17	++	n/a	++
2-18	++	n/a	-
2-19	++	n/a	++
2-20	++	n/a	-
2-21	++	n/a	++
non LChV2 infected	-	-	-

Legend: ++, strong positive reaction, +, weak positive reaction,
-, negative reaction

As previously indicated in this project, two other pathogens, namely LChV1 and Western X phytoplasma (WX) are associated with LCD. In summary, a total of 145 samples from symptomatic

trees showing small fruit were tested by RT-PCR for the presence of these two other pathogens along with LChV2. Results revealed 89 samples infected with LChV2, and 65 with WX and 14 with LChV1. Samples that contained only a single infectious agent were 71 for LChV2, 51 for WX and none for LChV1 (Table 3). It was previously presumed that WX is more confined along the southern regions of WA State (e.g. Yakima and Grant counties) whereas LChV2 is predominantly located in the northern part (e.g. Chelan and Douglas counties). The results of the 2014 testing reveal a dramatic increase in WX in the northern regions and LChV2 in the southern sweet cherry growing regions of the state. Specifically, the high percentage of samples infected only with WX is one of the reasons behind the seemingly failed detection of LChV2 in symptomatic samples; many negative samples were singly infected with WX and not LChV2.

Table 3. Summary of Little cherry virus 1 (LChV1), Little cherry virus 2 (LChV2) and Western X phytoplasma (WX) RT-PCR test

Pathogens	Number of positives per county				Total
	Chelan	Yakima	Grant	Benton	
LChV1	0	0	0	0	0
LChV2	38	23	0	10	71
WX	29	6	15	1	51
LChV1/LChV2	1	7	0	0	8
LChV1/WX	4	0	0	0	4
LChV2/WX	6	1	0	2	9
LChV1/LChV2/WX	2	0	0	0	2
Total					**145**

Even after the efficacy RT-RPA detection method was improved and the presence of other pathogens were accounted for, one symptomatic sample still remained negative for LChV2 in RT-RPA and even when using purified RNA preparation. The presence of LChV2, however, was confirmed by RT-PCR.

The possibility of a different genetic variant of LChV2 was examined by testing additional symptomatic samples by both RT-PCR and RT-RPA. Four symptomatic trees tested negative by RT-RPA but positive by RT-PCR. A unique genotypic variant of LChV2 was revealed from the nucleotide sequences of the RT-PCR amplicons from these four trees. A detailed examination of the nucleotide sequences showed that the currently employed probe in the RT-RPA kit would detect this variant of LChV2 with reduced sensitivity. A new probe was designed and is being evaluated to correct this situation.

Development of an RPA test for LChV1 and WX phytoplasma:
Since there was very limited existing sequence information available describing the genome of LChV1, the development of an RT-RPA assay for LChV1 was initiated by determining full to near full genome sequences of ten different LChV1 isolates by deep sequencing and identifying conserved regions therein. Using this information along with sequences available in public databases, target regions in the LChV1 genome were identified for potential use in an RT-RPA assay. A six DNA primer pairs were evaluated. Preliminary tests using the real time platform of RT-RPA with crude extracts from three LChV1 isolates yielded promising results. Currently, the lateral flow platform of RT-RPA, which is more suitable for field use, is being evaluated, along with selection of the best primers and probes. Similarly, two isolate of WX identified in WA State were subjected to deep sequencing leading to the design of four primers and corresponding probes from two regions of the WX genome. Selection of the best primers and probes for use in the RT-RPA assay is currently being pursued.

Report – November 2014
Prepared for the California Cherry Advisory Board

Project Title: Identifying *Drosophila suzukii* attractants from preferred fruits and yeast for improved monitoring and management

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Cooperating Personnel: Frank Zalom (Co-PI)
Dept. of Entomology and Nematology
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Davis, CA 95616.
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Key discoveries (enclosed with this report are two manuscripts resulting from the funding; One submitted to Proceedings of National Academy of sciences and the other being submitted to BMC Genomics):

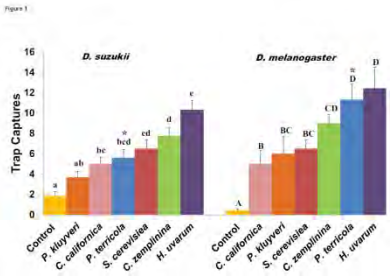
1- We successfully demonstrated the attraction in SWD to traps baited with yeasts that were derived from their guts.
2- We isolated and identified key constituents of yeast odors that were resolved as discreet entities by subjecting them Principal component Analysis (PCA).
3- Employing SWD antenna as the detector for biologically important odor constituents from the behaviorally active yeasts, we identified two chemicals, namely, isobutylacetate and isoamylacetate that induced significant attraction in SWD flies when tested in a baited trap under laboratory conditions.
4- We are in the process of testing these compounds in blends that can exclude the common vinegar fly, *D. melanogaster* flies from the baited traps.
5- An extensive analysis of chemosensory genome revealed expansions in the olfactory receptors, esp. those expressed in the trichoid sensilla that detect sex pheromone and other mating signals.

1- Traps baited with yeasts attract flies:

All the six yeast species tested for behavioral activity (*P. terricola, P. kluyveri, H. uvarum, C. californica, C. zemplinina,* and *S. cerevisiae*), elicited strong attraction from *D. suzukii* to yeast baited traps under controlled laboratory conditions. Regardless of yeast or fly species, yeast baited traps were consistently more attractive than control traps ($p < 0.02$). The robustness of our assays can be seen in the equal distribution of flies when both the traps were treated with the control bait, potato dextrose broth (PDB) ($p > 0.2$) (Table 1). A further investigation on the relative yeast preference of each fly species under multi-choice paradigm revealed that the discrimination is robust in *D. suzukii* (F=8.28; df=5; $p = 4.06 \times 10^{-6}$).

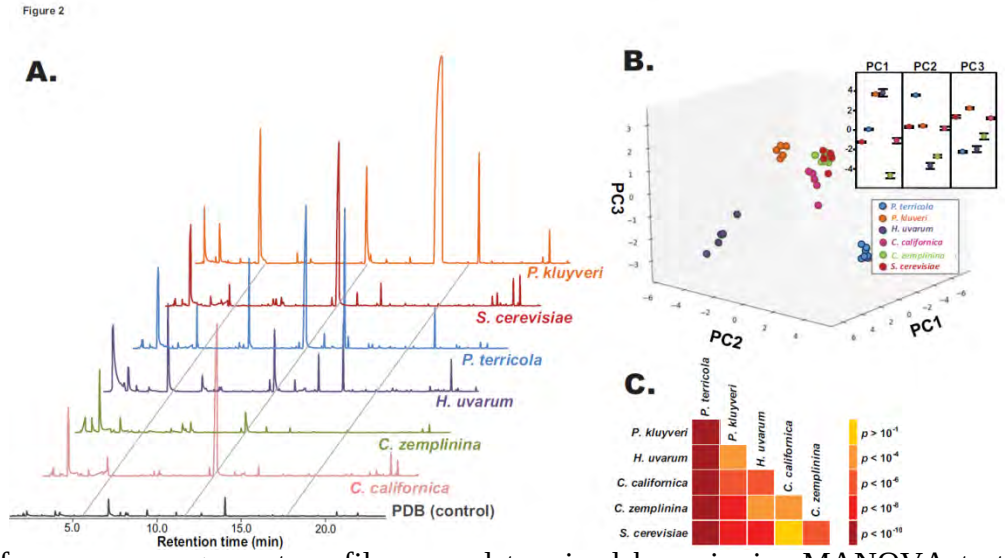
Table 1

Yeast spp.	Percent mean trap captures					
	D. suzukii			*D. melanogaster*		
	Treatment ± SEM	Control ± SEM	P-value	Treatment ± SEM	Control ± SEM	P-value
P. terricola	82.41 ± 4.06	17.59 ± 4.06	4.12E-04	88.32 ± 4.07	11.68 ± 4.07	1.75E-04
P. kluyveri	78.65 ± 2.90	21.35 ± 2.90	1.83E-04	73.63 ± 9.43	29.83 ± 9.43	1.90E-02
C. zemplinina	87.24 ± 1.33	12.76 ± 1.33	1.77E-04	91.40 ± 4.40	8.60 ± 4.40	6.58E-04
C. californica	75.93 ± 3.17	24.07 ± 3.17	1.75E-04	84.49 ± 5.03	15.51 ± 5.03	2.41E-04
H. uvarum	87.53 ± 2.79	12.47 ± 2.79	1.79E-04	71.47 ± 6.84	28.53 ± 6.84	1.48E-03
S. cerevisiae	86.37 ± 3.31	13.63 ± 3.31	4.07E-04	83.85 ± 4.99	16.15 ± 4.99	2.09E-04
Control x Control	53.14 ± 4.55	46.39 ± 4.55	7.59E-01	44.83 ± 6.49	55.17 ± 6.49	2.90E-01



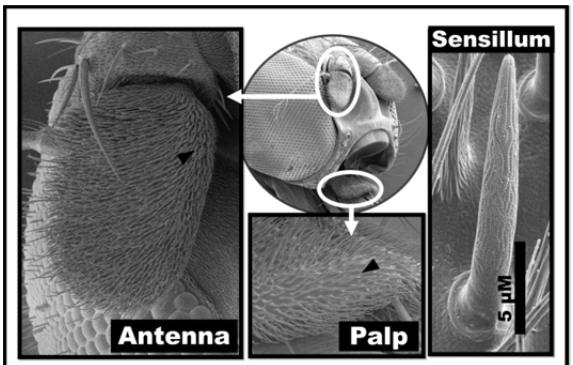
2- Yeasts are distinct in their chemical profile

Having established the relative attractancy of yeasts, we analyzed the chemical differences in order to see if some yeasts can be resolved more robustly than others. Analysis of Volatile Organic Compounds (VOCs) from all the yeast species revealed unique profiles. Finally, the statistical significance of the differences among yeast profiles was determined by pairwise MANOVA tests. Except for *C. californica* and *S. cervisiae*, all species were significantly different from each other (p = 0.0001), with *P. terricola* being the most significant from the rest.



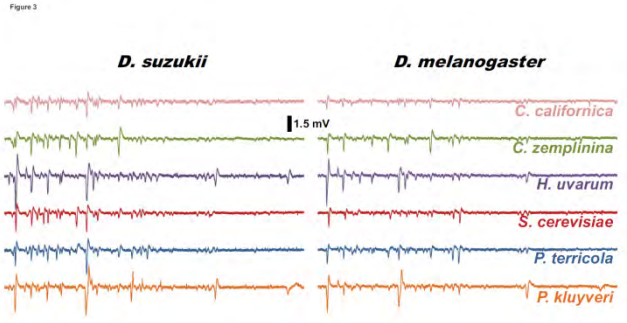
3- SWD antenna resolve yeast VOC profiles

Having established the strong preference of vinegar flies to yeast and their ability to discriminate among them, we investigated the sensory physiological basis of this attraction by using gas chromatography link electroantennographic detection (GC-EAD). Many of the yeast headspace odor constituents elicited electrophysiological responses of various intensities (Figure 3). To investigate whether *D. suzukii* and *D. melanogaster* can separate yeasts into discrete odor space based on the volatile bouquet and if this resolution is conserved between the species, we performed principal component analysis (PCA) on the induced olfactory response profiles and plotted them three dimensionally. This revealed a distinct sorting of the yeasts by the flies (Fig. 4A and B).

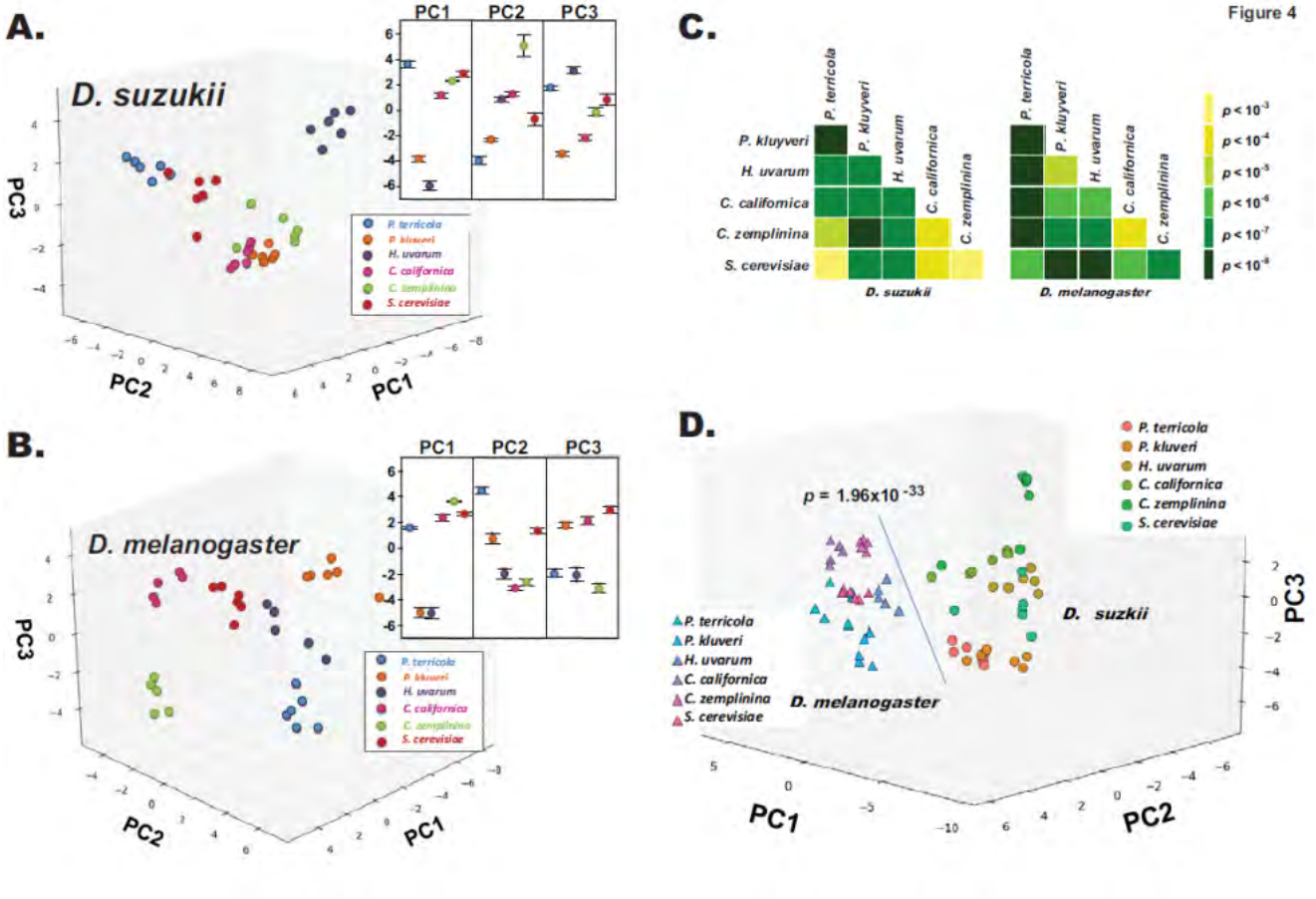


"Nose of a fly": Main olfactory structures of the fruit fly, *Drosophila suzukii*. Filled dark arrowheads on antenna and palps point to hair like structures called sensillum (enlarged on the right) that are adorned with many pores through which odors enter and bind to the olfactory proteins inside.

Finally, in order to determine the statistical significance of the odor separation represented in the PCA clustering (Fig. 4 A and B), pairwise MANOVA tests were performed between group responses. Responses elicited by all six yeast species were significantly different from each other in a pair-wise comparison performed on the two fly species (Fig. 4C). We note with great interest that *D. suzukii* could be effectively separated from *D. melanogaster* ($p = 1.96 \times 10^{-33}$)

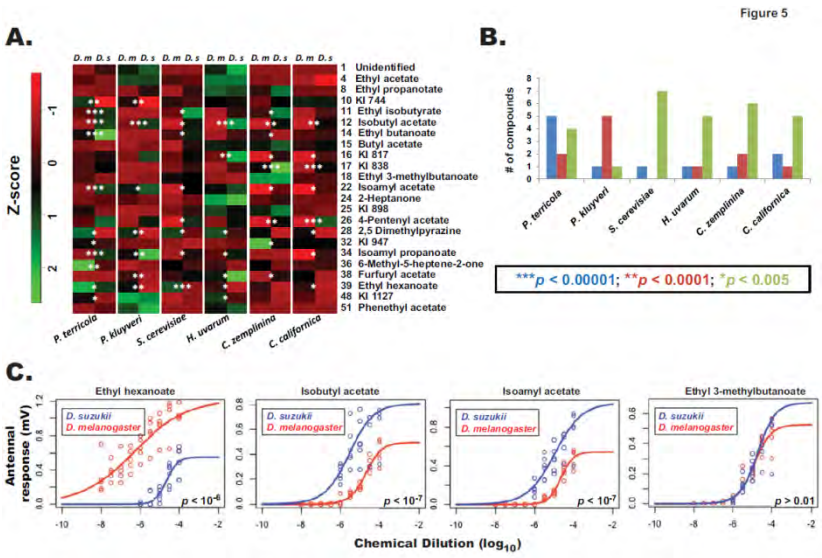


by subjecting yeast VOC induced responses from both fly species to a single PCA (Fig. 4D). The different space occupied by the two fly species in chemosensory space potentially explains the difference in overall behavioral discrimination. This strongly supports the hypothesis that yeast discrimination is at least in part due to physiological differences at the antennal level.



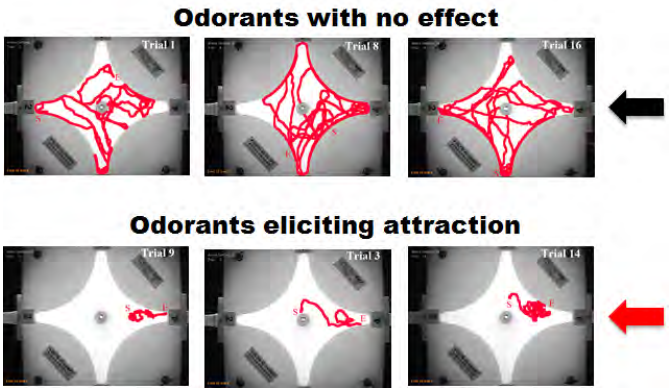
4-From complex yeast odors to a few compounds as potential baits

A paired wise comparison of responses elicited by each yeast constituent from two fly species revealed that only a handful of compounds are detected with differential sensitivity between two fly species. As a control, we used ethyl-3-methylbutanoate, which elicited comparable antennal responses from the two fly species for each of the yeasts tested (Fig. 5A). All four compounds elicited electrophysiological responses that were dose-dependent in both the species. As expected, the dose-response curves for the test compounds were significantly different between fly



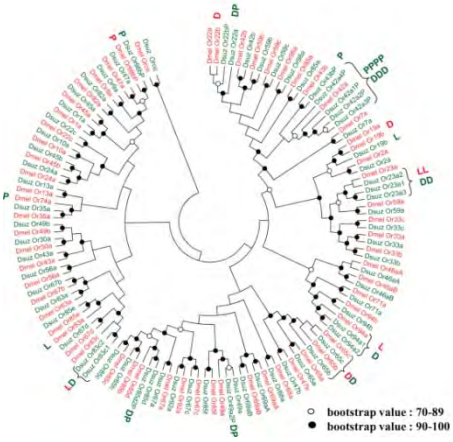
species ($p < 1.2x10^{-7}$), whereas ethyl-3-methylbutanoate (control) induced comparable depolarization (Fig. 5C). Additionally, while the straight chair ester, ethyl hexanoate elicited stronger responses (lower threshold) in *D. melanogaster*, the two other test compounds with a branched alkyl group induced responses with lower threshold in *D. suzukii*. This species-specific sensitivity confirms the critical role of a limited number of compounds potentially contributing to the behavioral differentiation.

By exposing flies individually to a 4-choice system wherein odor was added to one arm (bottom) and remaining three sides were contained clean air we found that odorants identified from yeast odors elicit attraction. The red tracks lines indicate tracks of SWD flies as they move inside the 4- arenas. Upper three panels represent random fly movements in clean air. In the lowe panelsare



5- Genome analysis reveals expansions in chemosensory genome and offers potentially new targets to develop focused attractants.

The novel habitats and food habits exploited by SWD seem to significantly alter the odorant receptors repertoire as compared to other fly species. A detailed comparison with a close neighbor, *D. melanogaster* revealed duplication of receptors that are expressed in trichoid sensilla, detectors for pheromones and sexual chemical signals.



In conclusion, we have identified odorants that are highly attractive to SWD under laboratory settings and we are now in the process of refining the blends to make them more robust and are expected to work in the field settings.

Ovicidal/larvcidal efficacy of Danitol and Malathion for the control of spotted wing drosophila

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Abstract: Malathion 57% exhibited excellent ovicidal/larvicidal activity while Danitol 2.4EC provided little ovicidal/larvicidal activity. However, in previous studies both materials provided excellent adulticidal activity.

Introduction: Spotted wing drosophila (SWD), *Drosophila suzukii*, exploded into California cherry production districts in 2009 causing significant economic loss for a number of growers. In 2010, the economic damage was kept to a minimum through repeated applications of insecticides. A consortium of scientists on the West Coast of the U.S. has evaluated the adult efficacy of most insecticides registered on cherry. However, few studies have been conducted on the ovicidal/larvicidal activity of these insecticides. Research reported here explores the ovicidal/larvicidal activity efficacy of Danitol 2.4EC (fenpropathrin) and Malathion 57% (malathion). Danitol 2.4EC and Malathion 57% were selected because both materials have a 3 day per harvest interval (PHI) and both materials are widely used.

Methods and Materials: Cherries were washed with Ivory soap, rinsed and dried. Sets of 18 cherries were placed in an ovipositional cage with laboratory reared SWD. After 15 minutes of exposure, the cherries were removed and the number of eggs determined in each fruit. Each cherry had a minimum of two eggs. The cherries were then held for 24 hours. After 24 hours, the infested cherries were dipped in experimental solutions and air-dried for 15 minutes. The fruit was held for another 72 hours. The cherries were held in a constant temperature cabinet at 23.5˚C with 16:8 (L:D) with relative humidity uncontrolled. After 72 hours, the number of larvae hatched was determined using the brown sugar floatation method (7 lbs brown sugar to 5 gal of water and several drops of defoamer). Cherries were crushed into the solution and larvae were removed and counted as they floated to the surface. Each cherry was evaluated for one hour of floatation. Data were analyzed using ANOVA and means were separated using Fisher's protected LSD, ($P \leq 0.05$).

Results and Discussion: There was no significant difference in the mean number of eggs deposited among the treatments in both the Danitol trial and in the Malathion trial (Table 1). There was no significant difference among the high and low label rate of Danitol 2.4EC and the untreated check. Thus Danitol 2.4EC does not appears to have ovicidal/larvicidal activity against SWD. Danitol is an effective pyrethroid insecticide when the adult SWD is target stage and is widely used by the cherry industry. In 2012 over 12,000 acres were treated with Danitol. This is a significant finding since Danitol 2.4EC has a 3-day PHI and is used shortly before harvest when the cherries are most susceptible to infestation by SWD. If oviposition occurs at this time then Danitol 2.4EC would not be able to stop the infestation. Because of the significance of this finding, additional verification is needed before a general recommendation can be made. Malathion 57% did result in a significant reduction in the number of larvae in both rates of application. However, there was no rate response. In fact the low rate of Malathion 57% provided greater mortality then the high rate. Thus Malathion that also has a 3-day PHI can be

applied shortly before harvest and provide excellent short-term adult mortality as well as ovicidal/larvicidal control. It would be most desirable to have multiple rates of application so that a dose morality line could be established for Malathion 57%. This would give us confidence in our recommendations.

Conclusions: Danitol 2.4EC at both high and low recommended levels did not provide ovicidal/larvicidal activity, while Malathion 57% provided excellent ovicidal/larvicidal activity.

Acknowledgements: I gratefully acknowledge Ms. Audrey Taylor whom conducted the above studies.

Table 1. Ovicidal/larvicidal efficacy of both high and low labeled rate of Danitol 2.4EC and Malathion 57%, Berkeley, CA – 2014

Treatment	Rate form 100/gal	Mean[a] no. eggs per cherry	Mean[a] no. larvae per cherry	Mean[b] percent corr. mortality
		Trial 1		
Danitol 2.4EC	10.3 fl. oz	4.9 a	3.1 a	0.0
Danitol 2.4EC	21.3 fl. oz	4.9 a	2.6 a	13.9
Untreated check	-----	6.1 a	3.4 a	-----
		Trial 2		
Malathion 57%	14.0 fl. oz	6.0 a	0.7 a	87.6
Malathion 57%	28.0 fl. oz	5.9 a	1.0 a	81.5
Untreated check	-----	6.5 a	5.8 b	-----

[a]Means followed by the same letter within a column within a trail are not significantly different (Fisher's Protected LSD, $P<0.05$).
[b]Mean percent corrected mortality calculated using Schneider-Orelli's formula.

Project Title: Developing a biopesticide to combat Spotted Wing Drosophila

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Keywords: Spotted Wing Drosophila, biopesticide, insecticide resistance, yeast

Budget:
Organization Name: University of California, Davis
Contract Administrator: Suzette Garcia
Phone: (530) 752-1879 Email: suzgarcia@ucdavis.edu

Item	2014-2015	2015-2016
Salaries	8664	9009
Benefits	113	117
Equipment	-	-
Supplies	7000	7000
Travel	-	-
Miscellaneous	5368	5904
Total	$21,145	$22,030

Footnotes:
Salary and Benefits: Graduate Student Researcher (33%)
Supplies: Laboratory supplies and reagents for DNA/RNA extraction, molecular cloning to generate yeast expression vectors, yeast transformation, yeast growth and maintenance, fly rearing and maintenance, quantitative real-time PCR assays, insecticide bioassays (for year 2)
Miscellaneous: Fees for Graduate Student Researcher (33%)

Project Overview and Justification:

Drosophila suzukii, Spotted Wing Drosophila (SWD) is a major threat to cherry and other fruit crop production. Since its detection in CA in 2008, crop losses have been reported not only in CA, but also in OR, WA, BC, and more eastern states among growers of small (e.g. caneberry) and stone (e.g. cherry) fruits [1]. In CA alone, the farmgate value of these crops is $1.932 billion. A UC Giannini Foundation of Agricultural Economics report attributes 2009 SWD-dependent revenue losses on CA fruit crops in the millions [2]. Untreated, these losses could increase dramatically as SWD populations grow and spread. Research on SWD biology is underway to develop effective management strategies to protect growers from substantial economic loss [e.g. 3-7]. Based on laboratory and field efficacy trials, chemical pesticides are more effective than organic pesticides and horticultural oils in combating SWD [8]. With the need to satisfy insect damage standards and to reduce crop loss, growers generally adopt high levels of insecticide usage for risk reduction and SWD control. Chemical pesticides can be extremely effective in controlling pest populations, but there are drawbacks to using them. Pesticides are usually non-specific, so all organisms, even beneficial organisms such as honey bees and natural enemies of SWD, are affected. Chemical pesticides can also contaminate water supplies and damage ecosystems. Moreover, continued use of pesticides will eventually lead to development of pesticide resistance in target insects, rendering the insecticide ineffective.

To ensure sustainability of crop production and provide an alternative approach for SWD control, the goal of this project is to develop an environmentally friendly, economical, and species-specific biopesticide that targets SWD. Our biopesticide uses transgenic yeast as a vector to deliver double-stranded (ds) RNA that knockdown genes critical for SWD survival, and has several key advantages over current control methods: 1) Yeast is a part of the natural diet and attractant to SWD [9], and flies will naturally seek out and consume the biopesticide; 2) yeast is easy and cheap to culture in large quantities; 3) dsRNA can be designed to be species-specific and unlike conventional insecticides, will not affect beneficial insects; 4) growers will not need to change crops to transgenic varieties as in other plant engineering technologies that use dsRNA. If successful, this technology can be extended to target other insect pests and have broad impact in fruit crop production.

Objectives:

1. Design and construct yeast expression vectors that produce dsRNA targeting essential genes necessary for survival of SWD.

2. Create yeast strains (*Saccharomyces cerevisiae* and *Hanseniaspora uvarum*) and evaluate production of dsRNA using quantitative real-time PCR.

3. Conduct SWD feeding assays to assess the efficacy of the yeast biopesticide in knocking down expression of target gene(s) and inducing SWD mortality.

4. Determine species specificity of yeast biopesticide.

Experimental Plan and Methods for Year 1:

Overview: This is designed as a two-year project. Although results from preliminary dsRNA feeding experiments in larvae and adult flies are encouraging and indicated that the dsRNAs we tested, which target SWD essential genes, may be effective in causing SWD mortality to some degree, Year 1 of the project was dedicated to testing a larger number of candidate essential genes for knock down to increase the toxicity of the biopesticide and induce maximum damage to SWD. All dsRNA biopesticide were tested using yeast feeding experiments as the results will be more relevant to our goal of eventually using the biopesticide in field setting. The objectives were performed concurrently (except objective 4), and new yeast strains were tested as they were generated. Objective 4 will be performed only if a biopesticide targeting a particular gene is found to elicit a high SWD mortality rate.

Objective 1: **Design and construct yeast expression vectors that produce dsRNA targeting SWD essential genes.**

***Rationale*:** In preliminary experiments, feeding SWD with our yeast biopesticide, i.e. dsRNAs that target *tubulin* and *vATPase,* has been shown to be effective in decreasing survival rates to some degree (around 10-20%). In year 1, we focused on increasing the toxicity of the biopesticide so that it can be a valid alternative or complement to chemical pesticides. The low mortality rate can be attributed to the fact the knockdown effect of the dsRNA we used was too low. For example, the SWD *tubulin* gene was only knocked down by 50% by the *tubulin* biopesticide. This suggests that if we can achieve higher level of gene knockdown, we will be able to induce higher SWD mortality.

***Methods*:** Using a standard yeast expression vector as a backbone (Figure 1), we used standard molecular cloning techniques to construct new yeast expression vectors that produces dsRNA that targets 8 essential SWD genes (Table 1). For 5 target genes, multiple regions of the gene sequences were targeted as previous studies have shown variable degree of knockdown when different regions of the same gene is used for dsRNA production. The FlyAtlas database [10] was used to select genes that are (1) essential for survival or are targets of chemical pesticides and (2) expressed in the fly gut as the route of biopesticide administration is by feeding. The species-specificity of the target sequences was carefully designed using bioinformatic and comparative sequence analysis that have already been completed in our SWD genome project [11]. Only gene sequences that are highly variable between SWD and other organisms was used. In addition to the use of an inducible GAL1 promoter that turns on dsRNA production in the presence of galactose, we also tried

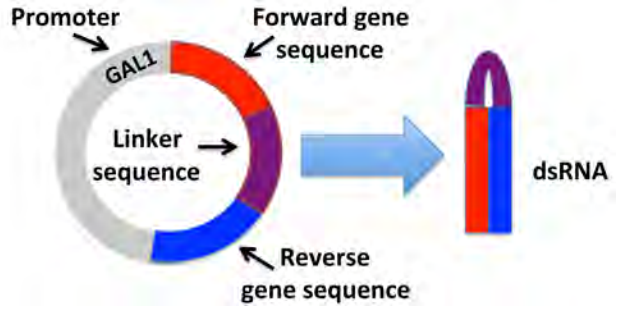

Figure 1: Design of the yeast expression vector. The design is modular and different parts of the construct, e.g. the promoter or target sequence, can be replaced by more effective substitute if necessary.

using a second yeast expression vector with a constantly active TEF1 promoter in the hopes of achieving higher levels of knockdown for all the targets.

Table 1: Target genes for dsRNA knockdown attempted in Year 1 of project

Target Gene Name	FlyBase ID	Gene Function	Fragments Used
Excitatory amino acid transporter	CG3747	synaptic transmission	2
Rutabaga	CG9533	synaptic transmission	2
Acetylcholine esterase	CG17907	removal of neurotransmitter	2
Synaptobrevin	CG12210	release of neurotransmitter	2
Bellwether	CG3612	proton transport	1
Ribosomal protein L19	CG2746	protein synthesis	1
Tubulin 23C	CG3157	cell structure and cytoskeleton	1
Vacuolar H+-ATPase	CG1088	proton transport	2

Objective 2: **Create yeast strains and evaluate production of dsRNA using quantitative real-time PCR**

Rationale: Expression vectors from Objective 1 were transformed into yeast. Transgenic yeast, which can be cultured easily and inexpensively, can be used as bio-factories for dsRNA production. Both *Saccharomyces cerevisiae* and *Hanseniaspora uvarum* were used. *H. uvarum* has been isolated as the most common yeast species in the gut of SWD and may be more attractive to SWD [12].

***Methods*:** Expression vectors were transformed by standard heat-shock protocol. Expression of dsRNA was induced by the addition of galactose into the yeast growth media if GAL1 promoter is used. Other nutrient supplements can be used to induce expression if an alternative promoter is used in the future. Expression level of dsRNA was assayed using quantitative real-time PCR (Biorad CFX96 located in Chiu Lab).

Objective 3: **Conduct SWD feeding assays to assess the efficacy of the yeast biopesticide in knocking down expression of target gene(s) and inducing SWD mortality.**

Rationale: The ultimate test for the utility of the biopesticide is to assay SWD survival post-yeast feeding. We plan to test the effectiveness of individual biopesticide expressing different dsRNA in (i) knocking down target genes; and (ii) inducing SWD mortality. We anticipate that high level of SWD mortality is likely if high level of gene knockdown by dsRNA can be achieved.

***Methods*:** Standard *Drosophila* diet was supplemented with either (A) control untransformed yeast or (B) transformed yeast expressing dsRNA. Fly survival as well as the survival of first generation progenies was examined. About 20 male and 20 female flies were used per trial. In addition, RNA was extracted from 12 flies (6 males and 6 females) per treatment and used to assess extent of target gene knock down using quantitative real-time PCR. At least 2 trials were performed for each biopesticide. Standard t-tests or ANOVA was used to assess significance.

Results:

Design and construction of yeast expression vectors for production of biopesticide

We designed yeast expression vectors that target eight essential genes for SWD survival (Table 1). For five of these genes, we inserted multiple regions of the gene into the expression vector, so a total of thirteen unique dsRNAs were tested in Year 1. As described in the methods, each fragment was cloned into two vectors independently: an expression vector that allows for constitutive (constant) expression of dsRNA, and a second vector with galactose-inducible expression. Both vectors were optimized for use with *S. cerevisiae.* There are no commercially available vectors that are designed specifically for *H. uvarum*, however, it is within the *Saccharomycetaceae* family, so we tested these vectors for compatibility with *H. uvarum*.

Target genes were selected by evaluation of two criteria: 1) Molecular function indicating that it is essential for SWD survival suggesting that RNA knockdown may lead to SWD mortality; and 2) midgut expression level and enrichment (see below). Many chemical pesticides such as pyrethroids and organophosphates act on the nervous system and induce mortality by disrupting neurotransmission. For this reason, we chose to target genes that are involved in synaptic transmission (*excitatory amino acid transporter 1, rutabaga, acetylcholinesterase, synaptobrevin, bellwether, and vATPase*). The molecular function of these genes includes proton transport, release of neurotransmitter, and removal of neurotransmitter from the synapse. *Tubulin* is a structural component of the cytoskeleton and was chosen because it has proven to be an effective target in other insect species. *Ribosomal protein L19* was selected because it has a critical function in protein synthesis. The midgut is the portion of the gastrointestinal tract where absorption of dsRNA is most likely to occur with yeast feeding since it lacks a keratinized lining. RNAi effects may not be systemic in *Drosophila* as opposed to in other insects, i.e. knockdown only occurs in cells where dsRNA is present, so midgut expression was considered as a key criteria for target gene selection. We used FlyAtlas [10] to ensure that the target genes are expressed at detectable levels in the midgut, thus allowing for knockdown. Additionally, we selected genes that are enriched in the midgut, which may indicate that the function of the target gene is particularly important in this tissue.

Transformation of dsRNA expression vectors into yeast:

Yeast expression vectors were transformed into *H. uvarum* and *S. cerevisiae* using standard heat shock protocols. All constitutive and inducible vectors were transformed into *S. cerevisiae,* and only constitutive vectors were transformed into *H. uvarum.* A

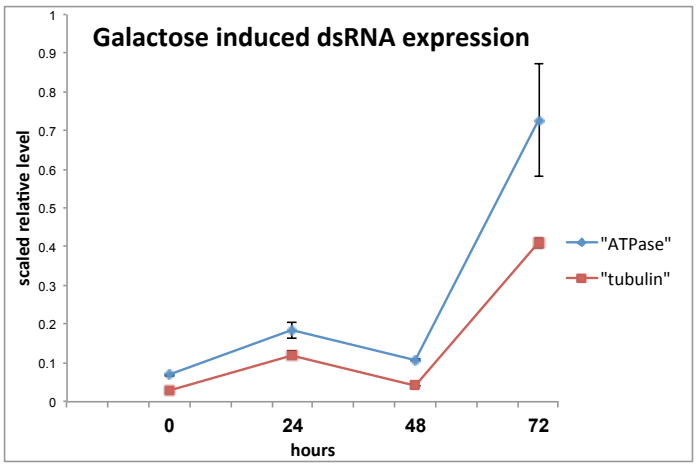

Figure 2: Quantitative PCR results showing production of dsRNA for 2 example target genes after addition of galactose in yeast culture media. A significant increase can only be observed after 72 hours after addition of galactose.

laboratory strain of *S. cerevisiae* with an auxotrophic marker was used so transformants could be selected by plating the yeast cells on nutrient deficient media (strain INVSc1, Invitrogen). A wild strain of *H. uvarum* that contains no selectable markers was used (strain 10-348, UC Davis Phaff yeast culture collection). Transformants were confirmed with colony PCR detection of the recombinant plasmids. Expression of the dsRNA was verified by quantitative real-time PCR, and was found to be highly expressed in *S. cerevisiae* under the constitutive TEF1 promoter (data not shown). Galactose induction was also tested and we found that dsRNA expression started to increase within 24 hours of the addition of galactose to the media but attained a high level after 72 hours after addition of galactose (Two examples are shown in Figure 2).

Spotted Wing Drosophila yeast feeding experiments :

We developed and optimized a yeast feeding assay to minimize accidental death of the flies due to drowning in fly food. All *H. uvarum* and *S. cerevisiae* biopesticides were fed to adult SWD in this optimized feeding assay. The control groups were fed transgenic yeast that does not produce dsRNA biopesticide. Survival of adults and progenies were examined over a two week period. As observed in our preliminary experiments, there were 10-20% mortality when SWD adults were treated with the yeast biopesticides, and the results vary depending on the target gene to be knocked down. The biopesticides that produce the most consistent SWD mortality rate (although low) was observed when targeting *bellwether, tubulin,* and *vATPase.* This was not observed in our control treatment.

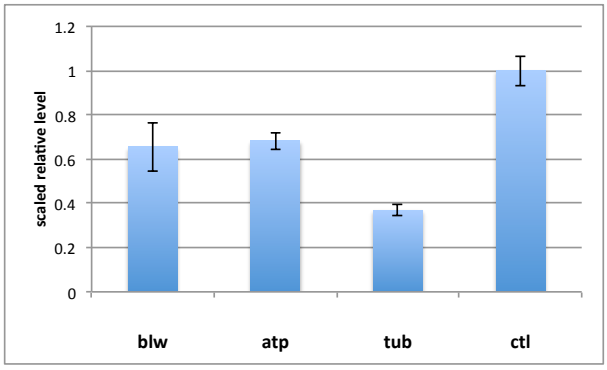

Figure 3: Quantitative PCR results showing varying levels of target gene knockdown using biopesticides as normalized to control (set at 1). The target genes are *blw (bellwether), atp (vATPase),* and *tub (tubulin).*

Target gene mRNA levels were measured in adult SWD to assess the extent of gene knock down. RNA was extracted from biopesticide-treated SWD and quantitative real-time PCR was performed to detect changes in the level of the target mRNA between the control group and the experimental group treated with biopesticide that caused a change in SWD mortality. This assay was conducted in SWD that had been fed transgenic *S. cerevisiae* containing

bellwether, tubulin, and *vATPase* dsRNAs. Each of these target transcripts were present at a lower level in the experimental group (knockdown at 30 to 60% depending on target), indicating that gene knockdown did occur (Figure 3). However, level of knockdown may not be high enough to produce high mortality rate.

Discussion and future directions for Year 2

Although so far the biopesticides tested have not led to high SWD mortality rate, it is encouraging that target mRNA levels were in fact reduced. To be effective, the biopesticide requires: 1) dsRNA production within the yeast, 2) consumption of the yeast by SWD, 3) absorption of dsRNA into the cells lining the midgut, and 4) sufficient knockdown of the appropriate transcript to induce mortality. Since the feeding of the yeast biopesticides to adult SWD did reduce the target transcript level by 30 to 60% (depending on target), this indicates requirements 1-3 are in fact being met and we have already optimized the biopesticide to meet many of the challenges this project presents. These results also indicate that dsRNA delivered by the yeast are capable of activating the insect RNAi machinery, which has not been verified until now.

A greater magnitude of gene knockdown was observed in our preliminary experiments where larvae were soaked in dsRNA produced *in vitro* than in our experiments where transgenic yeast was fed to adults. This could indicate that susceptibility of *Drosophila* to RNAi is varied at different life stages. Alternatively, stability and uptake of dsRNAs produced *in vitro* (in test-tubes) and delivered via soaking larvae in dsRNA solution could differ from that of dsRNAs produced and delivered in yeast cells. Stability and uptake of dsRNAs are likely important factors in efficacy of this biopesticide that has potential to be improved upon. For example, we plan to use a third yeast species, *Pichia pastoris*, that are also known to be associated with SWD [12].

We tested 13 dsRNAs that target 8 different essential genes. There are hundreds of other genes that have been identified as essential for survival in *Drosophila.* It is likely that other genes would be more suitable targets for this biopesticide. Screening of more target genes to find more susceptible targets could help improve the efficacy of this biopesticide. Moreover, we expect that a combinatory approach using treatment with multiple strains of transgenic yeast expressing different dsRNAs, leading to knock down of multiple essential SWD genes, may also result in higher mortality. This approach will be explored in future experiments in Year 2.

Finally, since our experiments from Year 1 indicated that we can knock down target genes using our biopesticides (although not achieving very high mortality rate), we plan to take advantage of this tool to knock down insecticide detoxification genes. We want to test if this will decrease the ability of SWD to metabolize specific classes of insecticides that are commonly used to combat SWD and render SWD more susceptible to chemical insecticides. This could pave the way to decrease insecticide use and delay development of resistance. Insecticide bioassays can be performed after treatment with biopesticides to test this hypothesis. We have recently performed a gene expression analysis to identify detoxification genes that are up-regulated upon treatment with

spinosad, zeta-cypermethrin, and malathion. These detoxification genes are expected to be important for metabolizing the specified insecticides, and will be ideal targets for these experiments.

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University of California
Division of Agricultural Sciences

PROJECT PLAN/RESEARCH GRANT PROPOSAL

Project Year 2014
Anticipated Duration of Project 1-2 years with possible extension
Project Leaders: Bruce A. Hay Ph.D., Omar S. Akbari Ph.D.
Location: California Institute of Technology, Pasadena, CA.
Cooperating Personnel: Katie Kennedy, Dept. of Biology and Biological Engineering, Caltech.

Project Title: **Engineered Transgenic *Drosophila suzukii* for wild population suppression and eradication: Production, Performance Assessment and Effective wild releases.**

Keywords: *Drosophila suzukii*, transgenic, eradication, suppression

Commodity(s) Sweet Cherry
Relevant AES/CE Project No.

Problem and its Significance:

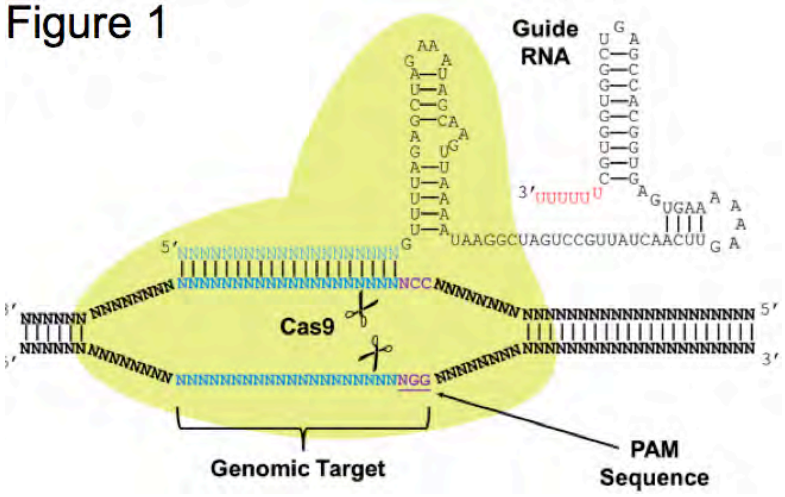
Spotted wing *Drosophila*, *D. suzukii,* is a pest for cherries, raspberries, blackberries, blueberries, and strawberries. It is a pest in many counties across California, as well as several other states. It damages these fruits by using its heavily sclerotized and serrated *ovipositor* to pierce fruits and lay eggs inside the fruit. Most of the damage caused by *D. suzukii* is a result of larvae feeding on fruit flesh. However, the insertion of the prominent ovipositor into the skin of the fruit can also cause physical damage to the fruit, as it provides access to secondary infections of pathogens - such as fungi, yeasts and bacteria - that may cause faster deterioration and further losses. These damages can result in severe crop losses, and the implications for exporting producers may also be severe, depending on the future quarantine regulations. In 2008, *D. suzukii* alone resulted in the loss of over 38.3 million dollars in cherry crop loss in California and this number is only increasing (Goodhue et al., 2011).

The current method of control of *D. Suzukii* include the use of expensive non-insect specific insecticides, for example malathion, which also kill beneficial insects like the pollinators: honeybees and useful predators: green lacewings which prey on various harmful insects, including black cherry aphids and small caterpillars. As an alternative to insecticides farmers can also attempt to trap *D. suzukii* using chemical attractants, however no *D. suzukii* specific attractants are currently available and this approach is not effective. Overall, this insect is thriving in California, suggesting these current methods of control are not effective enough.

An alternative approach, that would complement current control methods, would use genetically engineered *D. Suzukii* as a biological control agent. In brief, our goal is to engineer a *D. Suzukii* strain that could be released into the wild to suppress/eradicate the wild population of *D. Suzukii*, by eliminating the production of females, the sex that damages fruit. A primary appeal of such an approach is that it is 100% insect specific -only *D. Suzukii* would be targeted; Secondly, it is catalytic - modest numbers of engineered insects would need to be released into the wild population, and the elimination of females, and overall population suppression would occur as the relevant transgenes spread into the population. An important consequence of the fact that the system relies on the

engineered insects to do the work of suppression generation after generation is that it is cheap, with only a few releases would resulting in suppression of the species on an ongoing basis, as compared to the use of insecticides which need to be applied on a regular basis.

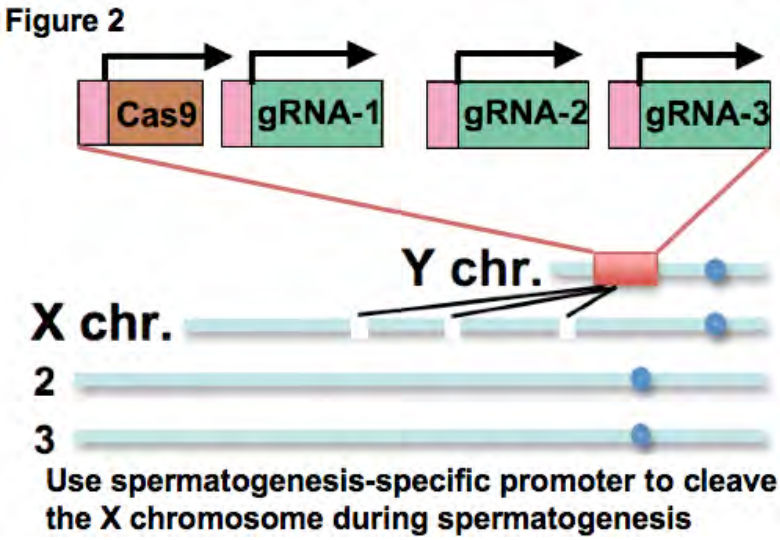
Figure 1



Our lab has extensive experience engineering synthetic selfish genetic elements designed to spread genes into populations and/or suppress them, using *Medea* (Akbari et al., 2012; Chen et al., 2007) and underdominance (Akbari et al., 2013), in *Drosophila melnogaster*, a closely related species to *D. Suzukii*. Most importantly for the purposes of this proposal, new molecular technologies (the Crispr/Cas system) have become available in the last year that provide an opportunity to bring about population suppression through the creation of all male populations. This system, known as Y-drive because it results in the spread of a transgene bearing Y chromosome, consists of two components, both located on the Drosophila Y chromosome. The first component is a DNA-cleaving enzyme (a nuclease) known as Cas9. The second component is a variable number of small guide RNAs (gRNAs) that provide sequence specificity to the target sites that Cas9 cleaves. In brief, Cas9 binds individual guide RNAs. Each guide RNA contains a region that can base-pair with a specific DNA sequence 20 nucleotides long. When the Cas9-guide RNA complex binds to a complementary piece of double-stranded DNA, Cas9 cleaves the DNA, breaking it into two fragments (Mali et al, 2013; Gratz et al., 2013) (Figure 1).

Expression of Cas9 and the guide RNAs will be driven by a spermatogenesis-specific promoter. Importantly, the sequences to be targeted are specific to the X chromosome. Therefore, the goal of the cassette located on the Y chromosome is to bring about destruction of the X chromosome through cleavage at multiple positions (Figure 2).

Figure 2

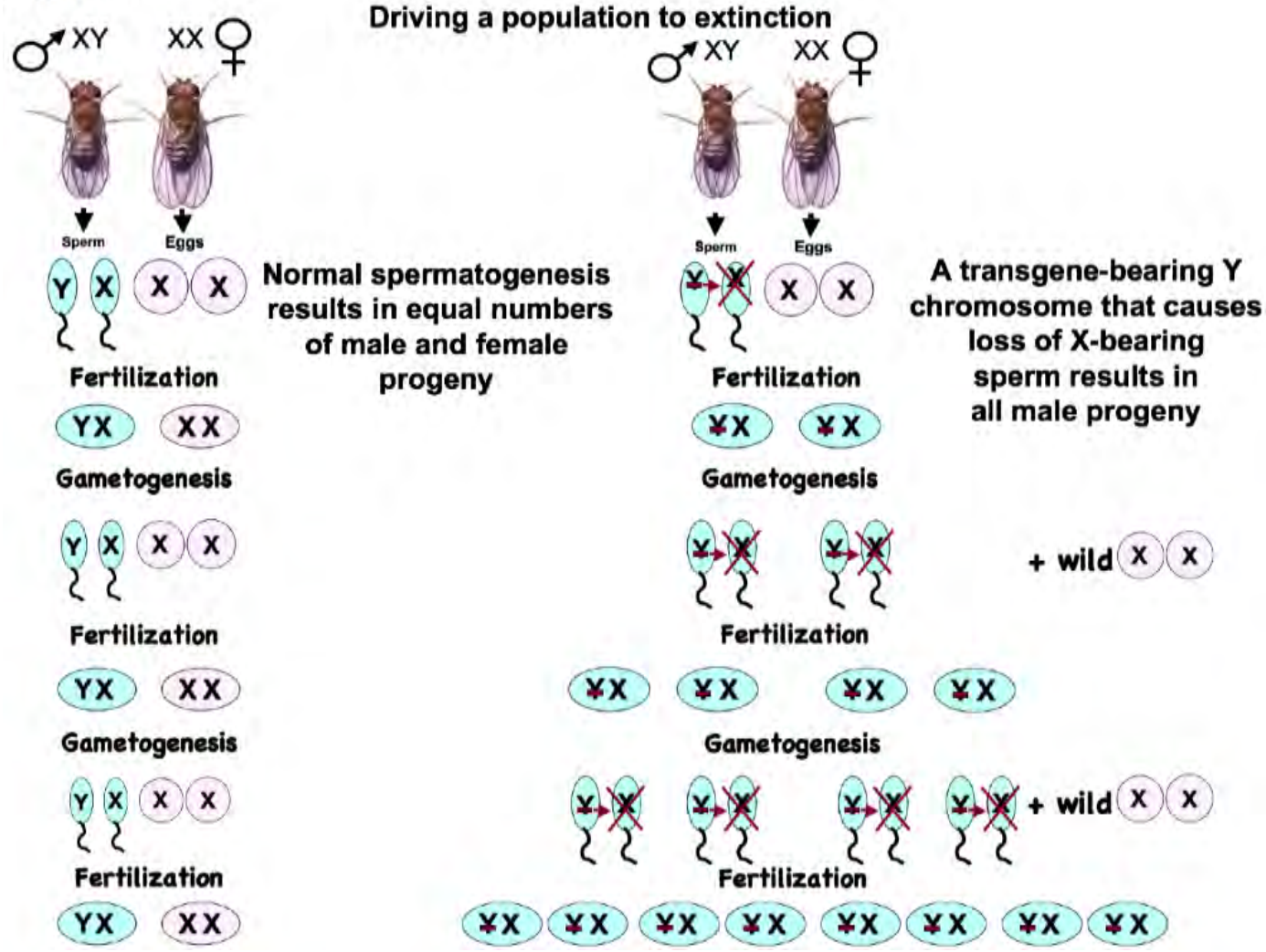


How can this cleavage bring about the creation of a male-only population? The basic idea is illustrated in Figure 3. Our hypothesis is that because males carrying the Cas9/gRNA cassette have cleaved the X chromosome into multiple pieces, they will only produce Y-bearing sperm. If this is the case, and sperm is in excess over the number of eggs laid by a female, then such a male will produce twice as many male progeny as a wildtype male (which produces equal numbers of male and female progeny). As illustrated in Figure 3, over multiple generations the Y-drive chromosome out-competes the wildtype Ychromosome, resulting in the creation of more and more males.

Importantly, both modeling and experiment support the idea that such an approach can lead to powerful population suppression, and in some cases extinction. First, modeling by Hamilton in the 1960s (Hamilton, 1967) showed that release of modest numbers of Y-drive males into a wildtype population is predicted to result in extinction of the population as the transgene-bearing Y

chromosome spreads (Figure 4). Second, work by Lyttle in the 1970s (Lyttle, 1977) showed that if a fragment of a Drosophila autosome that also results in loss of sperm that do not inherit it, is attached to the Y chromosome, this results in the loss of sperm that do not carry the Y chromosome. Importantly, using this model system he was able to drive population cages of Drosophila melanogaster to extinction.

Figure 3



Unfortunately, we still do not know exactly how this autosomal fragment of DNA works to cause loss of specific sperm. However, work in the mosquito has shown that a Y-drive chromosome exists in nature in these insects as well (Wood and Newton, 1991). We see these populations (they have not gone extinct) because not all X-bearing sperm are eliminated, with the sex ratio being between 80-95% male. Here, cytological experiments show that the X chromosome fragments into several pieces before X-bearing sperm are lost. These observations suggest that some set of genes on the Y in the mosquito is resulting in cleavage of the X, which then results (through mechanisms we still don't understand) in the loss of these sperm. Our idea is to take advantage of the fact that we can now bring about directed cleavage of the X chromosome using Cas9/guide RNAs to bring about a similar effect - the loss of X-bearing sperm - by design.

Here we propose to engineer a transgenic strain of *D. Suzukii*, carrying a Y-drive chromosome, which can be released into the wild, resulting in suppression and eventual eradication of the wild populations of this insect.

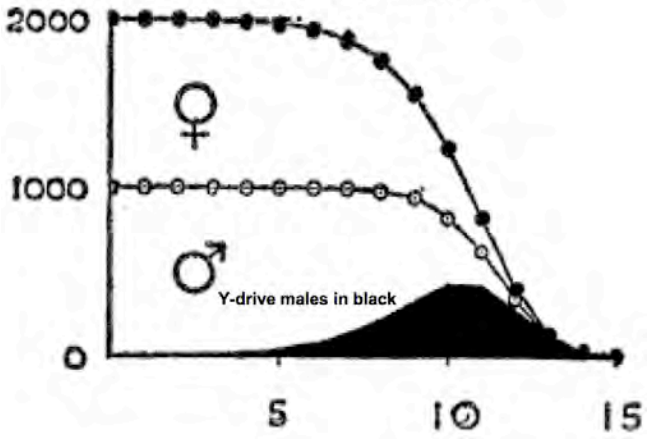
Goal:

To produce, assess the performance of transgenic *D. Suzukii* carrying the Y-gene drive system.

Objectives:
Establish a transgenic suppression line of *D. Suzukii, by* introducing a cassette expressing Cas9 and guide RNAs onto the Y chromosome that results in loss of X-bearing sperm.

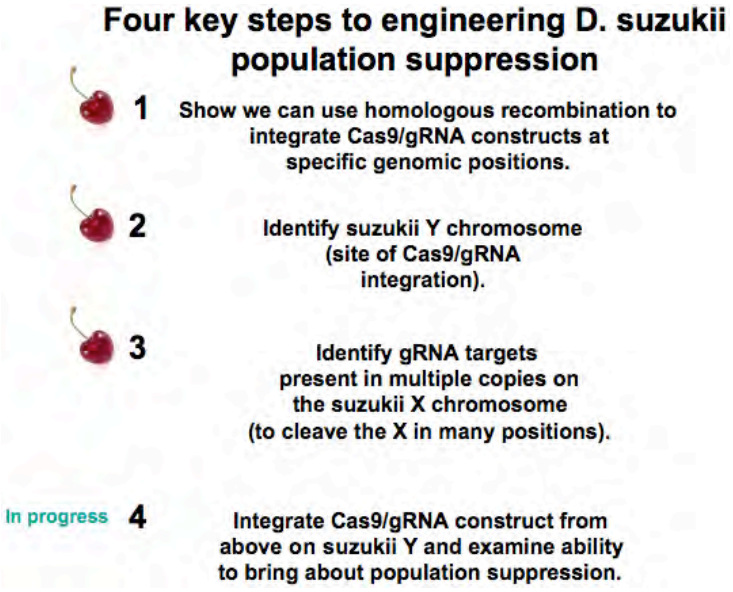
If the above transgenics show male-biased sex ratios we will conduct standard laboratory and field-cage assays to test the ability of Y-drive males released into a wild *D. Suzukii* population to suppress the population by preventing the production of females.

Figure 4
Introduction of Y-drive males into the wild is predicted to result in population extinction



Year 1 Progress report:

There are four steps to engineering *D. suzukii* population suppression through shredding of the X chromosome in males. These are outlined in the following two figures, and discussed in more detail below. To summarize, we have completed three of these steps, and are now preparing to carry out the final, key *D. suzukii* transgenesis experiment.



1. We needed to show that we could engineer a large transgene cassette containing male germline-expressed Cas9 and guide RNAs, get this to insert at specific positions in the genome, and cleave the appropriate target sequence.

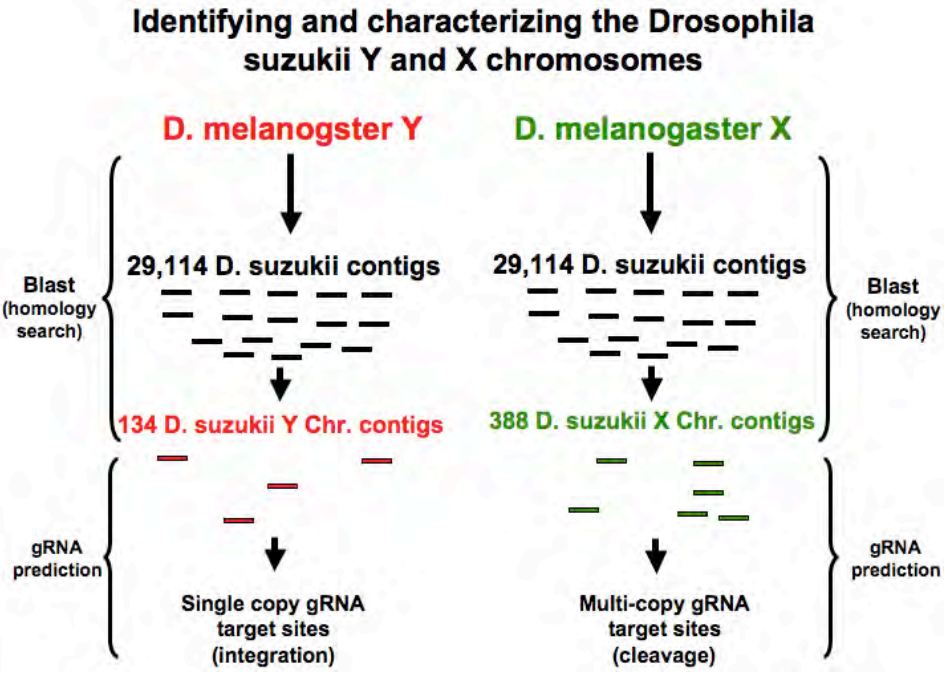
We have characterized strong Drosophila melanogaster male germline promoters, and have identified their *D. suzukii* counterparts. Two promoters of particular interest are B-tubulin, and members of the Mst gene family, both of which are male-germline specific.

We have also shown that we can take a Cas9/gRNA cassette and force it to insert at a desired position in the genome. In brief, we designed a large 10kb+ transgene that would insert into the genome of *D. melanogaster* via Cas9/gRNA mediated homologous recombination (we carried out this initial experiment in Drosophila melanogaster because for *D. suzukii* we first needed to identify the X and Y chromosome from the genome sequence, discussed below). We targeted this transgene to a gene required for female fertility gene as Yellow-G. Upon microinjection, we were able to efficiently target our transgene into the Yellow-G gene (unpublished work). Importantly, this very large cassette is able to "home" (copy itself from one chromosome to another) with appreciable frequency. This is important because it indicates that once Cas9/gRNA cassettes are integrated into the genome they continue to function, and can cleave its target sequence generation after generation.

2. With a solution to Cas9/gRNA integration in hand, we sought to solve a second problem, identification of the *D. suzukii* Y chromosome, which is where we want to insert our X-chromosome-cleaving Cas9/gRNA cassette. The current genome annotation of *D. Suzukii* version 1.0 (http://spottedwingflybase.oregonstate.edu) is 220Mb, which is 59% larger then D. melanogaster's

genome size of 130Mb, and is divided into 29,114 contigs (independent fragments that have not been brought together to make a clear linear sequence map of each chromosome). It is not even known which of these contigs comes from the *D. suzukii* Y chromosome.

To solve this problem we used a bioinformatic approach. We took the entire Drosophila melanogaster Y chromosome sequence and carried out a search for related sequences (a BLAST homology search) among the *D. suzukii* contigs; essentially, we looked for regions of *D. suzukii* that were nearly identical to those from the melanogaster Y chromosome, as these are likely to represent *D. suzukii* Y chromosome sequence.



To summarize, we identified a total of 134 contigs that had extremely high homology (E-value = 0) to the *D. melanogaster* Y-Chromosome. Given this high homology, we are very confident that these contigs are pieces of the *D. Suzukii* Y-chromosome. From this data we have identified several regions of the *D. Suzukii* that should be ideal locations for our Cas9/gRNA cassette (outside of any known transcribed regions, in unique, non-repetitive DNA).

3. The third problem that needed to be solved has two components: first, we needed to identify sequences from the *D. suzukii* X chromosome, as with the Y above; second, we wanted to identify potential gRNA sequences on the X that were present only on the X chromosome, and in multiple copies. We wanted to target sequences present in multiple copies on the X, as this would create more targets for cleavage, making destruction of the X chromosome more likely.

We identified 388 contigs from *D. suzukii* as being X-linked using the homology-based approach outlined above for the Y, starting with the known sequence of the Drosophila X chromosome. To identify potential gRNA sequences specific to the *D. suzukii* X chromosome, and present in multiple copies, we first developed a program to predict all possible Cas9 cleavage sites on the X-chromosome by searching for the PAM motif (XGG in the target sequence N(21)XGG). Once potential X-chromosome cleavage sites were identified, they were aligned to the rest of the genome (all the other non-X contigs) and those that showed a sequence match to these contigs were eliminated. The final output of this program was a conservative list of X-chromosome specific Cas9 cleavage sites. To identify target sites present only on the X, and in multiple copies, we designed the program to filter and choose target sites found on the X-chromosome in multiple locations.

From all of this, we conservatively predicted several potential target sequences repeated exclusively on the X chromosome in up to ten locations, making them ideal for the development of guide RNAs to cleave the *D. Suzukii* X-chromosome. We have narrowed down this list and identified and cloned several guide RNAs that are being used to generate the final *D. suzukii* Cas9/gRNA cassettes.

4. The final step in the development of a *D. suzukii* population suppression system is to put all of the above components: gRNA targeting the *D. suzukii* X in multiple positions, and Cas9 driven by a male germline-specific promoter, onto the *D. suzukii* Y chromosome, at a site identified through the

bioinformatic process outlined in **1.** This work is in progress and we hope to be generating transgenic males for testing in roughly the next three-four months.

If we are successful in these key lab-based experiments, our plan is still to continue with cage trials and ultimately wild releases, as outlined in our initial proposal.

Cage Trials

Laboratory and caged field trials will be conducted to determine mating competitiveness, longevity, and fitness compared to wild flies. This data will be used and fed into mathematical models to predict the introduction frequencies. Gene drive experiments will be initiated at various introduction frequencies to characterize the population suppression dynamics. Modeling work will occur in collaboration with Dr. John Marshall, a mathematical biologist with whom we have worked on a number of modeling studies

Wild Releases

The ultimate goal here is to develop a product (a genetically modified *D. Suzukii*) that can be mass-reared and deployed into the wild to catalytically suppress, and completely eliminate, the wild populations of *D. Suzukii*. Once the product is developed, how likely is it that regulatory bodies will ever permit its release? Our answer to this question is that we will follow the steps and guidelines that have been established, and utilized by others to secure permission for releases of transgene-bearing insects of a variety of species.

In brief, this involves by requesting a permit from USDA-APHIS BRS/PPQ. APHIS is responsible for issuing permits for the import, transit and release of regulated animals, animal products, veterinary biologics, plants, plant products, pests, organisms, soil, and genetically engineered organisms. The specific permit we will apply for is BRS 2000 (Application for Permit or Courtesy Permit for Movement or Release of Genetically Engineered Organisms). These permits have been successfully issued for the release of transgenic insects in the USA. For example, in 2009 the USDA approved the integration of genetically engineered pest insects (including pink bollworm moth (*P. gossypiella*), Mediterranean fruit fly (*Ceratitis capitata*), Mexican fruit fly (*Anastrepha ludens*), and oriental fruit fly (*Bactrocera dorsalis*)) into ongoing SIT programs (Reeves et al., 2012). These insects have been engineered to carry either a heritable marker gene, or a heritable marker gene and a repressible female lethality gene resulting in the production of only males. Transgenic insects have also been developed, and released into the wild, to prevent human disease. For example, a biotech company based in the UK, known as Oxitec, is commercially generating genetically modified mosquitoes and releasing them, in populated cities, in many countries including the Cayman Islands, Malaysia, and Brazil (Reeves et al., 2012). These GM mosquitoes are likely going to be released in the USA once permits are approved through the USDA (http://keysmosquito.org/modified-mosquito-release/). Therefore, the key point here is that obtaining regulatory approval for releasing transgenic insects in the USA, that are engineered to reduce wild populations and prevent crop damage, has been achieved in the past, and therefore we do not envision it to be a limitation with our approach.

Our approach is safe, species specific (horizontal transfer between species is not possible as *D. suzukii* is evolutionarily quite distant from North American species), results in the release of only males (crops will not be damaged). It is also ultimately reversible in the sense that transgenic males will disappear as wild females disappear. It is also worth noting that *D. suzukii* is a very recent invasive species in North America (2008), and therefore it simply cannot be the case that these represent an important part of the North American ecology. In short, there is no constituency that will advocate in support of its presence. Finally, it is also important to note that we can also create populations that are resistant to our Y-drive chromosome. This might be important if it was argued that our engineered *D. suzukii* might end up back in Asia, where it could play an important ecological role (though the literature does not support this). We can do this simply by altering the target sites on the X chromosome of the wild strain so that they are no longer recognized by the Cas9/guide RNAs on our

Y chromosome. In the absence of cleavage, equal numbers of male and female offspring will be created, and the Y-drive chromosome will not spread.

Once a permit is granted, a possible experimental path to utilization would involve mark-release-recapture studies, and collections at different times of year to create a picture of the structure of *D. suzukii* population and the extent of migration. Simulation models parameterized with these data and field cage competition assays would then be used to propose release strategies that could be cost effective and yield population suppression or eradication as quickly as possible in specific high value environments.

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2014 Annual Crop Report, California Cherry Board

Investigating Biological Controls to Suppress Spotted Wing Drosophila Populations

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Abstract. The spotted wing drosophila (SWD) has become a major cherry pest in California. To develop sustainable management options for this highly mobile pest, we investigated the potential of biological control of SWD thorough improved understanding of the pest's phenology and field biology, the impact of resident natural enemies and use of introduced natural enemies from the pest's native range in Asia. Our results showed that a wide range of crop and landscape plant species serve as alternative SWD hosts or refuges during non-cherry seasons. The fly can overwinter as an adult or later immature stages. Two generalist resident parasitoids readily attack SWD pupa but there is a complete lack of resident parasitoids attacking SWD larva, which emphasizes the need to introduce effective larval parasitoids from Asia. Working with material imported by Oregon State University Co-Investigators, we found that several larval parasitoids, imported from Korea, can readily attack SWD in UC Berkeley quarantine evaluation. Here, we report the major results on SWD's phenology, overwintering survival and use of alternative host fruits, field survey and evaluation of resident natural enemies, and introduction and evaluation of Asian parasitoids.

Introduction

Spotted wing drosophila (SWD), *Drosophila suzukii* Matsumura has established in all California cherry growing regions and become a major pest. The fly also attacks various other soft- and thin- skinned fruits. Adult flies are highly mobile and may move among different host plant species as they seek out susceptible (ripening) fruit. Current control programs rely on multiple insecticide sprays, trying to kill the adult SWD as they enter cherry orchards to lay eggs in the ripening fruit. Because a wide range of crop and landscape plant species may serve as SWD alternative hosts when cherries are harvested or refuges for overwintering, there will always be untreated SWD nearby to re-infest the cash crop and continue the need for future insecticide sprays. It is therefore crucial to suppress source populations on other cultivated or non-cultivated hosts in order to reduce pest pressure in susceptible crops at the landscape level. Self-perpetuating biological control agents, once established, may target the source populations in both cultivated crops and non-cultivated host fruits. Any reduction in the sizes of source populations surrounding the crop fields would greatly improve the efficiency of other control strategies, making it easier and more economical to manage the fly with a combination of other IPM methods in commercial cherry orchards.

However, information is lacking on the pest's seasonal biology as how it might overwinter and move among different crops as well as the impact of resident natural enemies. To develop sustainable management options for this invasive pest, the project was aimed to (1) understand SWD seasonal biology, including seasonal occurrence dynamics, host use, and overwintering biology; (2) determine the impact of resident natural enemies; and (3) investigate the potential of classical biological control of SWD. Here we report major results on these studies (additional results are attached as supplemental materials at the end of this report).

1. Seasonal population dynamics

Adult SWD were monitored weekly from April 2013 to May or July 2014 using apple cider vinegar traps in cherry orchards and surrounding other fruit crop or non-crop sites in Brentwood (Contra Costa County), Stockton (San Joaquin County), Courtland (Sacramento County), and Parlier (Fresno County).

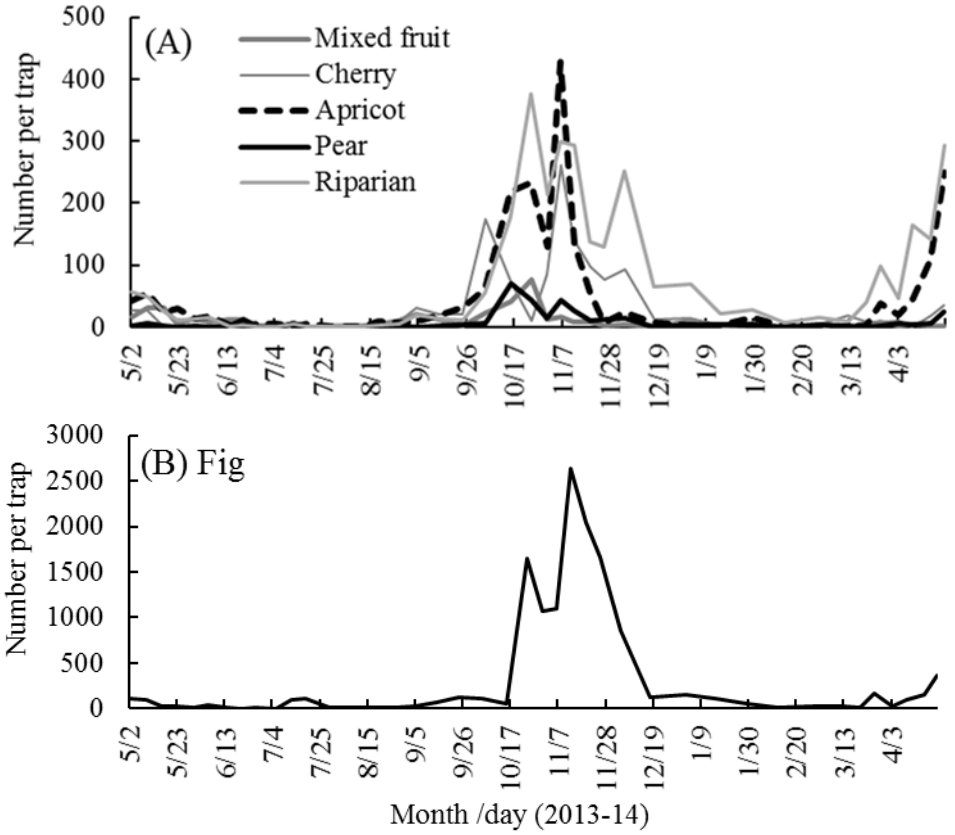

Fig. 1. Weekly mean SWD trap captures in (A) multiple crop and non-crop sites and (B) a fig orchard within an organic fruit and vegetable garden in Brentwood, CA.

SWD were captured in all monitored crop and non-crop sites throughout the seasons and the overall seasonal capture patterns were similar across sites and geographical locations, with capture peaks in the spring and fall (**Figs. 1-4**). The fall capture peak was much higher than the spring peak in all sites. Numbers of captured flies dramatically dropped during the hot summer or cold winter months. However, weekly mean number of captures varied largely among

different sites and locations; the traps captured the highest number of flies were in Brentwood, followed by Courtland, Stockton and Parlier.

During the cherry fruit seasons, the traps in cherry orchards always captured more SWD than the traps in other sites (**Figs. 1-4**). However, during non-cherry seasons, high numbers of SWD were captured in other crops or non-crop sites. For example, in Brentwood, as high as 2,632 SWD per trap per week were captured during the fall peak in a fig orchard within a multiple fruit (fig, lemon, apple, cactus, plum) and vegetable (tomato) crop organic garden (**Fig. 1**). High numbers of SWD were also captured in riparian sites especially in early spring in Brentwood. In Parlier, SWD were monitored in 14 different orchards within a 330-acre UC Kearney Agricultural Research Farm. Adult SWD populations seemed to highly aggregate in the citrus orchard during the later fall and early winter seasons (**Fig. 4**).

Correlation analyses showed that numbers of captured SWD were positively correlated between two close sites (< 2 km) or two subsequently available fruit crop orchards (**Table 1**). For example, in Courtland the numbers of captured SWD between a cherry and an adjacent pear orchard were highly correlated, suggesting possible local population movement.

Table 1. Correlation in captures of adult SWD between two close sites (< 2 km) or subsequently available fruit crop orchards in different locations

Location	Site 1	Site 2	Site distance (km)	Slope	r^2	df	*F*	*P*
Brentwood	Riparian	Cherry	0.82	0.583	0.434	1,39	30.0	< 0.001
		Mixed fruit	0.29	0.248	0.149	1,39	6.8	0.013
	Fig	Apricot	1.1	0.576	0.404	1,39	26.4	< 0.001
		Pear	1.3	0.455	0.452	1,39	32.2	< 0.001
	Apricot	Pear	0.2	0.464	0.386	1,39	24.5	< 0.001
Stockton	Cherry 2	Peach	1.29	0.199	0.109	1,39	4.8	0.036
Courtland	Cherry 1	Cherry 2	0.5	0.932	0.733	1,44	178.3	< 0.001
		Cherry 3	1.7	0.809	0.568	1,44	57.8	< 0.001
		Kiwi	Adjacent	0.997	0.802	1,44	120.5	< 0.001
	Cherry 2	Cherry 3	1.0	0.852	0.645	1,44	79.9	< 0.001
	Cherry 3	Pear	Adjacent	0.553	0.382	1,44	27.2	< 0.001
Parlier	Cherry	Apricot	0.42	0.444	0.503	1,63	63.8	< 0.001
		Fig	1.1	0.287	0.180	1,63	13.8	< 0.001
		Pomegranate	1.2	0.153	0.189	1,63	14.7	< 0.001
	Apricot	Fig	1.1	0.787	0.533	1,63	71.9	< 0.001
		Pomegranate	1.2	0.384	0.457	1,63	52.9	< 0.001
		Citrus	1.2	0.671	0.248	1,63	20.8	< 0.001
	Fig	Pomegranate	Adjacent	0.432	0.672	1,63	128.9	< 0.001
		Citrus	Adjacent	0.981	0.617	1,63	101.5	< 0.001
	Pomegranate	Citrus	Adjacent	1.314	0.309	1,63	20.8	< 0.001

Generalized Linear Model analyses showed that the weekly mean number of adult captures in most sites were affected by the accumulated degree-days over the adult fly's most active temperature range (10-30 °C), presence of susceptible (mature or overripe fruit), as well as the interactions of these two factors (see **supplemental Table 1**). This is particularly obvious, for example, in Courtland (**Fig. 3**).

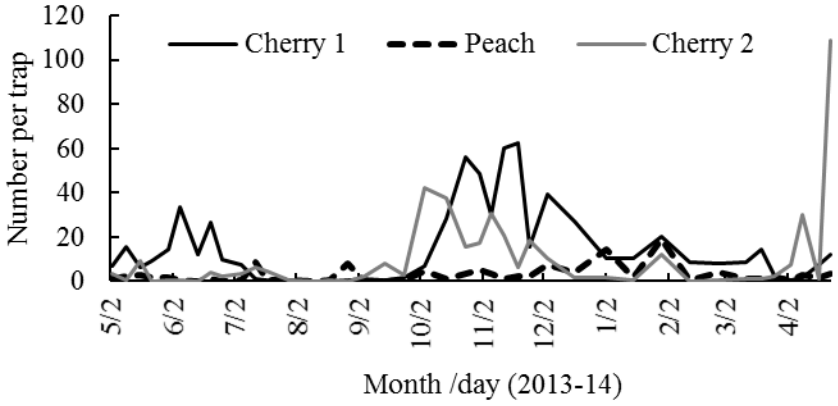

Fig. 2. Weekly mean SWD trap captures in two cherry and one peach orchards in Stockton, CA.

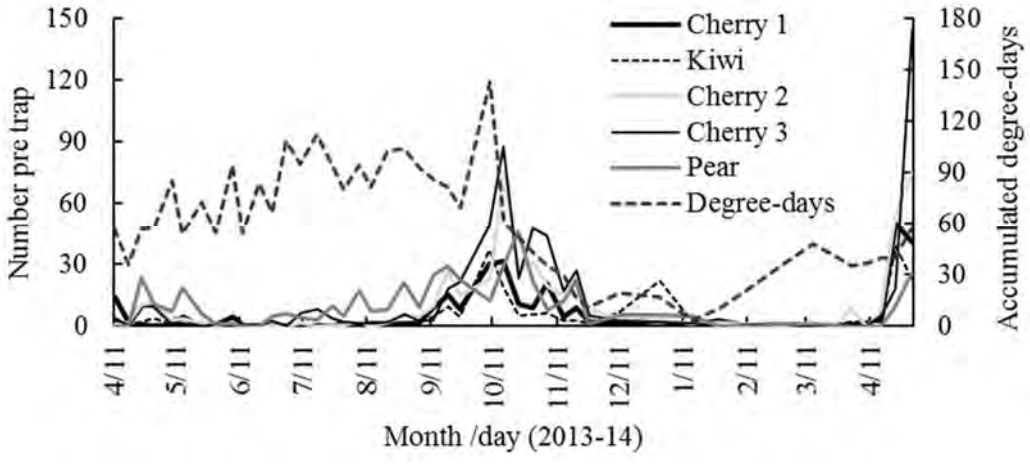

Fig. 3. Weekly mean SWD trap captures in three cherry and two adjacent kiwi or pear orchards in Courtland, CA. Accumulated degree-days were calculated based on adult fly's activity thresholds (10-30 °C).

A subsample of at least 10 randomly selected female SWD from each trap were dissected, when available, to determine the female's mature egg-load. A total of 1,992, 1,331 and 1,762 female SWD captured from Brentwood, Courtland, and Parlier were dissected. In all three locations, female SWD captured during the cherry fruiting seasons contained the highest numbers of mature eggs (**Fig. 5** for Brentwood data, see **supplemental Figs. 1-2** for results from other two locations). The number of mature egg load in captured females decreased when the season progressed and most females captured during the winter and early spring seasons did not contain mature eggs. This supports an early hypothesis that adult female SWD may enter reproductive diapause when host fruit is not available during late fall and winter. This also suggests that cherries can be very vulnerable as most SWD females contained mature eggs during the cherry fruit seasons. Of the dissected females, 50, 61 and 48 females from Brentwood, Courtland and Parlier were found to contain larvae inside their ovaries, i.e. ovoviviparity.

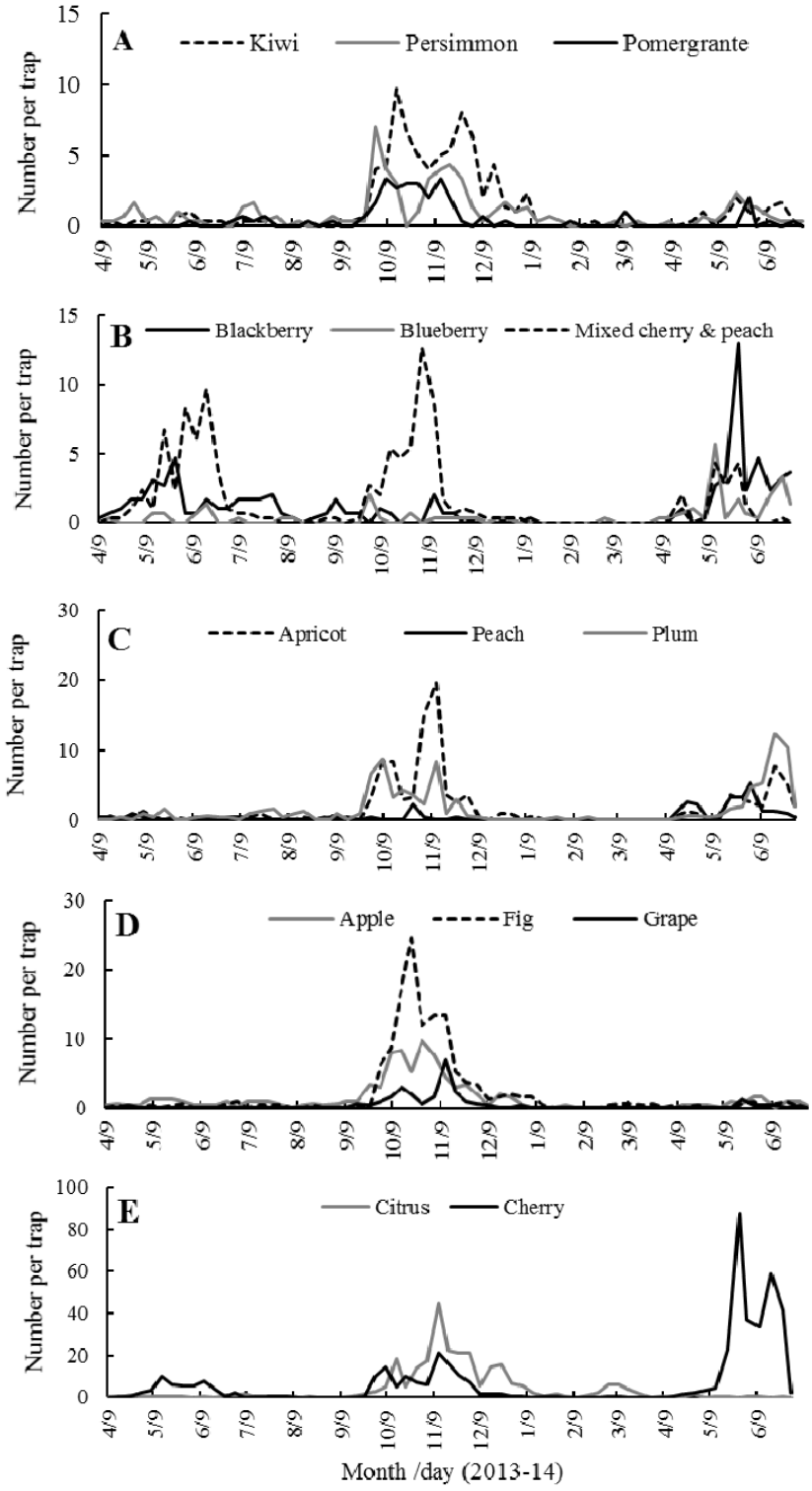

Fig. 4. Weekly mean SWD trap captures in multiples crops (A-E) at UC Kearney Research Farm in Parlier, CA.

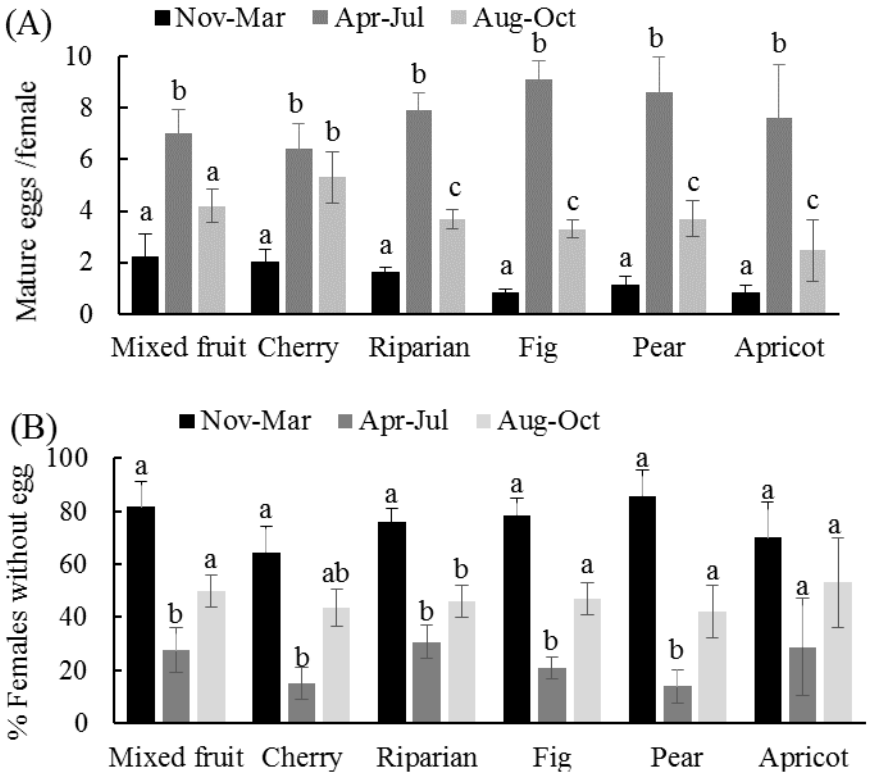

Fig. 5. Mean (± SE) number of mature eggs (A) and percentage of female SWD without mature eggs (B) from traps in different sites in Brentwood, CA. Data were pooled for the three different seasons, and different letters above the bars indicated significant difference among the three periods for each site (ANOVA and Tukey's HSD, $P < 0.05$).

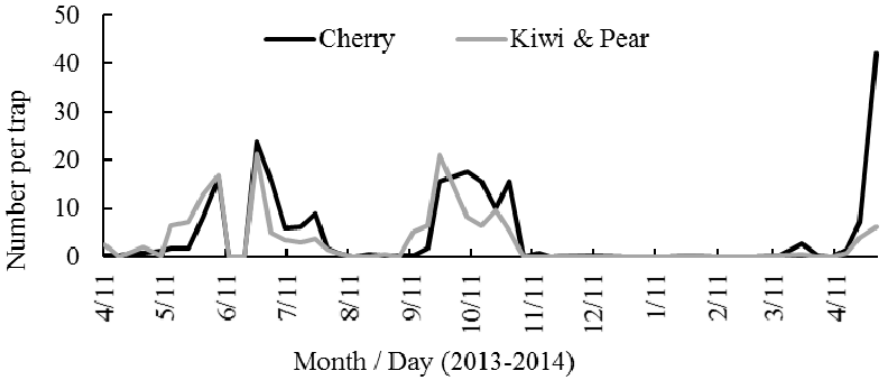

Fig. 6. Weekly mean drosophilid parasitoids captured in *D. suzukii* trap in Courtland, CA.

The cider vinegar SWD traps also captured some common drosophilid parasitoids (mainly the larval parasitoids *Leptopilina* spp.) that attack some common drosophilids such as *D. melanogaster*. These parasitoids were active except during the winter season, and the over

patterns of captures of parasitoids were similar across different sites and location (**Fig. 6**, Courtland for an example, data for other locations see **supplemental Fig. 3**). Laboratory tests found that some resident *Leptopilina* spp. could attack SWD but were unable to successfully develop due to the host's immune response that kills the parasitoids eggs or larvae through encapsulation. Occasionally, captured adult SWD were found to contain encapsulated parasitoids in their abdomen.

2. Overwintering survival

Field experiments were conducted to determine the survival rates of various immature (egg, larva and pupa) as well as adult SWD over the winter seasons in Parlier. Beginning once every two weeks from 22 November 2013 to 28 March 2014, laboratory reared fly eggs, larvae or pupae were transferred to drosophila vials with artificial diet and the vials were placed inside field cages. The cages were hung inside the canopies of citrus trees. Concurrently, vials with fly pupae were also buried 5-10 cm below the soil surface under the canopies of the same trees hanging the cages. Each treatment was consisted of 10 replicates and each replicate had 10 individual flies. All vials were monitored every 2-3 days to record the survival and development of the flies.

The adult survival test had four food provision treatments: (1) no food or water; (2) water only; (3) 10% honey water only; and (4) 10% honey water and a half orange that could serve as food or ovipositional media. The oranges were picked directly from the field and cut into halves. The adult test started on 22 November, once every month until March 28 (i.e., a total of 5 tests), each consisting of three replicates (i.e., three cages). About 25 females and 10 males that had newly emerged from the laboratory culture were released into each cage, and the cages were hung on the same citrus trees as for the immature fly tests. All adults were also checked every 2-3 days to record the survival of the flies until all flies died. The orange was replaced weekly and the number of eggs laid in the orange was counted in the laboratory.

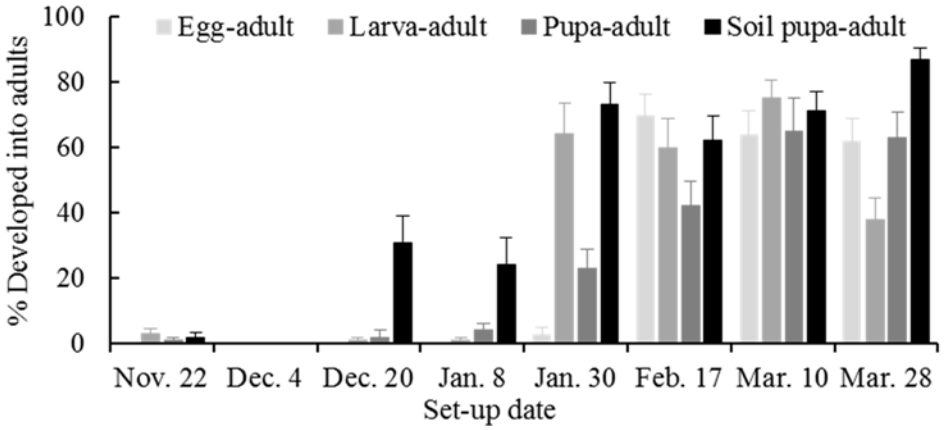

Fig. 7. Mean (± SE) percentage of various stages of immature SWD successfully developed into adults in the field-cage test.

Percentage of immature SWD successfully developed into adults in the field was affected by the set-up date of the test cage, fly developmental stage and the interaction between these two factors (date: $F_{7,288} = 163.7$, $P < 0.001$; stage: $F_{3,288} = 27.8$, $P < 0.001$; interaction: $F_{21,288} = 7.1$, $P < 0.001$). When the tests were launched in November and December, no eggs developed, but a low percentage of larvae or pupae still developed into adults (**Fig. 7**). In general, pupae from the

soil burial test had the highest percentage of successful development, while egg had the lowest percentage of survival and development. After January, the majority of larvae or pupae successfully developed into adults.

Without food or water, adult SWD died within 4 days and survived only about one week with water only (**Table 2**). When honey water or both honey water and orange were provided, flies survived a mean of 14.2 to 44.1 days, depending on the launching date of the test cages in the field (**Table 2**). The maximum longevity of the fly was about 3-4 months (see survival analysis for all test dates in **supplemental Fig. 4**).

Table 2. Maximum and mean ± SE survival days and number of eggs reproduced by adult female *D. suzukii* in field cage test

Set-up	No food		Water only		Honey water		Honey water + Citrus[1]		
date	Max	Mean	Max	Mean	Max	Mean	Max	Mean	Eggs /female
19 Nov	8	4.4 ± 0.2	10	6.7 ± 0.3	68	22.8 ± 1.9	118	39.3 ± 3.4	4.3 ± 0.5a
27 Dec	7	3.4 ± 0.9	19	4.8 ± 0.4	82	43.4 ± 3.0	87	44.1 ± 3.0	9.5 ± 2.5ab
22 Jan	-	-	23	7.2 ± 0.8	82	25.4 ± 2.9	90	32.2 ± 2.3	13.4 ± 0.3b
24 Feb	-	-	11	7.1 ± 0.2	57	23.6 ± 1.4	60	34.1 ±2.3	13.4 ± 1.6b
28 Mar	-	-	12	5.0 ± 0.3	25	16.6 ± 0.5	31	14.2 ± 0.8	9.9 ± 0.5b

[1]Different letters indicate a significant difference among treatment (ANOVA, $P < 0.05$).

When both honey water and orange were provided, the female survival rates were similar among the tests launched in different dates, except the last test that was launched in 28 March when the field temperature started rising (**Fig. 9**).

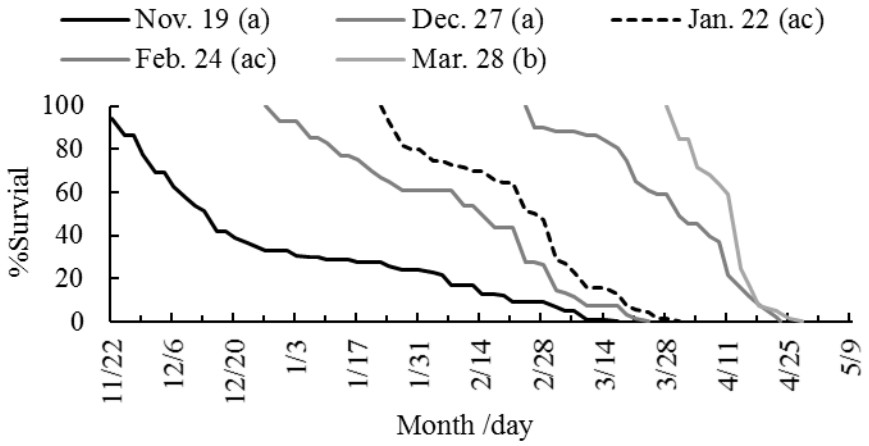

Fig. 9. Survival of adult female SWD in field cages launched from 22 November 2013 to 28 March 2014. Different letters to the right of the legend key indicate a significant difference of the survival curves among the different month releases (Survival Analysis, log-rank test, $P < 0.05$).

Surviving female SWD successfully laid eggs in the orange (**Table 2**). When female SWD emerged from the soil burial tests were released into field cages upon their emergence and provided with honey water and a half orange, these females successfully produced eggs. At least one female and one male fly were released into the cage (depending on the availability of the flies each time) and a total of 98 females and 98 males were tested, from 6 January to 4 April

2014 (a total of 11 releases). The females and males survived a mean of 21.8 ±1.7 and 25.3 ±2.1 day in the field cages and each female produced a mean of 4.7 ± 1.1 eggs over their lifetime (n =11). This suggest that SWD could survive a longer period in the field over the winter when food is available and successfully produce eggs, although these eggs are less likely to develop into adults. Oviposition likely occurs during the a few hour of warm period of the day in the winter (see **supplemental Fig. 5** for daily field temperature).

3. Use of alternative hosts

To understand how various fruits may sever as alternative developing hosts for offspring, or as food for adult fly survival, various fruits and fruit juices were tested.

3.1. Cherry and alternative hosts

First, we evaluated the effect of fruit size on host preference and suitability by SWD using the same cherry variety (cv. 'Bing'). In choice test, when two different-sized fruit (4.4 ± 0.08 g and 8.7± 0.09 g for small and large fruit, $n = 35$) were provided, adult female SWD preferred oviposition on the larger fruit ($F_{1,68} = 5.9$, $P = 0.016$) and also laid proportionally more female offspring in the larger fruit ($F_{1,48} = 9.8$, $P = 0.003$). But the percentage of eggs that developed into adults was not affected by the fruit size ($F_{1,59} = 0.1$, $P = 0.786$) (**Fig. 10**).

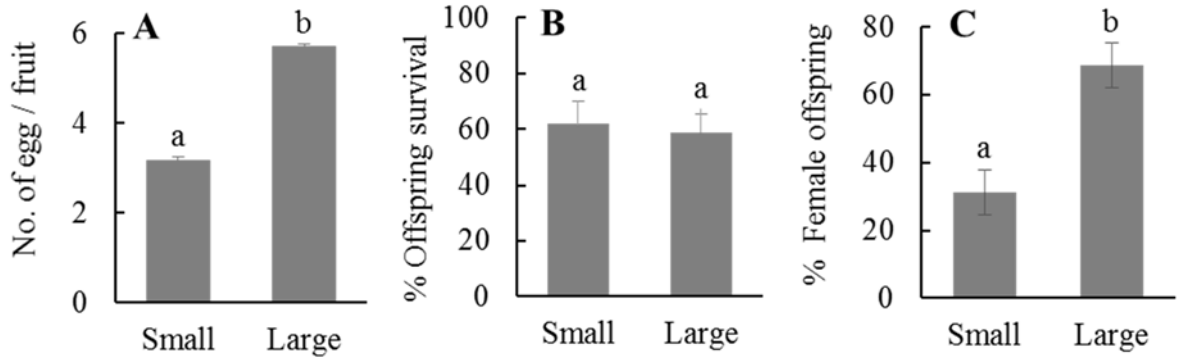

Fig. 10. Fruit size preference by adult female SWD and suitability for offspring development. Different letters over the bars indicate significant difference (ANOVA, $P < 0.05$).

Second, we evaluated the effect of host density on the fly's offspring survival in cherry (cv. 'Bing') using similar size fruit. Egg densities were manipulated through varying exposure times and ranged from 1 to 41 eggs per fruit. Percentage of eggs successfully developed into adults decreased with increased egg density per gram fruit unit (**Fig. 11**).

Finally, we evaluated the relative suitability of various cherry cultivars and other common cultivated fruits as well some non-cultivated fruits serving as developing hosts for SWD in them of offspring survival. Tested fruit included 10 different cherry cultivars, different types of grapes, pomegranate seed, mandarin, ornamental plum, wild plum and cactus (**Table 3**). Although some of these fruit such as pomegranate, mandarin and cactus are unlikely attacked by SWD when they are intact due to the thick or tough skin, split, damaged or overripe fruits could be attacked by SWD. Offspring survival rate was affected by host fruit species ($F_{17,481} = 14.1$, $P < 0.001$), egg density (i.e., number of eggs per gram fruit) ($F_{1,481} = 29.5$, $P < 0.001$) as well as their interaction ($F_{19,481} = 2.3$, $P = 0.002$). SWD developed comparably well in most alternative fruit

as they did on various cherry cultivars, except in grapes (**Table 3**). For example, one small pomegranate seed (0.4 g) could support the successful development of up to 4 individual flies. Grape seems to be not a goof host for SWD, and our preliminary studies suggest that high tartaric acid concentration can negatively affect the immature fly survival and fitness of developed adult flies.

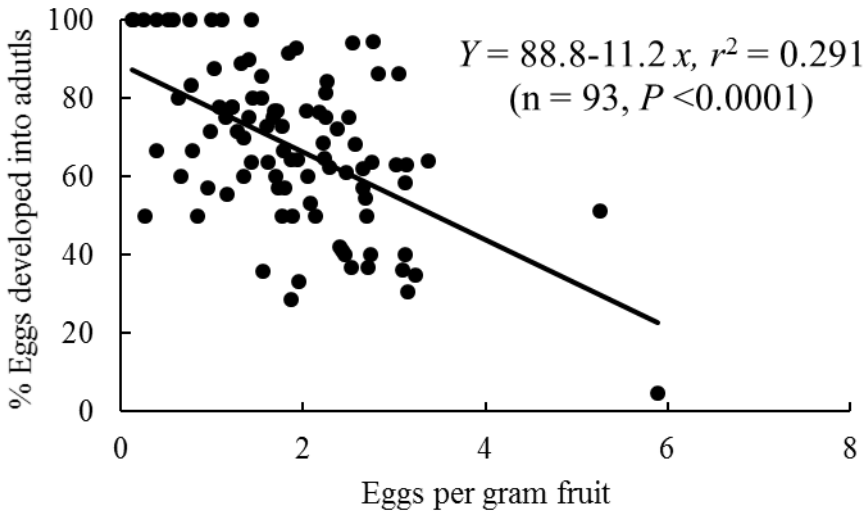

Fig. 11. Relationship between SWD egg density per gram cherry fruit and percentage of eggs successfully developed into adults.

Table 3. Suitability of various fruits as SWD developing hosts

Fruit	Weight of fruit unit (g)	Brix	*n*	Eggs per fruit unit	Eggs per gram fruit	Eggs developed to adults (%)
Cherry 1(Bing)	8.6	21.5	25	10.0 ± 1.4	1.2 ± 0.2	63.4 ± 6.3
Cherry 2	4.9	24.2	25	11.4 ± 1.4	2.4 ± 0.3	57.1 ± 5.6
Cherry 3	4.0	15.6	25	7.8 ± 1.1	2.0 ± 0.3	80.2 ± 4.6
Cherry 4	7.9	23.9	25	9.2 ± 1.2	1.2 ± 0.2	80.3 ± 4.9
Cherry 5	2.5	15.9	25	10.9 ± 1.5	4.4 ± 0.7	52.9 ± 5.7
Cherry 6	5.0	22.5	25	10.0 ± 1.4	2.0 ± 0.3	61.0 ± 6.7
Cherry 7	6.2	18.3	25	11.5 ± 1.5	1.9 ± 0.3	77.8 ± 4.8
Cherry 8	4.4	27.3	25	11.5 ± 2.3	2.7 ± 0.6	53.1 ± 6.0
Cherry 9	6.6	24.6	25	9.0 ± 1.2	1.4 ± 0.2	69.1 ± 6.4
Cherry 10	4.3	22.6	25	9.0 ± 1.2	2.2 ± 0.3	68.4 ±4.6
Wine grape	0.7	23.5	22	2.8 ± 0.5	4.8 ± 0.5	4.5 ± 3.1
Raisin grape	1.8	17.9	52	3.6 ± 0.4	2.2 ± 0.3	26.5 ± 4.5
Table grape	3.5	21.2	33	4.0 ± 1.0	1.1 ± 0.4	31.4 ± 6.1
Pomegranate seed	0.5	16.9	49	1.9 ± 0.1	4.1 ± 0.3	70.7 ± 5.9
Mandarin	2.4	11.5	53	3.9 ± 0.3	1.8 ± 0.3	53.9 ± 6.6
Wild plum	3.7	17.4	10	5.1 ± 1.2	1.5 ± 0.3	82.2 ± 6.4
Ornamental plum	9.7	10.1	25	10.7 ± 1.5	1.1 ± 0.2	53.1 ± 6.6
Cactus	7.2	11.5	22	11.5 ± 1.2	1.6 ± 0.1	64.1 ± 4.9

3.1. Adult food and feeding test

Fruit juices of cherry, apple, pomegranate, orange or grape were tested as SWD adult food for the fly survival. The test was consisted of provision of different juices to newly emerged adult flies in small cages. As control, test also included water only or 10% honey water only treatments. Each treatment had at least 3-8 replicates and each replicate had about 25 females and 10 males. Juice was provided consistently in soaked tissue paper in small containers filled with juice until all tested flies died. The longevity of female and males were recorded daily while the food was added if necessary. The food was replaced once every week and any the fly eggs and larvae were allowed to develop into adults in the old containers. Additionally, test was conducted to quantify the effect of a single meal of honey water on the female fly's longevity.

Table 4. Mean ± SE sugar concentration of tested fruit juice and offspring produced per female SWD in the food

Fruit juice or honey water	n	Brix[1]	No. of test	Total no. of females tested	Offspring produced per female[1]
Honey water	3	10.0 ± 0.0 a	3	31	6.7 ± 3.1 a
Orange	5	12.2 ± 0.6 ab	6	153	6.6 ± 1.6 a
Apple	6	14.8 ± 0.5 b	7	121	6.3 ± 0.5 a
Pomegranate	3	16.8 ± 0.6 b	3	31	20.5 ± 0.8 d
Grape	6	23.2 ± 0.9 c	7	113	5.6 ± 1.2 a
Cherry	3	25.5 ± 1.3 c	8	107	17.8 ± 4.4 cd
Cherry + water			8	144	14.8 ± 1.8 bc

[1]Different letters indicate a significant difference among treatment (ANOVA, $P < 0.05$).

On average, per female *D. suzukii* consumed 0.865 ± 0.034 µl honey water within 8.2 ± 1.1 min (n = 37) for a single meal. Female fly survived 5.3 ± 0.2 days after a single meal of honey water while flies deprived of honey water survived 4.3 ± 0.1; a single meal of honey water significantly increased the female's longevity ($F_{1,55}$ =11.4, $P = 0.001$).

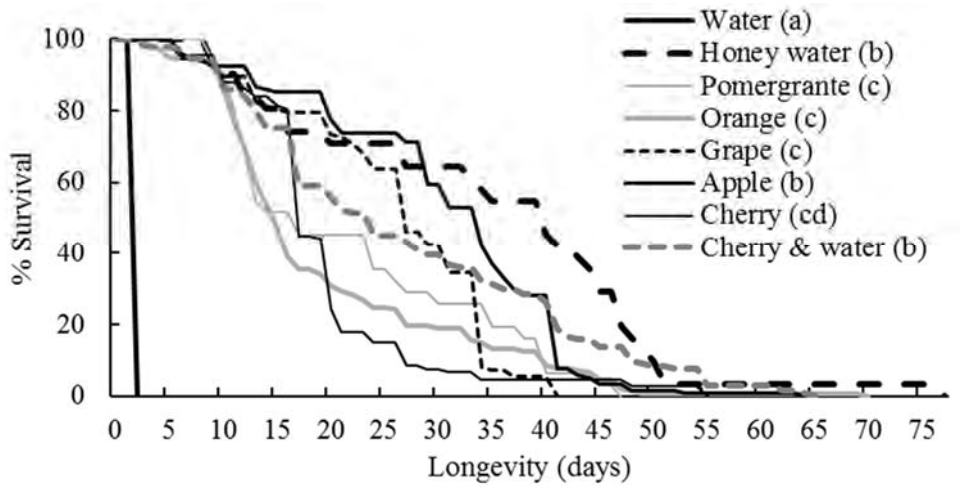

Fig. 12. Survival of adult female SWD under various food prevision conditions. Different letters to the right of the legend key indicate a significant difference of the survival curves among the different month releases (Survival Analysis, log-rank test, $P < 0.05$).

The sugar concentrations of different fruit juices were different ($F_{5,20} = 56.0$, $P < 0.001$) (**Table 4**). Female survived better when honey water or fruit juice with middle levels of brix were provided (**Fig. 12**). When cherry juice was provided together with water, the female flies survived better than cherry juice only. High sugar concentration in the fruit juices appeared not to favor adult fly's survival. However, more offspring were produced from the fruit juice with high sugar concentration, with the exception of grape juice ($F_{6,35} = 5.0$, $P < 0.001$) (**Table 4**).

When honey water or orange juice was provided as adult food along with artificial diet, female SWD survived 21.6 ± 2.4 days or 21.6 ± 1.5 (n = 20), produced 106.8 ± 14.1 or 98.5 ± 13.1 offspring, respectively; the life-time longevity (Survival analysis, log-rank test, $\chi^2 = 0.6$, df =1, $P = 0.108$) and fecundity ($F_{1,40} = 0.2$, $P = 0.669$) were not different between the two food provision treatments.

4. Survey of resident parasitoids

It may be prudent to understand the impact of resident natural enemies (mainly parasitoids) before considering introduction of exotic natural enemies for the control of SWD in USA. Therefore, we conducted surveys of resident *drosophila* parasitoids through direct samples of fruits, fruit baiting traps and sentinel pupal traps.

4.1 Field sampling of fruit

We conducted monthly field samples of various fruits from 2012 to 2014 in Parlier (most from Parlier), Brentwood, Courtland and Stockton, when the fruits were available. Cherries were randomly picked from trees while for other fruits (including peach, plum, apricot, apple, pomegranate, fig, persimmon, grape, pear, orange, mandarin, loquat and cactus), only split, damaged or overripe fruits were collected from trees or ground. SWD emerged from collected cherry, peach, fig, plum, apple, pomegranate, pear, apricot, persimmon, loquat and cactus, but not from grape, orange or mandarin. One sample of overripe fig and split pomegranate in Parlier, and one sample of damaged pear yield higher numbers of SWD. Most of these damaged fruit were infested by other drosophilids (see a full list of surveyed fruits, recovered flies and parasitoids in **supplemental Table 2**).

Cherries were often seriously infested by SWD. For example, a mean of 1.68 ± 0.20 (n = 50) adult SWD emerged per fruit from SWD-infested fruit. Several larval parasitoids *Leptopilina* spp. (likely *L. heterotoma* and *L. boulardi*) (Figitidae), and two pupal parasitoids *Pachycrepoideus vindemiae* (Pteromalidae) and *Trichopria* sp. (Diapriidae) emerged from field collected fruit. But all these larval parasitoids emerged from other drosophlids (none one from SWD). For example, rearing of 106 damaged cherries from ground recovered 1.79 ± 0.13 adult SWD flies per fruit and three *Leptopilina* spp. that all emerged other drosophila flies. These larval parasitoids are unable to develop from SWD, but can attack other drosophilids feeding on damaged fruit. Parasitism of other drosophilids by these larval parasitoids was as high as 50% in some locations. The two pupal parasitoids were recovered occasionally from SWD.

4.2 Banana-baited fruit trap

The same parasitoid species (*Leptopilina* spp., *P. vindemiae* and *Trichopria* sp.) have been collected from banana-baited traps. The banana bait appeared not very attractive to SWD, as most recovered flies were other drosophilids (**Table 5**).

Table 5. Parasitoids recovered from banana-baited fruit traps in different locations

Year	Location	Total no. of traps	SWD	Other drosophilids	No. of Parasitoids	Species
2010	Berkeley	5	0	715	55	*Leptopilina* spp.
2011	Berkeley	5	0	560	83	*Leptopilina* spp.
2012	Berkeley	5	0	865	90	*Leptopilina* spp.
2012	Parlier	25	4	158	17	*Leptopilina* spp.
2013	Parlier	65	0	1947	66	*Leptopilina* spp.
2014	Brentwood	40	0	970	6	*Leptopilina* spp.
					1	*Trichopria* sp.
2014	Parlier	80	14	4637	76	*Leptopilina* spp.
					37	*P. vindemiae*
					173	*Trichopria* sp.

4.3. Sentinel pupal traps

Because of the difficulty of soil sample soil to assess SWD parasitism, sentinel traps each baited with 20 SWD and 20 *D. melanogaster* pupae were used. The traps were set-up weekly from 19 March to 23 May 2014 in cherry, citrus and pomegranate orchards to assess early spring activity of these pupal parasitoids in Parlier. Each trail consisted of tests in two different orchards. Ten sentinel traps were placed in each orchard. After one week field exposure the traps were collected back and the pupae were kept in the laboratory until the emergence of flies or parasitoids.

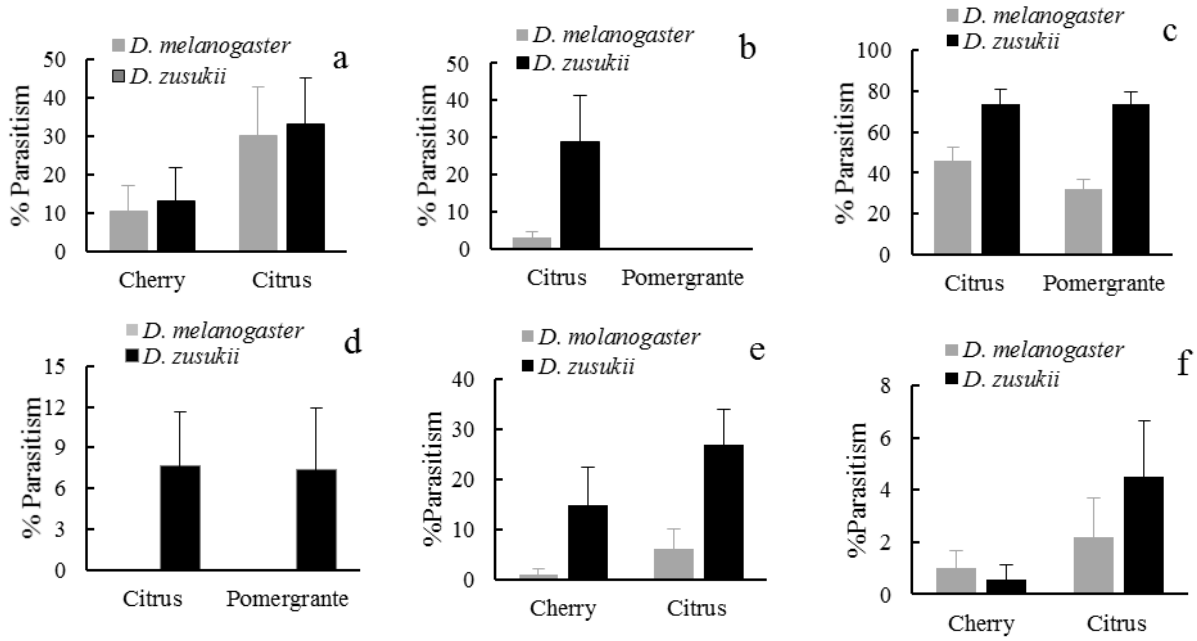

Fig. 13. Parasitism of SWD and *D. melanogaster* pupae by *P. vindemiae* in sentinel traps set-up in different orchards on (a) 19 March, (b) 3 April, (c) 11 April, (d) 17 April,(e) 9 May and (f) 23 May.

Two pupal parasitoids, *P. vindemiae* and *Trichopria* sp., were recovered from the sentinel traps. *P. vindemiae* was the dominant species while *Trichopria* sp was recovered only twice in

two traps. Overall, parasitism of SWD was higher than that of *D. melanogaster* (**Fig. 13**). The highest mean parasitism of SWD by *P. vindemiae* was 73.9% in the citrus orchard.

5. Evaluation of resident parasitoids

A series of experiments have been conducted to evaluate the potential of these two pupal parasitoids as biological control agents against SWD. We investigated their life-time fecundity, host stage preference, host species preference, relative efficiency and potential interspecific interaction. Observation on the parasitoids' searching behavior and field-cage release test of both parasitoids were also conducted, but data have not been analyzed yet.

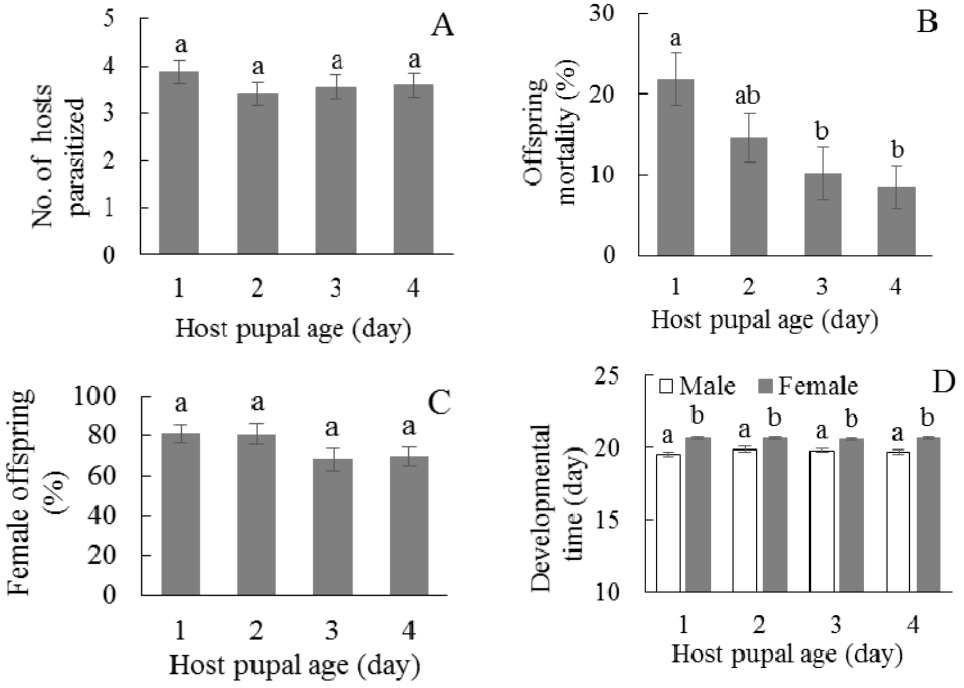

Fig. 14. Preference by and suitability to *P. vindemiae* of differently aged SWD pupae: (A) number of each stage parasitized; (B) percentage mortality of parasitoid offspring; (C) percentage of female offspring; and (D) immature developmental time. Values are mean ± SE and different letters above the bars indicate significant difference (ANOVA, $P < 0.05$).

5.1 Host stage preference and suitability

A choice test was conducted to determine if either *P. vindemiae* or *Trichopria* sp. would prefer to attack differently aged SWD pupae and the suitability of the different host pupal stages for the parasitoids' offspring survival and development. Five (for *P. vindemiae*) or 10 (for *Trichopria* sp.) of each of the four stages (i.e., 1, 2, 3 and 4 d old pupae) were provided to individual females in small dishes. The tests had 34 replicates (i.e. 34 females) for *P. vindemiae* and 26 replicates for *Trichopria* sp.. After a 24 h exposure, the differently aged host pupae were separately reared until the emergence of parasitoids or flies. After all parasitoids had emerged, all dead pupae were dissected to determine the presence or absence of recognizable fly or parasitoid cadavers (pharate adults or larvae). The number of parasitized hosts was estimated based on emerged adult parasitoids and dissected pupae containing a dead parasitoid, while offspring sex ratio was estimated based on emerged adult parasitoids.

Both parasitoids did not show a preference among the four different SWD pupal stages in terms of the number of each stage parasitized (**Figs. 14-15**). For *P. vindemiae*, offspring mortality generally decreased with host age ($F_{3, 125} = 4.9$, $P = 0.003$). There was no significant difference in the percentage of female parasitoids reared from the four different host stages ($F_{3, 124} = 1.3$, $P = 0.293$). Developmental time from egg to adult emergence was not affected by host stage but males developed faster than females in all host stages (host stage: $F_{3, 457} = 0.8$, $P = 0.465$; sex: $F_{1, 457} = 137.0$, $P < 0.001$; host stages × sex: $F_{3, 457} = 1.5$, $P = 0.227$). For *Trichopria* sp. there was no significant difference in offspring mortality ($F_{3, 100} = 1.4$, $P = 0.241$), female body size ($F_{3, 128} = 1.3$, $P = 0.539$) or the percentage of female parasitoids reared from the four different host stages ($F_{3, 100} = 0.7$, $P = 0.539$). Developmental time from egg to adult emergence increased with host age and males always developed faster than females (host stage: $F_{3, 636} = 5.1$, $P = 0.002$; sex: $F_{1, 636} = 219.0$, $P < 0.001$; host stages × sex: $F_{3,636} = 0.6$, $P = 0.636$).

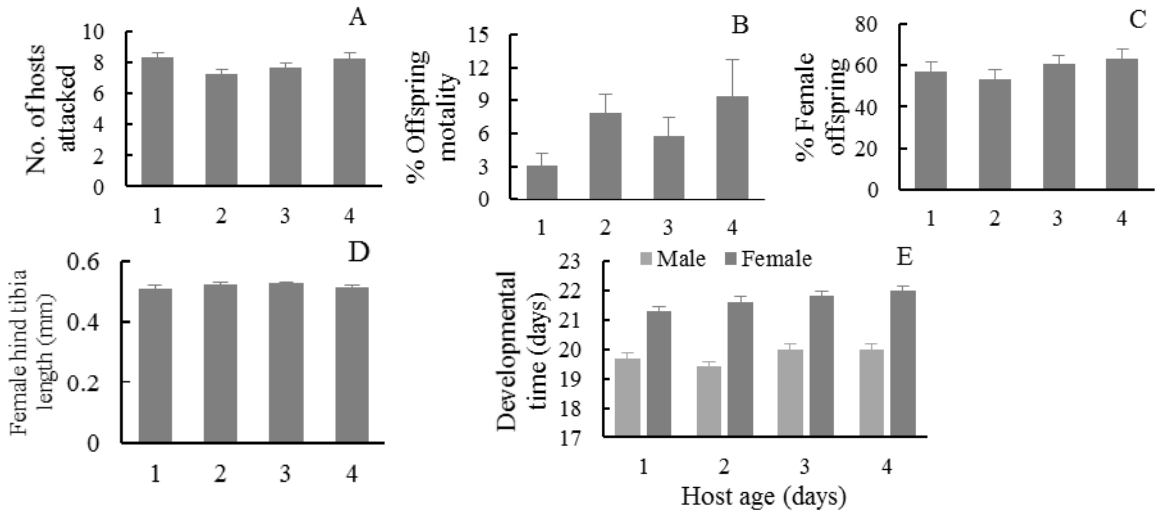

Fig. 15. Preference by and suitability to *Trichopria* sp. of differently aged *D. suzukii* pupae: (A) number of each stage parasitized; (B) percentage mortality of parasitoid offspring; (C) percentage of female offspring; (D) female body size, and (E) developmental time from egg to adult emergence.

5.2 Life-time fecundity and population parameters

The longevity and lifetime fecundity of female *P. vindemiae* and *Trochipria* sp. were determined using SWD pupae as the host at 23°C. Newly emerged (< 24 h after eclosion) female and male *P. vindemiae* were paired and provisioned with 10 pupae for every 2 d (Monday through Friday) or 15 pupae for 3 d (Friday through Monday) (i.e. 5 pupae per day) until the female parasitoid died. The number, sex, and developmental time (egg to adult) of emerged adult parasitoids were recorded. Twenty-eight female *P. vindemiae* and 50 female *Trochipria* sp. were tested and all dead females were dissected to determine mature egg load. Female longevity, numbers of hosts parasitized and offspring developed, offspring sex ratio and survival rate, and developmental time from egg to adult were calculated. From these data, life table fertility parameters were estimated, including net reproductive rate (R_o), intrinsic rate of natural increase (r_m), mean generation time (T), and doubling time (DT). Mean number of offspring produced per day was estimated based on the total number of offspring produced during each 2 or 3 d exposure.

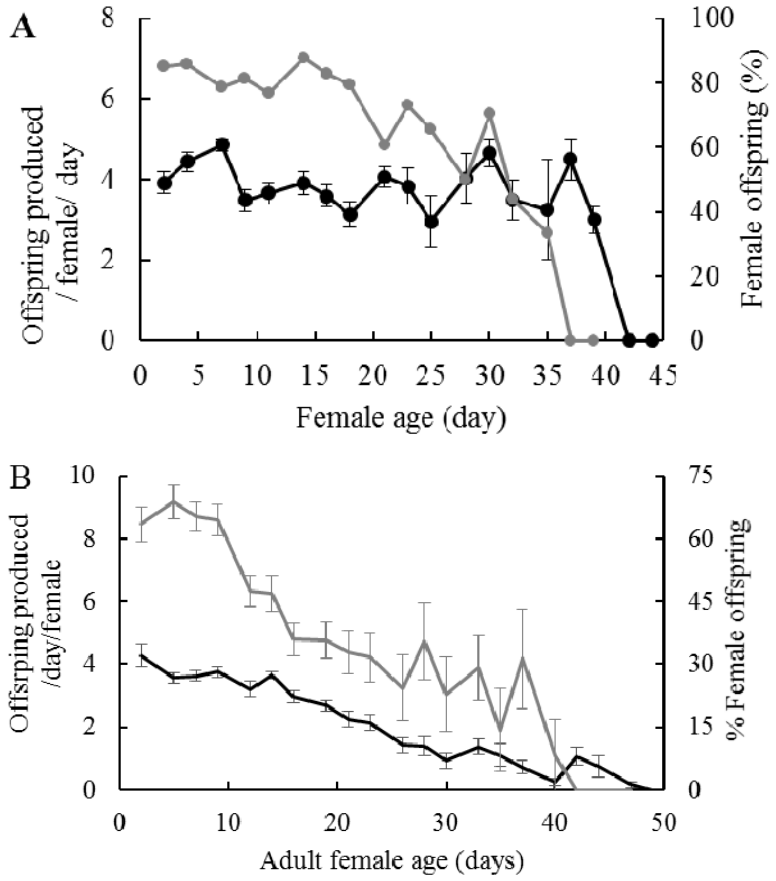

Fig. 16. Number of offspring produced per day (black line, bars refer to SE) and percentage of female offspring (gray line) over the life time of female *P. vindemiae* (A) and *Trichopria sp*. (B) on *D. suzukii* pupae at 23 °C.

Both female *P. vindemiae* and *Trochipria* sp. started oviposition within 2 days after emergence and mating. Female *P. vindemiae* produced similar number of offspring throughout most of the adult's lifespan and then sharply reduced the production before they died (**Fig.16A**). Adult female *P. vindemiae* survived 21.5 ± 1.7 d (ranged 5 to 44 days) when provided food and SWD pupae. Over the parasitoid lifespan, 79.8 ± 7.6 host pupae were parasitized and 70.0 ± 7.0 offspring successfully developed into adults, of which 80.4 ± 2.4 % were females. Offspring survival was high (87.1 ± 1.1 %). However, the percentage of female offspring decreased with adult female age (**Fig.16A**). Net reproduction rate was 51.6. The estimated intrinsic rate of natural increase was 0.1391. Mean generation time and double timing were 28.5 and 5.0 day, respectively. Dissection of tested females at their death found that each dead female contained 1.0 ± 0.3 mature eggs (ranged 0 to 7 eggs); i.e. most tested females run out of eggs at death

Number of offspring produced by *Trochipria* sp. decreased with increased age (**Fig. 16B**). Adult female *Trochipria* sp. survived 27.5 ± 1.5 days (ranged 12 to 49 days) when provided food and *D. suzukii* pupae. Over the parasitoid lifespan, 73.5 ± 2.4 host pupae were parasitized and 86.5 ± 2.3 offspring successfully developed into adults, of which 53.9 ± 2.5 % were females. However, the percentage of female offspring decreased with adult female age (**Fig. 16B**). Net

reproduction rate was 36.2. The estimated intrinsic rate of natural increase was 0.1115. Mean generation time and double timing were 32.2 and 6.2 days, respectively. Dissection of tested females at their death found that each dead female contained 1.7 ± 1.4 mature eggs (most of females run out of eggs at death).

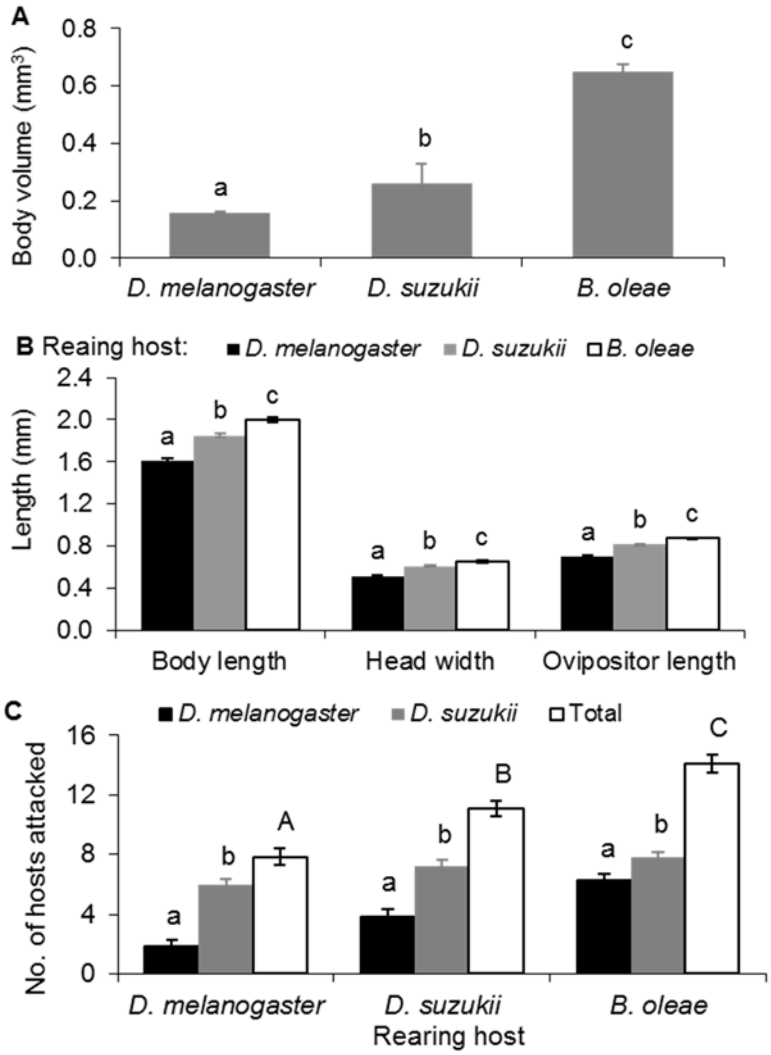

Fig. 17. Effects of host body size (A) on the body size (B) and parasitization efficiency (C) of developed female *P. vindemiae***.** Values are mean ± SE and different letters above bars indicate significant difference (ANOVA, $P < 0.05$).

5.3 Host species selection and fitness consequences

Since many host species are obviously different in their body size, especially SWD is larger than many other drosophilid species, we investigated the host species preference and fitness consequence of host species selection by both parasitoids. When *P. vindemiae* were reared on the three different-sized host species: SWD, *D. melanogaster* and *B. oleae* (the volume V of a prolate ellipsoid fly puparium with maximum body length l and width w was estimated on the formula: $V = 4/3\pi \cdot (l / 2) \cdot (w / 2)^2$) ($F_{2,57} = 687.2$, $P < 0.001$) (**Fig. 17A**), there was a positive correlation

between the size of emerged parasitoids and the size of their host fly species (**Fig. 17B**; body length: $F_{2,63} = 147.6$, $P < 0.001$; head width: $F_{2,63} = 93.6$, $P < 0.001$; ovipositor length: $F_{2,63} = 159.9$, $P < 0.001$). Regardless of its rearing host species, the parasitoids all preferred to attack the larger (SWD) to the smaller (*D. melanogaster*) host species when provided with a choice (parasitoids reared from *D. melanogaster*: $t_{1,30} = 80.7$, $P < 0.001$; parasitoids reared from SWD: $t_{1,30} = 28.9$, $P < 0.001$; parasitoids reared from *B. oleae*: $t_{1,30} = 7.7$, $P < 0.01$) (**Fig. 17C**). Large wasps parasitized more hosts than did small ones ($F_{2,59} = 30.6$, $P < 0.001$) (**Fig. 17C**).

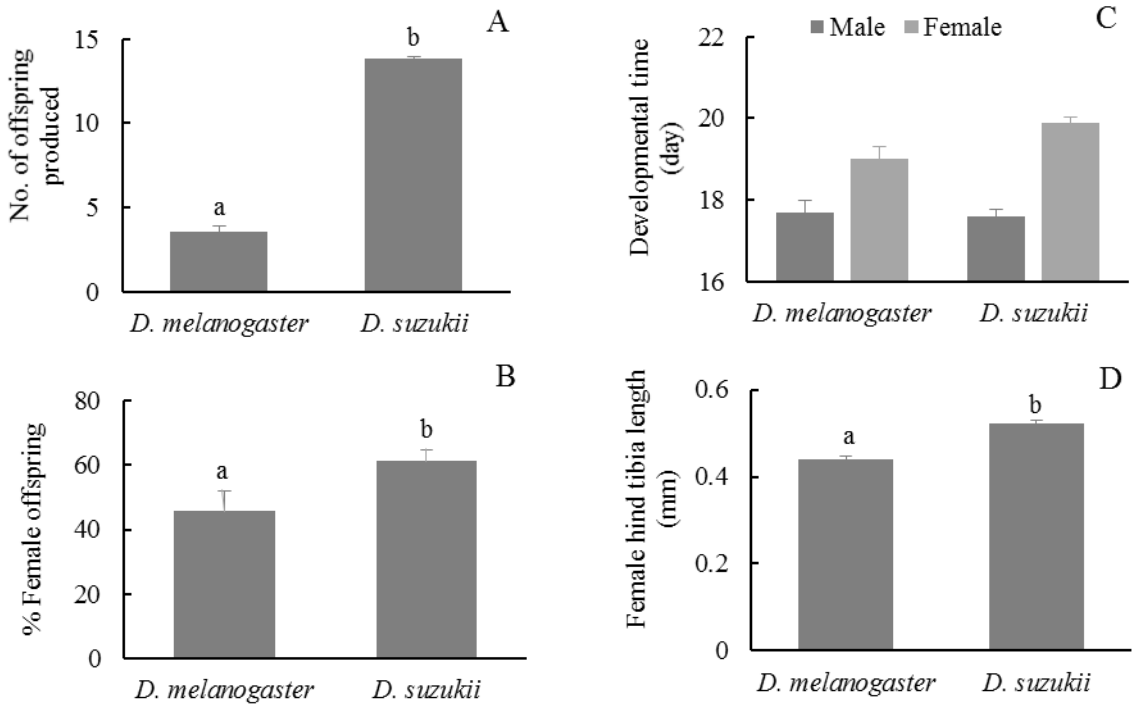

Fig. 18. Host species preference and fitness consequence of host species selection by *Trochipria* sp. Values are mean ± SE and different letters above bars indicate significant difference (ANOVA, $P < 0.05$).

Trochipria sp. also preferred to attack SWD over *D. melanogaster* ($F_{1,60} = 633.9$, $P < 0.001$) (**Fig. 18A**), and produced proportionally more female offspring from SWD ($F_{1,58} = 9.5$, $P = 0.003$) (**Fig. 18C**). Female parasitoids developed from SWD were larger than those from *D. melanogaster* ($F_{1,64} = 44.8$, $P < 0.001$) (**Fig. 18D**). Developmental time from egg to adult emergence was affected by host species, sex and the interaction of both factors (host species: $F_{1,60} = 4.1$, $P = 0.045$; sex: $F_{1,60} = 58.5$, $P < 0.001$; host species × sex: $F_{3,60} = 4.6$, $P = 0.032$) (**Fig. 18B**).

5.4 Functional response

To assess the daily reproduction potential of both parasitoids, we investigated the parasitoids' functional response at eight different host densities (**Fig. 19**). Both parasitoids showed a functional response of the type I; i.e. the number of hosts killed increases with the host densities linearly (**Fig. 19),** but *Trochipria* sp. attacked more hosts than *P. vindemiae* at the higher host

densities, suggesting possible egg limitation by *P. vindemiae* as dissection found tested female *P. vindemiae* contained less mature eggs than *Trochipria* sp..

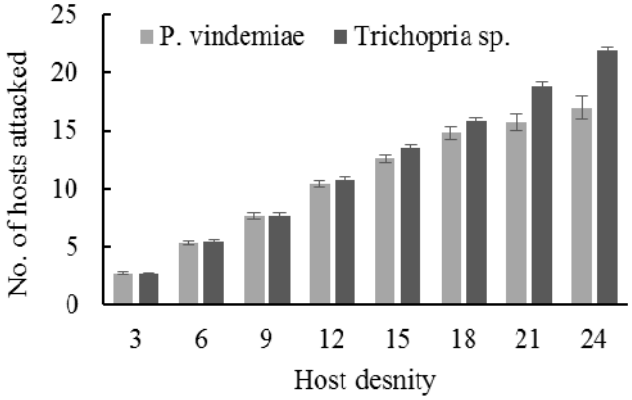

Fig. 19. Relationship between host density and the number of host attacked by *P. vindemiae* and *Trochipria* sp.

We also investigated the functional response of both parasitoids at different densities using *D. melanogaster* as host and the effects of female parasitoid's mature egg load, body size and exposure time on the functional response (data are not presented here).

5.5. Interspecific interaction

A series of experiments have been conducted to determine relative efficiency, interspecific interaction and host discrimination by *P. vindemiae* and *Trochipria* sp.. First, we assessed the possible effect of interspecific interactions on the ability of parasitoids to impact the host population under laboratory conditions. Twenty SWD pupae were exposed to one female parasitoid of either species alone or one female parasitoid of both parasitoids together for 24 hours. The observed levels of SWD mortality in the two species release treatment (*P. vindemiae* + *Trochipria* sp.) were compared to expected levels of SWD mortality calculated using data from the single species release treatments only. If interspecific interactions among parasitoid species have no effect on the host population (parasitoids have independent effects), the levels of host mortality should follow a multiplicative risk model:

$$H_{exp} = H_p + H_t - H_p \times H_t$$

H_{exp} is the expected host mortality by parasitoids *P. vindemiae* and *Trochipria* sp. together, H_p is the observed host mortality by *P. vindemiae* alone and H_t is the observed host mortality by *Trochipria* sp. alone. The observed and expected levels of SWD mortality were compared, for each replicate, using simple T-tests.

More hosts were attacked by *Trochipria* sp. than by *P. vindemiae* when they were alone ($F_{1,40} = 4.9$, $P = 0.032$) or together ($F_{1,40} = 36.6$, $P < 0.001$) (**Fig. 20**). Parasitism by *P. vindemiae* or *Trochipria* sp. alone and both species were 77.4 ± 3.6%, 87.4 ± 2.8%, and 64.5 ± 3.3%, respectively. The excepted host mortality if the two species acted independently was 97.1 ± 0.8% which was significantly higher than the observed morality ($F_{1,40} = 93.2$, $P < 0.0001$), indicating the interspecific competition.

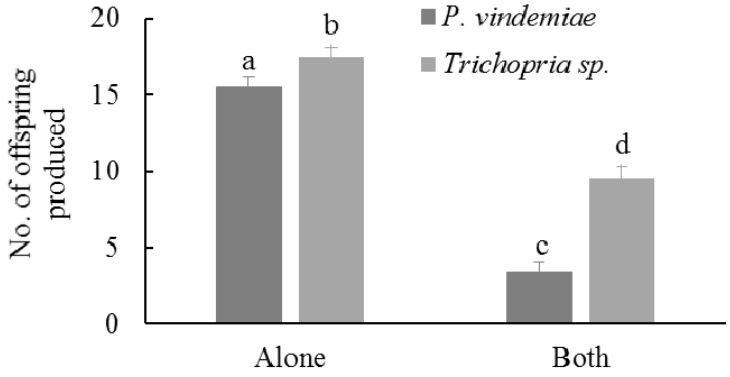

Fig. 20. Effect of interspecific interaction on the reproduction success by *P. vindemiae* or *Trochipria* sp. Values are mean ± SE and different letters above bars indicate significant difference (ANOVA, $P < 0.05$).

Second, we investigated host discrimination between the two parasitoids. Ten SWD pupae were exposed to each parasitoid specie for 24 hours, and then half of the exposed hosts and five unexposed hosts were presented to another parasitoid species for 24 hours. Following the subsequent exposure, the hosts were separately reared until the emergence of flies or parasitoids.

Table 6. Offspring produced when *P. vindemiae* or *Trochipria* sp. was exposed to half previously unexposed hosts and half previously exposed hosts to the other parasitoid

Exposure treatment [a]	No. of *P. vindemiae* emerged		No. of *Trichopria* sp. emerged	
	Previously unexposed host	Previously exposed host	Previously unexposed host	Previously exposed host
1) *P. vindemiae* only	2.60 ± 0.41			
2) Subsequently exposed to *Trichopria* sp.		1.48 ± 0.30	2.88 ± 0.30	1.04 ± 0.19
3) *Trichopria* sp. only			2.72 ± 0.28	
4) Subsequently exposed to *P. vindemiae*	1.84 ± 0.39	1.04 ± 0.21		1.92 ± 0.31

[a] Half of previously exposed hosts were subsequently exposed to another parasitoid tighter with the same number of unexposed hosts.

With this low host density, numbers of offspring produced by either parasitoid alone were not different ($F_{1,48} = 0.1$, $P = 0.015$). Both parasitoids preferred to attack previously unexposed hosts as more offspring was produced from the previously unexposed hosts (by *P. vindemiae*: $F_{1,48} = 3.3$, $P = 0.038$; by *Trichopria* sp.: $F_{1,48} = 26.2$, $P < 0.001$) (**Table 6**). Subsequent exposure reduced the number of offspring by the first parasitoid when compared to number of offspring developed from the exposure to one parasitoid only (by *P. vindemiae*: $F_{1,48} = 5.1$, $P = 0.015$; by *Trichopria* sp.: $F_{1,48} = 3.4$, $P = 0.032$) (**Table 6**). These results suggest both parasitoids can discriminate previously parasitized host by the other parasitoid, and multiple parasitism could reduce offspring survival of the parasitoids.

Third, we compared the relative efficiency between the two parasitoids under a more realistic setting; the parasitoids were released into cage with SWD-infested cherry fruit on cherry branch. Cherry branches were freshly cut from an unsprayed orchard. Each branch bearing 10 intact fruit (excessive fruit were removed) was inserted into a container filled with soil and water and the container was placed over a large petri dish with wet soil inside a screen cage (the branch height roughly matched the height of the testing cage, 30 x 30 cm). The container was positioned so that any fly larvae, if popped out, would drop and pupate into the soil dish. Ten female SWD were released into each cage and then removed 24 hours later. Five days later when the flies had developed into pupae, 10 female *P. vindemiae* or *Trichopria* sp. were released into each cage for three days. Following the exposure to parasitoids, all host pupae were collected from the fruit or soil and reared separately until the emergence of flies or parasitoid. Test with each species had 10 replicates (i.e., 10 cages).

A mean (± SE) of 60.9 (± 5.1) and 40.6 (± 5.1) SWD pupae were collected from the fruit and soil (the number in fruit was higher than in the soil, $F_{1,38} = 7.9$, $P = 0.008$). In general, parasitism on fruit was higher than in soil by both parasitoids and parasitism by *P. vindemiae* was higher than *Trichopria* sp., regardless the host pupation habitats (**Fig. 21**).

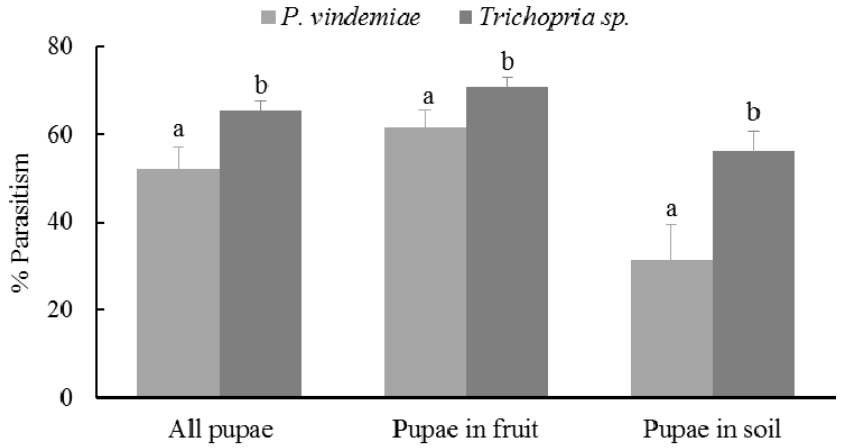

Fig. 21. Parentage parasitism of SWD pupated in cherry fruit or soil by both parasitoids (*P. vindemiae* or *Trichopria* sp.). Values are mean ± SE and different letters above bars indicate significant difference between two parasitoids (ANOVA, $P < 0.05$).

Finally, we investigated the possible interaction between these two parasitoids and two host species (SWD and *D. melanogaster*) using cherry as host fruit in cage. Host fruit on cherry branch were prepared as described above. In addition, another 10 artificially damaged cherries (1 cm long cut on the fruit surface to facilitate oviposition by *D. melanogaster*) were placed over a large soil petri dish along with the branch fruit inside the cage. Five female SWD and 5 female *D. melanogaster* were released into each cage and then removed after 24 hours. Five days later, 5 female *P. vindemiae* or *Trichopria* alone or together were released into the cages for 4-5 days. Following the exposure to the parasitoids, all fly pupae were collected and separately reared based on the fly species and pupation locations (fruit on branch, soil dishes). Each treatment had 15 replicates.

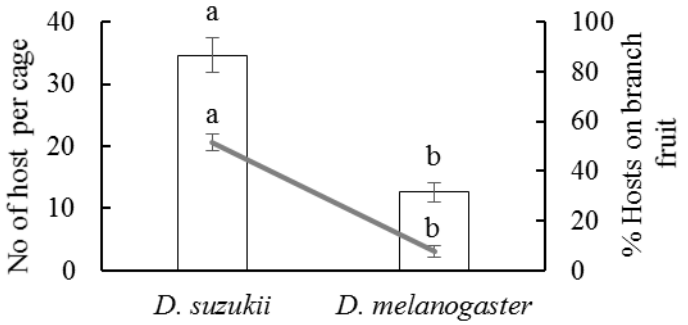

Fig. 22. Number of total pupae produced and percentage of pupae from branch fruit for each host species. Values are mean ± SE and different letters above bars indicate significant difference (ANOVA, $P < 0.05$).

SWD produced more pupae than *D. melanogaster* did ($F_{1,118} = 45.7$, $P < 0.001$). About half of the SWD pupae were collected from the branch (include the soil dish below the branch) while very few *D. melanogaster* pupae were collected from the branch fruit ($F_{1,118} = 107.6$, $P < 0.001$), suggesting that *D. melanogaster* rarely attacks intact cherry fruit (some may lay eggs into existing wounds created by SWD infestation) (**Fig. 22**).

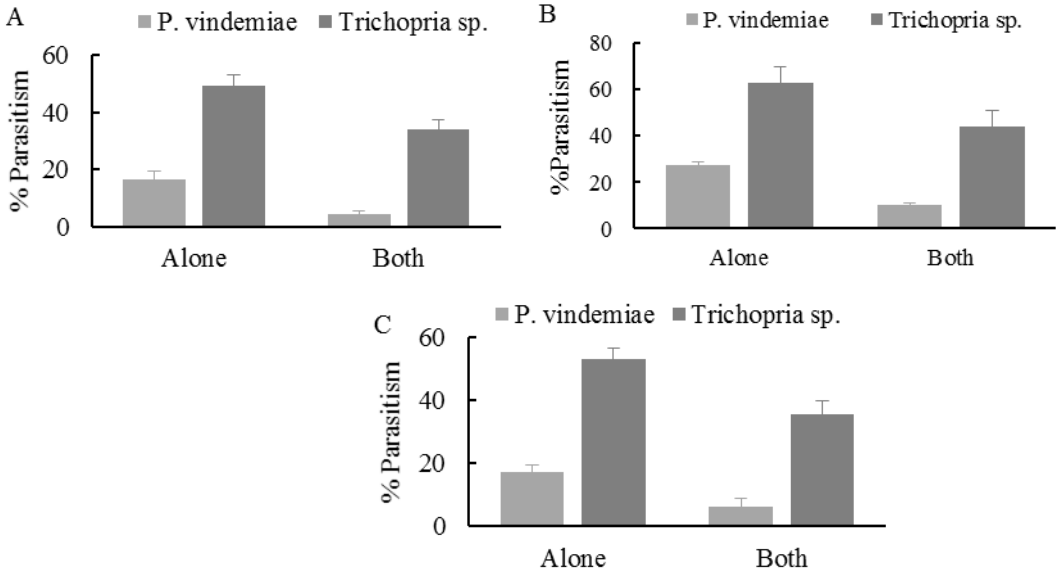

Fig. 23. Parasitism of (A) SWD, (B) *D. melanogaster* or (C) both host species by *P. vindemiae* or *Trichopria* when they were released alone or together.

Parasitism by *Trochipria* sp. was always higher than by *P. vindemiae* on SWD or *D. melanogaster*, or they were released alone or together (on SWD: $F_{3,56} = 24.7$, $P < 0.001$; on *D. melanogaster*: $F_{3,56} = 17.9$, $P < 0.001$; on both: $F_{3,56} = 26.3$, $P < 0.001$) (**Fig. 23**). The combined parasitism of both hosts by *P. vindemiae* or *Trochipria* sp. alone and together were 17.0 ± 2.6%, 53.1 ± 3.4%, and 41.6 ± 3.3%, respectively. The excepted mean parasitism (61.6 ± 2.6%) was

significantly higher than the observed parasitism ($F_{1,28} = 26.4$, $P < 0.001$), indicating the interspecific competition. In conclusion, *Trochipria* sp. seems to be more effective than *P. vindemiae* under the laboratory and semi-field conditions.

6. Introduction and evaluation of Asian *drosophila* parasitoids

In cooperation with colleagues at Oregon State University (Drs. Vaughn Walton, Jeff Miller, and Peter Shearer), we have introduced and started evaluation (in Quarantine) of novel parasitoids that may attack SWD. Our goal is to discover, import and select most specialized and effective parasitoids on SWD from the pest's native range (East Asia) for future field release to improve natural regulation of SWD in California

6.1 Foreign exploration and introduction

Asobara spp. (Braconidae)

Ganaspis spp. (Figitidae)

Trichopria sp. (Diapriidae)

Pachycrepoideus sp. (Pteromalidae)

Fig. 24. Parasitoid species collected in August-September 2013 from South Korea.

In 2013, a total of 3,266 individual fly pupae were collected from six different regions in South Korea (see **supplemental Fig. 6** for collection sites) using sentinel fruit traps baited with banana, peach, fig or blueberry, or direct samplings of blackberry, wild *Rubus*, apple, pear or grape, and imported to UC Berkeley's quarantine facility. At least two larval parasitoids *Asobara* spp. (Braconidae) and *Ganaspis* spp. (Figitidae), and two pupal parasitoids, *Pachycrepoideus* sp. (Pteromalidae), *Trichopria* sp. (Diapriidae) (**Fig. 24**) emerged from these collections. In total, 23

female and 9 male *Asobara* spp. emerged from SWD, *D. melanogaster* and other drosophila species; four female and two male *Ganaspis* spp. (Figitidae) emerged – most importantly these *Ganaspis* all emerged from SWD in collected fruits; three female *Pachycrepoideus* sp. emerged (all from *D. melanogaster*); and two female and three male *Trichopria* sp. emerged from SWD and *D. melanogaster.* Preliminary tests in the quarantine found that each of the four parasitoid species was able to attack and develop from SWD and *D. melanogaster* when the parasitoids were exposed to either host species in diet or blue berries (cherries were not available in the fall and winter in California) containing suitable host stages inside vials for 2-3 days (see **supplemental Table 3**).

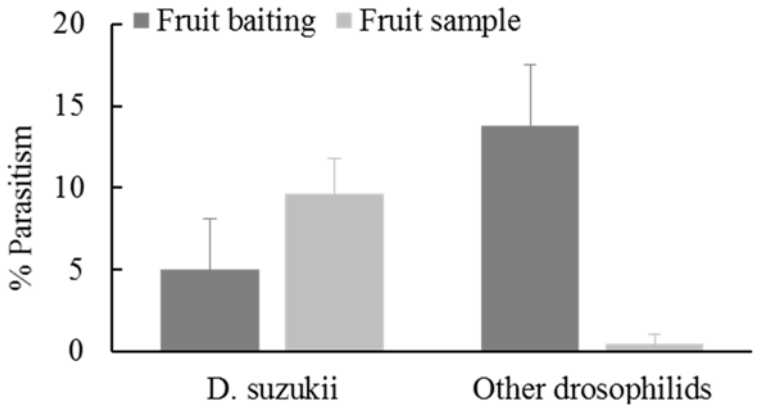

Fig. 25. Percentage parasitism of SWD and other drosophilids collected from fruits or sentinel fruit baiting traps.

Overall, parasitism of SWD was higher from fruit samples than sentinel fruit-baiting traps. In contrasts, parasitism of other drosophilids was higher from fruit baiting traps than fruit samples (**Fig. 25**). The highest parasitism of SWD was 33.3% from the collection in a blackberry field in Geochang, South Korea.

In 2014, the foreign exploration in South Korea was directed towards direct collection of suspected SWD host fruits. Fruits were collected from field and fly puparia were reared in the laboratory from these fruits. Consequently, the sampled and shipped parasitized hosts contained larval parasitoids as the hosts were likely attacked when they were at the larval stage in the field. A total of 18,840 pupae were collected from wild *Rubus*, cultivated blackberries, and raspberries in 14 different locations in South Korea. Two groups of larval parasitoids belonging to the Braconidae (*Asobara* spp.) and the Figitidae (*Leptopilina* spp. and/or *Ganaspis* spp) families were recovered from SWD and other *Drosophila* spp. At least 5 different fly species were recovered, most of the identified flies are SWD. Braconids emerged from 7 locations while Figitids emerged from 10 locations. The two parasitoid groups coexisted in 7 locations, and the Figitid presence was exclusive in other 3 locations. Four locations yield no parasitoids (**Fig. 26**). A total of 172 wasps emerged, including 113 (109 females and 4 males) *Asobara* spp. and 59 (37 females and 22 males) Figitids. Noteworthy is the difference in the field sex-ratio of these two parasitoid groups, i.e. 94.4% vs 62.7% of females for *Asobara* spp. and Figitids spp., respectively. The total mean parasitism was 3.20% (highest parasitism 38.4%), whereas it was

1.45 % and (highest parasitism 31.82 %) and 1.76% (highest parasitism 25.0%) for Braconids and Figitids, respectively.

Currently, 11 isofemale laboratory colonies of *Asobara* spp. (from 4 locations) and 7 Figitidae (from 4 locations) spp. colonies are being reared on SWD on artificial diet. Additionally, some entomopathogenic nematodes were recovered in one dish from the Poyeongsa location. Nematode samples have been sent to a specialist for ID and are currently being reared on SWD and artificial diet. Pilot experiments showed their ability to infect both SWD and *D. melanogaster* larvae and pupae in artificial diet.

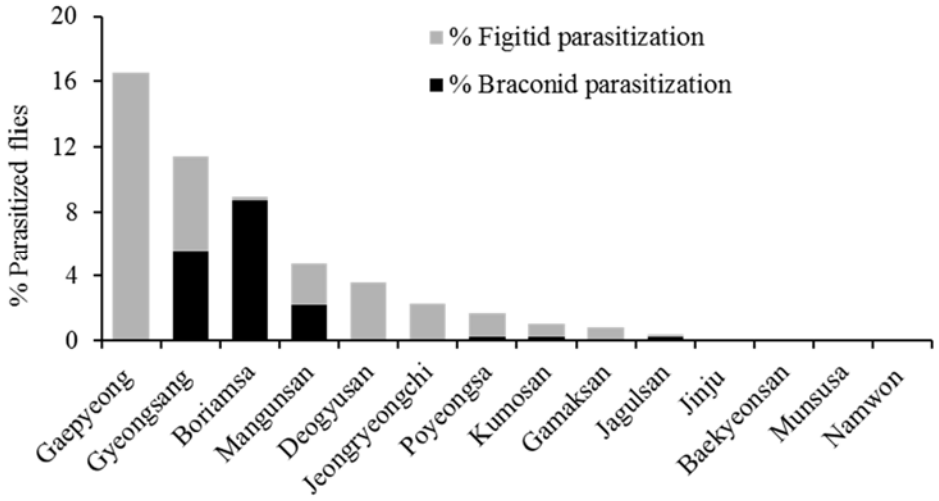

Fig. 26. Overall parasitization rates per parasitoid family and per sampled location from 2014 collections in South Korea. Numbers represent the number of isofemale colonies, per each species group, established and currently ongoing in the quarantine laboratory.

6.2 Evaluation of an Asian larval parasitoid for biological control of SWD

Laboratory bioassays were conducted in the quarantine to investigate the basic biological and ecological traits of the larval parasitoid species *Asobara* sp., collected in the summer of 2103 in Geochang, Gyeongsangnam-do province, South Korea. The laboratory experiments aimed at assessing the efficacy in controlling SWD and the potential non-target effect on other native fruit fly species.

Asobara sp. readily attack both SWD and *D. melanogaster*. When *Asobara* sp. was reared on those two different-sized host species under the same host density (15 hosts per female per 2 days) and room conditions, the number of offspring successfully produced (most were females) were similar from both hosts (**Fig. 27A**; $F_{1,37} = 1.1$, $P = 0.311$), but female parasitoids developed from SWD were larger than those reared from *D. melanogaster* (**Fig. 27B**; hind tibia width: $F_{1,50} = 28.4$, $P < 0.001$; ovipositor length: $F_{1,50} = 21.6$, $P < 0.001$). However, female parasitoids developed faster on *D. melanogaster* than on SWD ($F_{1,303} = 19.5$, $P < 0.001$) (Fig. **27C**).

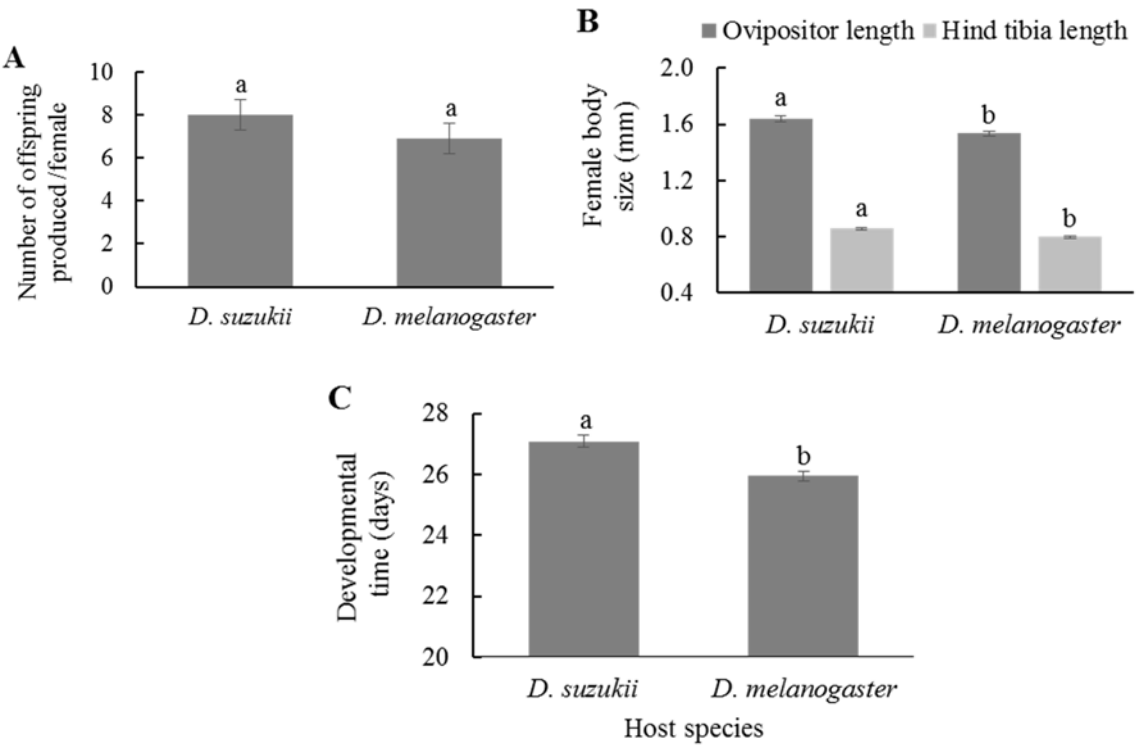

Fig. 27. Effects of host species on the parasitoid's (A) efficiency (i.e., number of offspring developed per female), (B) female offspring size and (C) developmental time. Different letters above the bars indicate significant difference between different host treatments (ANOVA, $P< 0.05$).

Using both SWD and *D. melanogaster* host species reared on the standard artificial diet, we studied the parasitoid's functional response at eight host densities, the parasitoid fitness (lifetime longevity, parasitization and fertility) and the host species preference and host switching capacity exposing the two hosts simultaneously at three host density combinations. Host preference were studied exposing parasitoid females, reared either on SWD or *D. melanogaster*, to cherry fruits, infested either with SWD or *D. melanogaster*. The response of parasitoid females to volatile cues emitted from SWD and DM infested fruits was also assessed through behavioral observation using a y-tube olfactometer device.

Parasitoids showed a functional response of the type I when parasitizing both fly species. The number of killed hosts increase with the host densities linearly (**Fig.28a**), whereas the proportion of attacked hosts is constant (**Fig 28b**). Parasitoid females lived 17.8 ± 1.4 and 15.9 ± 0.8 days when *D. suzukii* and *D. melanogaster* larvae were offered. During their lifetime, one parasitoid female killed an average of 230.0 ±14.9 *D. suzukii* and 223.5 ± 17.6 *D. melanogaster* larvae, whereas the offspring was 117.3 ± 10.1 (6.6 % males) and 95.5 ± 9.3 (7.2 % males) individuals on *D. suzukii* and *D. melanogaster*, respectively (**Fig. 29**).

When both hosts were offered to the parasitoid females on the artificial medium simultaneously, wasps did not show any host preference. Moreover, females developed either on *D. suzukii* or on *D. melanogaster* were able to switch host species successfully, i.e. females parasitize the host in which did not develop at the same rate and efficiency as the rearing host

(data not shown). *Asobara* sp. confirmed its ability to parasitize *D. suzukii* even on infested fruits. The estimated parasitism rates ranged from 30.0 to 36.4% in cherries and strawberries, respectively; while the percentages of host mortality due to the parasitoids were higher (**Fig. 30**). Much lower parasitism and host mortality rates were registered when cherries infested with *D. melanogaster* larvae were offered to the parasitoid, and this trend was even higher in the choice test (**Fig.31**), i.e. when fruits infested with the two hosts were offered simultaneously.

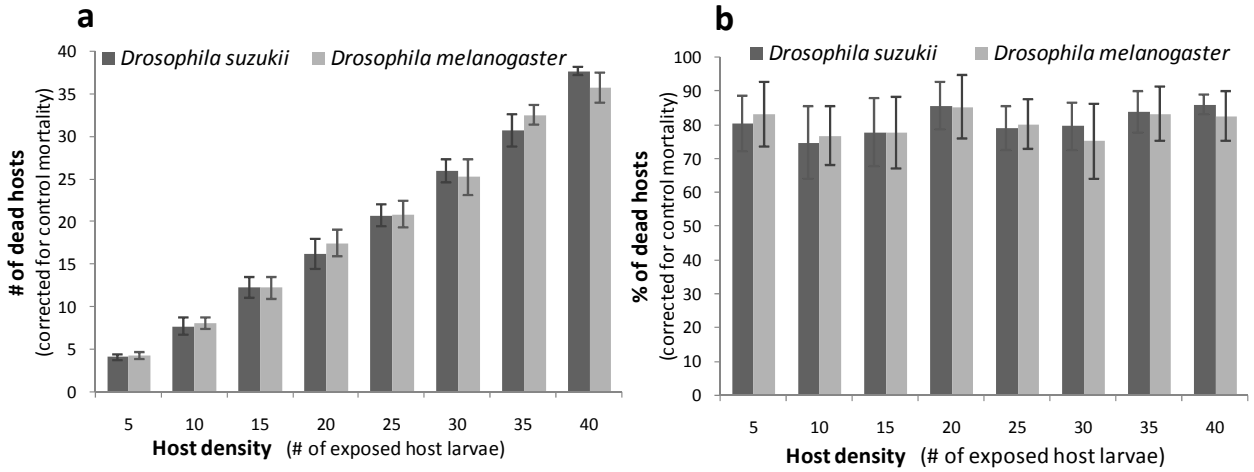

Fig. 28. *Asobara* sp. functional response on two drosophilid fly species. Means (± SE) of (a) number and (b) percentage of killed hosts by one parasitoid female at the eight host densities tested during 24h of exposure. Values are corrected for the mortality registered in the unreleased control.

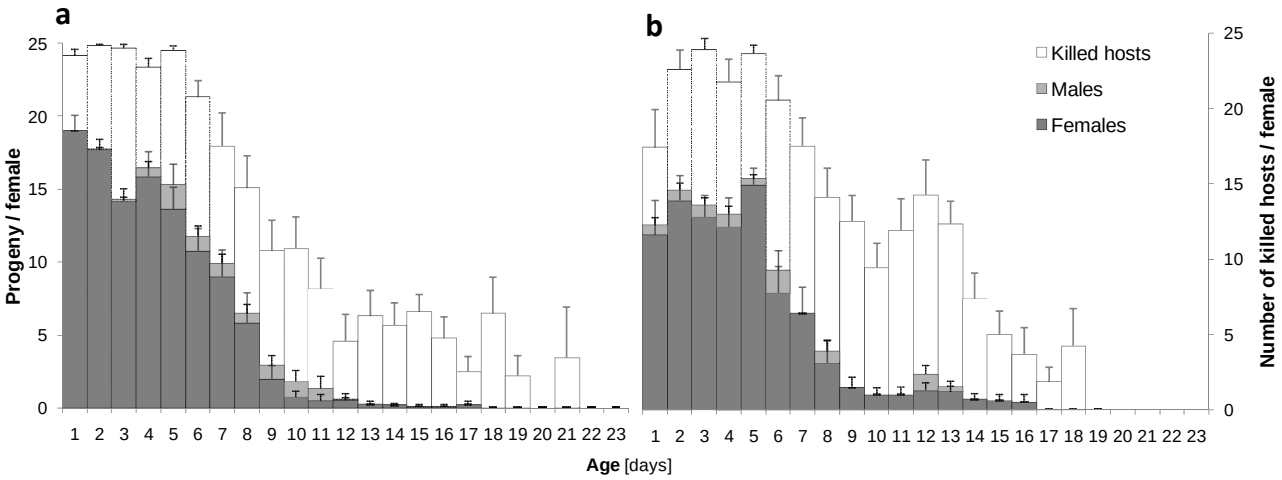

Fig. 29. *Asobara* sp. lifetime reproduction when parasitizing two drosophilid fly species. Means (± SE) of killed hosts (correct for control mortality), and of number of female and male progeny when 25 larvae a day of (a) *D. suzukii* and of (b) *D. melanogaster* were offered to one parasitoid female.

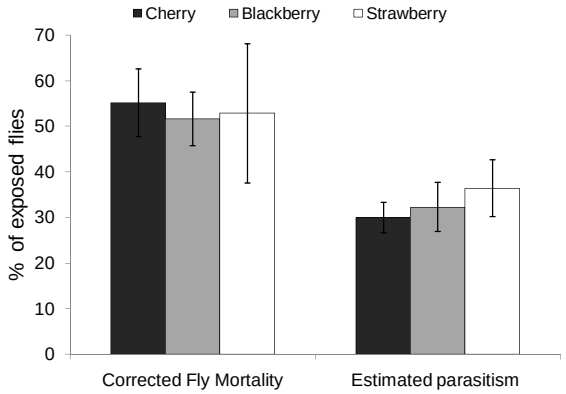

Fig. 30. *Asobara* sp. biocontrol potential on fruits artificially infested by *D. suzukii* larvae. Means (± SE) of % of killed (correct for control mortality) hosts and % of estimated parasitized hosts larvae.

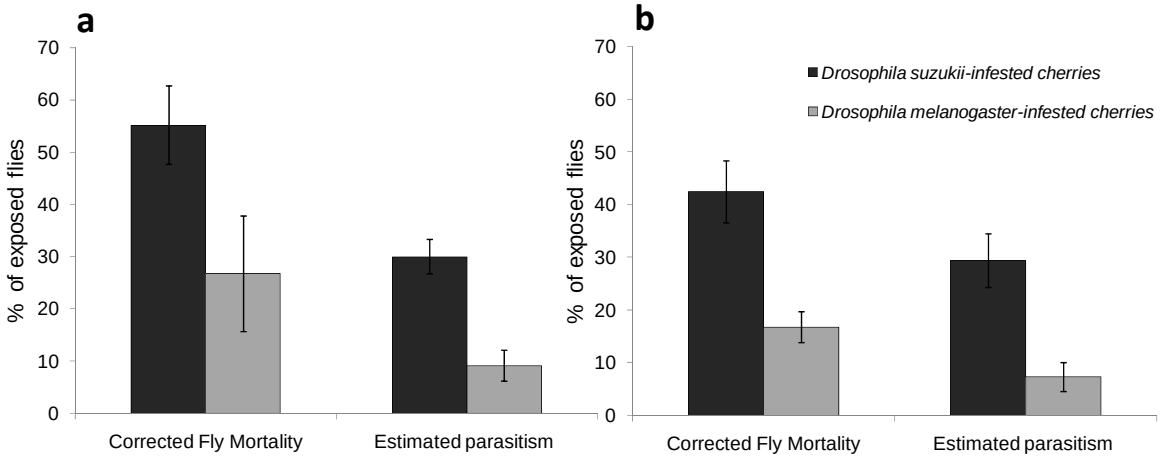

Fig. 31. *Asobara* sp. host preference between *D. suzukii*-infested and *D. melanogaster*-infested cherries. Means (± SE) % of killed (corrected for control mortality) hosts and % of estimated parasitized hosts larvae in the (a) no-choice and (b) choice bioassay.

Nevertheless, parasitoid females that were attracted by the volatile cues emitted by *D. suzukii*-infested vs healthy cherries did not show any preference between the volatile odors emitted by fruit infested with the two fly species (**Table 7**). These results were confirmed using both fly hosts and artificial diet and cherry fruits for rearing the experimental parasitoids. However females were significantly attracted by the volatiles emitted by *D. melanogaster*-infested vs healthy cherries only when they were reared using *D. melanogaster*-infested cherries (**Table 7**).

In conclusion, *Asobara* sp. showed a great potential of demographical increase (notably high female progeny production in the first week of female life) when parasitizing *D. suzukii* and *D. melanogaster* on artificial diet, but not when *D. melanogaster* infests cherry fruits. This species has a considerable potential an effective *D. suzukii* biological control agent, because it was demonstrated that it is able to detect the volatile cues emitted when it infest cherry fruits and to parasitize and develop on three different infested fruits.

Table 7. Response of *Asobara* sp. females reared on various hosts combinations to the volatile cues produced by healthy and *D. suzukii*- and *D. melanogaster*-infested cherries

Parasitoid hosts	Volatile odor sources and relative number of choices					
Drosophila suzukii on artificial diet	SWD-Infested **39**	Healthy 11	DM-Infested 31	Healthy 19	DM-Infested 24	SWD-Infested 26
Drosophila melanogaster on artificial diet	SWD-Infested **42**	Healthy 8	DM-Infested 30	Healthy 20	DM-Infested 31	SWD-Infested 19
Drosophila suzukii on cherries	SWD-Infested **49**	Healthy 1	DM-Infested 30	Healthy 20	DM-Infested 20	SWD-Infested 30
Drosophila melanogaster on cherries	SWD-Infested **48**	Healthy 2	DM-Infested **36**	Healthy 14	DM-Infested 20	SWD-Infested 30

Number of choices made by the females at the two-choice y-tube olphactometer devices. Values in bold represent significant preference at $P < 0.05$ following chi-square tests.

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Peer-reviewed publication (paper published manuscripts in review or preparation)

Stewart TE, Wang X-G, Molinar A, Daane KM. (2014) Factors limiting peach as a potential host for spotted wing drosophila. *Journal of Economic Entomology* 107: 1771-1779.

Wiman NW, Walton VM, Dalton DT, Anfora G, Burrack HJ, Chiu J, Daane KM, Grassi A, Miller B, Tochen S, Ioriatti C, Wang X-G (2014) Integrating temperature-dependent life table data into a matrix projection model for *Drosophila suzukii* population estimation. *PloS One* 9: e106909.

Miller B, Anfora G, Buffington M, Daane KM, Dalton DT, Hoelmer KM, Rossi Stacconi MV, Grassi A, Loni A, Miller J, Quantar M, Walton VW, Wiman N, Wang X-G, Ioriatti C**.** Resident field-sampled parasitoids associated with *Drosophila suzukii* and their seasonal occurrence in two small fruit production regions. Submitted to *Biological Control.*

Stacconi MVR, Buffington M, Daane KM, Dalton DT, Grassi A, Kaçar G, Miller B, Miller J, Baser N, Ouantar M, Ioriatti C, Walton VW, Wiman N, Wang X-G, Anfora G. Host stage preference, efficacy and fecundity of parasitoids attacking *Drosophila suzukii* in newly invaded areas. Submitted to *Biological Control*.

Daane KM, et al. Perspectives of biological control of *Drosophila suzukii* in Western USA.

Stewart et al. Seasonal occurrence and persistence of *Drosophila suzukii* in Californian interior fruit growing regions.

Stewart TE et al. Seasonal occurrence of *Drosophila suzukii* in Californian interior fruit growing regions.

Kaçar G et al. Functional response by two pupal drosophilid parasitoids.

Kaçar G et al. Use of alternative hosts and overwintering biology of *Drosophila suzukii* in California.

Biondi A, et al. Biology of *Asobara* sp. when parasitizing the invasive spotted wing drosophila and the common fruit fly.

Biondi A, et al. Functional response of *Asobara* sp. on two drosophilid fly species.

Biondi A, et al. Preference and host switching in a larval parasitoid attacking the common fruit fly and the spotted wing drosophila.

Biondi A, et al. Biocontrol potential of an imported larval parasitoid against the invasive *Drosophila suzukii*.

Wang X-G et al. Evaluating *Trichopria drosophilae* as a major resident parasitoid of *Drosophila suzukii.*

Wang X-G et al. Interactions between two resident pupal parasitoids attacking *Drosophila suzukii*

Wang X-G et al. Host species preference and fitness consequence by *Pachycrepoidues vindemiae*.

Papers presented at regional and national conferences

Daane KM, Wang X-G, Kacar G, Biondi A, Miller B, Miller JC, Shearer PW, Hoelmer KM, Walton VM, Guerrieri E, Giorgini M. Biological control of *Drosophila suzukii* in the US: current status and perspectives. ESA's 62nd Annual Meeting, Portland, Oregon November 16-19, 2014.

Biondi A., Wang X-G, Daane KM, Miller JC, Miller B, Shearer PW, Walton VM. Pre-introduction assessment of an Asian larval parasitoid for classical biological control of *Drosophila suzukii* in the US. ESA's 62nd Annual Meeting, Portland, Oregon November 16-19, 2014.

Wang X-G, Kacar G, Daane KM, Miller B, Walton VM. Evaluating the potential of two resident parasitoids for the biocontrol of *Drosophila suzukii*. ESA's 62nd Annual Meeting, Portland, Oregon November 16-19, 2014.

Stewart T, Daane KM, Wang X-G, Kacar G. Use of alternative host fruit and overwintering biology of spotted wing drosophila (*Drosophila suzukii*) in California. ESA's 62nd Annual Meeting, Portland, Oregon November 16-19, 2014.

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Walton V, Dalton DT, Wiman NG, Tochen SL, Miller B, Burrack HJ, Daane KM, Wang X-G, Shearer PW, Ioriatti C, Anfora G, Grassi A, Neteler M. *Drosophila suzukii* population estimation and development of a real-time risk model. ESA's 62nd Annual Meeting, Portland, Oregon November 16-19, 2014.

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Hoelmer K., Daane KM, Wang X-G, Walton V, Guerrieri E, Giorgini M. Prospects for classical biological control of spotted wing drosophila. ESA's 62nd Annual Meeting, Portland, Oregon November 16-19, 2014.

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Hoelmer K., Daane KM, Wang X-G, Walton V, Guerrieri E, Giorgini M. Prospects for classical biological control of spotted wing drosophila. EAS Eastern Branch Annual Meeting, Williamsburg, VA, March 16 2014.

Supplemental tables and figures

Table 1. Generalized Linear Model analyzing the effects of accumulated degree-days, presence of mature fruit, as well as their interaction on weekly captures of adult *D. suzukii* in different sites and geographical locations

Location	Site	Variable	Residual deviance	χ^2	*P*
Courtland	Cherry 1	Presence of fruit (F) [1]	253.7	16.8	< 0.001
		Accumulated Degree-days (DD)		25.6	< 0.001
		F × DD		191.6	< 0.001
	Cherry 2	Presence of fruit (F)	247.6	70.3	< 0.001
		Accumulated Degree-days (DD)		494.	< 0.001
		F × DD		167.9	< 0.001
	Cherry 3	Presence of fruit (F)	311.1	85.9	< 0.001
		Accumulated Degree-days (DD)		1.9	0.168
		F × DD		117.9	< 0.001
	Kiwi	Presence of fruit (F)	217.3	116.4	< 0.001
		Accumulated Degree-days (DD)		27.1	< 0.001
		F × DD		111.7	< 0.001
	Pear	Presence of fruit (F)	165.6	142.7	< 0.001
		Accumulated Degree-days (DD)		0.1	0.760
		F × DD		1.2	0.281
Stockton	Cherry 1	Presence of fruit (F)	316.7	82.3	< 0.001
		Accumulated Degree-days (DD)		50.5	< 0.001
		F × DD		4.0	0.045
	Cherry 2	Presence of fruit (F)	242.5	46.7	< 0.001
		Accumulated Degree-days (DD)		90.2	< 0.001
		F × DD		193.7	< 0.001
	Peach	Presence of fruit (F)	32.9	5.5	0.039
		Accumulated Degree-days (DD)		4.3	0.020
		F × DD		1.2	0.282
Brentwood	Cherry	Presence of fruit (F)	998.4	788.6	< 0.001
		Accumulated Degree-days (DD)		20.5	< 0.001
		F × DD		17.7	< 0.001
	Pear	Presence of fruit (F)	270.3	240.2	< 0.001
		Accumulated Degree-days (DD)		37.8	< 0.001
		F × DD		4.7	< 0.001
	Fig	Presence of fruit (F)	21291	6022	< 0.001
		Accumulated Degree-days (DD)		4762	< 0.001
		F × DD		54.4	< 0.001
	Apricot	Presence of fruit (F)	452.3	111.4	< 0.001
		Accumulated Degree-days (DD)		189.4	< 0.001
		F × DD		96.1	< 0.001
	Riparian	Presence of fruit (F)	1540	126.8	< 0.001
		Accumulated Degree-days (DD)		490.7	< 0.001

		F × DD		9.6	< 0.001
Parlier	Apple	Presence of fruit (F)	76.2	54.4	< 0.001
		Accumulated Degree-days (DD)		2.6	0.107
		F × DD		9.2	0.002
	Apricot	Presence of fruit (F)	26.8	10.2	0.001
		Accumulated Degree-days (DD)		13.1	< 0.001
		F × DD		10.7	0.001
	Blackberry	Presence of fruit (F)	48.6	14.7	< 0.001
		Accumulated Degree-days (DD)		5.7	0.017
		F × DD		0.01	0.772
	Blueberry	Presence of fruit (F)	9.5	6.5	0.011
		Accumulated Degree-days (DD)		0.1	0.884
		F × DD		< 0.1	0.926
	Cherry	Presence of fruit (F)	156.5	15.3	< 0.001
		Accumulated Degree-days (DD)		14.0	< 0.001
		F × DD		26.3	< 0.001
	Citrus	Presence of fruit (F)	273.8	49.3	< 0.001
		Accumulated Degree-days (DD)		5.6	0.024
		F × DD		1.7	0.189
	Fig	Presence of fruit (F)	250.3	98.2	< 0.001
		Accumulated Degree-days (DD)		14.0	< 0.001
		F × DD		4.7	0.031
	Grape	Presence of fruit (F)	52.2	18.1	< 0.001
		Accumulated Degree-days (DD)		0.4	0.541
		F × DD		4.4	0.035
	Kiwi	Presence of fruit (F)	130.1	70.7	< 0.001
		Accumulated Degree-days (DD)		1.1	0.285
		F × DD		0.2	0.626
	Peach	Presence of fruit (F)	36.1	5.7	0.016
		Accumulated Degree-days (DD)		2.0	0.153
		F × DD		33.5	< 0.001
	Persimmon	Presence of fruit (F)	45.2	31.4	< 0.001
		Accumulated Degree-days (DD)		2.8	0.092
		F × DD		1.6	0.212
	Plum	Presence of fruit (F)	51.5	39.1	< 0.001
		Accumulated Degree-days (DD)		0.1	0.796
		F × DD		6.1	0.014
	Pomegranate	Presence of fruit (F)	36.1	28.9	< 0.001
		Accumulated Degree-days (DD)		3.3	0.071
		F × DD		3.3	0.071

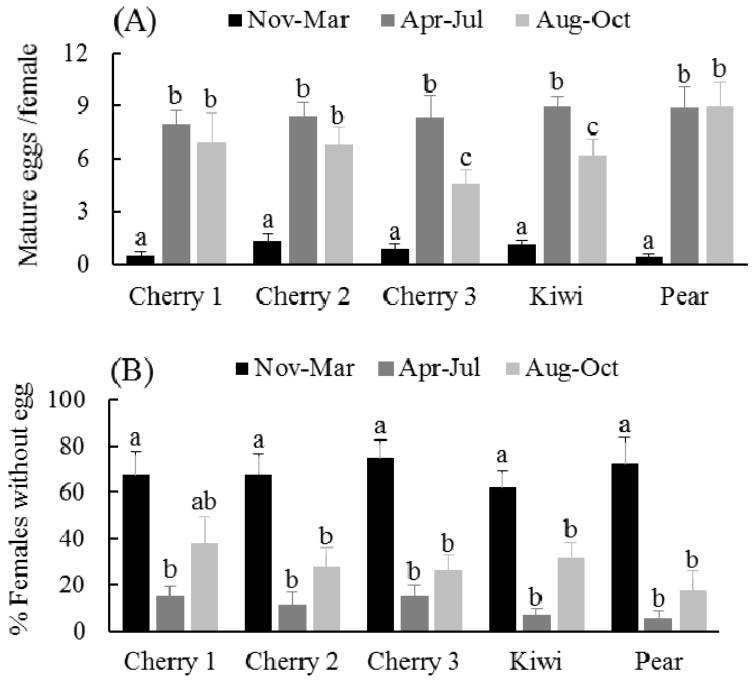

Fig. 1. Mean (± SE) number of mature eggs (A) and percentage of female *D. suzukii* without mature eggs (B) from traps in different sites in Coutland, CA. Data were pooled for the three different seasons, and different letters above the bars indicated significant difference among the three periods for each site.

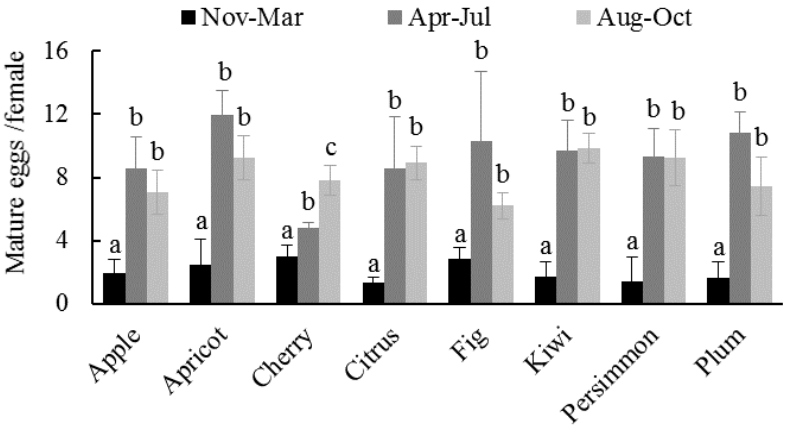

Fig. 2. Mean (± SE) number of mature eggs (A) and percentage of female *D. suzukii* without mature eggs (B) from traps in different sites in Coutland, CA. Data were pooled for the three different seasons, and different letters above the bars indicated significant difference among the three periods for each site.

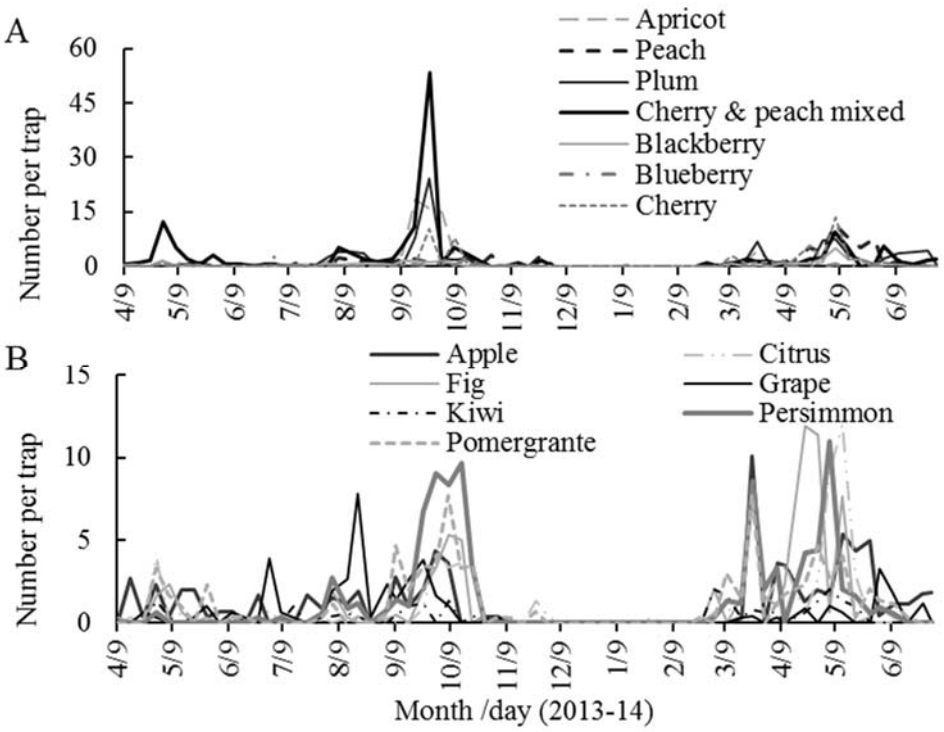

Fig. 3. Weekly mean drosophilid parasitoids captured in *D. suzukii* trap in different crops (A-B) in Parlier, CA.

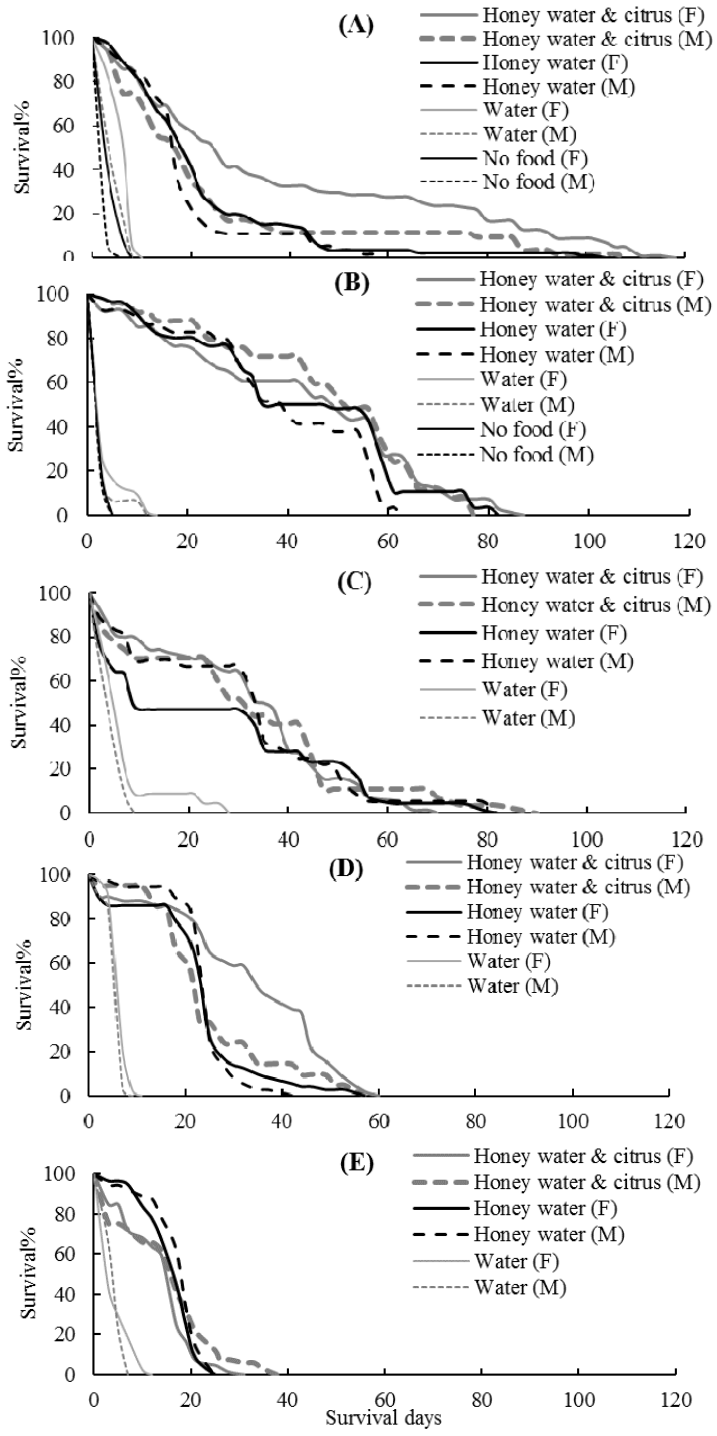

Fig. 4. Percentage of survival of adult female (F) and male (M) *D. suzukii* in test cages under different food provision conditions when the test cages were launched on (A) 19 Nov. (B) 27 Dec. (C) 22 Jan. (D) 22 Feb. and (E) 28 Mar.

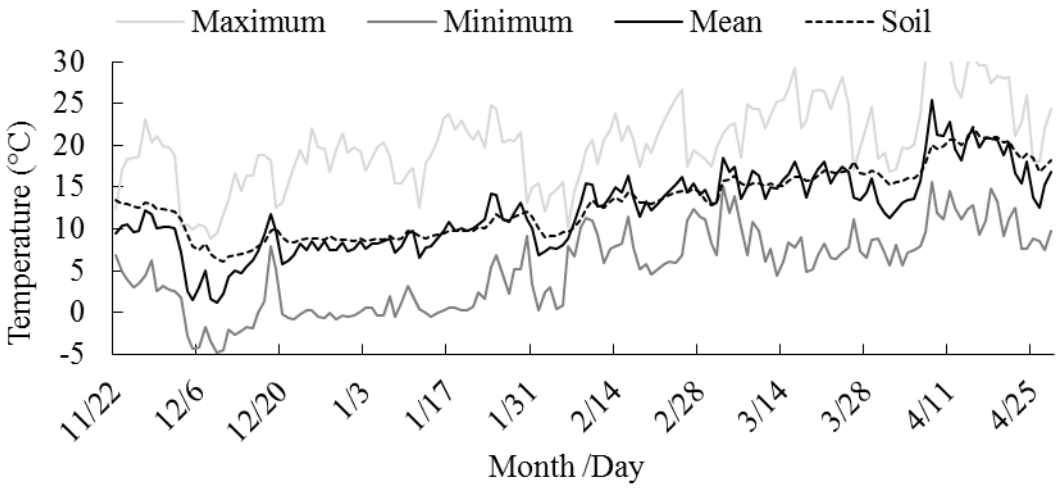

Fig. 5. Mean daily temperature (November 2013 to April 2014) in Parlier, CA

Table 2. Emerged flies and parasitoids from field collections of fruits

Year	Month	Fruit species	Status	Habitat	#Fruit	SWD	Other drosophilids	Parasitoids
Parlier								
2012	May	Cherry	Random	Tree	174	217		
		Cherry	Damaged	Tree	75	97	1	
	Jun	Cherry	Random	Tree	183	158		
		Cherry	Damaged	Tree	301	274	56	3L
		Cherry	Rotting	Ground	60		60	26L
		Nectarine	Rotting	Ground	10		500	
	Jul	Peach	Damaged	Tree	15	9	1153	38L
	Aug	Peach	Rotting	Ground	10		374	72L
		Fig	Overripe	Tree	20	5	155	
2013	Mar	Mandarin	Damaged	Ground	15		156	
	Apr	Mandrain	Rotting	Ground	10			
		Apple	Rotting	Ground	10		151	
	May	Cherry	Random	Tree	353	11		
	Jun	Peach	Damaged	Tree	10	2	100	
	Sept	Fig	Overripe	Tree	20	110	17	
		Grape	Overripe	Tree	500		15	
		Plum	Overripe	Tree	10		120	
		Apple	Damaged	Tree	15		130	
		Peach	Damaged	Tree	10		2	
	Oct	Plum	Rotting	Tree	20	2	67	2P
		Plum	Rotting	Ground	10	3	58	10P
	Nov	Apple	Damaged	Tree	20		90	2T
		Grape	Dry	Tree	500			
		Peach	Dry	Ground	10			
		Fig	Dry	Tree	10		1	1L
		Pomegranate	Dry	Tree	10		85	
	Dec	Apple	Rotting	Tree	20	3	27	8P
		Persimmon	Damaged	Ground	10			
		Plum	Dry	Ground	20		2	2P
		Pomegranate	Damaged	Tree	10	12	161	4P
2014	Jan	Apple	Damaged	Tree	20		15	
		Apple	Damaged	Ground	20	1	108	
		Persimmon	Damaged	Ground	25	1	310	
		Pomegranate	Damaged	Tree	10		161	1L
		Pomegranate	Damaged	Ground	10		40	
	Feb	Apple	Damaged	Ground	10		511	5P
		Pomegranate	Damaged	Tree	10		1013	163P
		Pomegranate	Damaged	Ground	10		415	377P, 7T
	Mar	Pomegranate	Dry	Ground	20		64	28P, 31T
		Mandarin	Rotting	Ground	20		1154	49P, 254T
		Apple	Rotting		20		37	1P, 2T
	Apr	Pomegranate	Dry	Ground	10		171	20P
	May	Mandarin	Rotting	Ground	10		41	1T
		Orange	Damaged	Ground	10		324	26P, 26T
		Cherry	Random	Tree	310	8		1T
		Cherry	Random	Ground	235	2	2	1T

		Orange	Rotting	Ground	10			
	Jun	Cherry	Random	Tree	318	15		
Courtland								
2013	Sept	Pear	Damaged	Tree	15		63	10L
Brentwood								
2013	May	Loquat	Damaged	Tree	25	5	102	3L
		Peach	Damaged	Tree	5		20	
		Apricot	Damaged	Tree	5	1		
		Cherry	Random	Tree	18	2		
2014	July	Pear	Damaged	Ground	5	103		
		Plum	Ripe	Tree	5			
		Fig	Ripe	Ground	20			
		Wild plum	Ripe	Tree	5			
		Peach	Ripe	Tree	5			
		Nectarine	Ripe	Tree	5			
	Sept	Pear	Damaged	Tree	5	3	63	29T, 7P
		Peach	Rotting	Ground	5		56	
		Fig	Dry	Ground	20			
		Cactus	Damaged	Tree	20	1		
Stockton								
2014	Jul	Nectarine	Ripe	Ground	5			
		Peach	Ripe	Ground	5		4	

L = *Leptopilina* spp.; P = *P. vindemiae* T = *Trichopria* sp.

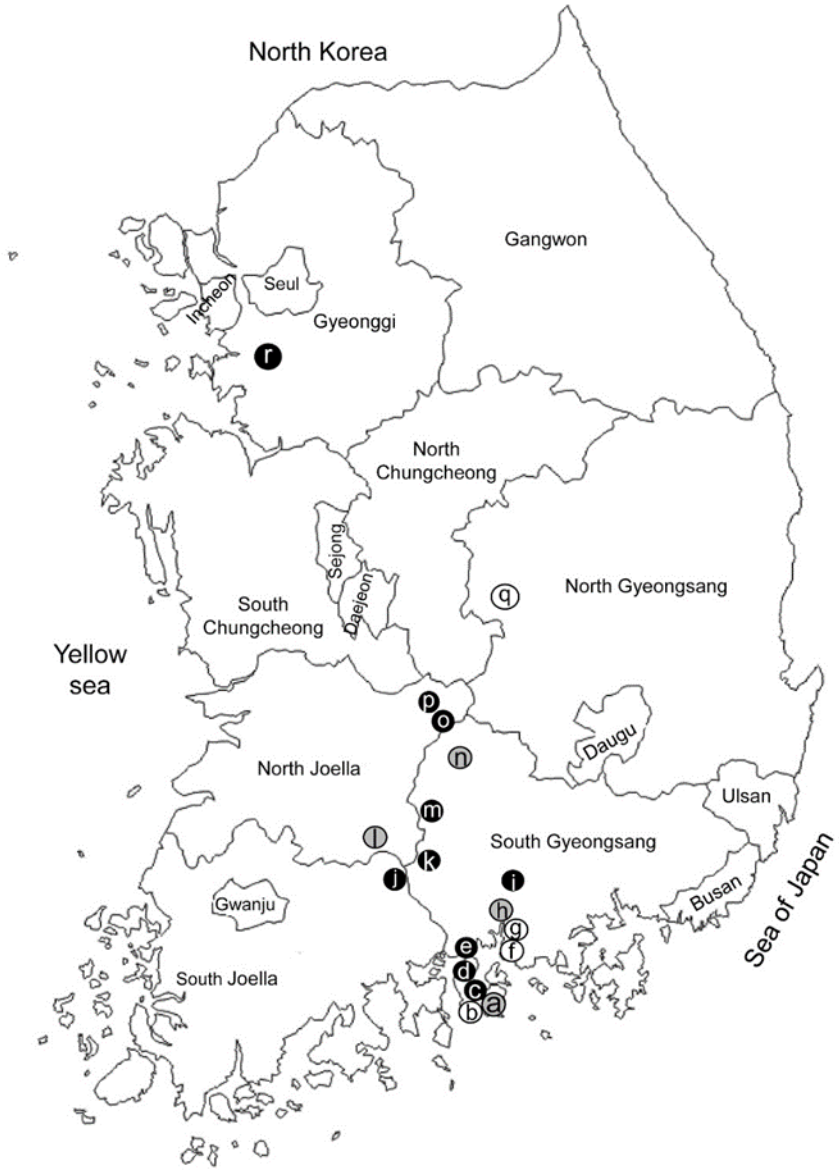

Fig. 6. South Korean sampling locations. White, black and gray circles represent sampling carried out during the summer of 2013, 2014 and both years, respectively. (a) Boriamsa; (b) Namhae Marina; (c) Namhae Marina; (d) Mangunsan; (e) Kumosan; (f) Sacheon; (g) Guam-Imdo; (h) Jinju; (i) Jagulsan; (j) Munsusa; (k) Jeongryeongchi; (l) Munsusa and Namwon; (m) Baekyeonsan; (n) Geochang; (o) Muju; (p) Jeoksangsan; (q) Sangju; (r) Gunjae Bong and Ban Suk San.

Table 3. Individual numbers of flies and parasitoids that emerged in parasitism experiments

Parasitoid species	Host species	Host medium	Wasps / vial	*n*	Emerged flies	Emerged parasitoids
Asobara spp.	*D. melanogaster*	Diet	2♀1♂	6	390	80
	D. suzukii	Blueberry	2♀1♂	11	6	14
		Diet	2♀1♂	9	14	32
Ganaspis spp.	*D. melanogaster*	Diet	1♀1♂	2	0	7
	D. suzukii	Blueberry	1♀1♂	2	2	2
		Diet	1♀1♂	2	2	6
Pachycrepoideus sp.	*D. melanogaster*	Diet	1♀	3	58	28
	D. suzukii	Blueberry	1♀	2	0	2
		Diet	1♀	2	3	3
Trichopria sp.	*D. melanogaster*	Diet	1♀1♂	8	205	90
	D. suzukii	Blueberry	1♀1♂	4	14	3
		Diet	1♀1♂	4	1	22

California Cherry Marketing and Research Board
RESEARCH GRANT – 2014
Progress Report

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

Project Title: Phytosanitary Irradiation Using a Cabinet X-ray Tube Machine

Funding: $11,100

Overall Objectives:

To develop and test a low-cost cabinet x-ray system to control quarantine insect pests that fits in a packing line. Irradiation treatment using this machine will allow movement of sweet cherries out of quarantine areas or into foreign markets by controlling quarantine pests. In year 1, sweet cherry varieties will be irradiated using a specialized x-ray emitter and evaluated for quality to demonstrate proof on concept.

Summary of workplan or methodology:

Concept designs for a packing line scale x-ray tube irradiation system have been developed in collaboration with Applied Energy Devices, LLC. The x-ray tube will be tested for dose uniformity, dose rate, and product treatment efficiency. California sweet cherries will be irradiated using the specialized x-ray emitter and fruit quality will be evaluated.

Progress:

Two sweet cherry irradiation tests were conducted. In the first test, 'Bing' cherries from Sambado & Sons were shipped to Atlanta and irradiated at Rad Source using two 4-Pi X-ray tubes mounted on a frame in a lead-lined treatment cabinet. This is a similar configuration to what is envisioned for a cabinet X-ray system, except the product was held stationary instead of moving on a conveyor belt (Fig. 1). The boxes was irradiated from one side then flipped and treated from the other side. Dosimetry showed that the X-rays easily penetrated the 18 lb box of fruit and had good dose uniformity. Fruit were repacked and shipped back to Sambado & Sons for evaluation, but the packed fruit experienced temperature abuse during shipping and the test was a bust.

In the second test, export quality 'Skeena' cherries were shipped under cold storage from Oregon to San Francisco, irradiated at Nutek (e-beam irradiation) in Hayward, and driven immediately to Sambado's for quality evaluation. The 'taste test' panel consisted of 15 employees at the packinghouse and included field and office workers, and management personnel. Each tester was presented with 3 bowls each containing 4-5 cherries and labeled A, B and C. Cherries in bowl A had been irradiated at 150 Gy, bowl B at 400 Gy, and bowl C were left untreated (0 Gy). Each taste tester was given three evaluation sheets (A, B, C), each with a

22 cm line on it with the word ‘dislike’ at the left end and ‘like’ at the right end. Testers were asked to sample (judge appearance, texture, taste) the cherries in each bowl and place a mark on the line corresponding to their overall degree of liking for each sample.

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I-----------------------------------------------------------I
Dislike                                                  Like
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The distance from the start of the line on the left to the mark was measured for each sample (A, B, and C) for each of the participants and averaged. The taste test panel was unable to distinguish between irradiated and unirradiated fruit, or between the two levels of irradiation (Fig. 2). Fruit were held for an additional 1 week in the refrigerator and a casual taste test by several Sambado & Sons employees was still unable to distinguish among treatments. Therefore, irradiated cherries were indistinguishable from untreated cherries. This result (no effect of irradiation on quality) is consistent with previous studies in Washington and Australia.

Fig. 1. Experimental set-up using 4-Pi X-ray tubes to irradiate boxes of sweet cherries.

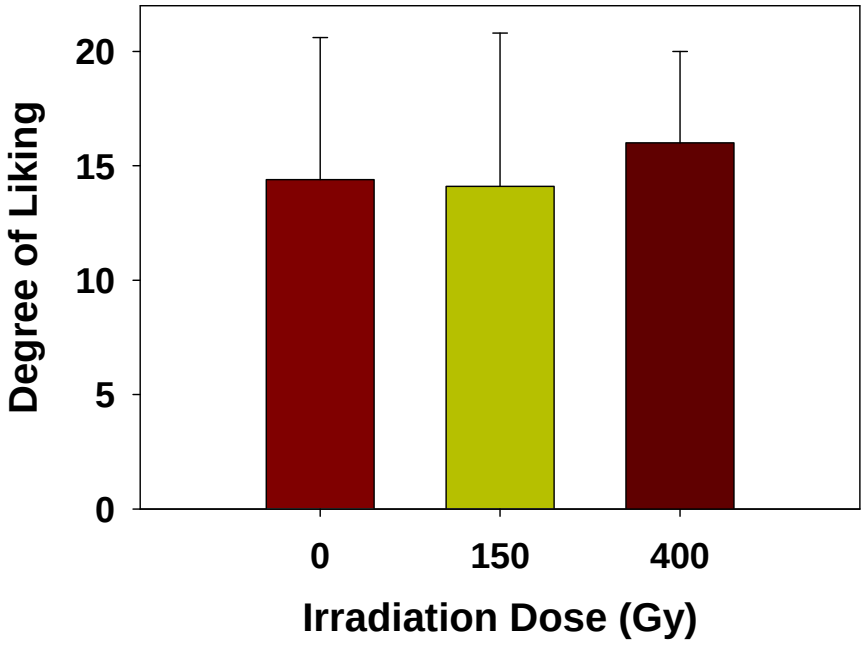

Fig. 2. The taste test panel could not differentiate between treatments, indicating that irradiation had no detectable effect on overall cherry quality (taste, texture, appearance). The 400 Gy treatment had a higher numerical average, indicating higher preference, but the results were not statistically different for any treatment.

Postharvest treatment of sweet cherries with cylinderized phosphine to control Oriental fruit fly, *Bactrocera dorsalis*, and Mediterranean fruit fly, *Ceratitis capitata*

by

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Abstract.

Phosphine (PH3) chamber fumigations were evaluated for postharvest control of Oriental fruit fly (OFF), *Bactrocera dorsalis*, and Mediterranean fruit fly (MFF), *Ceratitis capitata* in fresh sweet cherry exports from Western USA. A series of exploratory fumigations were conducted to identify the relative tolerance of OFF and MFF at ages spanning egg through larval life stages to ~1000ppmv phosphine (PH3) at treatment temperatures of 1.1 (± 1.5) ºC. In separate confirmatory fumigation trials for both species, infested cherries containing the most PH3-tolerant developmental timespan of Oriental fruit fly, 72- to 96-h old specimens (ca. 40% 2nd instar) (pre-treatment age), or Mediterranean fruit fly, 24- to 48-h old specimens (ca. 98% eggs). Specimens were buried amongst uninfested fruit in fruit bins consistent with commercial practice at load factors of ~50%, equilibrated for 12 h at fumigation temperatures of 1.1 to 4.4 (± 1.5) ºC, and then the fruit bins were fumigated at applied doses of 1000 to 1200 ppmv PH3 for 144 and 168 h for Oriental fruit fly and Mediterranean fruit fly, respectively. Fumigations resulted in 18 survivors out of 14,000 ± 1,020 ($\bar{x} \pm s$) treated Oriental fruit fly (Probit 7.9, 99.82% mortality) and 164 survivors out of 27,414 ± 7,127 ($\bar{x} \pm s$) treated Mediterranean fruit fly (Probit 7.5, 99.93% mortality) when treatment efficacy was diagnosed by the percentage of survivors emerging as pupae from fumigated cherries relative to that from non-fumigated controls.

Materials and Methods.

Insects and Fruit infestation.

Adult Oriental fruit flies (OFF), *Bactrocera dorsalis* (Hendel), and adult Mediterranean fruit fly (MFF), *Ceratitis capitata* were obtained from the Pacific Basin Agricultural Research Center in Hilo, Hawaii. Approximately 1000 females and 1000 males, ages 12- to 18-d old were placed in species respective wooden enclosures (61cm l x 41cm w x 32 cm h). Bing variety sweet cherries of varying sizes (8, 9, or 10 row) were obtained from commercial sources in Chile (ca. Dec. to Feb.), California USA (ca. May to June), or Washington USA (June to August). To simulate a naturally occurring infestation scenario, three hundred cherries (unwashed) were placed on the bottom of each enclosure in a single layer. Enclosures containing adults and sweet cherries were maintained at 24.1 ± 1.0 °C ($\bar{x} \pm s$), 61 ± 2% RH ($\bar{x} \pm rsd$), and 16:8 [L:D] h photoperiod for 24 h (12pm to 12pm).

Following the 24-h infestation period, during which there was opportunity for oviposition into

the sweet cherries, an enclosure was transferred to a walk-in cooler maintained at ~ 4.5°C. Once rendered immobile, adults were removed from the surface of each cherry using a brush before the cherries were removed from the enclosure. Infested cherries were transferred in pairs into a stainless-steel mesh ball cage (5.1-cm diameter). Caged cherries respective to an infestation were randomly selected, placed inside a pull-string cloth bag (~20 per bag), and transferred to a room for incubation at 26.6 ± 1.0 °C, 60 ± 2% RH, and 16:8 [L:D] h photoperiod to facilitate consistent progression of development across fumigation trials (ref).

For the exploratory fumigations, bags containing OFF-infested cherries were incubated for 0, 24, 96, or 120 h to yield ages, respectively, of 0- to 24-h, 24- to 48-h, 96- to 120-h, and 120- to 144-h old specimens at the start of a 12-h pre-fumigation period of temperature equilibration (i.e., tempering). Bags containing MFF-infested sweet cherries were similarly incubated for 0, 48, 96, or 120 h to yield ages, respectively, of 0- to 24-h, 48- to 72-h, 96- to 120-h, and 120- to 144-h old specimens. Infestations and subsequent incubations were synchronized so that the all ages described above, which spanned egg through larval life stages for both OFF and MFF, concurrently entered the tempering period that preceded fumigation.

For the confirmatory fumigations, infestation and incubation was planned to only yield the most-MB tolerant age of either species as determined in the exploratory fumigations (*vide infra*). Bags containing sweet cherries infested with OFF and MFF were incubated respectively 96 and 48 h prior to tempering (12 h), which yielded 72- to 96-h old OFF specimens and 24- to 48-h old MFF specimens.

Exploratory fumigations: most MB-tolerant life stage of OFF and MFF

A series of experiments was conducted determine the treatment duration, ranging from 12 to 144 h, to control egg through larval life stages of both OFF and MFF at headspace concentrations ≥ 1.5 mgL^{-1} (1000ppmv). Fumigations were conducted in modified Labonco® 28.32-L vacuum chambers housed in a walk-in environmental chamber with programmable temperature and humidity (USDA, 2010) set to treatment temperature of 1.1 ± 1.5 °C ($\bar{x} \pm s$). By varying the duration of incubation following the 24-h infestation period, five age groups of both species were isolated (*vide supra*) and then fumigated concomitantly within a chamber (8 bags per chamber). Alternatively, bags of infested cherries respective to the five age groups of both species were not fumigated and held as untreated controls to estimate the number of individuals treated during fumigation. To allow for temperature equilibration between fruit, insects, and the environmental chamber, bags of test specimens and untreated controls were transferred into the chamber 12 h prior to fumigation.

A pressure of approximately 70 mmHg was established in each chamber. Gas-tight super-syringes (Hamilton ® 500, 1000, or 1500 mL) were filled with a volume of fumigant from a cylinder of 1.6 % (v/v) PH3 balanced with nitrogen (Cytec Canada, Inc., Niagara Falls, Ontario, Canada) to achieve the requisite applied dose of ~ 1.65 mgL^{-1} (1100ppmv) as predetermined in preliminary calibration studies. A syringe was fitted to a LuerLok ® sampling valve, which was subsequently opened so that fumigant was steadily drawn into the chamber. The syringe was then removed and normal atmospheric pressure was established in each chamber before the valve was closed; this marked the beginning of the exposure period. Gas samples (40 mL) were taken temporally at standard intervals from the chamber headspace through a LuerLok® valve using a

B-D® 100 mL gas-tight syringe and quantitatively analyzed for PH3 with GC-PFPD. Fumigant concentrations were measured and exposures, expressed as concentration × time product ("CT"s) calculated by the method of Monro (1969), were tracked.

After completion of the exposure, chamber valves were opened to atmosphere and vacuum was pulled for 30 min to aerate the chamber. Chamber lids were opened and the bags of treated and non-treated specimens were collected. Sweet cherries were removed from the stainless steel mesh cages and placed on a 22 cm x 14.5 cm x 3.5 cm plastic tray (Kitahara Industries, Japan) with 0.5 L of pre-moistened OFF or MFF diet. Each tray held ~25 to 40 cherries. A tray was placed on sand layered to a depth of 0.25-cm inside of a modified fiberglass container 50cm x 31.5cm x 14.5cm with screened side windows for airflow. The container was sealed with a 1" masking tape over the seam between the cover and the top edges of the container. Containers were transferred into a rearing room maintained at ambient temperature. Containers housing the non treated control specimens were examined daily and 8 days after the first observation of pupae, the sand was screened to isolate pupae. Pupae were collected, counted, recorded, and placed into small cages and monitored for emergence of adults.

Confirmatory fumigations.

Fumigations were conducted in a steel chamber having a volume ($V_{chamber}$) of ~241.9L housed in a walk-in environmental incubator as described above set to treatment temperature of 1.1 to 4.4 (± 1.5) ºC ($\bar{x} \pm s$). Bags containing sweet cherries infested with either OFF, 48- to 72-h old specimens (ca. 70% 1st instar), or MFF, 24- to 48-h old specimens (ca. 98% eggs) were buried amongst uninfested cherries in wooden fruit bins (xl × xw × xh cm, V_L = 64.0), which were constructed out of 1.3-cm thick plywood as scaled-down replicates of those used in industry. The chamber was first loaded with four 0.5 ft^3 sand bags each wrapped in plastic packaging that displaced ~28.3 L total of chamber volume. A single fruit bin, filled to a level of ~75% capacity with bags and unifested fruit, was loaded into the chamber. The chamber load was estimated as a fractional percentage, 46.9 ± 0.7% ($\bar{x} \pm s$), of the volume occupied by the bin load relative to the displacement-corrected chamber volume (i.e., V_L $(V_{chamber} - 28.3)^{-1}$ x 100) (Monro, 1969).

Chambers loaded with a bin containing infested and uninfested sweet cherries, cherries infested with control specimens, source-gas cylinders, and gas-tight syringes were acclimated to fumigation temperature, or tempered, for 12 h prior to treatment. Fruit pulp temperature was confirmed prior to fumigation by each of three probes (YSI scanning tele-thermometer) that recorded the respective pulp temperature in three uninfested fruit distributed at different locations within bins of the infested cherries undergoing treatment. Temperature probes were then removed and chamber lids clamp-sealed in preparation for treatment.

The LuerLok ® sampling valve was opened and a stream of fumigant from a cylinder of 1.6 % (v/v) PH3 balanced with nitrogen (Cytec Canada, Inc., Niagara Falls, Ontario, Canada) was metered at 2 $mLmin^{-1}$ into the chamber to achieve the requisite applied dose of ~ 1.65 mgL^{-1} (1100ppmv) as predetermined in preliminary calibration studies (~ 2 min application time). After delivery of fumigant, the valve was closed to mark the beginning of the exposure period. Fumigant exposures were calculated as above; gas samples (40 mL) were taken from the chamber headspace through a LuerLok® valve using a B-D® 100 mL gas-tight syringe and

quantitatively analyzed for PH3 with GC-PFPD. 5 min following the dosing (i.e., initial concentration) and every day thereafter, with a 144h and 168 h final sampling for OFF and MFF, respectively. After completion of the exposure, chamber valves were opened to atmosphere and vacuum was pulled for 2 h to aerate the chamber. Chamber lids were opened, the treated and non-treated specimens collected, and the post-fumigation rearing was as described above.

Mortality evaluation.

The cumulative number of pupae that emerged in each container was counted and normalized to the number of infested fruit contained in the chamber, thus enabling an estimation of the number of untreated control specimens associated with a specific age group for a particular trial, or series of trials. Specifically, an average ($\bar{x}$) emergence from each infested fruit in the untreated control group was calculated along with a standard deviation ($\pm s$). The number of (pre-fumigation age) specimens ($n \pm s$) that were treated was estimated by multiplying the number of infested fruit treated in each trial, or series of trials, by the respective average emergence from each fruit that was infested and untreated ($\bar{x} \pm s$). The total number of age group-specific specimens that were treated across trials was estimated by summing the number from each respective trial and propagating the respective standard deviations.

To be detailed in a forthcoming publication, OFF and MFF specimens were more likely to survive and there was greater certainty in diagnosing survivorship after the treatment if incubated as described above rather than if refrigerated post-fumigation at < 5 °C under simulated commercial transport conditions, which confound the effect of a fumigation event on mortality. In general, refrigeration increases in the mortality of all OFF and MFF life-stages, the length of the developmental periods of each life-stage, and heterogeneity in the times required for completion of development within each life-stage.

Chemical Analysis and Calibration of Standards.

A 300-lb cylinder of 1.6 % (v/v) PH3 balanced with nitrogen was obtained from Cytec Canada, Inc. (Niagara Falls, Ontario, Canada) and used as the source for gas chromatography calibrations as well as fumigations.

PH3 levels in headspace of fumigation chambers were measured using gas chromatography; retention time (PH3, $t_r = 3.2 \pm 0.2$min) was used for chemical verification and the integral of peak area, referenced relative to liner least-squares analysis of a concentration – detector response curve, was used to determine concentration. Detector response and retention indices were determined each day in calibration studies by diluting known volumes of gases into volumetric gas vessels. PH3 analyses were with a Varian 3800 and splitless injection (140 °C) using a gas sampling port with a 10 µL-sample loop, a Teflon column (L = 2 m, OD = 2 mm) packed with Porpak N (80/100 mesh) held at 130 °C for 10 min, and a PFPD detector (13 mL/min H_2, 20 mL/min air, and 10.0 mL/min N_2 make-up) at 250 °C that received only 10% of the 15 ml He/min column flow.

Results and Discussion.

Most MB-tolerant life stage of OFF or MFF.

Cherries infested with OFF and MFF were fumigated for varying times with ~1000ppmv PH3 at NAP and 1.1 (± 1.5) ºC, "benchmark" conditions for the PH3 fumigation of fresh fruit. Probit regressions of the time-mortality response were modeled using Polo Plus (LeOra Software, 2002-2007) and used to quantify the relative efficacy of PH3 fumigations toward OFF and MFF in isolated groups over the ages 0- to 144-h old (pre- tempering age), ages that span the egg through larval life stages for both species. Figure 1 and Figure 2 respectively show for OFF and MFF the number of specimens treated, the number of control specimens included as a natural response in the model, the regression heterogeneity (H), the projected times to cause 50, 95, and 99% mortality in the treated population (respectively LT_{50}, $LT_{99,}$ and LT_{P9}), and the corresponding estimates of the upper (UL) and lower limits (LL) at the 95% level of confidence (LOC) (Finney, 1944 & 1971).

Lethal exposure ratios (LERs) were calculated with 95% LOC intervals and used to identify difference in PH3-tolerance within species. For OFF, likelihood ratio-based hypothesis testing of equality and parallelism were rejected (equality: $P < 0.05$, $\chi^2 = 0.281$ E +04, $df = 6$; parallelism: $P < 0.05$, $\chi^2 = 848$, $df = 3$), indicating that the slopes and the intercepts were not the same. The greater tolerance of 72- to 96-h old (pre- tempering age) OFF toward PH3 relative to 24- to 48-h, 48- to 72-h, and 96- to 120-h old specimens was established, as the LERs were >1 for all exposures projected to cause > 90% mortality in the treated populations. For MFF, ratio-based hypothesis testing of equality and parallelism were again rejected ($P < 0.05$, χ^2: 16.67, df: 6; parallelism: $P < 0.05$, $\chi^2 = 14.33$, $df = 3$). The greater tolerance of 24- to 48-h old (pre- tempering age) OFF toward PH3 relative to, 48- to 72-h, 72- to 96-h, and 96- to 120-h old specimens was established, as the LERs were >1 for all exposures projected to cause > 90% mortality in the treated populations.

On five separate occasions as presented below in figure 3, the probability of a OFF or MFF life stage being present just before fumigation was determined by dissecting samples of infested sweet cherry until the life stage of ~ 1000 specimens was evaluated ($\bar{x} \pm s$; egg, 0.011 ± 0.006; 1st, 0.058±0.018; 2nd, 0.226 ± 0.036; 3rd, 0.651 ± 0.035; pupa, 0.051 ± 0.015). Fumigation (and corresponding pre-treatment equilibration) of SWD-infested sweet cherries conducted at temperature < 13.9 °C resulted in the occurrence of relatively fewer pupae at time of dissection.

Confirmatory fumigations.

In separate confirmatory fumigation trials for both species, infested cherries containing the most PH3-tolerant developmental timespan of Oriental fruit fly, 72- to 96-h old specimens (ca. 40% 2nd instar) (pre-treatment age), or Mediterranean fruit fly, 24- to 48-h old specimens (ca. 98% eggs). Specimens were buried amongst uninfested fruit in fruit bins consistent with commercial practice at load factors of ~50%, equilibrated for 12 h at fumigation temperatures of 1.1 to 4.4 (± 1.5) ºC, and then the fruit bins were fumigated at applied doses of 1000 to 1200 ppmv PH3 for 144 and 168 h for Oriental fruit fly and Mediterranean fruit fly, respectively. Fumigations resulted in 18 survivors out of 14,000 ± 1,020 ($\bar{x} \pm s$) treated Oriental fruit fly (Probit 7.9, 99.82% mortality) and 164 survivors out of 27,414 ± 7,127 ($\bar{x} \pm s$) treated Mediterranean fruit fly (Probit 7.5, 99.93% mortality) when treatment efficacy was diagnosed by the percentage of

survivors emerging as pupae from fumigated cherries relative to that from non-fumigated controls.

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Walse, SS, Krugner, R, & Tebbets, JS 2012bThe postharvest treatment of sweet cherries with methyl bromide to eliminate the spotted wing drosophila, *Drosophila suzukii*. California Cherry Advisory Board and Northwest Horticulture Council report for USDA-APHIS and Biosecurity Australia, February 17, 2012.

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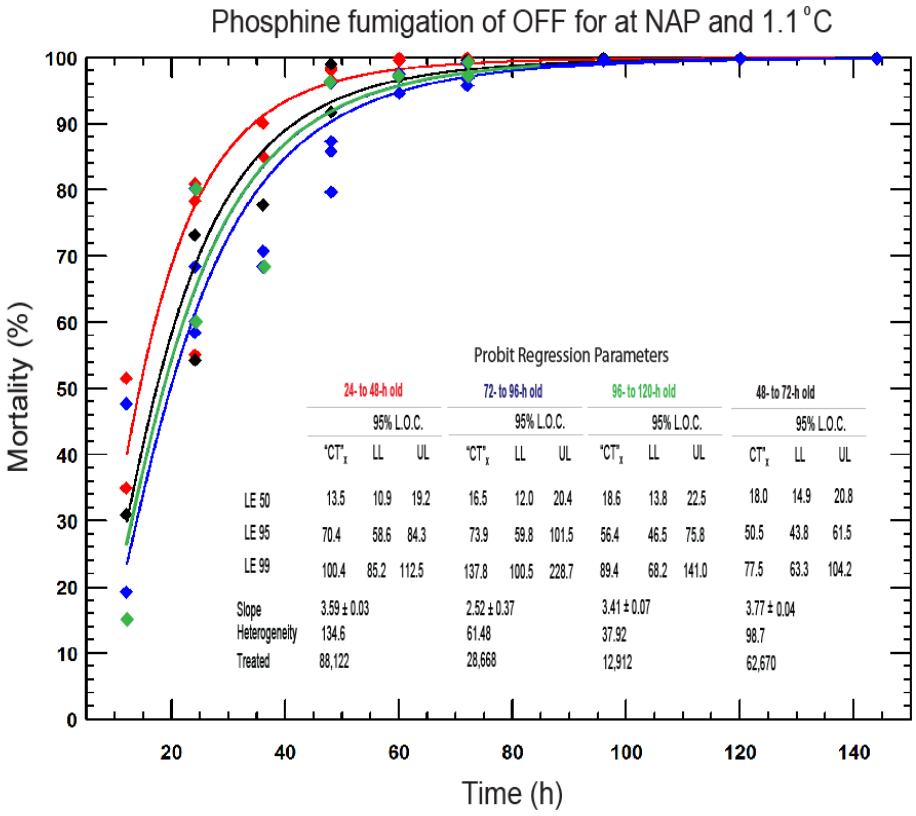

Figure 1. Mortality of Oriental fruit fly (OFF) following phosphine (PH3) fumigation at 1.1 ± 1.5 °C and normal atmospheric pressure (NAP) with corresponding probit regression analyses (Polo Plus, LeOra Software, 2002-2007) of the time-mortality response for distinct age groups showing the number of specimens treated, the regression heterogeneity (H), the projected CT exposures to cause 50, 95, and 99% mortality in the treated population (respectively LE_{50}, LE_{90}, and LE_{99}), and the corresponding estimates of the upper (UL) and lower limits (LL) at the 95% level of confidence (LOC).

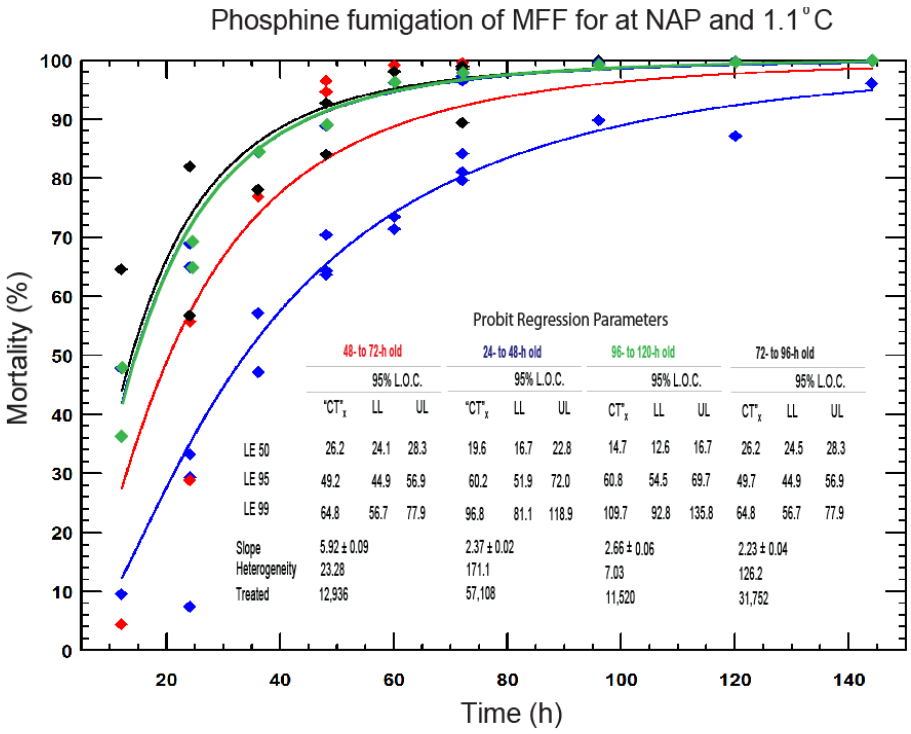

Figure 2. Mortality of Mediterranean (MFF) following phosphine (PH3) fumigation at 1.1 ± 1.5 °C and normal atmospheric pressure (NAP) with corresponding probit regression analyses (Polo Plus, LeOra Software, 2002-2007) of the time-mortality response for distinct age groups showing the number of specimens treated, the regression heterogeneity (H), the projected CT exposures to cause 50, 95, and 99% mortality in the treated population (respectively LE_{50}, LE_{90}, and LE_{99}), and the corresponding estimates of the upper (UL) and lower limits (LL) at the 95% level of confidence (LOC).

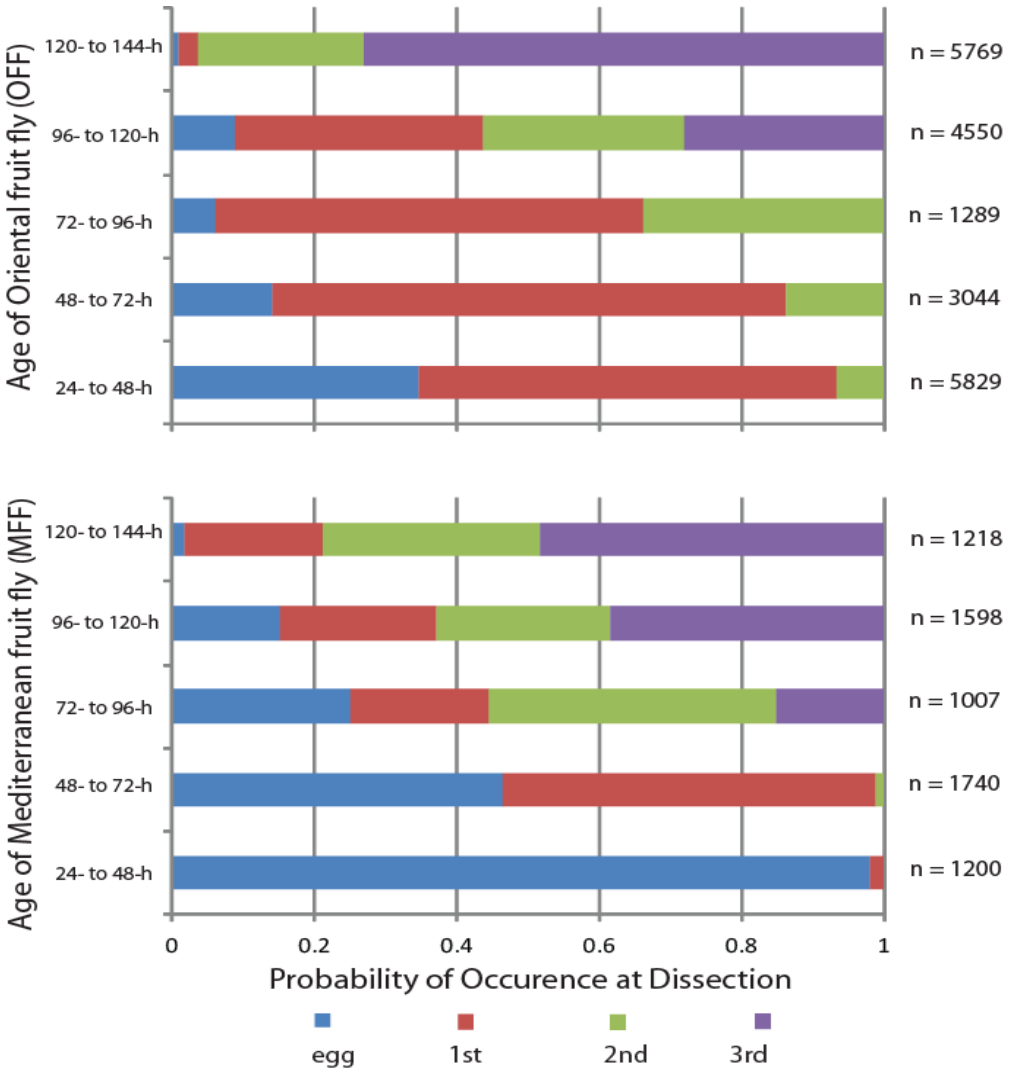

Figure 3. Sweet cherries were removed from OFF and MFF-containing enclosures after a 24-h ovipositional period and maintained under rearing conditions (24-27 °C, 80% RH, 16:8 [L:D] h) for an additional time so that fruit was infested with discrete ages corresponding to egg through 3rd instar larvae life stages Prior to conducting fumigation, the average probability of each life stage present in infested cherries was determined by dissecting samples of infested fruit until ~1000 specimens were evaluated.

CALIFORNIA CHERRY BOARD

PROJECT PLAN / RESEARCH GRANT REPORT

Work group / Department: USDA-ARS-SJVASC, Crop Protection and Quality Unit

Project Year: 1 (2014) Anticipated Duration of Project: 2 year

Postharvest systems-based treatment of California sweet cherries for Brown Marmorated Stink Bug

Project Leader:

Spencer S. Walse: USDA-ARS-SJVASC, 9611 S. Riverbend Ave, Parlier, CA 93648,
(559) 596-2750, fax (559) 596-2792, spencer.walse@ars.usda.gov

Cooperating Individuals:

Current Funding Request: $7,198

BACKGROUND/JUSTIFICATION

Although the economic consequence(s) of this pest are unknown, brown marmorated stink bug (BMSB) is likely to be a pest of concern to tree fruit growers in California USA. The purpose of this investigation is to verify elimination of BMSB from the marketing channel following the commercial packing process.

Long-term research goal. The overreaching goal of this project is to ensure pest-free high-quality prunes are channeled to markets.

Short-term research goal. Prove that postharvest cleaning and packing of BMSB-infested sweet cherries effectively remove this pest from the marketing channels.

2013 OBJECTIVES:

This project is planned in phases as indicated below. Each phase will have its own goals and these goals will feed those of the following phase.

Phase I. Establish a BMSB colony at UC Davis Contained Research Facilty with the throughput necessary to routinely conduct fumigation studies.

Timeline: Work already accomplished.

Phase II. Insects will be exposed to elements of postharvest processing individually and in series; statistically robust data on removal and/or mortality will be generated. Specifically, BMSB eggs will be deposited on leaves that will then be traced through a packing operation. BMSB postembryonic life stages will be placed on fruit and leaves and traced through a packing operation.

Timeline: 2014 production season

Systems approach for control of brown marmorated stinkbug, *Halyomorpha halys*, in California sweet cherries

by

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Abstract.

Brown marmorated stink bug (BMSB), *Halyomorpha halys*, is an insect of concern to certain countries that import California sweet cherries. The removal and/or mortality of BMSB as cherries are harvested, cleaned, packed, fumigated, and shipped was evaluated in support of a systems-based approach to meet the requirements of quarantine security. Post-embryonic life stages of BMSB (1^{st}-5^{th} instar & adult) were removed from fruit that is dunked or soaked, as part of commercial protocols for cleaning and packing California cherries. Moreover, the efficacy of postharvest methyl bromide fumigation was evaluated and indexed relative to exposures typically observed for exports to Japan, Korea, and Australia. Future work will evaluate the cumulative effect of consecutive postharvest cleaning, fumigation, and packing events as "systemic" joint probabilities of BMSB removal and mortality prior to the entrance of fruit into export marketing channels. This research can be provided to regulators and trading partners to quantify the reduction in risk/threat of BMSB as sweet cherries are moved from production areas through packing operations toward export markets.

Keywords: Brown marmorated stink bug, systems-approach, methyl bromide fumigation

Materials and Methods.

***Insects and Mortality*.**

BMSB (*Halyomorpha halys*) eggs were obtained from the laboratory colony of Dr. Tracey Leskey (USDA-ARS-Kearneysville, WV) that originated from wild specimens captured in small fruit and orchard crops in West Virginia, USA. Upon receipt of the BMSB eggs in Oct. 2012 at the Contained Research Facility at UC Davis (Davis, CA), a BSL-III agricultural quarantine facility, all specimens were transferred to an environmentally-controlled chamber set at 26 °C, 65% RH and a 16:8 diurnal light cycle until a strong colony of mixed life stages could be established. A second shipment of eggs was received from Dr. T. Leskey in March 2013 to supplement the CA colony.

Currently, all rearing is conducted within a series of 0.65-m^3 Bug Dorm™ (Bug Dorm 2400 series) enclosures containing live bush bean and cowpea plants (ca. 2 of each species) and trays filled with a various mixtures of almonds, pumpkin seeds, sunflower seeds, and walnuts. Enclosures are maintained inside a greenhouse at 26 °C and 65% RH. Upon reaching maturity, adults are removed and transferred to an oviposition enclosure, where adults of all ages are reared concurrently at an average of 200 adults per enclosure, though numbers fluctuate. Adult females lay an average 13.3 eggs per day on plant leaf surfaces and/or sheets of wax paper, or an average of 93 egg clusters total each week. Eggs are collected every 48 to 72 h, to prevent egg predation, over a course of a 7-d period and transferred to a separate enclosure, thereby yielding an enclosure with immature stages over a specific range of ages.

Dunking.

The removal of BMSB (1^{st}-5^{th} instar & adult) from the surface of fruit was examined after fruit were submerged into water, or dunked. BMSB eggs were not included in these studies because they are localized on leaves, which do not enter the export channels of sweet cherries. In a series of preliminary studies it was recognized that the ability of BMSB to remain on the surface of the fruit was inversely related to size (and age), therefore only the relatively small 2^{nd} instar life stage was used in subsequent studies. BMSB (n = 5) were collected into 15-dram clear plastic vials. Specimens were gently tapped from the vial onto the surface of a wet cherry, causing them to loosely stick to the surface. Infested fruit were submerged into soak tank water (~100 ppm calcium hypochlorite and 3% sodium bicarbonate), held for either 1 s under water, removed from the water, and then evaluated for the efficiency of BMSB removal.

Soak tanks.

To simulate packing house soak troughs used in the commercial cleaning and routing of cherries, at least with respect to protocols used in California, two 31-gallon plastic storage bins (“Rugged Tote”, Centrex Plastics LLC, model number 314141) were modified (Figure 3). The ends of each tank were outfitted with bulkhead fittings (Grainger Inc., item # 1MKH7) with 3/4 inch male barb threads to attach clear Tygon□□hose (3/4 “id, 1” od, Saint-Gobaine AJC00053) secured with band clamps. Inside the tank, 90 degree “L”-fittings were attached to the bulkheads to circulate flow, and the floating fruit, as in a packing house scenario. A utility transfer pump (ZOELLER model 314-0002, portable, self priming, 115 volt AC motor, Grainger Inc. item number: 4HEX4), equipped with 3/4 inch male barb/threaded fittings as above, joined the in-flow and out-flow hoses of each tank and had a maximum flow rate of ~20 gpm (gallons per minute). The tanks were also equipped as necessary with an in- line ‘point of use’ water heater (American Water Heaters brand, 110V, “Tiny Titan” model) in series between the out-flow hose and the recirculation pump. Tanks were fitted with white polywall vinyl coverings, the inside of which was coated with a thin layer of Tangle-Trap (Tanglefoot Inc.) using a putty trowel. The purpose of these “sticky-lids” (Figure 3) was to trap BMSB that attempted to escape the tank.

Tanks were filled with solutions of either 100 ppm chlorine (calcium hypochlorite) and 3% sodium bicarbonate or tap water that were maintained at ~35°F. Once the solutions were added to respective tank, the circulation

pumps were turned on, cherries (ca. 100 to150) were added to each, and groups of 15 BMSB were collected into a 15- dram clear plastic vial. One vial containing BMSB was submerged and shaken to remove insects and then the tank was immediately covered with the "sticky lid". Specimens were not introduced on the fruit surface as described above for dunking because in preliminary studies ~800 specimens were removed within 10 s of introduction. After 1 hour, the lids were detached and the ability of the BMSB to escape the solution was assayed by recording the number of specimens found on the lid and/or inside walls ("sides"). Between assays, BMSB were removed from the system whenever visible.

Fumigation.

Six life stages (1^{st} – 5^{th} instar nymphs, and adults) of BMSB were evaluated in the exploratory fumigations. Nymphs (1^{st} – 5^{th} instars) and adults were randomly collected from respective rearing cages described above. Samples from each life stage were then isolated in 15-dram vials with mesh-screen covered openings on the top, bottom and sides (2 locations) and placed into cloth bags (8" x 12"; ULine, Waukegan IL). Several pumpkin seeds and a wetted cotton wick were placed in to the cages to serve as food and water sources, respectively. Cloth bags containing vials of the life stages to be treated, were placed inside the environmental room, housing the fumigation chambers, for tempering.

Exploratory fumigations were performed in modified Labonco® 28.32-L vacuum chambers housed in a walk-in environmental incubator with programmable temperature and humidity (USDA, 2009). A series of experiments was conducted determine the relative tolerance of BMSB life stages to methyl bromide (MB) at headspace concentrations 12.0 ≤ [MB] ≤ 144 $mgL^{-1}h$ at 10.4 ± 0.5°C ($\bar{x} \pm s$). Chambers loaded with BMSB cages (treated specimens), untreated control specimens, source-gas cylinders, and gas-tight syringes were equilibrated to treatment temperature (i.e., tempered) for at least 12 h prior to fumigation. Chamber temperature was confirmed prior to fumigation by a HOBO data logger (HOBOware version 2.7). Temperature probes were then removed, circulation fans internal to the chamber were turned on, and chamber lids clamp-sealed in preparation for treatment.

A slight vacuum of approximately 76 to127 mmHg was established in each chamber. Gas-tight super-syringes were filled with a volume of MB to achieve the requisite dose (8 to 72 mgL^{-1}) as predetermined in preliminary studies. Requisite syringes were fitted sequentially to a LuerLok ® sampling valve, which was subsequently opened so that MB was steadily drawn into the chamber. After the addition of MB from the final syringe, the syringe was removed from the valve and normal atmospheric pressure (NAP) was reestablished in each chamber before the valve was closed; this marked the start of the fumigation and the beginning of the exposure period. Gas samples (40 mL) were taken from the chamber headspace through a LuerLok® valve using a B-D® 100-mL gas-tight syringe and [MB] was quantified with GC-FID, as described above, at temporal intervals. Fumigant exposures were expressed as concentration (*C*) × time (*t*), *Ct*, cross products and calculated by the method of Monro (1969).

After the exposure period, chamber valves were opened to atmosphere and vacuum was pulled for 2 h to aerate the chamber. Chamber lids were opened; the treated as well as untreated control specimens were collected and transferred into an incubator at 27.0 ± 1.0 °C and 80 ± 2% RH ($\bar{x} \pm s$). Mortality of specimens was assessed at daily intervals for 7 days following treatment. Mortality was diagnosed visually by lack of locomotion or by prodding-induced motion. Treated specimens were categorized as moribund if the mortality was inconclusive. Control mortality was diagnosed similarly and was assumed to be equal to that in fumigation trials and was treated numerically using Abbott's method (1925) as described by Finney (1944 and 1971).

Chemical analysis and calibration of standards.

A 50-lb cylinder of compressed MB, Meth-o-gas 100, was obtained from Cardinal Professional Products (Woodland, CA, USA). MB concentration in headspace of fumigation chambers, [MB], was measured using gas chromatography (GC) with flame ionization detection (FID) (GC-FID); retention time (MB, t_r = 3.2 ± min) was used for chemical verification and the integral of peak area, referenced relative to liner least-squares analysis concentration plotted versus detector response, was used to determine concentration. Detector response and retention indices were determined each day in calibration studies by diluting known volumes of gases into

volumetric gas vessels. Analyses were with a Varian 3800 GC and splitless injection (150 °C) using a gas sampling port (110 °C) with a 1 mL-sample loop, a 2 mm id x 2 m Teflon® column packed with 10% OV-101 on Gas-Chrom Q® (100/120 mesh) held at 100 °C for 10 min, and 15 $mlmin^{-1}$ He carrier flow. The FID detector was at 275 °C with respective flows of 20 $mLmin^{-1}$ H_2, 250 $mLmin^{-1}$ air, and 5.0 $mLmin^{-1}$ N_2 make-up.

Results and Discussion.

Dunking.

The effect of bin drenches on BMSB removal was simulated by dunking infested fruit in soak tank water for 1 s and all but 7 out of 200 specimens were removed. Results suggest that drenching a bin will not completely remove BMSB, or at least the 2nd instar life stage, from the surface of a fruit in the bin load (Table 1).

Soak tank.

BMSB were circulated in sealed soak tanks containing fruit and either ambient chlorine solution or ambient tap water with 2.0 or 1.5%, respectively, found and presumed alive on the sides or lid of the soak tank (Table 2). Only treated individuals having potentially escaped the surface tension of the bulk solution during 1 hour exposures were accounted for in post treatment evaluations, indicating that the wash solution itself was the primary reservoir for BMSB in these studies. These results support the conclusion that treated individuals sink and/or are physically destroyed by the crushing and circulating mechanics of the soak tank system. The difference in physical distribution and mortality of BMSB in ambient chlorine versus ambient tap water were tested for significance against the null hypothesis that the solution composition was unimportant. At the 95% level of confidence, the results were not significantly different using analysis of variance (Prob > $F \geq 0.05$). Results support the conclusion that the efficacy with which soak tanks remove BMSB from the surface of cherries, and the resulting mortality, result from physical entrapment by the water and drowning, rather than toxicological properties of the solution.

It is critical to note that no BMSB were found on the fruit in any of the soak tank scenarios, which indicates soaking infested fruit was effective at removing BMSB from the fruit surface as well as eliminating the return of BMSB to the fruit surface over the course of soaking.

Fumigation.

Phytosanitary protocols to permit the international movement of a commodity typically specify that treatment efficacy be demonstrated on the most treatment-tolerant life stage of the offending pest (Jang and Moffitt 1994, NAPPO 2011). To identify the relative MB-tolerance of BMSB life stages were fumigated concomitantly in exploratory fumigations conducted for 2 h at 10.4 ± 0.5 °C ($\bar{x} \pm s$). Exposure-mortality regressions were modeled using Polo Plus (LeOra Software, 2002-2007) including the response over the range, 7 to 128 mg L^{-1} h (Figure 1). The estimated number of specimens treated, the number of control specimens included as a natural response in the model, the regression heterogeneity (H), the projected *Ct* exposures to cause 50, 95, and 99% mortality in the treated population (respectively LE_{50}, LE_{95}, LE_{99}), and the upper (UL) and lower limits (LL) of the 95% level of confidence (LOC) are shown in Figure 1 (Finney, 1944 & 1971). Likelihood ratio-based hypothesis testing of equality ($P < 0.05$, $\chi^2 = 54.2$, $df = 10$) and parallelism ($P < 0.05$, $\chi^2 = 24.5$, $df = 5$) were rejected, indicating that the slopes and intercepts of the respective regression lines were not similar. Lethal exposure ratios (LERs) were calculated with 95% LOC intervals and used to identify difference in MB-tolerance across life stages. At 10.4 ± 0.5 °C, LERs paralleled a ratio of 1 (± 0.5) across exposures projected to cause 10 to 99% mortality, indicating that these all life stages are of equivalent MB-tolerance.

Sweet cherries exported from Western USA to Japan, Korea, and Australia are fumigated with MB to control spotted wing drosophila (SWD). Walse et al. (2012b) reported the relationship between the applied MB dose, corresponding "CT" exposures, and treatment efficacy for fumigations lasting 2 h with load factors of 30%. To maintain a threshold of treatment efficacy for SWD ≥ 99.9968 %, applied doses (mg L^{-1}) were increased incrementally as treatment temperatures (T) were lowered across the range frequently used by industry per the

schedule: 40 mg L^{-1},T ≥ 17.2 ºC ; 48 mg L^{-1}, 17.2 ºC ≥ T ≥ 13.9 ºC; 56 mg L^{-1}, 13.9 > T ≥ 12.2 ºC; 64 mg L^{-1}, 12.2 > T ≥ 10.6 ºC; and 72 mg L^{-1}, 10.6 > T ≥ 8.3 ºC. It is critical to note that fumigation of sweet cherries for 2 h at 10.4 ± 0.5 °C ($\bar{x} \pm s$), the conditions used in the exploratory fumigations, yield an exposure of 109.0 ± 12.0 $mgL^{-1}h$. Results suggest that > 99% mortality of BMSB would result from such a fumigation, regardless of the life stage present.

Cumulative systems evaluation.

Future work, year 2 of the proposed project, will focus on developing the above data into a systems approaches to quarantine security. Systems approaches to quarantine security have been defined as "the integration of those pre- and post-harvest practices used in production, harvest, packing and distribution of a commodity which cumulatively meet the requirements of quarantine security" by Jang and Follett (Jang and Moffitt, 1994). The general rule for the multiplication of probabilities, expanded in the seminal work of Finney (1948) and Rosenthal (1978) on combining results (probabilities) of independent events, can be used to quantify the cumulative effect of consecutive postharvest cleaning and packing events on the "systemic" joint probabilities of BMSB removal and mortality.

For each cleaning and/or packing "event", the observed likelihood (expressed as a percentage) of finding a live BMSB after treatment, the theoretical percentage of BMSB removal and/or mortality can becalculated at the 95% LOC by the method of Couey and Chew (1986), along with the associated probability, the respective Probit values at the 95% LOC, and the confidence interval associated with Probit 9 treatment efficacy as calculated by Liquido and Griffin (2010).

In the case where one event, E_1, has no effect on the probability of the other(s), the joint probability of BMSB removal/mortality associated with multiple treatment events, $P(E_{1+}E_{2+}En)$, can be calculated from the multiplication of the simple probability of each event (Finney, 1948):

$$P(E_{1+}E_{2+}En) = 1-(1-P(E_1))(1-P(E_2))(1-P(En)) \qquad \text{(Eq.1)}$$

Given equation 1, the special multiplication rule for independent events, the probability of live BMSB remaining on the surface of fruit following the joint occurrence of two or more treatment events can be calculated for numerous scenarios directly applicable to commercial citrus cleaning and packing procedures used in California (Couey and Chew 1986; Follet and Neven 2006).

An alternative approach to calculating the joint probability of multiple treatments, $P(E_b|E_a)$, involves multiplying the simple probability of the first event times the conditional probability of the second event, E_b, given the first, E_a :

$$P(E_b|E_a) = \frac{P(E_a \text{ and } E_b)}{P(E_a)} \qquad \text{(Eq. 2)}$$

It is critical to note that even greater mortality and/or removal of BMSB would be expected if a pair or series of events was evaluated conditionally (equation 2) versus independently (equation 1), because treatments often render biological effects whereby those surviving treatment are not fully healthy, and are thus more susceptible to the subsequent treatment (Finney, 1948).

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Table 1. Dunking the infested fruit in water for 1 s removed all but 7 out of 200 specimens.

Dunking fruit			
Trial Number	Number of BMSB Used	Number Still on Fruit	Number LIVE on Fruit
1	5	0	0
2	5	0	0
3	5	0	0
4	5	1	1
5	5	0	0
6	5	0	0
7	5	0	0
8	5	0	0
9	5	1	1
10	5	0	0
11	5	0	0
12	5	0	0
13	5	1	1
14	5	0	0
15	5	0	0
16	5	0	0
17	5	0	0
18	5	0	0
19	5	1	1
20	5	0	0
21	5	0	0
22	5	0	0
23	5	0	0
24	5	0	0
25	5	0	0
26	5	0	0
27	5	0	0
28	5	1	1
29	5	0	0
30	5	1	1
31	5	0	0
32	5	0	0
33	5	1	1
34	5	0	0
35	5	0	0
36	5	0	0
37	5	0	0
38	5	0	0
39	5	0	0
40	5	0	0
TOTALS:	200	7	7
PERCENTS:		3.5	3.5

Table 2: Efficacy of BMSB removal from fruit subjected to recirculation soak tanks containing tap water or a solution of 100 ppm chlorine (calcium hypochlorite) at 34°F (operation temperature).

Ambient Chlorine		
Trial Number	Number of BMSB Used	BMSB Escaping (lid + live sides)
1	5	0
2	5	0
3	5	0
4	5	0
5	5	1
6	5	0
7	5	0
8	5	0
9	5	0
10	5	0
11	5	0
12	5	0
13	5	0
14	5	1
15	5	0
16	5	0
17	5	0
18	5	0
19	5	0
20	5	0
21	5	0
22	5	0
23	5	0
24	5	0
25	5	1
26	5	0
27	5	0
28	5	0
29	5	0
30	5	0
31	5	0
32	5	0
33	5	0
34	5	1
35	5	0
36	5	0
37	5	0
38	5	0
39	5	0
40	5	0
TOTALS:	200	4
PERCENTS:		2.0

Ambient Water Only		
Trial Number	Number of BMSB Used	BMSB Escaping (lid + live sides)
1	5	0
2	5	0
3	5	0
4	5	0
5	5	0
6	5	1
7	5	0
8	5	0
9	5	0
10	5	0
11	5	0
12	5	0
13	5	0
14	5	0
15	5	0
16	5	0
17	5	0
18	5	0
19	5	0
20	5	0
21	5	0
22	5	0
23	5	0
24	5	0
25	5	1
26	5	0
27	5	0
28	5	0
29	5	0
30	5	0
31	5	0
32	5	0
33	5	0
34	5	0
35	5	1
36	5	0
37	5	0
38	5	0
39	5	0
40	5	0
TOTALS:	200	3
PERCENTS:		1.5

Figure 1. Probit analysis of the exposure-mortality response for brown marmorated stink bug life stages following exploratory fumigations with methyl bromide (MB) for 2 h at treatment temperature of 10.4 ± 0.5°C ($\bar{x} \pm s$).

Probit Regression Parameters

	1st instar			2nd instar			3rd instar			4th instar			5th instar			adult		
		95% LOC			95% LOC			95% LOC			95% LOC			95% LOC			95% LOC	
	Ct_x	LL	UL	Ct_x	LL	UL	Ct_x	LL	UL	Ct_x	LL	UL	Ct_x	LL	UL	Ct_x	LL	UL
LE50	43.2	34.1	51.3	41.0	29.4	47.1	47.8	33.3	54.3	39.4	33.2	45.0	31.4	27.1	35.7	38.9	32.4	44.3
LE95	91.4	75.7	122.8	61.2	52.9	91.9	74.8	64.6	124.5	68.7	58.3	92.7	74.3	61.9	97.6	61.3	52.6	83.4
LE99	124.5	98.0	189.5	72.3	60.0	136.5	90.1	73.5	202.9	86.6	70.1	131.5	106.1	83.5	154.4	73.9	60.9	114.4
Slope	5.1 (+/-) 0.5			9.5 (+/-) 2.4			8.5 (+/-) 2.2			6.8 (+/-) 1.1			4.4 (+/-) 0.5			8.3 (+/-) 1.3		
Heterogeneity	2.7			1.3			1.3			1.2			1.1			1.8		
Treated (cont.)	498 (79)			357 (52)			211 (34)			186 (30)			245 (37)			259 (44)		

Past, present and future *Drosophila suzukii* distribution, impact and management in United States berry fruits

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Abstract

***Drosophila suzukii*, Spotted Wing Drosophila (SWD), is a key insect pest threatening the long-term sustainable production of commercial small fruits in the United States of America. *D. suzukii* is a key pest in all major production areas in the USA. Fruit damage because of SWD larval development has resulted in a major change in current production practices of berry fruit in the USA. These changes have resulted in significant increases in production costs of berry fruit. *D. suzukii* is highly adaptable and can be found to survive in a range of climates as represtend by key production regions. Extensive studeis have been conducted on monitoring techniques, however trapping and fruit monitoring provide little value as an early warning tool. Population estimations have resulted in an additional tool that may be used as such a tool and these estimates can also be used to direct timing and impact of different management trechniques. Current biological control studies using pupal and larval parasitoids are emerging. These studies however show limited impact of such parasitioids in affected production regions. Studies in native regions of *D. suzukii* occurrence however indicate the promise of future use of such parasitoids.**

INTRODUCTION

Drosophila suzukii Matsumura (Diptera: Drosophilidae, SWD) is an economic pest of small and stone fruit in major and specialty production areas including North America, Asia and Europe (Cini et al., 2012; Goodhue et al., 2011; Walsh et al., 2011).

Female *D. suzukii* lay eggs in suitable ripening fruits enabled by a serrated ovipositor (Walsh et al., 2011). Generally other drosophilids do not have this capability, which lay their eggs in overripe or previously damaged fruit (EPPO 2013; Tochen et al 2014). Often multiple developing fruit fly larvae render affected fruit unmarketable and reduce processed fruit quality. In Western US production areas, *D. suzukii* damage may cause up to $500 million in annual losses assuming 30% damage levels (Goodhue et al., 2011), and $207 million in Eastern US production regions (NCCE, 2014). Worldwide, the potential economic impacts of this pest are staggering. United States cultivated blueberry, blackberry, raspberry, strawberry, and sweet cherry are valued in aggregate at over $4.367 billion (USDA NASS, 2013). National crop loss from SWD in the United States has been estimated to potentially exceed $718 million annually, and costs directly related to management practices are estimated to vary between $129 and 172 million (6 to 8% of farmgate value), annually (Bolda et al., 2010; SWD SCRI Stakeholder Workshop 2013). These records clearly illustrate the importance of *D. suzukii*. The purpose of this paper is to provide a short review of overwintering and seasonal phenology in differing climates, highlight currently used monitoring methods, describe the current conventional management techniques employed by producers, and the current status of biocontrol by resident parasitoids.

Seasonal population patterns based on trapping

As expected, seasonal trap captures using apple cider vinegar in clear deli cups differ depending on the production region. In mild regions like the San Joaquin Valley (SJV) of California, trap counts start earlier than cooler regions like the Willamette Valley (WV) (Dalton et al., 2011; Wiman et al., 2014). High mid-summer temperatrues in the SJV result in population decreases. During the latter portion of the season in the SJV the trap counts again start to increase, and eventually decrease at the onset of the cooler winter period. Unlike the SJV, in the WV SWD populations increase to a peak during the latter portion of the season and then decrease during October during the onst of the cooler winter period. In regions where colder winter temperatures are experienced such as Eastern Washington (EW) and Michigan (MI). *D. suzukii* are found in traps much later during the growing season. When comparing EW and MI, *D. suzukii* were caught in traps much later than EW, as well as the WV and the SJV. caught in traps in ifferent seasonal patterns. When comparing trap captures between the WV and much milder winter climates found in Wilmington, North Carolina (NC) (Wiman et al 2014), early season *D. suzukii* pressure as indicated by trap counts is comparable to that of the SJV. In NC pest populations however continue to increase as indicated by both trap counts and population modeling (Wiman et al., 2014). Preliminary studies in Oregon and California suggest that SWD overwinter as adults, although detailed overwintering biology is still unclear (Dalton et al., 2011; Walsh et al., 2011). There is increasing evidence that *D. suzukii* may seek refuge in more suitable as a mechanism to avaoid unsuitable cold winter temperatures. Preliminary work on cold-adaptation indicate that this phenomenon may aid the survival of adult flies during unsuitable periods.

Monitoring

Effective sampling methodology for *D. suzukii* is lacking despite extensive efforts to improve trap methodology or determine effective fruit infestation sampling protocols. Trapping should aid growers in the timing of management, and enable more judiciuos use

of insecticides. Most growers and scientists use traps baited with apple cider vinegar or a combination of sugar-water and yeast to monitor adult *D. suzukii* flight (Cha et al., 2012; Landolt et al., 2012; Lee et al., 2012). Trapping is however not standardized and it is questionable how much is gained from a management timing perspective. Monitoring, using traps is labor intensive and in many cases the costs do not justify the benefits for many growers. Trap data has often not provided a reliable warning against *D. suzukii* attack (Wiman et al., 2014), especially for susceptible crops in high-density population areas (Lee et al., 2011; 2012). Monitoring fruit infestation levels to guide management may also be impractical as it is unclear how many samples would be needed to accurately determine infestation levels. Additionally, by the time that larvae are detected in fruit, it may be too late to manage as damage has already occurred. No detailed studies could be found using monitoring for fruit infestation for this pest, and precision of sampling methodology is currently unavailable.

Chemical and cultural control

Pesticide applications have been a key control tactic for *D. suzukii* in North America and in Europe. Effective materials are used to target gravid females and include pyrethoids, carbamates, and spinosyns (Cini et al., 2012, Beers et al., 2011, Bruck et al 2011). Applications are timed to prevent oviposition on ripening host crops (Beers et al., 2011; Bruck et al., 2011). Many growers in the Pacific Northwest schedule spray intervals at 4-7 days (Beers et al., 2011; Bruck et al., 2011). This prophylactic use of insecticide is unsustainable as growers have a limited selection of products and mode of action. These limitations could ultimately lead to *D. suzukii* resistance development and may result in secondary pest outbreaks because of negative non-target effects on beneficial organisms. Furthermore, production costs have increased substantially in susceptible crops where *D. suzukii* are managed (Walsh et al., 2011). There are no registered insecticides that will control maggots within fruit, (Beers et al., 2011; Bruck et al., 2011). In particular, organic production is seriously threatened because there are few effective organically approved SWD insecticides (Walsh et al., 2011; Van Timmeren and Isaacs, 2014). Chemical control also presents difficulties in timing insecticide applications for many crops because of pre-harvest interval concerns (Walsh et al., 2011). Several exporters to Asia have lost fruit consignments due to unacceptably high pesticide residue levels as a result of the need to control SWD close to harvest (USDA SCRI Stakeholder feedback November 2012, J. DeFrancesco, pers. comm.). Current cultural management practices include timely and costly repeated harvests to remove fruit before it reaches peak susceptibility to SWD (Lee et al., 2012). Efforts to optimize chemical control is currently focusing on increased efficiency which includes electrostatic sprays, border sprays and alternate row sprays.

Population estimation

Degree-day (DD), or phenology models, are tools used for integrated pest management in temperate regions and are used to predict the life stages and time management. Degree-day models work best for pests with a high level of synchronicity and few generations (Wiman et al., 2014). *D. suzukii* temperature-related parameters and population modeling (Tochen et al., 2014, Wiman et al., 2014) suggest that *D. suzukii* has short generation times, high reproductive levels, and high generational overlap. Given these factors, it is believed that population models represent an additional and more

applicable tool for modeling *D. suzukii* pressure. *D. suzukii* population estimates can be improved by using additional tools such as mark-recapture and analytical or individual-based techniques (Williams et al., 2008). It is highly advantageous if fruit producers, can make predictions of *D. suzukii*.. The major factors affecting survival, fecundity and population dynamics of drosophilids include temperature, humidity, and the presence of food resources (Mitsui et al, 2006). Temperature-dependent fecundity and survival data (Tochen et al., 2014) was used in a matrix population model to describe relative *D.* population pressure and age structure (Wiman et al., 2014). This is a modification of Leslie matrix population models. is presented as a way to examine how insect populations interact with the environment, and has application as a predictor of population density. For *D. suzukii*, we examined the use of this model. As case studies, we examined model predictions in fruit production regions in the United States and Italy. These regions have differing climates. In general, patterns of adult *D. suzukii* trap catches broadly resembled seasonal population phenology predicted by the model using mean daily temperatures as an input. Age structure data from this work suggest that trap and fruit infestation data are often of limited value. Although there are many factors affecting population dynamics of *D. suzukii* in the field, temperature-dependent survival and reproduction are believed to be the main drivers for *D. suzukii* populations.

Biological control

Parasitoid biological field data is currently limited, despite wide spread establishment of SWD and the significant level of damage that can be caused by this pest (Goodhue et al., 2011; Rossi Stacconi et al., 2013). Efforts are initiated to develop SWD biological controls, and one pupal ectoparasitoid, *Pachycrepoideus vindemiae* (Hymenoptera: Pteromalidae) has been reported to attack SWD in Oregon and Italy (Rossi Stacconi et al., 2013). Over 50 hymenopteran parasitoids attack drosophilids globally (Carton et al., 1986). These parasitoids mainly belong to four families: Braconidae, Figitidae, Diapriidae and Pteromalidae. Such *Drosophila* parasitoids result in a high rate of mortality in their hosts. The average rate of parasitism of drosophilids can reach 90% (Fleury et al., 2004). In *D. suzukii's* natural range in Japan, Mitsui et al. (2007) found 15 parasitoid species attacking Drosophilidae that attack fruit. The most commonly found larval parasitoids include *Asobara*, and *Leptopilina* and *Ganaspis* species. These parasitoids are however not true specialists. Mitsui et al. (2007) also reported two pupal parasitoids, *Trichopria* sp. and *P. vindemiae.*
Parasitoid species that occur in the US, include *L. heterotoma, L. boulardi, Ganaspis* sp., *Trichopria* sp., and *P. vindemiae* (Kacsoh and Schlenke, 2012). The species composition, distribution, and host range of *D. suzukii* parasitoids are however poorly documented in North America. Kacsoh and Schlenke (2012) tested 15 species of parasitoids and found that only 7 were able to develop on *D. suzukii*. Thi is due to a strong immune response by *D. suzukii* against the parasitoid eggs. The parasitoid showing most promise was the Japanese species *Asobara japonica* (Mitsui et al., 2007; Ideo et al., 2008). The only other larval parasitoid able to develop from SWD was *Ganaspis* sp., an undescribed species collected in Florida and Hawaii (Kacsoh and Schlenke, 2012). Chabert et al. (2012) similarly tested five European parasitoid species , including three larval parasitoids, *A. tabida, L. heterotoma* and *L. boulardi*. Two generalist pupal parasitoids, *Trichopria* sp. and *P. vindemiae* were also trialled, Only the two pupal parasitoids successfully developed on SWD, while *L. heterotoma* and *L. boulardi* not. In more recent field studies in Oregon and Italy, *P. vindemiae* and *Leptopilina heterotoma* Thomson (Hymenoptera:

Figitidae), were commonly collected from sentinel traps containing *D. suzukii*, but laboratory studies only confirmed successful attack of *D. suzukii* by *P. vindemmiae* in the USA. *L. heterotoma* in Italy was able to parasitize SWD in addition. The impact of these parasitoids on pest populations of *D. suzukii* is however believed to be limited as indicated by a parasitism index from the sentinel traps (Miller et al. in prep).

CONCLUSIONS

D. suzukii is clearly an important cosmipolitan insect pest. This pest has resulted in significant increases of production costs and have become a key pest in most affected major production production regions. Current management of SWD focuses heavily on chemical control which targets adult ovipositing individuals. Population modeling and age structure however indicate that these management techniques only target a small portion of the total population. Larvae emerging from fruit after a short period will again result in renwed pressure, necessitating additional tightly spaced pesticide sprays. This management technique is requiring high input costs. This is clearly unsustainable, particulrly given the narrow range of effective active ingredients against *D. suzukii*. Future studies on resistance management against *D. suzukii* is warranted.

Studies on *D. suzukii* monitoring methdology have been extensive. Despite some advances made in these studies there are several shortcomings. Monitoring using various baited traps however do provide an idea of relative seasonal abunce of adult *D. suzukii* populations. It is believed that monitoring is of limited value in its current state as monitoring cannot be used as an early warning tool.
Population estimation studies based on temperature-related longevity and fecundity do provide some insights, but this work need to be verified and refined in order to be of additional value to producers. Data from population studies can be of value to direct IPM management techniques. *D. suzukii* population estimation has application for use as a virtual laboratory where 'what-if' statements can be suggested and answered prior to management action.Work on biological control using parasitoids is emerging, but have highlighted the limited impact to manage *D. suzukii* pest populations in two key production regions. Studies in native ranges of *D. suzukii* are however encouraging. This information stresses the importance of studies for classical biological control of SWD.

This paper is by no means an exhuastive summary of all disciplines studying *D. suzukii* but provide basic information of the current impact in the USA and the currently used management techniques used by growers. We described seasonal phenology in various production regions. Population modeling, integrated with ecophysiological and cultural management techniques are suggested as one avenue to manage this important pest.

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