2013 California Cherry Research Reports

California Cherry Board
University of California
USDA-ARS

UC Cooperative Extension
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CALIFORNIA CHERRY BOARD

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2013 CCB RESEARCH PROJECTS Approved Funding (4/23/2013)

ITEM	CCB PROJECT NUMBER	PROJECT LEADER	PROJECT TITLE	FIRST FUNDED PROJECT YEAR	ORIGINAL ESTIMATED DURATION	CURRENT YEARS REMAINING	TOTAL COST TO DATE	2012 FUNDING	2013 PROPOSED FUNDING	2013 REQUESTED FUNDING
PRE-HARVEST PROPOSALS										
1	10-12-99	Dr. Doug Gubler UCCE, Specialist	Diagnosis, Epidemiology and Control of Canker Diseases in Sweet Cherry.	2010	2 years	N/A	$82,700	$30,200	$34,059	**$40,000**
2	13-13-107	Dr. Zainulabeuddin Syed University Notre Dame	Identifying Drosophila Suzukii Attractants from Preferred Fruits and Yeast for Improved Monitoring and Management	2013	2 years	1 year	$53,872	$0	$53,872	**$53,872**
3	12-12-102	Kassim Al-Khatib	UC IPM Update and Maintenance for Cherries	2012	1 year	N/A	$8,014	$4,122	$3,892	**$3,892**
4	13-13-112	Dr. Todd Einhorn Oregon State University	Early Season Estimation of Fruit Set and Size Potential	2013	1 year	N/A	$10,000	$0	$10,000	**$10,000**
5	13-13-106	Yan Wang Oregon State University	Extending Storage/Shipping Life and Assuring Good Arrival of Sweet Cherry	2013	1 year	N/A	$8,000	$0	$8,000	**$8,000**
6	13-13-108	Dr. Bob Vansteenwyk UC Berkeley	Monitoring, Biology, and Control of Spotted Wing Drosophila	2013	2 years	N/A	$48,228	$27,488	$20,740	**$20,740**
7	13-13-109	David Haviland IPM Advisor, UCCE, Kern County	Evaluation of Spirotetramat as a Post-Plant Nematicide in Cherries	2013	3 years	2 years	$16,862	$0	$16,862	**$16,862**
8	13-13-110	David Haviland IPM Advisor, UCCE, Kern County	Management of Spotted Wing Drosophila in the Lower San Joaquin Valley	2013	3 years	N/A	$19,341	$0	$19,341	**$19,341**
9	13-13-111	Dr. Kent Daane UC Berkeley	Investigating Biological Controls to Suppress Spotted Wing Drosophila Populations	2013	1 year	N/A	$28,405	$0	$28,405	**$28,405**
POST-HARVEST PROPOSALS										
10	ARS 12-12-104	Dr. Spencer Walse USDA/ARS, Parlier	Postharvest treatment of sweet cherries with Methyl Bromide to Control Spotted Wing Drosophila	2013	1 year	N/A	$14,000	$0	$14,000 ***Funded by USDA TASC Grant***	**$14,000**
11	ARS 12-12-105	Dr. Spencer Walse USDA/ARS, Parlier	Phosphine-Oxygen Mixtures for Postharvest Treatment to Control Spotted Wing Drosophila/Oriental Fruit Fly	2012	2 years	1 years	$0	$0	$0 ***Funded by USDA TASC Grant***	**$0**
JOINT PROPOSALS										
12	08-13-95	Dr. James Adaskaveg UC Riverside Joe Grant UCCE, Farm Advisor	Management & Epidemiology of Pre & PostharvestFoliar and Fruit Diseases of Sweet Cherry	2008	4 years	On going	$218,500	$28,000	$37,500	**$37,500**
								TOTAL	$246,671	**$252,612**

First two digits indicate year project began.
Second two digits indicate current year or year project concluded.
Third two digits indicate unique project number, in numeric order from 1987.

CALIFORNIA CHERRY BOARD
2013 FINAL RESEARCH REPORTS

Project year: 2013

Project leader: W. Douglas Gubler, C.E. Specialist
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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.**

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

Objective 2. Determine the role of other fungi in cherry cankers.

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.

In Linden CA, one orchard is experiencing severe canker disease. While *E. lata, L. persoonii,* and *C. pulchella* have been isolated from this orchard, other fungi are being isolated more frequently from the cankers. Of particular interest is the isolation of *Alternaria arborescens.* It is unclear if this fungus is causing the lesions or is entering the wood following infection from another fungus. *Alternaria* species have been associated with cankers in a wide variety of crops, including grape (Urbez-Torres *et al.*, 2009), apple (Brown and McManus, 2000), and kiwi (Tsahouridou and Thanassoulopoulos, 2000).

The objectives of this study are to (i) identify fungi associated with cherry cankers in CA, (ii) determine the role of *Alternaria arborescens* and other fungi in cherry cankers, and (iii) implement chemical control methods against Calosphaeria canker, Eutypa dieback, and Leucostoma (Cytospora) canker.

Growth Chamber Experiment

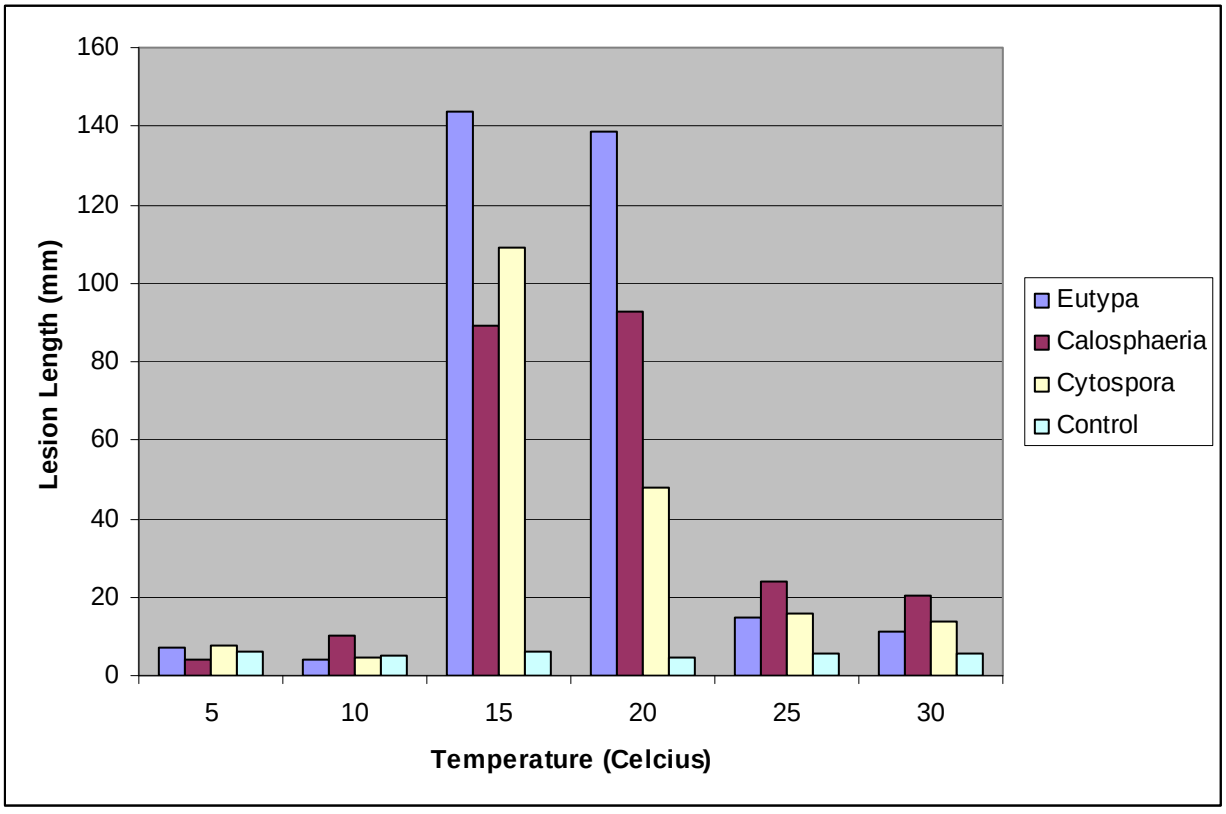
Procedure

Growth chamber trials were established to evaluate the effect of temperature on infection and lesion expansion caused by *Eutypa lata*, *Leucostoma persoonii* (Cytospora), *Calosphaeria pulchella*. Small branches from sweet cherry trees were cut into 12-inch segments and all leaves were removed. The branches were soaked for 15 minutes in a 10% bleach solution and then rinsed with sterile distilled water, and then the ends dipped in paraffin wax to prevent desiccation. Near the middle of each branch, a 4 mm wound was made. Mycelial agar plugs of the fungi were placed into wounds. The inoculated area of the branches were wrapped with parafilm and placed in 12 X 9 inch plastic boxes with lids in place (crispers). The crispers were placed in growth chambers at 5, 10, 15, 20, 25 and 30°C. Each isolate was inoculated on four different twigs per temperature.

Results

Temperatures of 15-20 °C were ideal for fungal growth (Figure 1). Using ANOVA, these differences were significantly different at $p<.001$. While Cytospora, Eutypa and Calosphaeria all grew best at these temperatures. Cytospora's growth was slower at 20 °C.

Figure 1. Lesion length development of *Eutypa lata, Calosphaeria puchella and Leucostoma personii* (Cytospora) at different temperatures.



Pruning Wound Protection Trials
Several field trials have been conducted evaluating fungicide efficacy against canker pathogens. Field trials were established in Davis, Brentwood, Stockton 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 application 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.

Davis Fungicide Trial - June

Procedure
Fresh pruning wounds were made on two to three year-old wood in Davis in June 2013. Liquid formulations of Scholar(.38 g/500ml), Tilt (.63 ml/500ml), Rally (.45g/500 ml), Mertect (.75 ml/500ml), Topsin M (1.99 g/500ml), Trichoderma, Vitiseal, Vitiseal 1:10, Vitiseal 1:10+Tilt (.63 ml/500ml), Vitiseal 1:10+Rally(.45g/500 ml)+Topsin(1.99 g/500ml), Vitiseal 1:10+Scholar(.38 g/500ml), Luna Experience (.47 ml\500 ml) and Cannonball (.53 g/ml) were sprayed in a single application with 500 ml spray bottles immediately after pruning. Pruning wounds were inoculated with mycelial plugs of Cytospora, Eutypa or Calosphaeria, approximately one hour after fungicide treatments. After three months tree 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
All pathogens were reduced by fungicide treatment. (Figures 2-4). As shown in Figure 2, stub cuts treated with Rally, Topsin, Trichoderma, Tilt, Vitiseal 1:10, Vitiseal 1:10+Tilt, Mertect and Luna Experience were not infected by Calosphaeria. For Eutypa, Topsin, Cannonball, Vitiseal 1:10, Vitiseal 1:10 +Rally and Topsin, Vitiseal 1:10 + Scholar, Vitiseal 1:10 +Tilt, and Mertect When data for all 3 pathogens were combined, treatment means were significantly different ($p<.0023$). As shown in Table 1, all treatments significantly reduced canker formation. The most effective treatments were Topsin M and Vitiseal 1:10+Tilt. All treatments significantly reduced disease incidence.

Figure 2. Percent of stub cuts developing cankers following fungicide applications and inoculation with *Calosphaeria puchella* in Davis fungicide trial, June 2013.

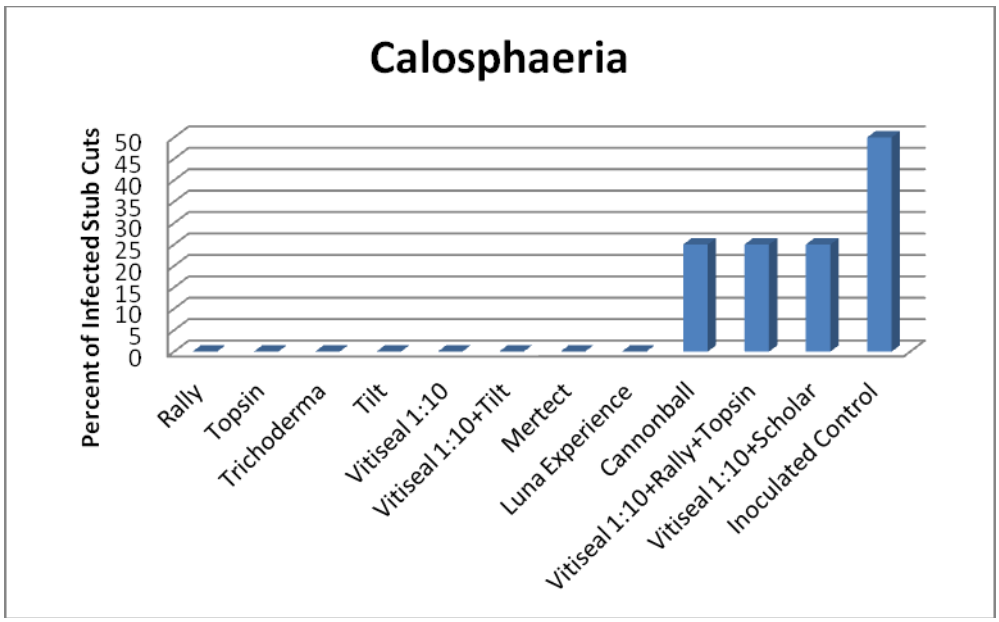


Figure 3. Percent of stub cuts developing cankers following fungicide applications and inoculation with *Eutypa lata* in Davis fungicide trial, June 2013.

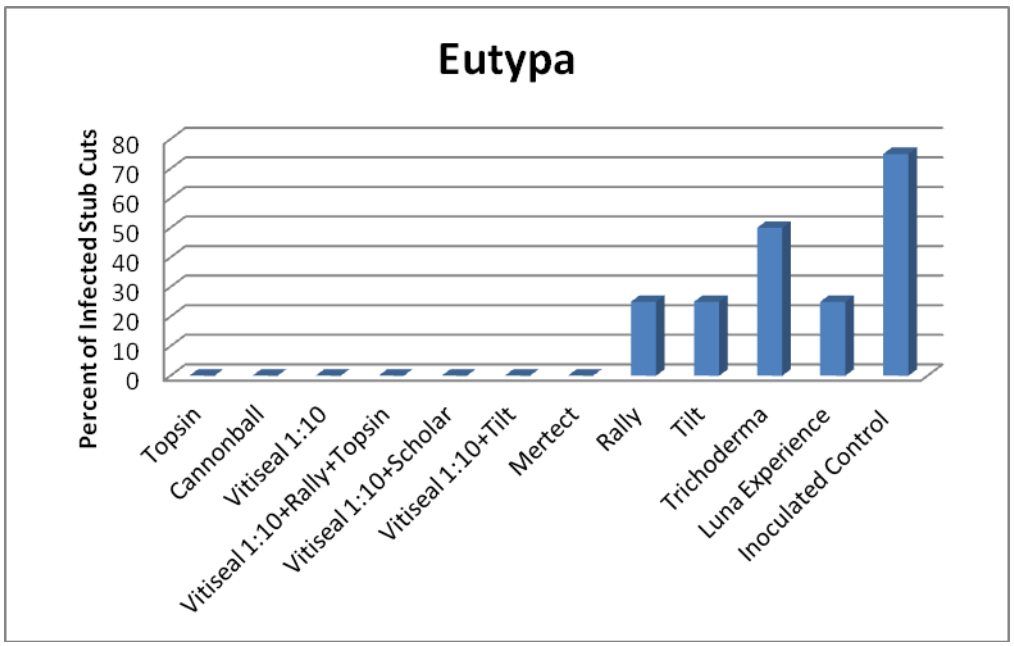


Figure 4. Percent of stub cuts developing cankers following fungicide applications and inoculation with *Leucostoma persoonii* (Cytospora) in Davis fungicide trial, June 2013.

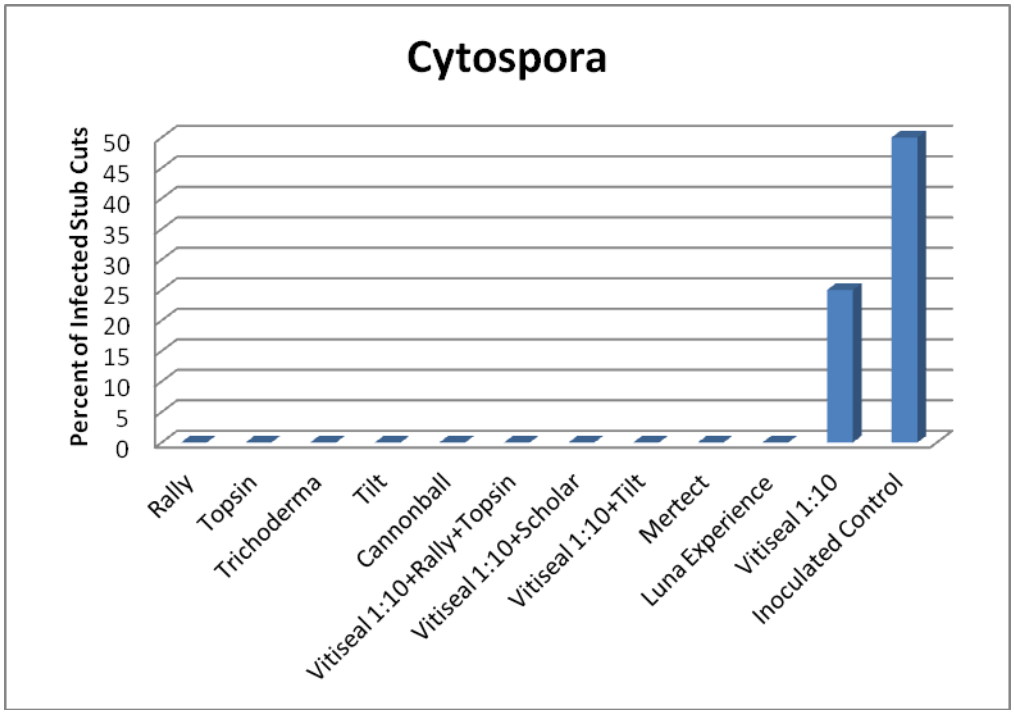


Table 1 . Average incidence (percent) of cankers formed for different treatments in Davis fungicide trial, June 2013. Treatment means followed by the samel letter are not significantly different.

Treatment	**Incidence (%)**
Inoculated Control	58 a
Vitiseal 1:10	25 bc
Vitiseal	17 bc
Trichoderma 1	17 bc
Cannonball	8 bc
Luna Experience	8 bc
Mertect	8 bc
Rally	8 bc
Tilt	8 bc
Vitiseal 1:10+Rally+Topsin	8 bc
Vitiseal 1:10+Scholar	8 bc
Topsin	0 c
Vitiseal 1:10+Tilt	0 c

Davis Fungicide Trial – August 2013

Procedure

In August 2013, fresh pruning wounds were made on two to three year-old cherry wood in Davis. Liquid formulations of Scholar(.38 g/500ml), Tilt (.63 ml/500ml), Rally (.45g/500 ml), Topsin M (1.99 g/500ml), Vitiseal 1:10, Vitiseal 1:10+Tilt (.63 ml/500ml), Vitiseal 1:10+Rally(.45g/500 ml), Vitiseal 1:10+Scholar(.38 g/500ml), Luna Experience (.47 ml\500 ml) and Cannonball (.53 g/ml) were sprayed in a single application with 500 ml spray bottles immediately after pruning. Pruning wounds were inoculated with mycelial plugs of Cytospora, Eutypa or Calosphaeria, approximately one hour after fungicide treatments. After three months tree 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

Figures 5-7 show treatments were effective in reducing disease. Stub cuts treated with Vitiseal 1:10, Vitiseal 1:10+Tilt and Vitiseal 1:10 + Scholar prevented Calosphaeria growth (Figure 5). Stub cuts treated with Vitiseal 1:10, Vitiseal 1:10 + Tilt, and Vitiseal 1:10+Scholar prevented Eutypa growth (Figure 6). Cytospora growth was prevented by Topsin, Cannonball, Vitiseal 1:10 and Vitiseal 1:10 + Scholar. When the data is combined for all three pathogens, the results were significantly different ($p<.001$). As shown in Table 2, all treatments significantly reduced canker formation. The most effective treatments were Vitiseal 1:10 and Vitiseal 1:10+Scholar. This trial differed from the June trial because temperatures were cooler and more suitable for fungal growth. More of the inoculated controls developed cankers in this trial.

Figure 5. Percent of stub cuts developing cankers following fungicide applications and inoculation with *Calosphaeria puchella* in Davis fungicide trial, August 2013.

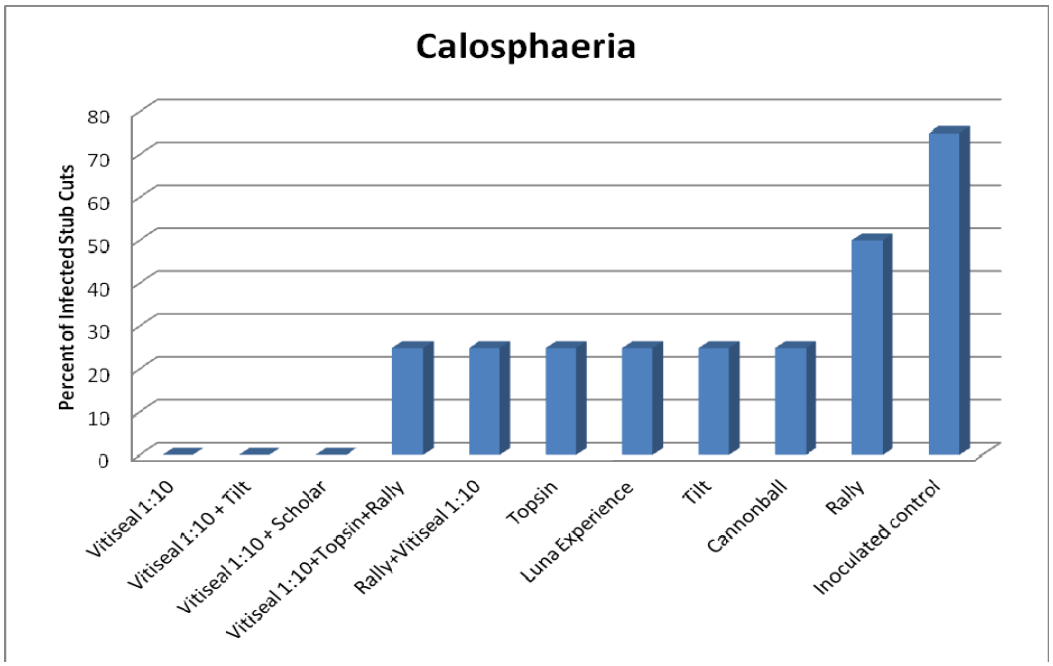


Figure 6. Percent of stub cuts developing cankers following fungicide applications and inoculation with *Eutypa lata* in Davis fungicide trial, August 2013.

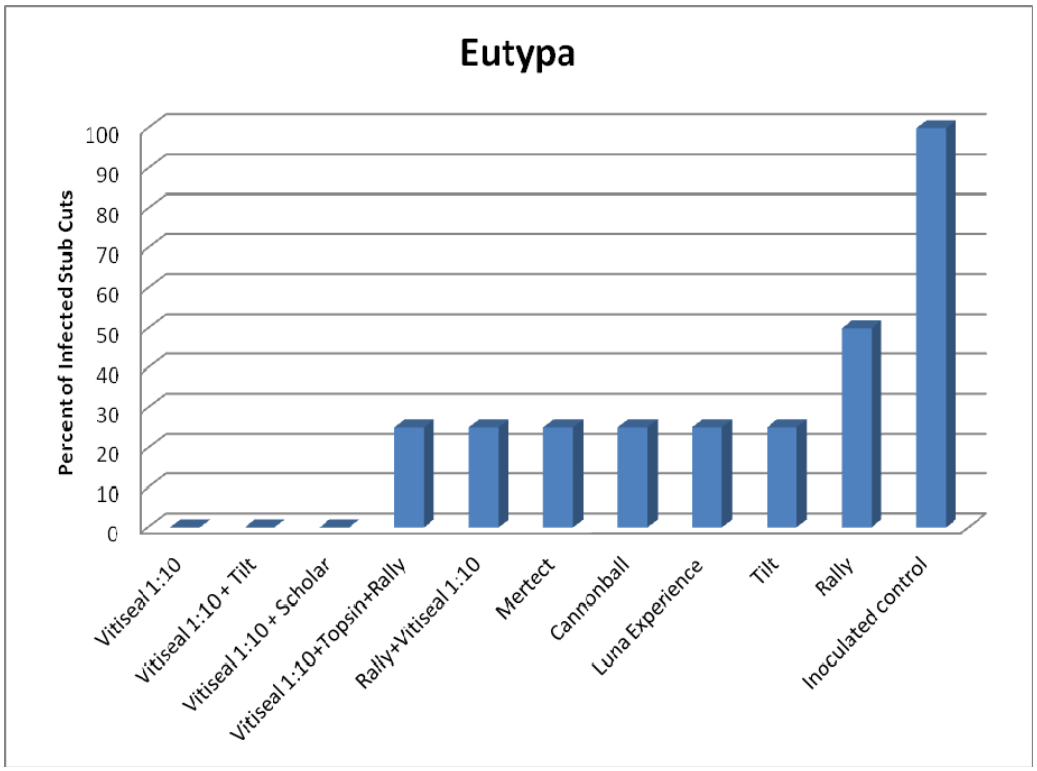


Figure 7. Percent of stub cuts developing cankers following fungicide applications and inoculation with *Leucostoma persoonii* (Cytospora) in Davis fungicide trial, August 2013.

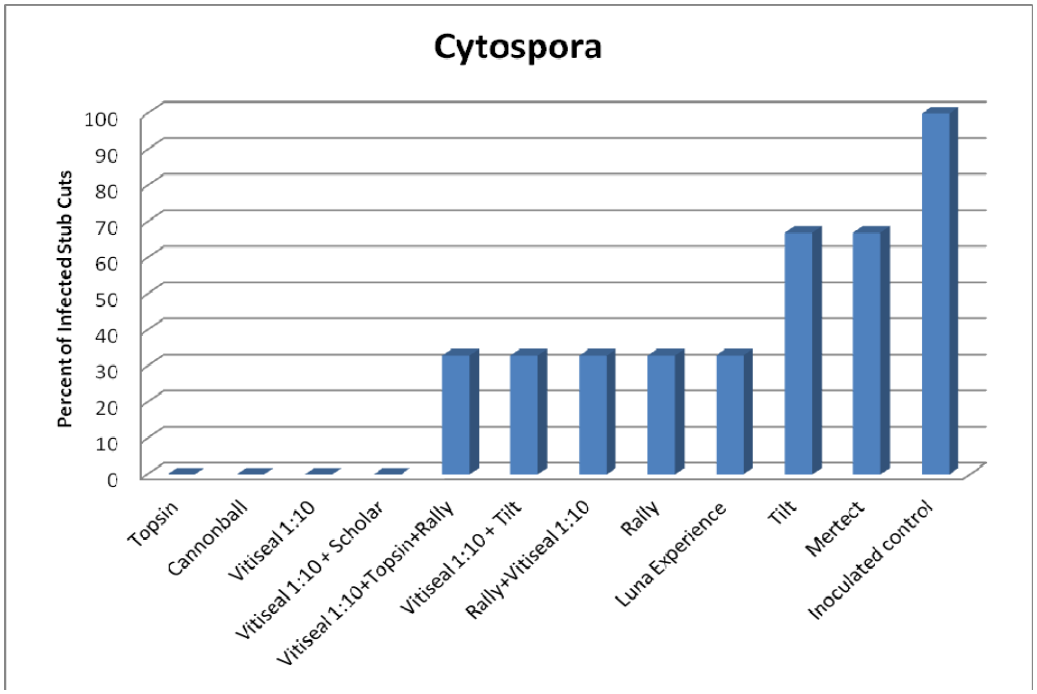


Table 2. Average incidence (percent) of cankers formed for different treatments in Davis fungicide trial, August 2013. Treatment means followed by the same letter are not signifcantly different.

Treatment	**Incidence (%)**
Inoculated control	90.91 a
Rally	54.55 b
Rally+Vitiseal 1:10	36.36 bc
Tilt	36.36 bc
Luna Experience	27.27 bcd
Vitiseal 1:10+Topsin+Rally	27.27 bcd
Mertect	25 bcd
Cannonball	18.18 cd
Topsin	9.09 cd
Vitiseal	9.09 cd
Vitiseal 1:10 + Tilt	9.09 cd
Vitiseal 1:10	0 d
Vitiseal 1:10 + Scholar	0 d

Cherry Canker Kickback Trials

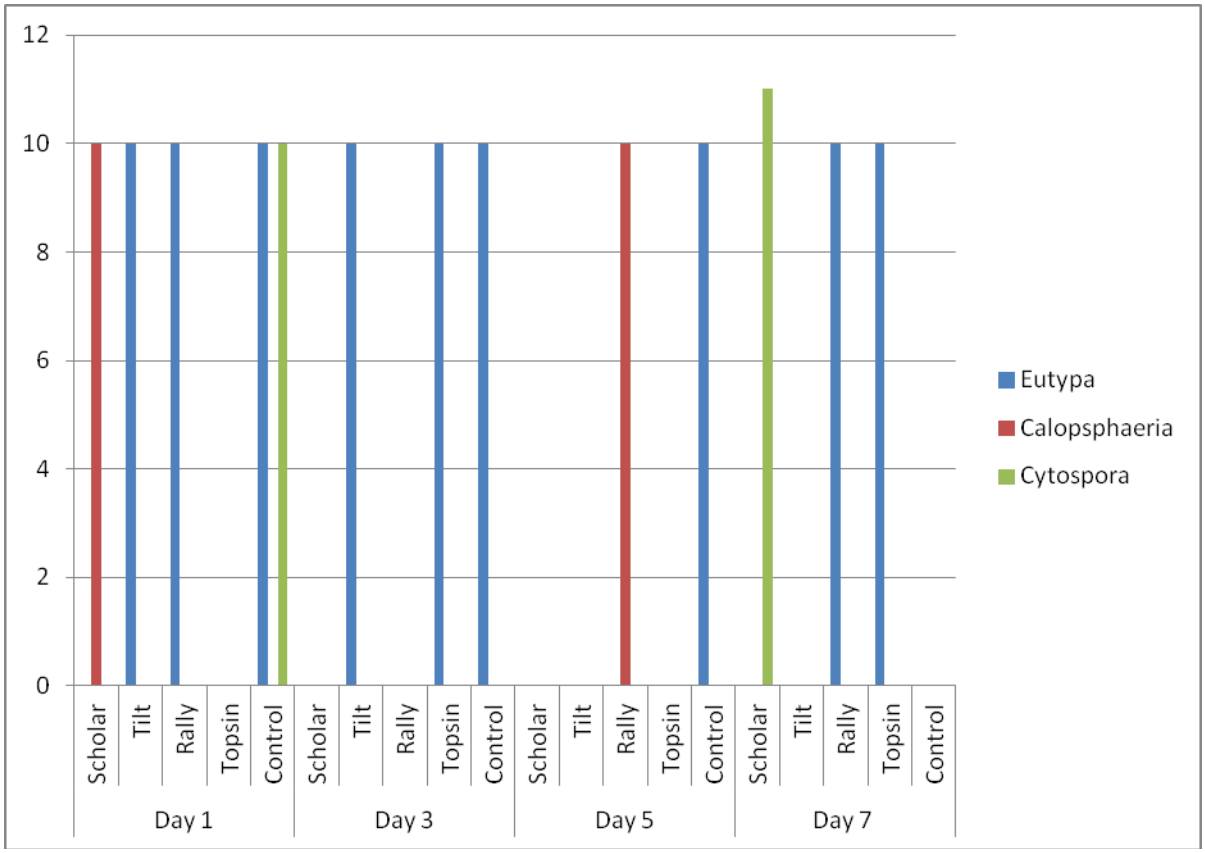
Procedure

Two trials were established in Stockton and Davis, CA to look at fungicide efficacy following a rain event on newly pruned branches. Stub cuts were made one day prior to a rain event. Scholar (.38g/500ml), Tilt (.63g/500ml), Rally (.45g/500ml) or Topsin M (1.99g/500ml) were sprayed either1, 3, 5, or 7 days following rain event onto pruning wounds, using 500 ml spray bottles. Fifteen branches were sprayed each day for each fungicide. 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

Results were not statistically significant. Infection rates were low and did not differ between days 1, 3, 5, and 7 following a rain event (Figure 8). Eutypa was the most common pathogen.

Figure 8: Percent of stub cuts developing cankers following fungicide applications at 1, 3, 5 or 7 days after rain event in March 2013 trials established in Stockton and Davis, CA.

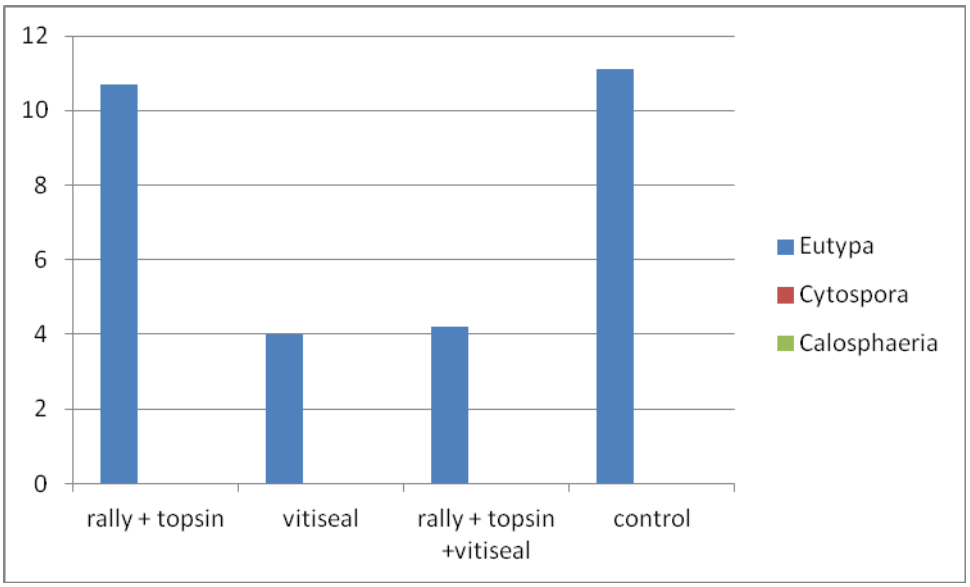


Natural Inoculum Fungicide Trial Stockton

Procedure
Stub cuts were made in March 2013 in Stockton, CA. For each treatment, 25 stub cuts were treated. Treatments were sprayed using 500 ml spray bottle. Treatments were Rally 0.45g/500 ml + Topsin 1.99g/500 ml, Rally 0.45g/500 ml + Topsin 1.99g/500 ml +Vitiseal RTU, Vitiseal RTU, and a control. 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
Eutypa was primarily reisolated from stub cuts. All treatments had fewer cankers than control (Figure 9). Stub cuts treated with Vitiseal 1:10 or Vitiseal 1:10 + Rally and Topsin had lower infection rates although the results were not significant.

Figure 9. Percent of stub cuts forming cankers in fungicide trial using natural inoculum in Stockton in March 2013.



Natural Inoculum Fungicide Trial Two Stockton

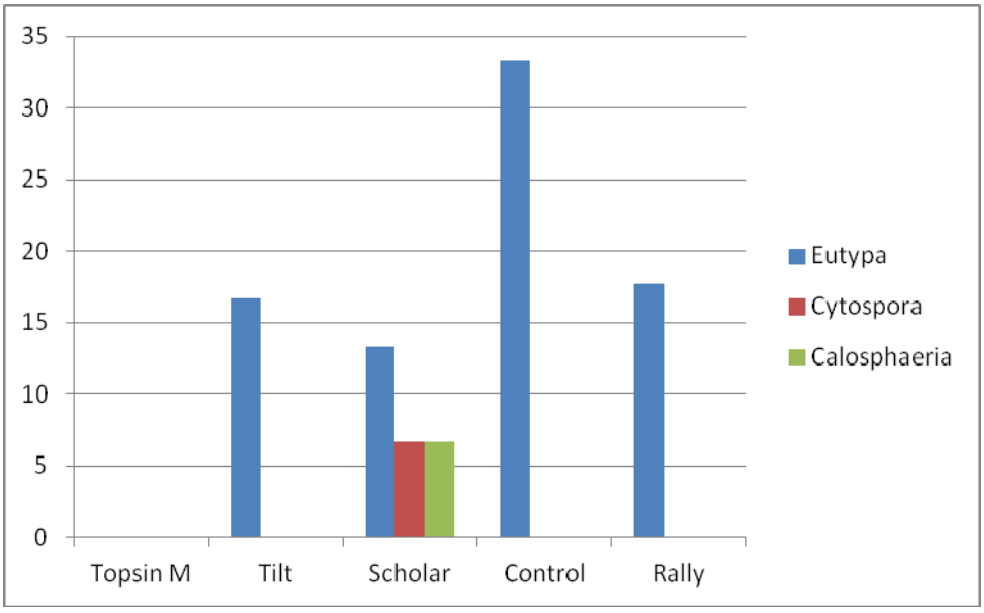
Procedure
Stub cuts were made in March 2013 in Stockton, CA. Treatments included Scholar (.38g/500ml), Tilt (0.63 ml/500ml), Rally (0.45g/500 ml), Topsin (1.99g/500 ml), and a control. Seventy five stub cuts were made. Fifteen stub cuts were sprayed for each treatment listed. After several months, treated 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

Topsin M was significantly different from control in preventing cankers using Pearson's test ($p<.0236$). The control had higher levels of Eutypa than other treatments. Topsin M prevented all pruning wound infections. Stub cuts treated with Scholar, Tilt or Rally had few cankers than the control (Figure 10).

Figure 10. Percent of stub cuts forming cankers in fungicide trial using natural inoculum in Stockton in March 2013.



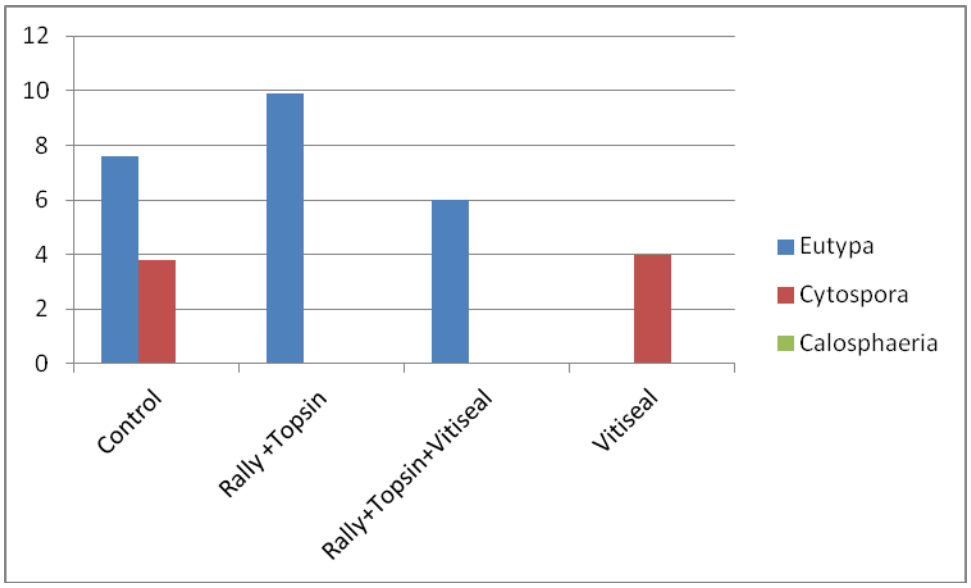
Natural Inoculum Cherry Canker Trial Davis

Procedure

In March 2013, 120 stub cuts were made in Davis, CA. Thirty stub cuts each were sprayed with the following treatments Rally (0.45g/500 ml) + Topsin (1.99g/500 ml), Rally (0.45g/500 ml) + Topsin (1.99g/500 ml) +Vitiseal RTU, Vitiseal RTU, and a control. 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
Eutypa was the most common pathogen reisolated (Figure 11). In this trial, fungicides did not significantly lower canker formation but overall, canker formation was low. Stub cuts treated with Vitiseal did not develop any infections from Eutypa.

Figure 11. Percent of stub cuts forming cankers in fungicide trial using natural inoculum in Davis in March 2013.



Natural inoculum Trial Linden

Procedure
In January, on a drizzly day where the following fungicides were used to treat ten stub cuts each: Scholar (.38g/500ml) ,Orbit (0.63 ml/500ml), Mertect (0.63 ml/500ml), Rally (0.45g/500 ml), Topsin (1.99g/500 ml), Luna Exp (.47 ml/500ml), Cherry Trichoderma spore solution, Vitiseal RTU, Topsin (1.99g/500ml) + Orbit (0.63 ml/500ml), Mertect (0.63 ml/500 ml), Orbit (0.63 ml/500ml), Topsin (1.99g/500ml) + Rally (0.45g/500ml), Vitiseal, and control. This trial was repeated a few days later on a sunny day. After several months, treated branches were collected and returned to the laboratory for assessment of fungal colonization and wound protection. Stub cuts were treated as previously reported.

Results
Disease incidence was low for both trials. On the sunny day Eutypa and Cytospora were the two pathogens isolated (Figure 12). Eutypa was only isolated from the control and Cytospora was only isolated from the Vitiseal 1:10 treatment. On the foggy day, Calosphaeria was the only pathogen isolated from the control, Eutypa was isolated from the Topsin + Orbit treatment and Cytospora was isolated from the Luna Experience treatment (Figure 13). Variations seen between fungicides in the trials were not statistically significant. Weather did not affect infection rates. Infection rates were similar on foggy and sunny days.

Figure 12. Percent of stub cuts forming cankers in fungicide trial using natural inoculum in Linden, applied on a sunny day, 50-55 degrees F in January 2013.

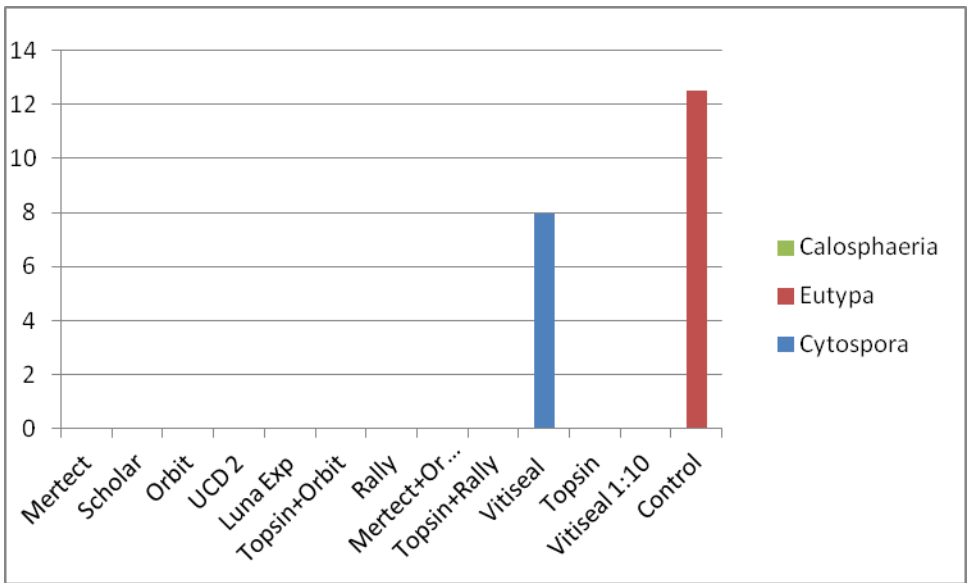
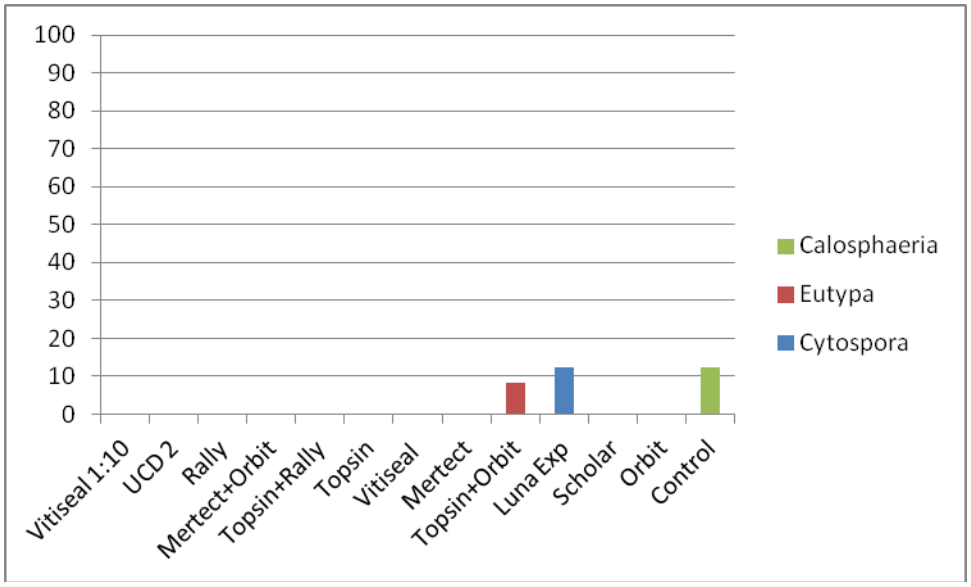


Figure 13. Percent of stub cuts forming cankers in fungicide trial using natural inoculum in Linden, applied on a foggy, damp day, 55 degrees F in January 2013.



Brentwood Vitiseal Trial

Procedure
Cuts were made in March 2013 to two year old trees and the Vitiseal RTU was applied to cuts on half the trees. The cuts were heading cuts on the primary scaffolds. Three rootstock/variety combinations were treated: Coral on Geisla 6 (8 X 5 tree replicates = 40 treated trees and 40 untreated trees), Coral on Geisla 12 (6 x 5 tree replicates = 30 treated and 30 untreated trees), and Lapins on Geisla 12 (6 x 5 tree replicates = 30 treated and 30 untreated trees). In June, the cuts were visually evaluated for canker formation.

Results
The results shown in Table 3 are statistically significant ($p<.0041$). Vitiseal reduced canker formation in Coral/G6 and Lapins/G12.

Table 3. Canker formation in stub Cuts treated with Vitiseal RTU (diluted 1:9) in Brentwood.

SUMMARY		No. Cankers	No. cuts	% cankers
Coral/G6	Vitiseal	18	173	10.40%
	Control	35	162	21.60%
Coral/G12	Vitiseal	7	67	10.45%
	Control	7	76	9.21%
Lapins/G12	Vitiseal	10	76	13.16%
	Control	23	72	31.94%

Davis Wood Age Field Trial

Procedure
One, two and three year old wood was pruned and a mycelial plug from Calosphaeria, Eutypa or Cytospora was placed on the stub cut and covered with parafilm and aluminum foil. Five branches of each wood age were inoculated for each of the three fungi for a total of 45 branches. A randomized block design was used. After three months, infected branches were removed and lesion lengths measured.

Results
Variation in lesion length between wood of different ages was not significant, $p<.753$.

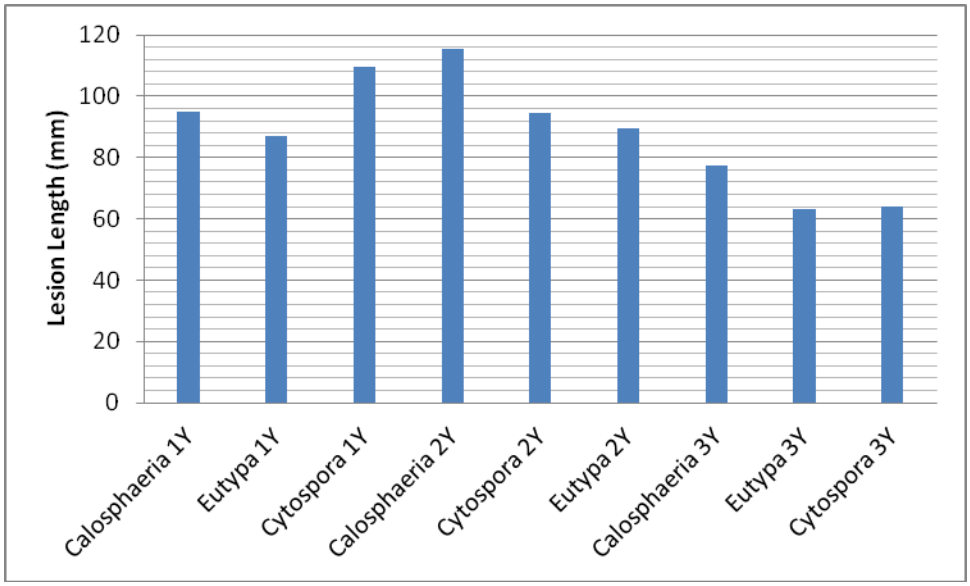
Davis Wood Age Crisper Trial

Procedure
Twelve inch pieces of one, two and three year old wood were pruned. Wood was surface sterilized in a 10% bleach solution for fifteen minutes. Five pieces of each wood age were inoculated with one of three fungi: Calosphaeria, Eutypa and Cytospora for a total of 45

branches. Branches were placed in crispers at room temperature for six weeks. Lesion lengths were measured.

Results
The crisper trials showed lesion length expansion was greater in one and two year old wood (Figure 14). Three year old wood had significantly shorter lesions for all three species. ($p<.0001$).

Figure 14. Lesion length formation in cut wood branches in Davis lab trial ages 1,2 and 3 years inoculated with Calosphaeria, Eutypa or Cytospora in July 2013.

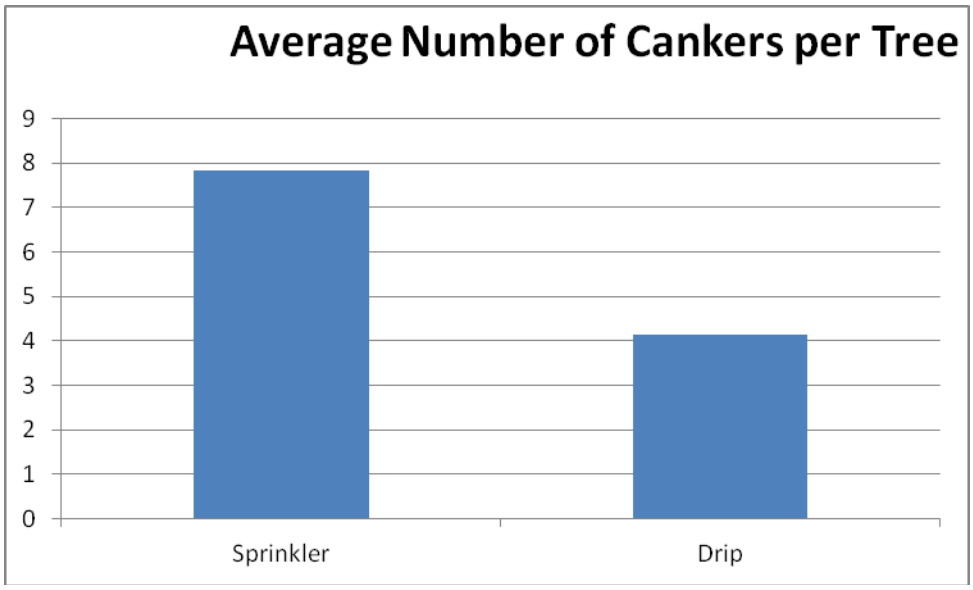


Drip vs Sprinkler Trial

Procedure
Drip and Sprinkler orchards were surveyed in San Joaquin Valley to look at average number of cankers. In each orchard, forty trees were surveyed for numbers of visible cankers. The trees were chosen at random.

Results
Drip orchards had significantly fewer cankers than sprinkler orchards, $P<.001$ using an ANOVA (Figure 15).

Figure 15. Average number of cankers per tree in drip vs sprinkler irrigated orchards.



Pruning Wound Susceptibility Trial

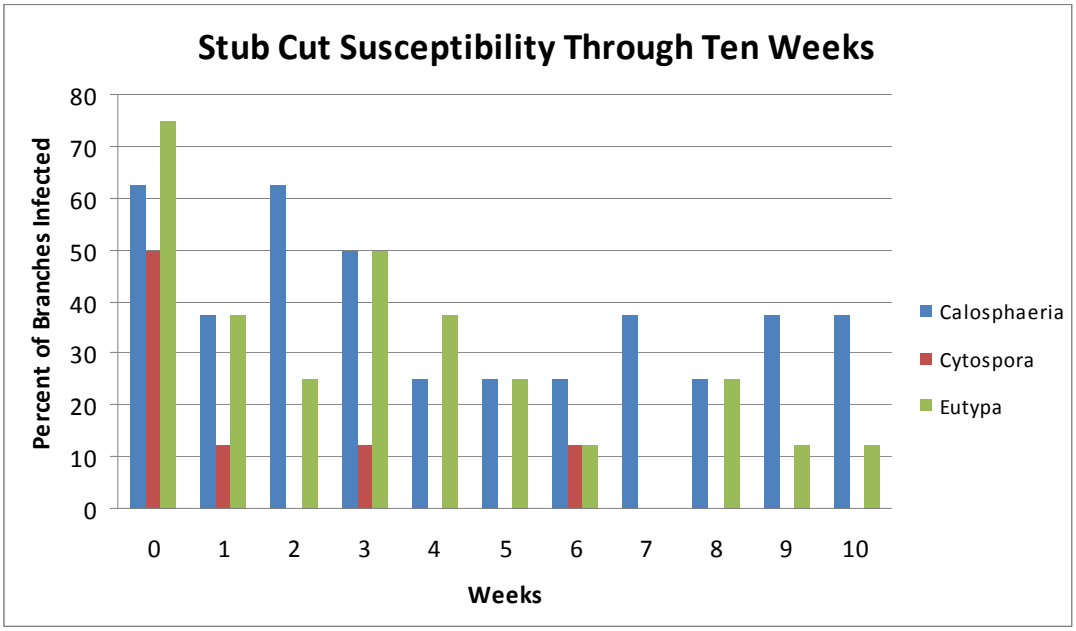
Procedure

Three hundred eighty four pruning wounds were made July 1, 2013 in Davis. Every week, eight branches each were inoculated with Eutypa, Cytospora, or Calosphaeria for a total of 32 branches. Mycelial plugs were placed on the pruning wounds and covered with parafilm. This process was repeated every week for twelve weeks. Two months after the last pruning wounds were inoculated, branches were be 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 ten week old pruning wounds. However, susceptibility declined over the ten week period for both pathogens. Cytospora was able to infect only about 10% of pruning wounds out to six weeks (Figure 16).

Figure 16. Stub cut susceptibility to Calosphaeria, Cytospora and Eutypa through ten weeks.



In Vitro Fungicide Trial

Procedure

A bottle trial was established to assess fungicide efficacy on canker causing fungi in a completely controlled environment. Two and three year old cherry branches were cut in about one inch pieces and autoclaved twice. Scholar (.75 g/L), Mertect (1.25 ml/L), Luna Sensation (.94 ml/L), Rally (.9 g/L) + Topsin (3.98 g/L), Scholar (.75 g/L) + Vitiseal RTU, Mertect (1.25 ml\L) + Vitiseal RTU, Luna Sensation(.94 ml\L) + Vitiseal 1:10, Rally (.9g/L) + Topsin (3.98g/L) + Vitiseal RTU and Vitiseal were the nine treatments tested in this trial. *Eutypa lata*, *Leucostoma persoonii* (Cytospora), *Calosphaeria pulchella,* were cultured in bottles containing PDA tetracycline medium. Five replications of each fungus/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

Rally+Topsin+Vitiseal treated wood had 0, 1.4, and 2.4% mycelial growth of *Leucostoma persoonii* (Cytospora), *Calosphaeria pulchella, and Eutypa lata* respectively. Rally+Topsin, Scholar+Vitiseal, and Mertect+Vitiseal treated wood were found to have various effects on fungal colony growth (0 to 24.4%) depending on the fungal species. Luna Sensation treatment resulted in 100% wood colonization by *E. lata*, 75% *C. pulchella,* and 67% *L. persoonii.* Vitiseal, Scholar, Mertect, and Luna+Vitiseal generated various wood colonization percentages by the three fungi ranging from 22.3% to 96% (Fig. 17 and 18).

Wood with Rally+Topsin+Vitiseal treatment was found to have a significantly lower fungal growth on them for all three fungi by the end of the sixth week of the trial (Fig. 18). As a result

of this treatment (Rally+Topsin+Vitiseal) the cherry wood had no sign of colonization by *Leucostoma persoonii*. Moreover, this treatment had the lowest percentage of mycelial growth of *Calosphaeria pulchella, and Eutypa lata* among nine treatments. As illustrated in Figure 18, the sum of the mycelial growth of the three fungi in Rally+Topsin+Vitiseal treatment is 3.8%, which is 16.2% less than Rally+Topsin treatment. This result shows the effectiveness of the addition of Vitiseal 1:10 in the efficacy of the treatment. The same pattern occurs in the addition of Vitiseal 1:10 to Scholar, which results in 124.4% decrease (from 156.8% to 32.4%) of mycelial growth of the three fungi on treated wood. According to Figure 18, the addition of Vitiseal 1:10 to Scholar decreased *E. lata* mycelial coverage from 62% to 11%, *C. pulchella* from 71.4% to 21%, and *L. persoonii* from 23.4% to 0.4%. The positive effect of the addition of the Vitiseal 1:10 to Luna Sensation and Mertect are also notable in the Figure 18. As a result of this addition, mycelial growth on Luna treated wood was reduced by about 50% for all three fungi. Mertect+Vitiseal had 35.6% reduction in *E. lata* , 68% reduction in *C. pulchella,* and 1% reduction in *L. persoonii* wood colonization compared to Mertect treated cherry wood.

The survey from number of cherry orchards in different parts of California indicates all three canker causing fungi that are used in this trial are found abundantly in cankered trees, regardless of the location of the orchard. Therefore, the applied treatment has to control all three canker causing fungal pathogens effectively. As discussed in previous section (The Effect of Adding Vitiseal 1:10 to fungicides) and illustrated in Figure 18, the Rally+Topsin+Vitiseal treatment was the most effective treatment towards all three fungi. Rally+Topsin, Scholar+Vitiseal, and Mertect+Vitiseal treatments were the next most effective treatments after Rally+Topsin+Vitiseal treatment respectively. The cumulative mycelial growth of three fungi on treated wood with Luna+Vitiseal, Mertect, Scholar, Vitiseal, and Luna were more than 100% (ranging from 104.45% to 242%), which indicate the low efficacy of these treatments against *L. persoonii* (Cytospora), *C. pulchella, and E. lata*.

Figure 17: Percent coverage with fungal mycelium of three canker causing fungi on woods treated with fungicide six weeks after inoculation.

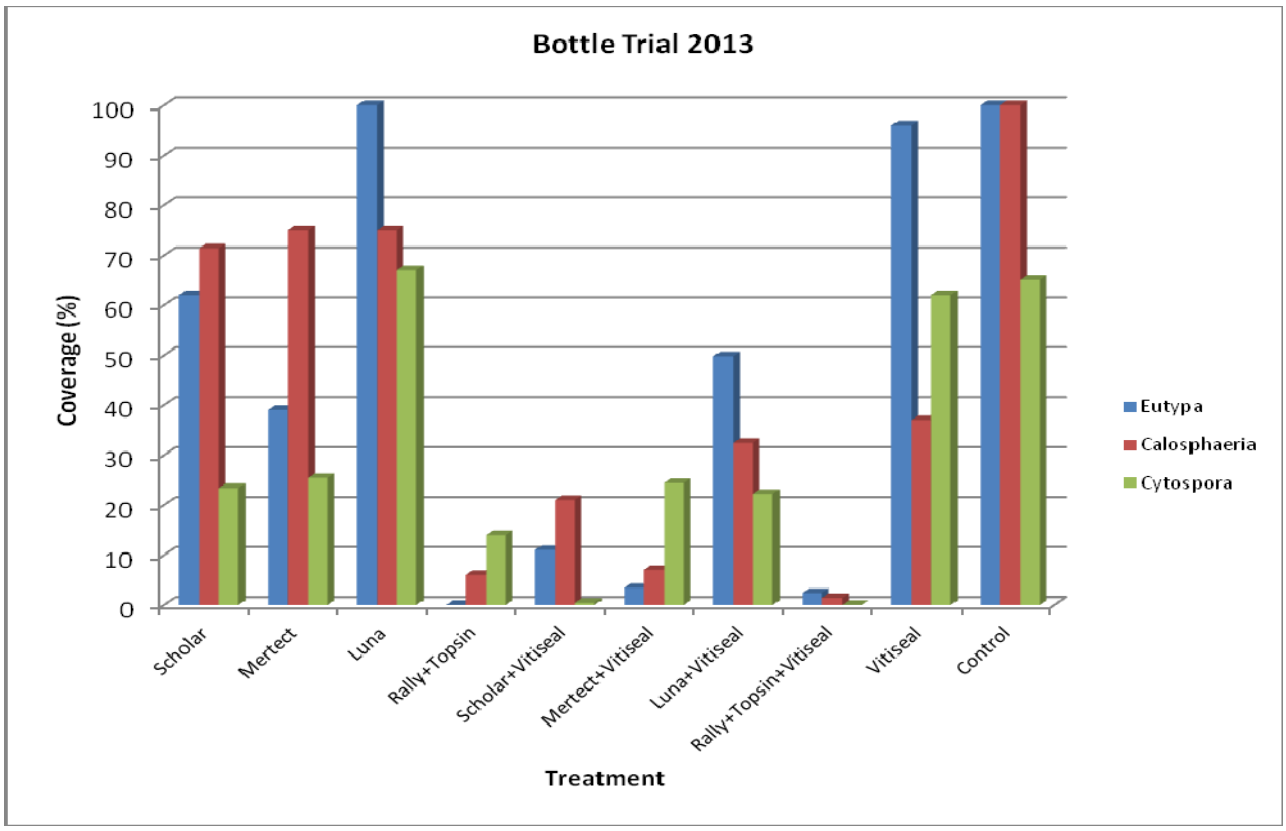


Figure 18: Cumulative percent colonization with fungal mycelium by three canker causing fungi on cherry wood treated with fungicide six weeks after inoculation.

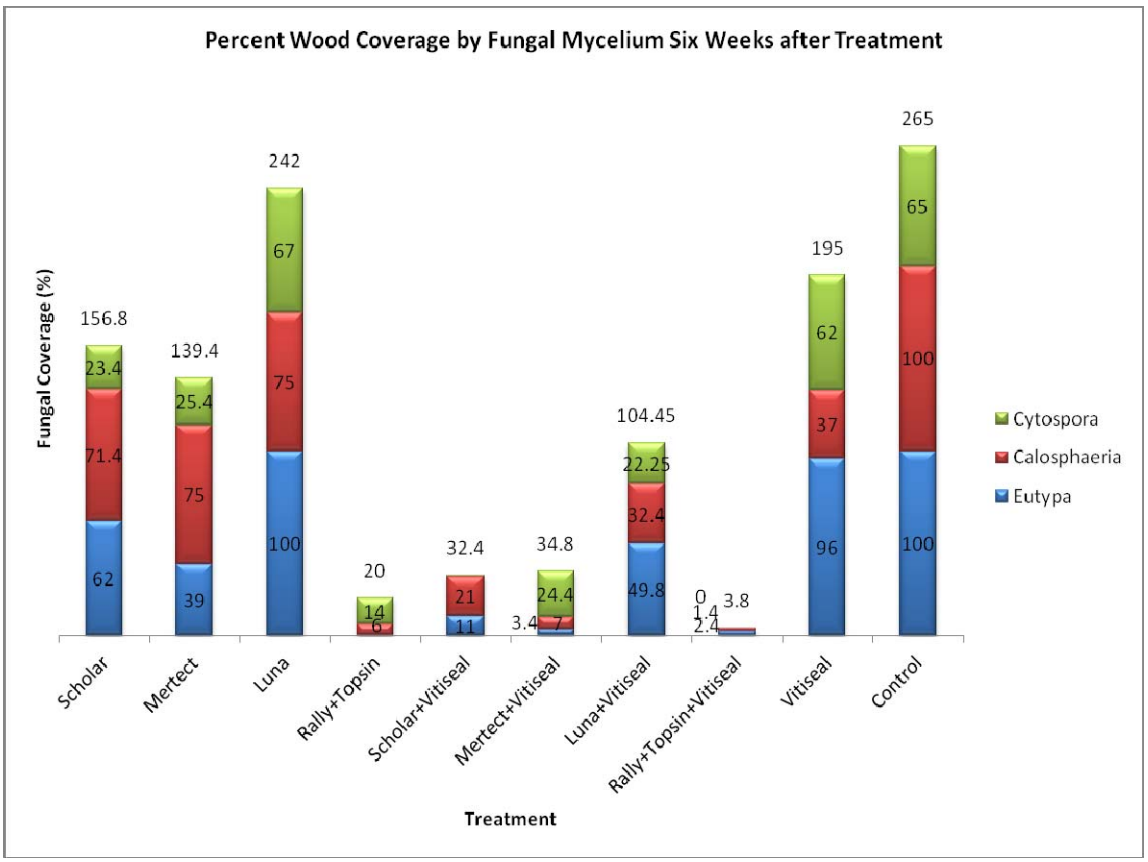


Figure 19-21 illustrate the fungal growth pattern of three canker causing fungi; *Leucostoma persoonii* (Cytospora), *Calosphaeria pulchella, and Eutypa lata* on cherry wood during the six week period of this experiment. The best fit line, the coefficient of determination (R^2), and the fungal growth rate (% growth/week) associated with each treatment is presented in tables 4-6. The results in table 4-6 indicate the efficacy of the treatments with or without Vitiseal.

Calosphaeria pulchella growth rate on cherry wood treated with Luna Sensation had the highest value (15.85% wood coverage/week). The fungal growth rate on Luna+Vitiseal treated wood was 6.98%/wood colonization week, which is a 55.9% reduction in growth rate compared to Luna alone. The Scholar treatment allowed 13.5% fungal growth/week.. Scholar+Vitiseal protected wood by 73.3%. Mertect+Vitiseal treatment caused 90.8% reduction in *C. pulchella* growth rate compared to Mertect. The growth rate associated with Rally+Topsin+Vitiseal was a negative number, which could be observation error due to presence of Vitiseal stains on the wood. The trend of the fungal growth presented in Figure 19 indicates that Rally+Topsin+Vitiseal treatment caused a lower fungal growth throughout the experiment.

Fungal growth rate on the wood treated with Vitiseal was lower than Mertect, Luna Sensation, and Scholar.

As shown in figure 20, *Eutypa lata* growth rate treated with Mertect+Vitiseal, Luna+Vitiseal, and Scholar+Vitiseal was 94.8%, 33.7% and 84.9% less than Mertect, Luna Sensation, and Scholar treated wood. The addition of Vitiseal to Rally+Topsin did not have a significant difference on fungal growth rate. *E. lata* growth rate on wood treated with Vitiseal was the fastest growth rate among the nine treatments and twice as fast as the growth rate of *Calosphaeria pulchella.* As it was shown in Figure 18, by the end of the sixth week of the trial, wood treated with Vitiseal was 96% covered with *E.lata,*..

Leucostoma persoonii growth rate on cherry wood treated with Mertect+Vitiseal was 8% higher than Mertect treated wood (Figure 21). The fungal growth rate had a spike in week four in Mertect+Vitiseal treatment, resulting in overall higher growth rate compared to Mertect treatment. Despite the spike in the growth rate, the final (week six) mycelial coverage of the wood treated with Mertect+Vitiseal was lower than wood treated with Mertect (Fig. 18). The fungal growth rate on wood treated with Luna Sensation, Scholar, and Rally+Topsin were 58.3%, 99.7%, and 96.5% higher than the same treatments mixed with Vitiseal. Vitiseal treated wood had a 10.94% mycelial coverage/week. Overall, the addition of Vitiseal to fungicide had a significant effect on the efficacy of the treatment *in vitro.* Further investigation of the efficacy of this amendment is required, and currently pursued, in field conditions.

Figure 19: Calosphaeria fungal growth rate (% colonization/week) during the six week period on cherry wood treated with fungicide.

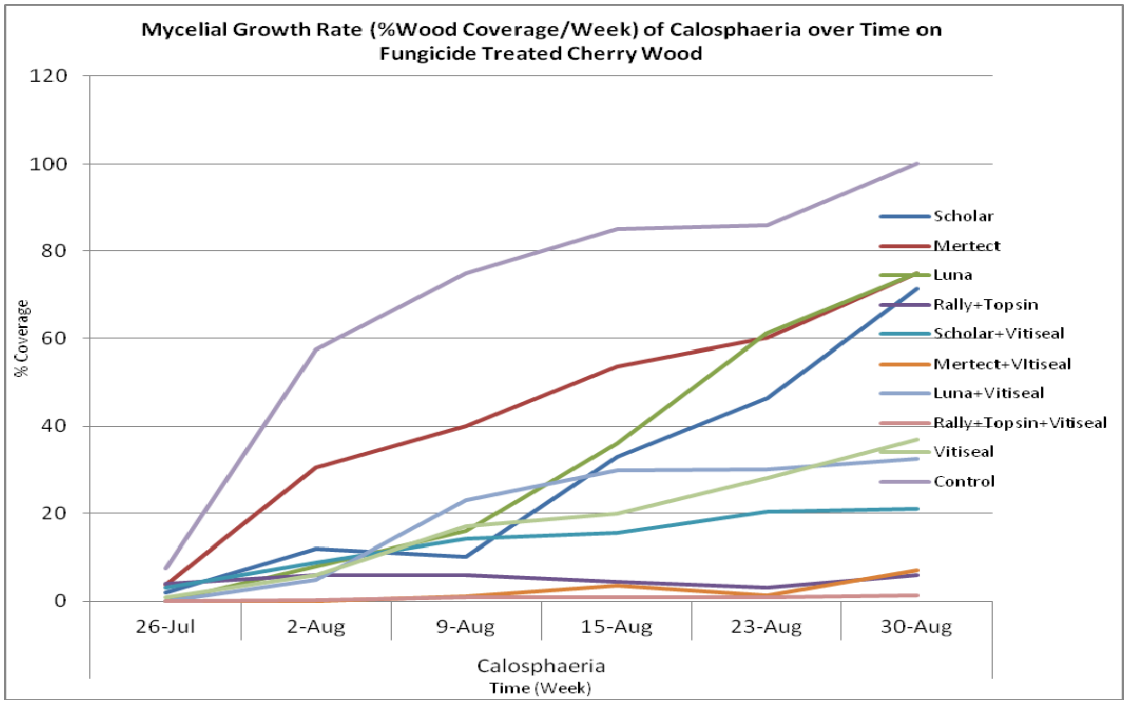


Table 4: The best fit line, the coefficient of determination (R^2), and *Calosphaeria pulchella* growth rate (% growth/week) associated with each treatment.

Calosphaeria pulchella			
Treatment	Best Fit Line Equation	R^2	Fungal Growth Rate (%colonization/week)
Mertect	y = 13.1x - 2.0167	0.9594	13.1
Mertect+Vitiseal	y = 1.1886x - 1.96	0.6807	1.188
Luna Sensation	y = 15.85x - 22.767	0.9613	15.85
Luna+Vitiseal	y = 6.9829x - 4.44	0.8621	6.9829
Scholar	y = 13.514x - 18.133	0.9164	13.514
Scholar+Vitiseal	y = 3.6x + 1.2	0.9459	3.6
Rally+Topsin	y = -0.0171x + 4.96	0.0006	-0.017
Rally+Topsin+Vitiseal	y = 0.2686x - 0.1733	0.8568	0.2686
Vitiseal	y = 7.1143x - 6.7333	0.9854	7.1143

Figure 20: Eutypa fungal growth rate (% colonization/week) during the six week period on woods treated with fungicide.

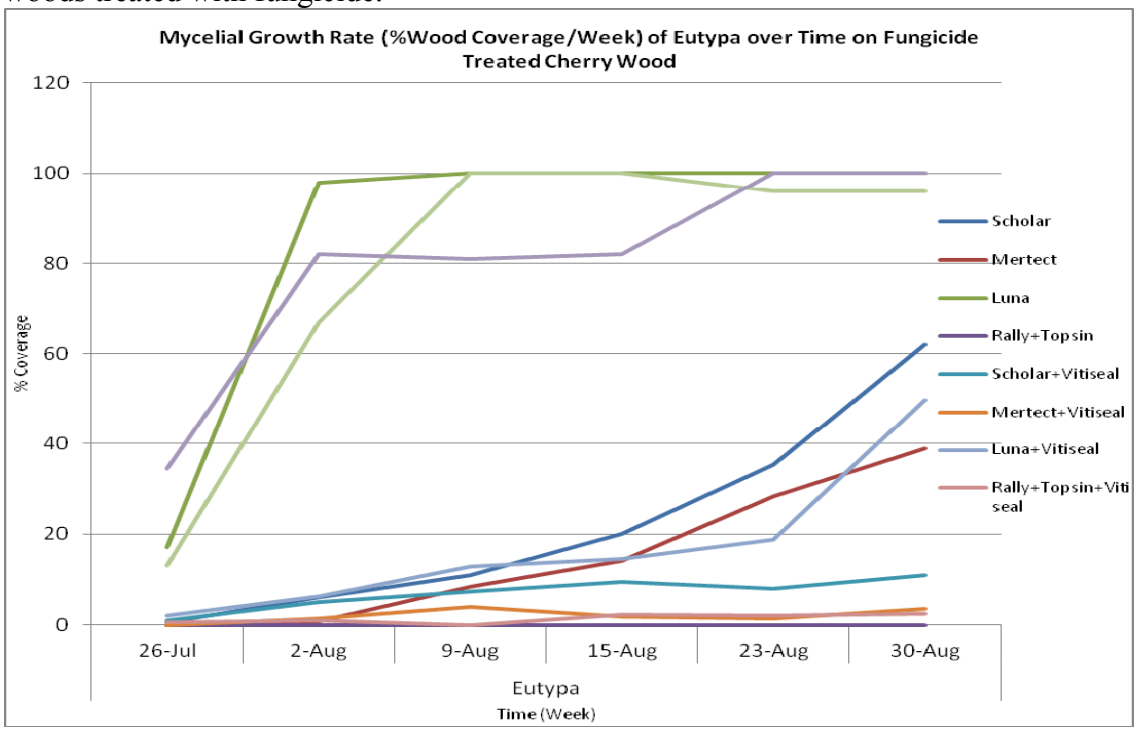


Table 5: The best fit line, the coefficient of determination (R^2), and *Eutypa lata* growth rate (% growth/week) associated with each treatment.

Eutypa lata			
Treatment	Best Fit Line Equation	R^2	Fungal Growth Rate (%colonization/week)
Mertect	y = 8.1029x - 13.227	0.937	8.1
Mertect+Vitiseal	y = 0.4229x + 0.52	0.2919	0.42
Luna Sensation	y = 12.029x + 43.733	0.4451	12
Luna+Vitiseal	y = 7.96x - 10.493	0.7683	7.96
Scholar	y = 11.563x - 17.987	0.8926	11.56
Scholar+Vitiseal	y = 1.7429x + 0.8667	0.8449	1.74
Rally+Topsin	y = 0	#N/A	0
Rally+Topsin+Vitiseal	y = 0.42x - 0.12	0.6281	0.42
Vitiseal	y = 14.343x + 28.467	0.6041	14.34

Figure 21: Calosphaeria fungal growth rate (% colonization/week) during the six week period on woods treated with fungicide.

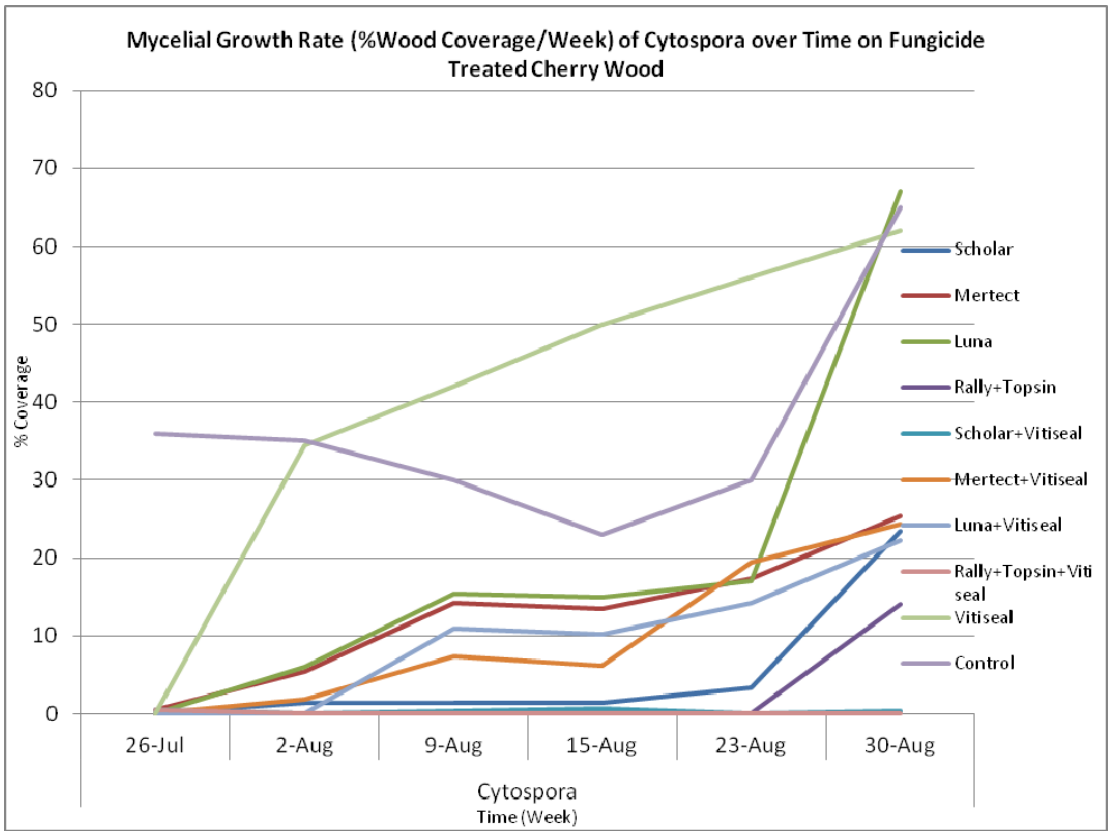


Table 6: The best fit line, the coefficient of determination (R^2), and *Leucostoma persoonii* (Cytospora) growth rate (% colonization/week) associated with each treatment.

Leucostoma persoonii (Cytospora)			
Treatment	Best Fit Line Equation	R^2	Fungal Growth Rate (%growth/week)
Mertect	y = 4.5629x - 3.2533	0.9384	4.56
Mertect+Vitiseal	y = 4.96x - 7.4933	0.8894	4.96
Luna Sensation	y = 10.503x - 16.693	0.6749	10.5
Luna+Vitiseal	y = 4.3786x - 5.7	0.9109	4.38
Scholar	y = 3.5143x - 7.1333	0.5339	3.51
Scholar+Vitiseal	y = 0.0086x + 0.3467	0.0039	0.009
Rally+Topsin	y = 2x - 4.6667	0.4286	2
Rally+Topsin+Vitiseal	y = 0.0714x + 0.3333	0.4286	0.07
Vitiseal	y = 10.937x + 2.4533	0.8468	10.94

Fungal Isolations

Procedure

Branches with canker lesions were collected from several areas of California. Approximately thirty orchards throughout the state (in Wasco, Arvin, McFarland, Salida, Linden, Stockton, Lodi, Clarksburg, Brentwood and Davis) have been visited and canker samples have been collected to identify fungi associated with cankers. Wood chips from necrotic lesions or vascular discoloration at least one cm below the pruning wounds were plated onto PDA-tetracycline plates. Fungi were subcultured and identified using morphological and molecular techniques. Pathogenicity tests of isolated fungi were conducted at UC Davis Armstrong Farm. Symptoms were assessed and pathogens re-isolated to PDA medium.

Results

The following fungi have been isolated from pruning wounds: *Eutypa lata*, *Alternaria alternata, Calosphaeria puchella, Leucostoma personii, Aspergillus niger, Fusarium sp., Truncatella angustata, Alternaria arborescens, Botryosphaeria sp., Diaporthe neotheicola, Trametes versicolor and Lasiodiplodia theobromae.*

The fungi isolated from pruning wounds for each location are listed below.

Arvin: *Calosphaeria puchella, Leucostoma persoonii, Lasodiplodia theobromae, Aspergillus niger, Eutypa lata, Alternaria alternate, Fusarium sp.* and *Diaporthe neotheicola*

Wasco: *Eutypa lata, Trametes versicolor* and *Alternaria alternata*

McFarland: *Calosphaeria puchella, Alternaria alternata, Leucostoma persoonii, Eutypa lata* and *Aspergillus niger*

Lodi: *Calosphaeria puchella, Alternaria alternata, Eutypa lata, Aspergillus niger Leucosoma persoonii* and *Diaporthe neotheicola*

Stockton : *Calosphaeria puchella, Leucostoma persoonii, Alternaria sp.,Fusarium, sp. Aspergillus niger* and *Eutypa lata*

Salida: *Calosphaeria puchella, Leucostoma persoonii, Alternaria sp.* and *Eutypa lata*

Linden: *Calosphaeria puchella, Leucostoma persoonii, Lasodiplodia theobromae, Aspergillus niger, Eutypa lata, Alternaria alternata, Truncatella angustata, Fusarium oxysporum, Botryosphaeria sp.*and *Diaporthe neotheicola*

Brentwood: *Eutypa lata, Calosphaeria puchella, Leucostoma persoonii and Alternaria sp.*

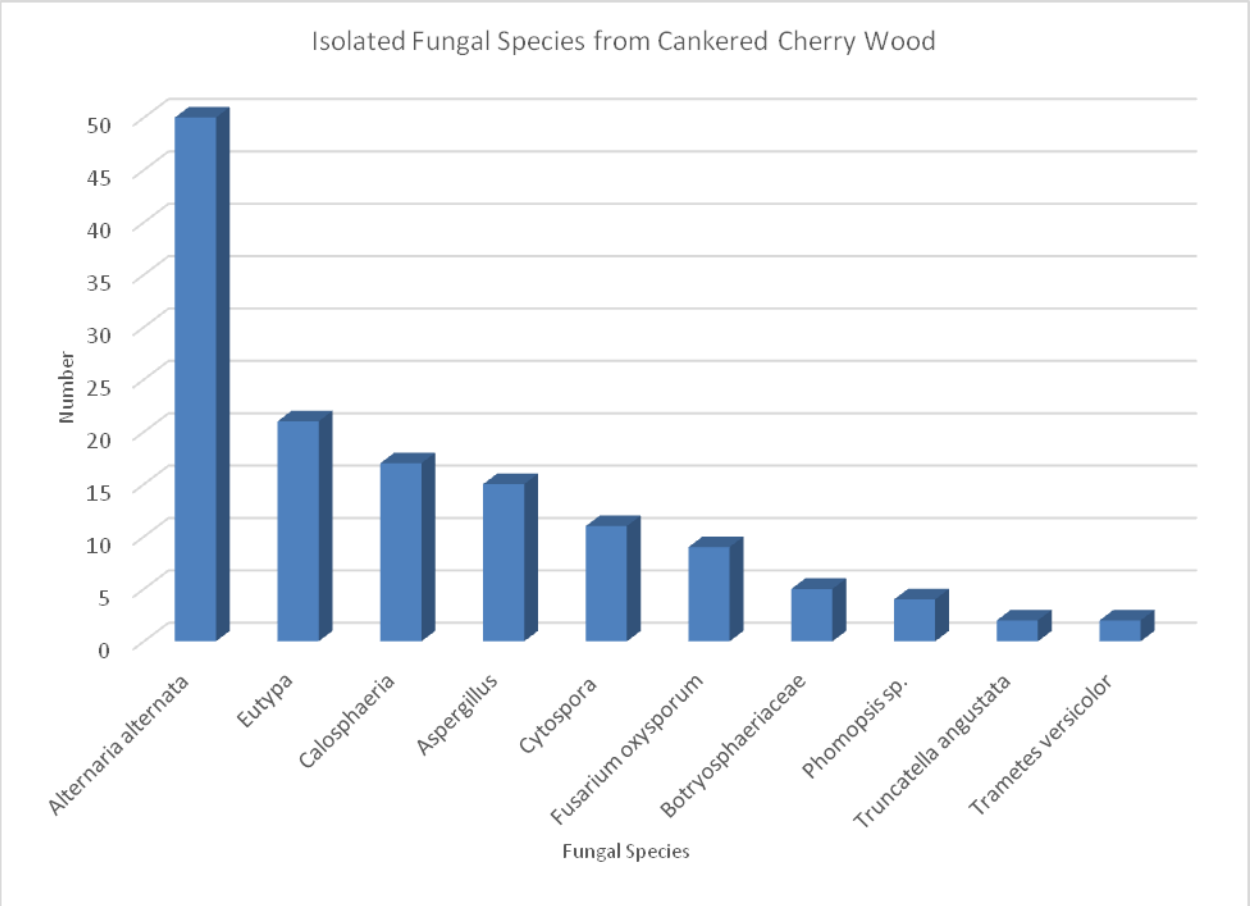
Of this group of fungi, the following are known to be pathogens; *Cytospora, Eutypa, Calosphaeria, Diaporthe, Lasiodiplodia and Botryosphaeria.*

Survey of Fungi Isolated from Linden Cankers

Procedure
Fungal isolates from 135 cankered branches collected from Linden orchard were identified using morphological and molecular methods. For molecular identification, the fungal DNA was extracted from the pure fungal isolates using DNA extraction kit. The internal transcribed spacer (ITS) region was amplified using the universal primers ITS 5 and ITS 4. The sequence results of the DNA samples (ITS amplicons) were analyzed using BLAST software.

Results
Figure 22 shows the fungi isolated from cankers. *Alternaria alternata* was the primary fungus isolated, followed by *Eutypa* and *Calosphaeria*.

Figure 22: Fungi Isolated from Cankered Cherry Branches.



Current Trials

Brentwood Natural Inoculum Vitiseal Trial

Procedure
Large pruning wounds were made 8/13/13. A randomized block design was used. Approximately 50 trees with 1-6 pruning wounds each were treated for each of the following treatments for a total of 400 trees. The treatments were Vitiseal RTU, Vitiseal RTU + Rally (.047g/500 ml), Vitiseal RTU+ Topsin (.300 g/500 ml), Vitiseal RTU + Rally (.047g/500 ml) +.Topsin M .(300g/500 ml), Vitiseal RTU + Rally (.112 g/500 ml), Vitiseal RTU + Topsin (.450g/500 ml), Vitiseal RTU + Rally (.112g/500 ml) +Topsin M (.450g/500 ml and a control).

Air Blast Fungicide Trial

Procedure
Pruning wound protection against invading canker pathogens were tested using commercial air blast or backpack airblast sprayers in a mature orchard at the Plant Pathology research station in

Stockton. Treatments included Endure KD, Rally 40W and Topsin M (70 WP) in combination with HiWett, and a water control. The experiment was setup using a randomized complete block design with four replications (four blocks). Experimental units consist of one tree. Each experimental unit received twenty pruning wounds made randomly on one to three year-old twigs across the tree canopy. Fungicides and control treatments were applied (one-time application) directly after pruning using an air blast backpack-mounted sprayer. No inoculation of pruning wounds were made in this test. We will depend on natural inoculum for infection. Pruning wound tissue will be collected and taken to the laboratory to be examined for canker formation. After six months, branches will be removed. Wood samples will be surface sterilized using ethanol and flaming. Wood chips from necrotic lesions or vascular discoloration just below the pruning wounds will be plated onto PDA-tetracycline plates to look for fungal growth.

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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Interim Report - September 2013

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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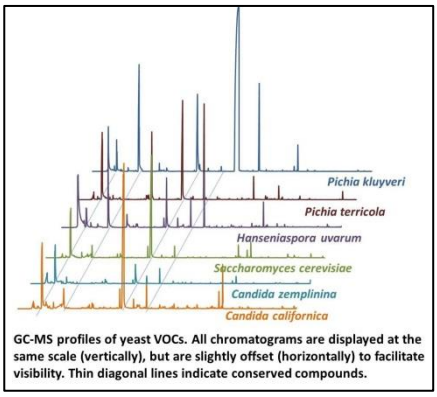
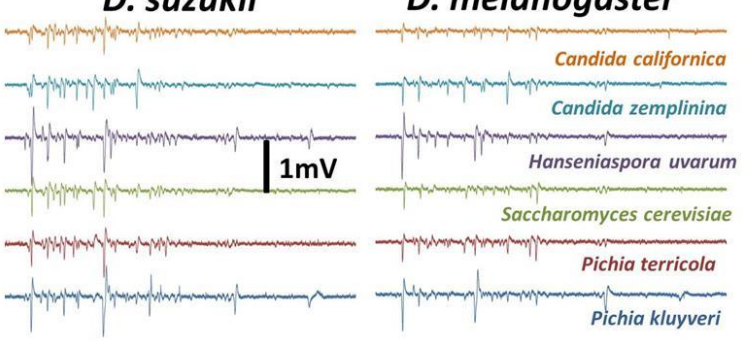
We just finished detailed investigations into the constituent odorants (Volatile Organic Compounds, VOCs) from all the major yeast species associated with spotted wing drosophila (SWD) that are implicated in the fruit/host-choice. Analysis of VOCs was performed on yeasts grown on minimal or normal potato dextrose media to compare and contrast media effects. We raised yeasts to comparable densities so as to preclude effect of varying densities on VOC quantities, and collected VOCs either on a high affinity adsorbent that was later eluted in an organic solvent to yield a solvent extract or using Solid Phase MicoExtraction (SPME) method that offered the advantage of collecting a higher amount of VOCs.



GC-MS profiles of yeast VOCs. All chromatograms are displayed at the same scale (vertically), but are slightly offset (horizontally) to facilitate visibility. Thin diagonal lines indicate conserved compounds.

Analysis of VOCs on a high resolution Gas Chromatography (GC) column employing Mass spectrometry (MS) revealed: No significant qualitative differences in VOCs collected from minimal or PD media; Higher VOC quantities were collected from PD; A set of VOCs, such as isoamylalcohol, isoamylacetate, phenylethylalcohol and phenylethylacetate were consistently identified in higher amounts across many of the yeast species, whereas species specific VOCs were also found in other yeast strains.

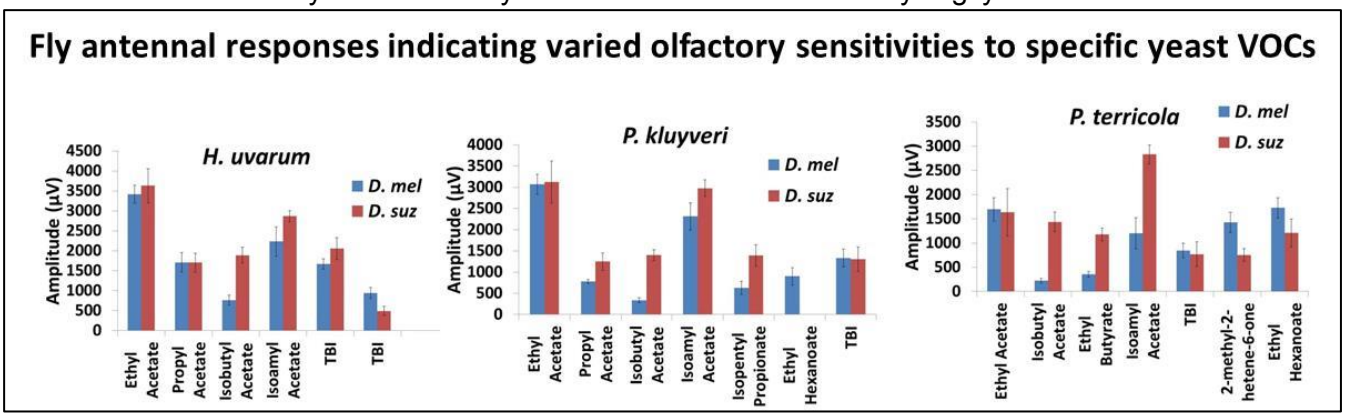
Electrophysiological responses indicating varied olfactory responses from flies to yeast VOCs



The VOC extracts were subjected to isolation and identification of biologically active constituents using a live, restrained SWD fly's antenna as sensing elements to detect constituent odorants as they emerge from the chromatography column. This method, described as Gas Chromatography linked electroantennographic detection (GC-EAD) revealed intriguing results: Overall, SWD were more sensitive to VOCs from yeasts compared to *D. melanogaster*, and that a few compounds elicited specific responses

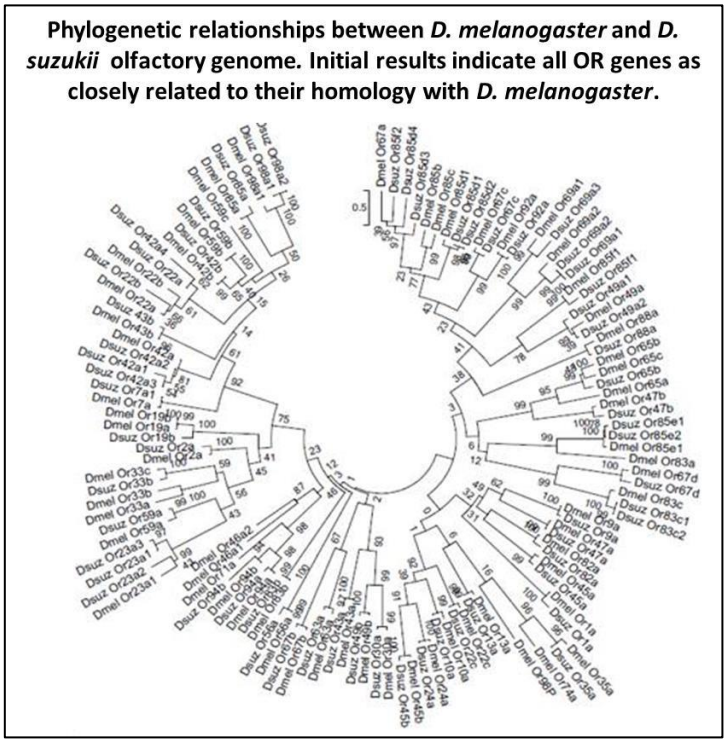
from SWD and *D. melanogaster*.

We are poised to compare and contrast the behavioral correlates for the electrophysiological differences induced by VOCs from yeasts. We have started analyzing yeast-VOC extract induced



behaviors. We aim to formulate blends of biologically active yeast constituent VOCs (identified from GC-EAD analysis) that will provide effective and selective SWD attractive blends.

Exploiting the recently finished genome of SWD at UC-Davis, we made a detailed phylogenetic analysis of the proteins that define attraction in flies to hosts and mates. Olfaction in flies is mediated by a large family of proteins, olfactory receptors (ORs) that are expressed in the antenna. We initially obtained the OR sequences from *D. melanogaster*, that has 60 functionally characterized ORs. We searched for sequence homology for individual genes in SWD. TmPred software was used to analyze multiple transmembrane domain characteristics of ORs. Based on previous results, a low cutoff of 369 bp was used as a basis for annotating a given sequence as a functional OR. Sequences below 369 bp were annotated as pseudogenes. The results indicate extensive gene loss and duplications in *D. suzukii.* The average gene length of OR in *D. suzukii* is 474bp compared to 395bp in *D. melanogaster* indicating extensive OR evolution in *D. suzukii*. This is clearly depicted in number of gene duplications and losses. A total of 20 gene duplication and 14 gene losses were recorded in *D. suzukii*. Of the 20 gene duplications, 7 were pseudogenes (<369bp in length) and 13 are putative functional *Ors* making the total number within the functional OR repertoire 53. Some of the interesting facts of the OR gene repertoire of *D. suzukii* are: There is one functional copy of *Or42* in *D. melanogaster* compared to four gene duplications in *D. suzukii,* and all four of those are pseudogenes; The *Or85e,* classified as a pseudogene in *D. melanogaster* has an ortholog in SWD and it is functional, thus offering an exciting possibility that this OR is a potential candidate in detection of SWD specific VOCs.



California Cherry Marketing and Research Board Progress Report

Title: Maintaining the UC IPM Pest Management Guidelines for Cherry (2013-14)
PI and Contributors: Kassim Al-Khatib, Tunyalee Martin, Romy Basler
Expected Completion: April 7, 2014

Results
The *UC IPM Pest Management Guidelines: Cherry* (UC ANR Publication 3440) is the University of California's official guidelines for managing pests in cherries. http://www.ipm.ucdavis.edu/PMG/selectnewpest.cherries.html

- General properties of fungicides and Fungicide management sections updated in March 2013.
- Botrytis blossom blight, brown rot blossom and twig blight, powdery mildew, and ripe fruit rot updated March 2013
- Spotted wing drosophila updated
- The fungicide efficacy, timing, and resistance management general information sections will be updated for 2013.
- The annual call for updates is planned for November 2013.

Coordinator, Crop Team, and Authors
PMG Coordinator Romy Basler coordinates the process and edits material for clarity and completeness and to conform to format and style. The Crop Team helps to manage the overall direction of the Pest Management Guidelines and, with the authors, provides scientific content.

Crop Team
Romy Basler (PMG Coordinator)
Janet Caprile (Crop Team Leader)
James Adaskaveg
William Coates
Kevin Day
Joseph Grant
John Roncoroni

Authors
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William Coates
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Joseph Grant
John Roncoroni
Robert VanSteenwyk
Becky Westerdahl

Update Process
The annual call for updates requests that authors submit changes to the PMG Coordinator and may result in changes to pesticides and monitoring or management methods. New pests can be added as well. PMG Coordinator Romy Basler will release the call in November. The process:

1. The PMG Coordinator sends an email to the authors asking them to submit changes within a month.
2. The PMG Coordinator makes these changes, reconciling them to one another and going back to the authors for clarification; edits the manuscript for flow and style; and updates the fungicide general information tables. The PMG Coordinator returns the resulting manuscript to authors for review.

3. The authors review the updated manuscript and either approve or make additional changes. If additional changes are needed, they are incorporated by the PMG Coordinator and reviewed by the authors; this process continues until the authors approve.
4. The PMG Coordinator submits the manuscript to the UC ANR Office of Pesticide Information and Coordination to ensure the pesticide information is correct.
5. The PMG Coordinator then works with the UC IPM Production Team to get the manuscript prepared and posted to the UC IPM Web site.

CONTINUING REPORT **YEAR: 1of 2**

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

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Cooperators: Matthew Whiting

Total Project Request: **Year 1:** $59,910 **Year 2: $60,964**

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	28784	29648	
Benefits	18064	18604	
Wages	3520	3520	
Benefits	352	352	
Equipment			
Supplies	2310	1960	
Travel	1000	1000	
Miscellaneous			
Plot Fees			
Total	54030	**55084**	

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) 2000 to 3000 ovaries sampled randomly at 15 to 18 days after bloom were sufficient for crop estimates by dry weight per ovary

2) Bee exclusion bagging of limbs provided reference values for the growth of unfertilized ovaries

3) Ovary length to width ratios improved detection of potential fruit versus developmentally failed fruit

4) Crop estimates improved every five days, up to 30 days from bloom

5) Potential fruit size at harvest was determined 30 to 35 days from bloom

6) Fresh weight to dry weight ratios of ovaries differ between Fruit and Failures as early as 10 days from bloom and may lead to a new method using density of ovaries for crop estimates

7) Some bagged ovaries grew similar to fruit, especially in 'Sweetheart' indicating some self-fertilization in the absence of pollinators

8) '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

9) Early season application of Cytokinin increased fruit size at the pit hardening stage

Growth Analysis of Sweetheart. Calendar days versus Degree days

Growth analyses are necessary to objectively compare cherry growth behavior between different grow sites and seasons in order to develop predictive models that will inform growers and marketers of factors influencing cherry fruit quality. We performed such analyses for 'Sweetheart' at three grow sites with historical differences in bloom and harvest timing (fig.1).

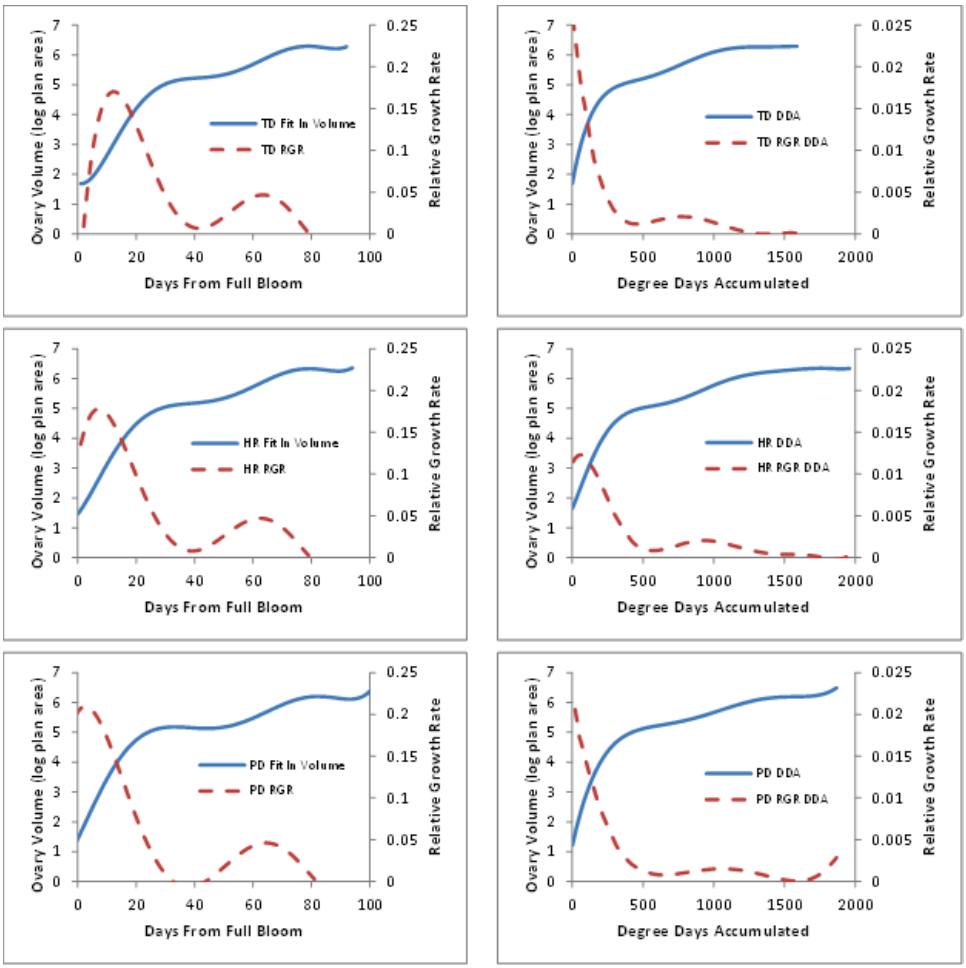

Figure 1. Growth analysis of 'Sweetheart' in three locations; TD (The Dalles) top panels, HR (Hood River) center, and PD (Parkdale) lower panels. Calendar date (left panels) and Degree Day (right panels). Ovary volume and Relative Growth Rates (RGR) are shown for comparison. Degree Days Accumulated (DDA) were calculated on a 43° F baseline.

Of great importance in producing growth models is the elimination of growth from unfertilized ovaries which lead to failed fruit development. Models which do not separate fertilized fruit from unfertilized fruit in the first 30 days from bloom will grossly underestimate fruit growth. This is the case since, on average, 25%-40% of the initial flowers set fruit (Table 1). Therefore, 60%-75% of the fruit in a random sample collected during the first 30 days from bloom will not be carried through to harvest, severely underestimating the potential growth rate and underscoring the importance of eliminating unfertilized growth from such analyses.

In this portion of the project we compared growth of open pollinated ovaries to ovaries enclosed in bee exclusion bags seeking to determine the size and shape differences that could be used to distinguish between fruitful ovaries and failed ovaries collected randomly. We measured size (plan area) and shape (length and width) photographically from approximately 300 ovaries at five day intervals for Chelan, Bing, Rainier and Sweetheart. The size and shape factors determined from the

bagged ovaries (data not shown) were then used to perform statistical cluster and discriminate analyses of the open pollinated ovaries, thereby eliminating unfertilized growth.

Fruit growth of sweet cherry is dependent upon temperature and thus can be modeled by growing degree units (fig 1). In 2013 there was approximately a two week difference in bloom timing, and a one week difference in the fruit development period between bloom and harvest when comparing The Dalles (TD) and Parkdale (PD). The timing for Hood River split the difference. TD site had the coolest temperatures at bloom and a delayed peak of relative growth rate (RGR), but the warmest summer resulting in the fewest days from bloom; whereas PD had the warmest temperatures at bloom with the most rapid increase in RGR but a cooler summer resulting in the longest time until harvest. Differences between the RGR curves, especially soon after bloom, and differences in the total degree days accumulated (DDA) at harvest time, indicate the baseline temperature used to calculate DDA should be adjusted downward.

Similar growth studies will be repeated next season. Each season and location that can be added to this study will add confidence to a DDA dependent model of cherry fruit growth.

Fruit Set Analysis. Size of fruit versus failures provides an early estimate of *Marketable Fruit*

Prediction of the potential crop and expected fruit size would aid growers in understanding and assessing the environmental factors and horticultural practices that limit fruit from reaching their predicted potential. Furthermore, prompt crop estimates would inform marketing strategies. It is important to note that most crop estimates neither account for aborted fruit that drop after 30 days nor for fruit that remains until harvest only to be culled at the packing house for lack of size. In the event that unfertilized ovaries or fertilized fruit that suffer some limitation in their development within the first 20 days from bloom, their growth rate will be reduced, and this measure can be used in crop estimates. We were able to generate these data and determine several groupings of fruit based on their weights (fig. 2). Dry weight was used (as opposed to fresh weight) because it provides the actual carbon gain of the fruit and, as a technique, it eliminates fruit weight loss (and measurement error) when significant time is required for analyzing fresh samples- as was the case for the processing and individual weighing of over 50,000 fruit in our 30 d sampling period.

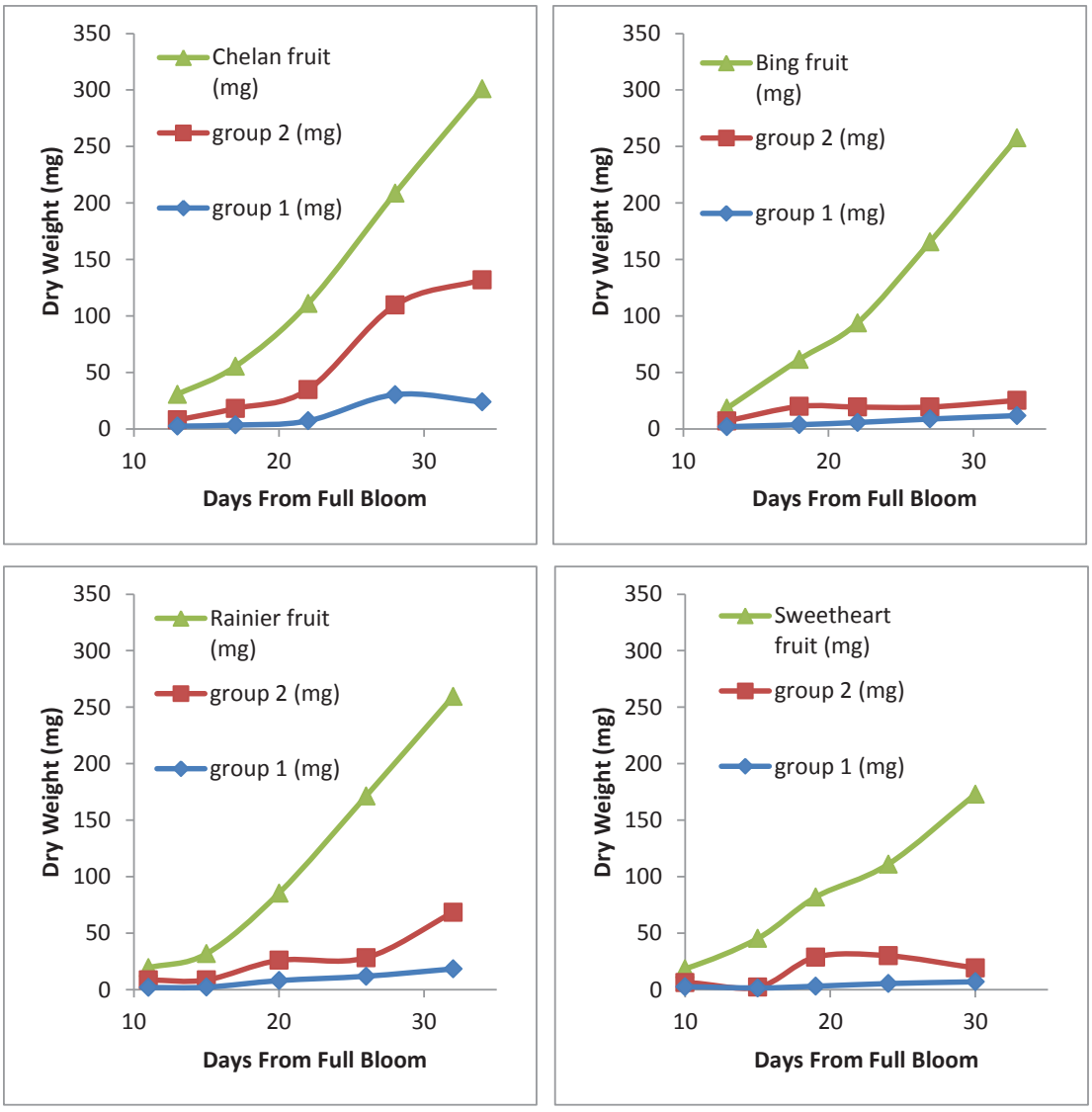

Figure 2. Growth analysis and fruit set of four varieties in The Dalles. Ovary dry weights were classified into three groups easily detected with statistical cluster analysis. Group 1 ovaries expand little beyond their size at bloom. Group 2 ovaries grow to the size of a 10 or 20 DFFB cherry then fail and drop. Chelan appears to have the greatest extent of growth of the Group 2 ovaries which explains the 'apparent' continual or 'late' drop often observed with this variety.

We also examined the relative water content of fruit during development (derived from fresh and dry weights) and have noted that a density difference distinguishes Fruit from Failures early in their development. A simple bucket procedure using liquids of varying density should allow Failures to float. Producers would then have a rapid test to estimate their crop in the orchard. We propose to develop this assay in 2014.

Table 1. Developing and undeveloped cherries from both unfertilized and fertilized ovaries were sampled at ~5 d intervals beginning ~15 d after full bloom. Ovaries were dried to constant weight in an oven and weighed individually (expressed in table as ovaries examined). These data were then subjected to statistical analyses to estimate the percentage of total fruit on the tree that will remain to harvest over time.

Variety	Days From Full Bloom	Ovaries Examined	Crop Estimate of Market Sized Fruit Remaining on the Tree	Fruit Set on Selected Limbs as a Percent of Bloom Count not Corrected for Market Size
	(days)	(no.)	(%)	(%)
Bing	18	2567	36%	
	22	2403	48%	
	27	1910	68%	
	33	1909	78%	
	39			38%
	50			30%
Chelan	17	2391	51%	
	22	2423	34%	
	28	1792	53%	
	34	1670	76%	
	41			30%
	52			24%
	62			24%
Rainier	15	2528	71%	
	20	2026	71%	
	26	1776	84%	
	32	1645	90%	
	39			41%
	50			36%
	78			39%
Sweetheart	15	3289	24%	
	19	2730	30%	
	24	1915	70%	
	30	1674	94%	
	36			36%
	47			32%

Ovary size was measured by dry weight allowing us to harvest several thousand ovaries on multiple dates. Ovaries collected from the field were brought to MCAREC where we removed styles and stems before drying the ovaries slowly in ovens. When dry, individual ovaries were weighed on an analytical balance connected to a computer for data acquisition.

PGR Experiments.

Eight single-tree reps in a 'Lapins' block were treated with various PGRs at 5 dafb with a pressurized hand gun. Treatment timing was based on our previous work, which identified early season maximum growth rates of sweet cherry fruit (irrespective of cultivar) to occur within the first week from bloom. Cytokinin (CPPU; KimBlue) and GA (ProGib) alone or in combination (Promalin) were applied to determine if fruit size could be increased at harvest. Fruit, randomly sampled at pit-hardening stage were significantly larger when treated with Promalin or CPPU, indicating a positive effect of cytokinin on early fruit growth (fig 3). This effect was not influenced by cropload and PGR treatments did not affect fruit set (Table 2). That GA (when applied on its own) did not have a positive effect on fruit growth suggests that this compound may not have a role in early fruit development at the rates applied. Zhang and Whiting (2011) observed a GA-induced increase in the

size of fruit at harvest when applied in a lanolin paste to the stems of cherry fruit at 9 dafb; however, in their study GA rate was 200 ppm. Though our applications were less direct (sprayed to entire canopies), GA clearly was taken up as shown by the significant enhanced stem growth (stems 13% longer) relative to other treatments (Table 2).

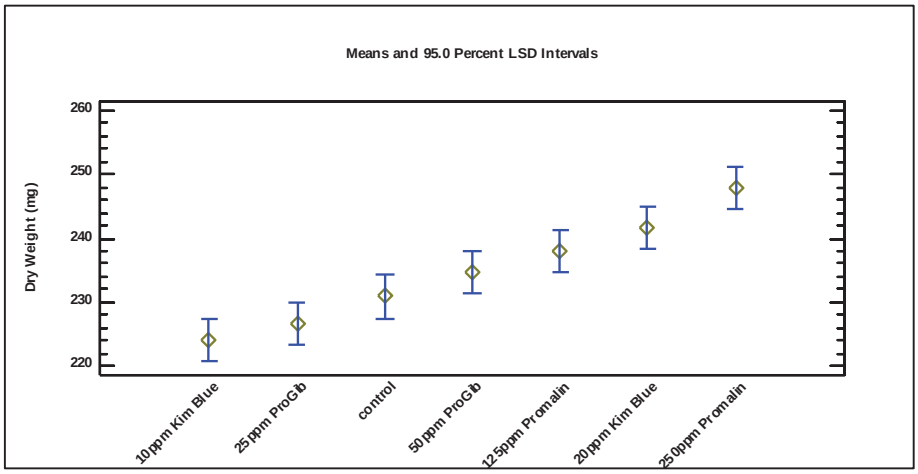

Figure 3. Fruit size (expressed as dry weight) was determined at pit hardening following PGR applications to whole trees 5 days after full bloom. Means are based on 240 fruit per treatment.

Table 2. Effect of PGRs applied to whole canopies of Lapins 5 days after bloom.

Treatments	Fruit set (%)	Yield (lbs/tree)	Fruit diameter (mm)	Fruit wt. (g)	FF (g/mm)	Stem length (mm)	SS (%)	TA (%)
Control	58	158.3	28.7	10.4 abc	213	45.8 c	16.6	0.58
GA (25 ppm)	62.7	196.4	26.8	9.1 c	217	49 ab	16.1	0.59
GA (50 ppm)	58.1	170.3	29	11.1 a	208	51.7 a	15.6	0.6
Promalin (125 ppm)	58.4	168.9	28.3	10.7 ab	194	50.8 a	16.5	0.59
Promalin (250 ppm)	67.9	140.9	28	10.1 bc	197	48.9 ab	17.3	0.6
CPPU (10 ppm)	72.2	171.3	28.2	10.2 bc	209	47.5 bc	16.3	0.6
CPPU (20 ppm)	59.2	150.7	28.2	10.3 abc	212	46.1 bc	16.2	0.58

data are means of 8 single tree reps; n=100 for fruit diameter, fruit weight, FF, and stem length; a segment of 2 and 3-year-old wood (1 per tree) was selected at bloom to determine fruit set. Flowers were counted at bloom and fruit (per segment) were counted at 40 dafb.

In fact, the influence of GA from Promalin treatments promoted increased stem length. At harvest no positive effects from PGRs were apparent on any of the fruit quality attributes analyzed; however, we feel that several key factors contributed to the apparent 'disappearance' of an early-season growth effect. Late-season climatic conditions were unfavorable, and likely adverse to cherry fruit growth. Between 28-June and 3-July daily maximum temperatures exceeded 95 °F, with maximum temperatures above 102°F on 30-June. These high temperatures followed a ~1/2 inch rain event the previous week. Marked splitting and sunburn injury was visually apparent at harvest (unaffected by PGRs) and fruit were exceptionally soft (~200 g/mm fruit firmness; Table 2), indicating severe heat stress. Fruit firmness over the past several years has averaged 270 g/mm in that block. Further evidence that development was impaired by these environmental factors was evident by low SS and TA, relative to past years. This was, in part, due to an earlier season; however, skin color at harvest was 5.5 on a ctifl color scale, which was similar to past years and suggests that similar fruit maturation was attained on the tree. We propose to expand our early-season PGR evaluations by selecting alternative sites (and cultivars) in 2014.

CONTINUING PROJECT REPORT **YEAR**: 2 of 3
WTFRC Project Number:

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

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City/State/Zip: OR97031

Cooperators: Todd Einhorn, Lynn Long, David Felicetti (Pace International LLC), Ryan Durow (Orchard View Farm), Kumar Sellakanthan (Amcor), Ray Clarke (Apio Inc.), Xingbin Xie

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 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 is to minimize pitting, splitting, acid loss, dull color, and stem browning, therefore improve shipping quality of the PNW sweet cherry through (1) selecting the right modified atmosphere packaging (MAP) liner and zipper-lock bags/clamshells, (2) implementing calcium (Ca) in hydro-cooling and flume water, and (3) edible coatings and GRAS compounds.

The key objectives are to:

1. Understand the dynamics of cherry respiration physiology influenced by cultivars, ripeness, temperature, O_2 and CO_2 – an essential knowledge for improving shipping quality.

2. Determine efficacy of the major commercial MAP liners and the optimum MAP parameters (O_2, CO_2) for improving shipping quality of the major PNW and California cultivars at typical shipping conditions.

3. Optimize perforation ratios of zipper-lock bag and clamshell to maintain stem quality.

4. Study the mechanism and practical postharvest Ca treatments to minimize postharvest pitting, splitting, and stem browning.

5. Evaluate edible coatings and GRAS compounds applied post-harvest on shipping quality of PNW sweet cherries.

Goals, activities, and anticipated accomplishments for the next year:

- Determine the effect of simulated temperature fluctuations during commercial shipping on MAP efficacy, and optimize MAP parameters at typical shipping conditions.
- Optimize postharvest Ca application protocols on increasing Ca uptake, reducing pitting and splitting, and improving shipping quality of different PNW cultivars.
- Optimize application protocols of edible coatings and GRAS compounds to increase shipping quality of PNW cultivars.

SIGNIFICANT FINDINGS (year 2)

1. Respiration Dynamics

- At shipping temperatures (i.e., 32-40 °F), respiration rate of the major PNW and California cultivars was affected very little by reduced O_2 from 21 to 10%, but declined logarithmically from 10 to ~1%.
- Estimated fermentation induction points determined by a specific increased respiratory quotient (RQ) were <1% and 3-4% O_2 for most of the major cultivars at 32 and 68 °F, respectively.
- 'Skeena' has a higher Q_{10} from 32 to 50 °F and a higher RQ at elevated temperatures (i.e., 40 °F) than 'Lapins', 'Regina', and 'Sweetheart'. 'Skeena' fruit stressed by heat have a higher respiration rate and could show pitting on trees or after harvest without mechanical damage.

2. MAP Technologies

- The major commercial MAP liners (7) have extremely varied equilibrium O_2 and CO_2 concentrations for 'Bing', 'Regina', 'Skeena', 'Lapins', 'Sweetheart', and 'Coral' (California cultivar) at simulated commercial shipping conditions (i.e., 32-36 °F).
- **O_2 concentration affected flavor.** MAP liners with equilibrium 5-8% O_2 at 32 °F could reduce respiration rate and therefore maintain titratable acidity (TA) and flavor of the major

cultivars at commercial shipping temperatures (i.e., 32-40 °F). MAP liners with O_2 > 10% at 32 °F did not maintain flavor. MAP liners with O_2 < 5% at 32 °F may cause anaerobic fermentation due to temperature fluctuations during commercial storage/shipping.

- **CO_2 concentration affected fruit dull color.** MAP liners with equilibrium CO_2 > 10% at 32 °F could maintain the shiny fruit color at simulated shipping temperature (32-40 °F). MAP liners with CO_2 < 8% at 32 °F have little beneficial effect on maintaining fruit shiny color.
- 'Skeena' is more susceptible to anaerobic fermentation at elevated temperatures, therefore, needs MAP liners with relatively higher gas permeability (i.e., equilibrium 10-15% O_2 at 32 °F) to avoid anaerobic fermentation in commercial shipping.

3. Consumer packaging (see continuing report year-1)

4. Postharvest Ca application

- Adding Ca (0.2-0.5%) in hydro-cooling water efficiently increased fruit tissue Ca concentration and fruit firmness (FF), reduce pitting susceptibility, maintained stem quality and TA, and reduced decay of 'Lapins' and 'Sweetheart'. Ca application rate and temperature gradient between fruit pulp and solution are the key factors determining efficacy of the Ca treatments. Higher Ca rates (1.0-2.0%) damaged stems.
- Adding Ca in flume water at proper rates (i.e., 0.2-0.5%) reduced postharvest splitting and improved shipping quality (FF, total antioxidant capacity [TAC], stem quality, TA, and decay) of 'Skeena' and 'Sweetheart'. Higher Ca rates (i.e., 1.0-2.0%) damaged stems.

5. Edible coatings and GRAS compounds

- Semperfresh™ at appropriate rates (i.e., 0.5% active ingredient [a.i.]) reduced moisture loss, maintained stem quality, and reduced pitting expression of 'Chelan', 'Lapins', and 'Sweetheart' packed in clamshells. However, Semperfresh™ at its label rate of 1.0% a.i. increased pitting expression of 'Sweetheart'. Pitting expression seems to be associated with moisture loss and localized O_2 deficiency.
- Postharvest applications of salicylic acid (SA) and oxalic acid (OA) tended to reduce respiration rate and maintain higher TA, but did not affect total antioxidant capacity (TAC) of PNW cultivars following cold storage/shipping.

METHODS

1. Respiration Dynamics

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.

2. MAP Trials

Seven commercial MAP liners (ViewFresh, Xtend, LifeSpan, Breatheway, and Primpro, PEAKfresh, FreshLOK) with distinct technologies were obtained from 7 manufactures internationally (OVF, StePac, Amcor, Apio, Chantler, PEAKfresh USA, and Shields Bag and Printing CO.). fruit of different cultivars were either obtained from packinghouses shortly after packing or harvested from 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, anthocyanin, SSC, and TA immediately after cold storage and plus 2 days at 68°F. Fifty fruit were randomly selected for evaluations of pitting, splitting, stem quality, and decay. Ten fruit were randomly selected from each box for 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 Applications

1) Hydro-cooling water. Ca (Opti-CAL^{TM}) solutions at 0, 0.2, 0.5, 1.0, and 2.0% were cooled to 32 °F before treatments. 'Lapins' and 'Sweetheart' 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.

2) Flume water. Ca (Opti-CAL^{TM}) solutions at 0, 0.2, 0.5, 1.0, and 2.0% were cooled to 32 °F before treatments. 'Skeena' and 'Sweetheart' fruit harvested at commercial maturity from MCAREC were air-cooled with fruit pulp temperature at 35 °F and then dipped in the cold Ca solutions for 30 min to simulate the commercial on-line processing procedures.

4. Postharvest Applications of edible coatings and GRAS Compounds

Semperfresh^{TM}, 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 logarithmically from ~10% to ~1% significantly for 'Bing', 'Sweetheart', and 'Coral'. Estimated fermentation induction points determined by a specific increased RQ were less than 1% and 3-4% O_2 for both 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).

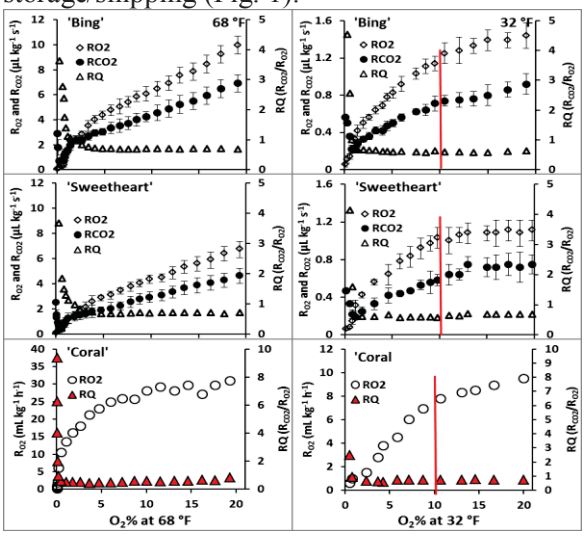

Fig. 1. Respiration dynamic of sweet cherry affected by variety, temperature, O_2 and CO_2.

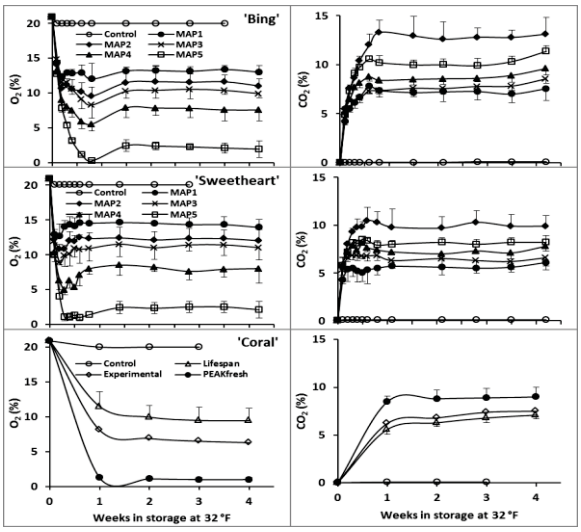

Fig. 2. O_2 and CO_2 concentrations in different MAP liners for 'Bing', 'Sweetheart', and 'Coral' at 32°F.

2. MAP Technologies

1). Gas permeability and efficacies of different MAP liners on maintaining fruit shipping quality.

The seven most popular 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. 2). O_2 ranged from 2-15% and CO_2 ranged from 5 to 13% for 'Bing', 'Lapins', 'Skeena', 'Regina', 'Sweetheart', and 'Coral'. While all the MAP liners maintained higher FF and reduced decay, only the MAP liners with lower O_2 permeability (i.e., equilibrated at 2-8% O_2 + 7-10% CO_2)

reduced fruit respiration rate and maintained TA and flavor of sweet cherries compared to the standard macro-perforated PE liners after 2-6 weeks of cold storage. In contrast, MAP liners that equilibrated with atmospheres of 10-15% O_2 + 5-13% CO_2 had little effect on inhibiting respiration rate and TA loss and maintaining flavor during cold storage.

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

Elevated transit temperatures from 32 to 41 °F reduced O_2 significantly (Fig. 3) but did not change CO_2 too much in MAP liners (data not shown). 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, respectively (Fig. 3). At 36 °F, the equilibrium O_2 was 4.5% and 1% in MAP4 and MAP5 during 2 weeks of cold storage and 'Sweetheart' fruit had no fermented flavor after 2 weeks of cold storage. At 41 °F, 'Sweetheart' fruit was tasted as fermented flavor in MAP5, but not in MAP4 after 2 weeks of storage. In conclusion, MAP with appropriate gas permeability (i.e., equilibrated at 5-8% O_2 at 32 °F) may be suitable for commercial application to maintain flavor without damaging the fruit through fermentation, even if temperature fluctuations, common in commercial storage/shipping, do occur.

'Skeena' has a higher RQ at elevated temperatures and is more sensitive to anaerobic fermentation due to temperature fluctuations during shipping (Fig. 4). MAP liners with equilibrium 10-15% 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' and 'Regina' fruit stressed by heat in the field had higher respiration rates and were more susceptible to anaerobic injury.

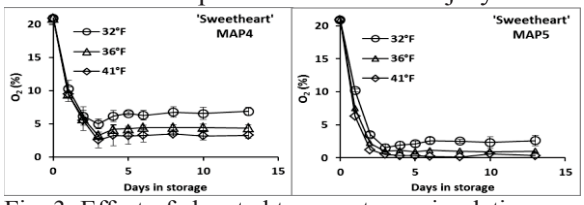

Fig. 3. Effect of elevated temperatures simulating commercial shipping on O_2 and CO_2 in MAP liners.

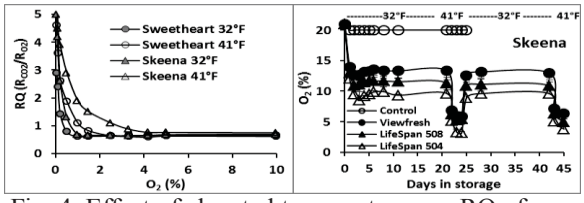

Fig. 4. Effect of elevated temperatures on RQ of 'Skeena' and O_2 and CO_2 in MAP liners.

3. Postharvest Ca Application in Hydro-Cooling Water and Flume Water

1) Hydro-cooling water

Adding Ca at 0.2-2.0% in hydro-cooling water efficiently increased Ca concentration in fruit tissue of 'Sweetheart' (Fig. 5) and 'Lapins' (Fig. 6). Fruit pulp temperature affected tissue Ca uptake, the greater the temperature gradient between fruit pulp and Ca solution, the higher the uptake rate of Ca into the tissue (data not shown). Fruit treated with Ca solutions maintained higher FF, reduced pitting susceptibility, reduced respiration rate, maintained higher TA, and maintained higher total antioxidant capacity (TAC) during 4 weeks of cold storage (Fig. 5&6). Stem quality of 'Lapins' and 'Sweetheart' were maintained by Ca at 0.2% and 0.5%, but damaged by Ca at 1.0% and 2.0% during 4 weeks of cold storage (Fig. 5&6&7).

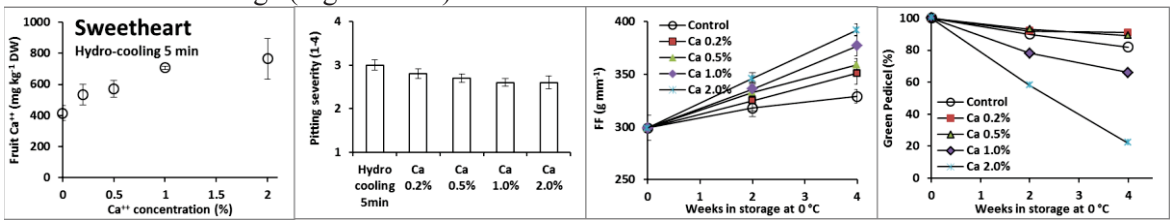

Fig. 5. Effect of Ca in hydro-cooling water on fruit tissue Ca content and shipping quality of 'Sweetheart'.

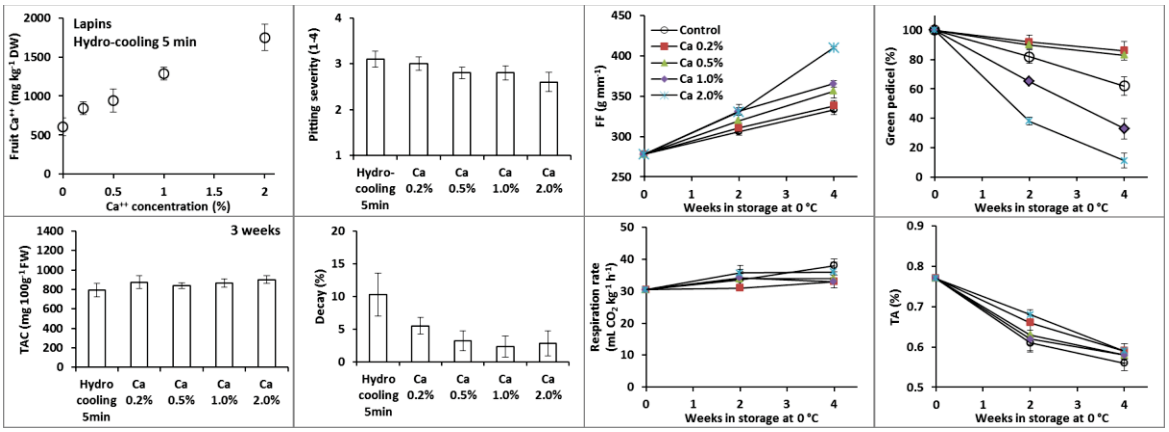

Fig. 6. Effect of Ca in hydro-cooling water on fruit tissue Ca content, shipping quality, and total antioxidant capacity (TAC) of 'Lapins'.

Moisture content after 4 weeks of cold storage:

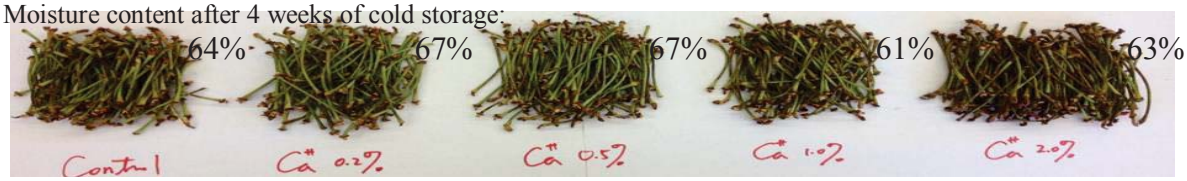

Fig. 7. Effect of Ca in hydro-cooling water on stem moisture content and color of 'Lapins' after 4 weeks in cold storage.

2) Flume water

Adding Ca at 0.2-2.0% in flume water increased Ca concentration in fruit of 'Skeena' (Fig. 8) and 'Sweetheart' (Fig. 10). Ca in flume water reduced postharvest splitting, increased FF, reduced respiration rate, maintained higher TA, and enhanced TAC of both cultivars during 4 weeks of cold storage. Stem quality was maintained by Ca at 0.2% and 0.5%, but damaged by 1.0% and 2.0% in flume water (Fig. 8&9&10). Ca in flume water did not affect water uptake but reduced soluble pectin compounds releasing from fruit of 'Skeena' and 'Sweetheart' into flume water (data not shown). In conclusion, Ca at 0.2-0.5% in flume water can reduce postharvest splitting, improve shipping quality, and enhance TAC of 'Skeena' and 'Sweetheart'.

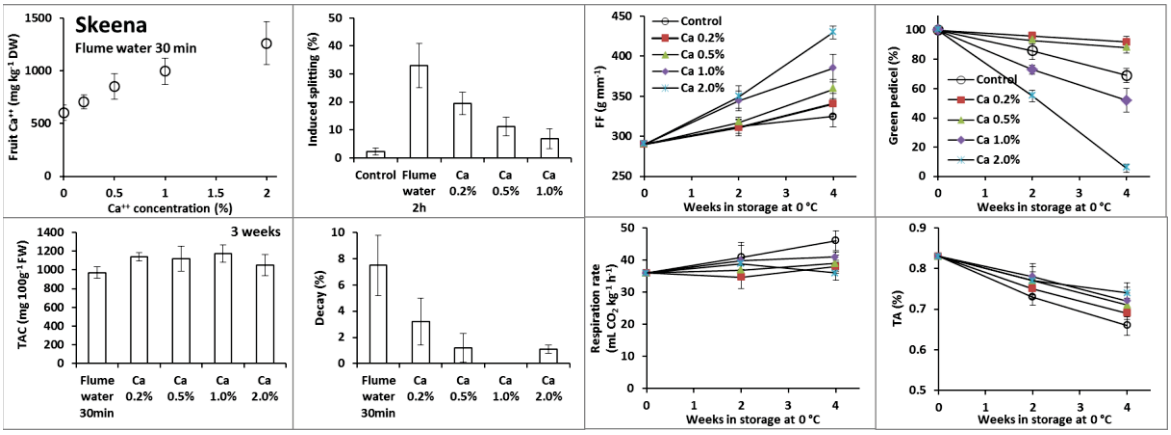

Fig. 8. Effect of Ca in flume water on fruit tissue Ca content, shipping quality, and total antioxidant capacity (TAC) of 'Skeena'.

Moisture content after 4 weeks of cold storage:

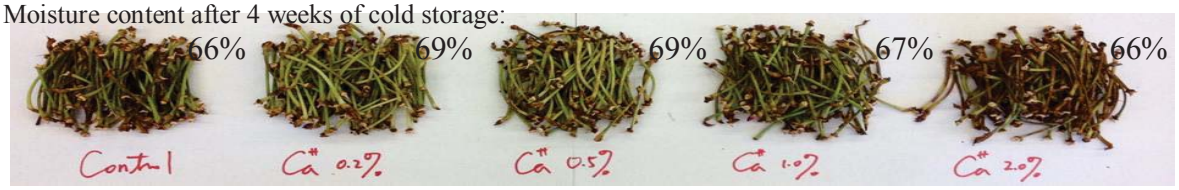

Fig. 9. Effect of Ca in flume water on stem moisture content and color of 'Skeena' after 4 weeks of cold storage.

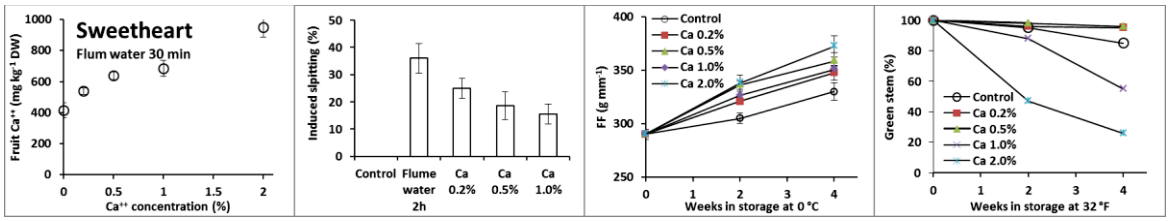

Fig. 10. Effect of Ca in flume water on fruit tissue Ca content and shipping quality of 'Sweetheart'.

4. Postharvest Treatments with GRAS Compounds and edible coatings

1) SA, OA, HBR,

Postharvest applications of SA and OA tended to reduce respiration rate and maintain TA of PNW cultivars packed in clamshells during storage (Fig. 11). It was reported that both SA and OA enhanced 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. 11). Postharvest treatment with HBR at 5 ppm had no effect on shipping quality of 'Lapins' and 'Skeena' (Fig. 11).

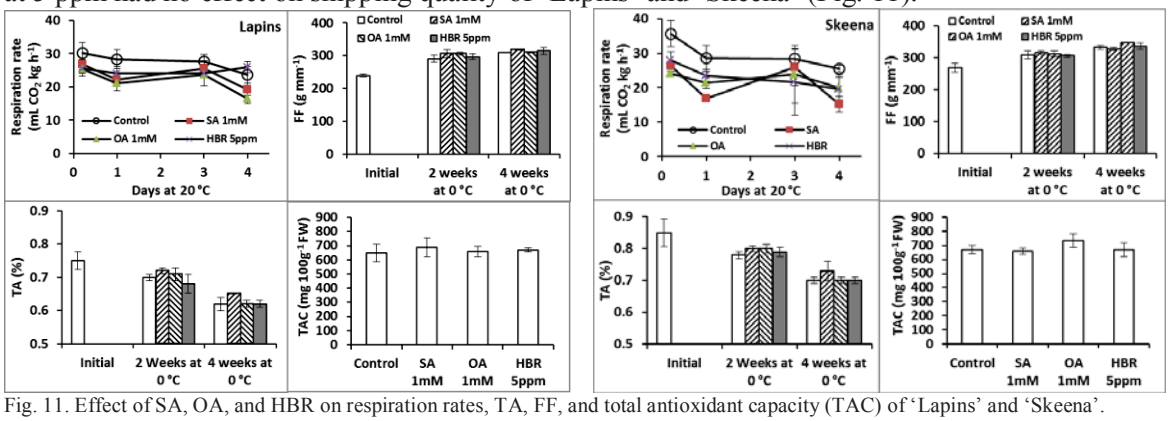

Fig. 11. Effect of SA, OA, and HBR on respiration rates, TA, FF, and total antioxidant capacity (TAC) of 'Lapins' and 'Skeena'.

2) Semperfresh™, GA_3

Semperfresh™ at 0.5% a.i. reduced moisture loss and maintained green stem of 'Chelan' and 'Lapins' packed in clamshells at simulated marketing conditions (Fig. 12). GA_3 at 100ppm did not affect shipping quality of 'Chelan' and 'Lapins'. Semperfresh™ 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. 13). Pitting formation seems to be associated with moisture loss and localized O_2 deficiency.

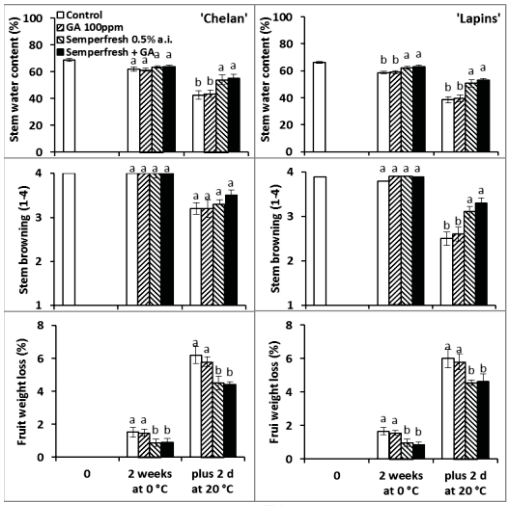

Fig. 12. Effect of Semperfresh™ and GA_3 on shipping quality of 'Chelan' and 'Lapins' at simulated marketing conditions.

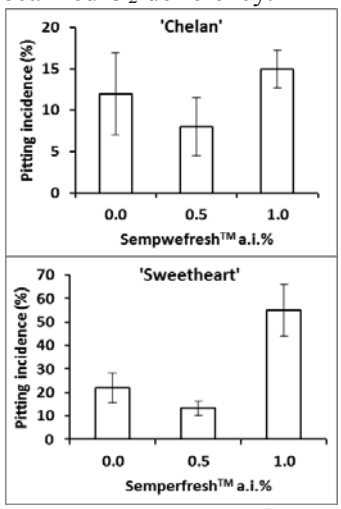

Fig. 13. Effect of Semperfresh™ on pitting incidences of 'Chelan' and 'Sweetheart' after 2 weeks of cold storage.

Monitoring, Biology, and Control of Spotted Wing Drosophila 2013

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Abstract:

This report details progress on the monitoring, biology and control of spotted wing drosophila (SWD), *Drosophila suzukii,* in cherry orchards. Research reported here is: assessment of baits for both field monitoring and for the development of an 'Attract and Kill' strategy, evaluation of the 'Attract and Kill' strategy in multi-orchard field studies, SWD infestation by canopy height, post-harvest insecticide efficacy, and diel periodicity.

The combination of vinegar and wine was more attractive than vinegar alone. The 3:2 vinegar:wine ratio is the recommended ratio. The side wire trap was more effective then the top wire trap. The side wire trap is recommended over top wire trap for the monitoring of SWD. It also recommended to coat the walls of the trap with a liquid Teflon material. A 1:0.75:0.75 Monterey Insect Bait (MIB): apple cider vinegar (ACV):water mixture showed the most promise for use as an attractant solution in "Attract and Kill" SWD management strategies. Baits containing fermented or fermentable products are the most attractive. In the 'Attract and Kill' foliage exposure trial all toxicants had significantly greater mortality than the check. In the 'Attract and Kill' field trials trees treated with an attractant and Danitol or Malathion had significantly fewer larvae than the untreated check. However, severe phytotoxicity was observed where the attractant contacted the foliage. SWD infestation in relation to canopy height appears to be greatly affected by tree density and growth characteristics. Dense, inter-grown canopies result in even distribution of infestation by height. In insecticide efficacy trials higher rates of CHA-062, a new malathion formulation, performed as well as Danitol, Mustang, Venom + Danitol and Gladiator, which all had higher mortality than Movento. SWD flight activity is crepuscular and related to temperatures during period of flight activity.

Spotted Wing Drosophila Monitoring: Monterey Insect Bait Attractant Development Trial I

Materials and Methods: This trial was conducted in a Tango mandarin citrus orchard located near Newman, CA. Nine treatments were replicated six times in a randomized, complete block design (RCB design). Each replicate was a single trap, with at least one buffer tree and row between each replicate. Tree spacing was 16 ft. between rows and 18 ft. between trees. Each trap consisted of a white opaque plastic 1 qt container (Consolidated Plastics Company, Inc., Stow, OH) filled with approximately 4 oz liquid bait and with a $1/8^{th}$ inch screen mesh top. All traps were also fitted with card stock rain shields (Pherocon® 1C Trap top, Trécé Inc, Adair, Oklahoma) to prevent flooding or bait dilution in event of precipitation (standard trap).
In all bait trials the apple cider vinegar (ACV) in all trials was 4% acidity (Amerifoods Trading Co., Los Angeles, CA). Monterey Insect Bait (MIB) (Brandt Consolidated Inc., CA) is primarily corn steep liquor and provided sugar for the yeast fermentation. Baker's Yeast was added at 1.25 oz per half gallon of warm bait solution. All baits included 4 ml color- and fragrance-free dish soap (Palmolive brand "pure+clear" concentrated liquid dish soap) per gallon of bait solution. In all SWD bait or trapping trials, all SWD were sexed and all other drosophila were counted, but not sexed, under magnification in the laboratory at UCB. Traps were placed on 4 December 2012 and monitored weekly through 31 January 2013. The trap baits were replaced and trap locations rotated weekly.

Results and Discussion: In the season mean total SWD, the two best performing baits were 1:1.5 MIB:ACV (21.4/trap/wk) and 1:4 MIB:ACV (20.8/trap/wk). These were significantly better than 1:1.5 MIB:water (6.6/trap/wk), 1:4 MIB:water (4.4/trap/wk) and 1:4 MIB:water + Yeast (4.4/trap/wk) (Table 1 and Fig.1). The 1:1.5 MIB:water bait was the most selective for female SWD (68.5%), and was very closely followed by 1:4 MIB:water + Yeast (67.8%). These two baits captured significantly higher percent females that all baits other than 1:4 MIB:ACV (59.4%) (Table 2 and Fig. 2) and were more selective for SWD over other *Drosophila* spp. 1:1.5 MIB:water was significantly more selective than all baits other than 1:4 MIB:water and 1:4 MIB: water + Yeast (Table 3).

Conclusions: Combining MIB with ACV in both a 1:1.5 and 1:4 ratio results in numerically higher SWD catches than MIB alone. Additionally, the dilution of MIB resulted in a solution that is less viscous and does not dry out in the field. The 1:0.75:0.75 MIB:ACV:water was as attractive as the mixtures with ACV alone, slightly less acidic, as well as requiring the least amount of ACV to make the solution. Thus, of the three most attractive baits, the 1:0.75:0.75 MIB:ACV:water mixture shows the most promise for use as an attractant solution in "Attract and Kill" SWD management strategies. The addition of sugar and yeast to the solution may increase the attractiveness of the bait to target female SWD prior to fruit damage. Further research will be conducted to determine the viability and efficacy of such a combination.

Spotted Wing Drosophila Monitoring: Wine and Vinegar Attractant Development

Materials and Methods: This trial was conducted in a Tango mandarin citrus orchard located near Newman, CA. Seven treatments were replicated six times in a RCB design. Each replicate

was a single standard trap, with at least one buffer tree and row between each replicate. Tree spacing was 16 ft. between rows and 18 ft. between trees.

All bait ingredients are commercially available. Rice Vinegar (RV) was 4.5 % acidity (Kong Yen Foods, Taipei City 106, Taiwan), the Merlot and Chardonnay were 12% abv (Peter Vella Vineyards, Modesto, CA) and the Sake was 15.6 % abv (Gekkeikan Sake (USA), Inc., Folsom, CA). Traps were placed on 4 December 2012 and monitored weekly through 31 January 2013. The trap baits were replaced and trap locations rotated weekly.

Results and discussion: ACV was consistently the least attractive bait with the exception of 2:3 ACV: Chardonnay on 11 and 18 December (Tables 4 and 5). The two Merlot baits were significantly more attractive compared to all other baits except those containing Sake. Among the two Merlot baits, the 3:2 ACV:Merlot mixture was more attractive than the 2:3 ACV:Merlot mixture. Merlot was the most attractive wine in this trial, but Merlot is not conducive for field monitoring due to its burgundy color obscuring the flies in the trap. Sake captured significantly more SWD than Chardonnay when both were combined with ACV and Sake captured numerically more SWD compared to Chardonnay when both wines were combined with RV. The 2:3 ACV:Sake captured 107% more total SWD throughout the trial than 2:3 ACV:Chardonnay. There was a greater impact of wine on SWD captured compared to either ACV or RV. The 2:3 RV:Chardonnay captured only 77% more flies than the 2:3 ACV:Chardonnay. When mixed with Sake, no significant difference was observed between ACV and RV in total SWD over the season. There was no significant difference among treatments in the percent female SWD. However, the percent of female SWD captured increased through the trial. The percent females was 46.1 % female on 11 December and rises steadily to 60.8 % females by 31 January (Table 6). There were significantly greater mean percent SWD of all Drosophila spp. captured in traps baited with Sake and ACV or Sake and RV as compared to ACV alone, 3:2 ACV:Merlot, 2:3 ACV:Chardonnay and 2:3 RV:Chardonnay (Table 7).

Conclusions: Vinegar and wine together are more attractive than vinegar alone. The combination of vinegar and Merlot in a 3:2 ratio caught more flies than the 2:3 ratio. The 3:2 ratio is the recommended ratio. Merlot is slightly more attractive than Sake, but is not viable for field monitoring due to its deep purple color. Both Merlot and Sake are significantly more attractive than Chardonnay. Sake is the recommended wine to use in field monitoring. Sake captured the higher percent SWD as compared to total drosophila. There was no difference in trap captures with RV or ACV. However ACV is less expensive than RV. The recommended bait for trapping is 3:2 ACV:Sake

Spotted Wing Drosophila Monitoring: Trap Improvement

Materials and Methods: This trial was conducted in a citrus orchard located near Newman, CA. Four treatments were replicated six times in a RCB design. Each replicate was a single trap, with at least one buffer tree and row between each replicate. Tree spacing was 16 ft. between rows and 18 ft. between trees. The trap in treatment 1 consisted of a standard trap. The traps in treatments 2-4 consisted of deli containers (Fabri-Kal, Plastics Place, Kalamazoo, MI) with side wire (1/8 in. x 1/8 in. wire openings). The total area of wire screens in both top and side openings were equal, at 12.6 in^2. Top wire traps had a radius of 2 in., while side wire traps had openings that measured 5.2 x 2.4 in. Fluon AD-1 (Northern Products, Woonsocket, RI), a liquid Teflon product, was applied in an even single thin coat using a smooth paper moistened with

Fluon, after which traps air dried for 1 hr. All traps were filled with approximately 4 oz ACV. The bait for treatments 1-3 consisted of soaped ACV. The bait for treatment 4 consisted of ACV combined with 2 ml/gal Fluon. Traps were placed on 4 December 2012 and monitored weekly until 31 January 2013. Throughout the trial trap baits were replaced and trap locations rotated weekly.

Results and Discussion: Side wire traps captured numerically more total SWD than the top wire traps and captured significantly more on 27 December, 24 January and in the season average (Table 8). The side wire traps captured an average of 81% more SWD than the top wire traps during the study. The addition of Fluon to the walls of the side wire trap increased the average catch as compared to traps without Fluon. Side wire traps with Fluon on the walls captured 13% more SWD than side wire traps without Fluon on the walls but the difference was only statistically significant on 4 Jan. The addition of Fluon to both the wall of the side wire traps and the bait appeared to suppress SWD capture throughout the majority of the trial. Side wire traps with Fluon only on the walls captured significantly more flies in the seasonal average than side wire traps with Fluon on the walls and in the bait (Figure 3). There was no significant difference among trap type for selectivity for SWD or SWD sex ratios.

Conclusions: The side wire trap is recommended over top wire trap for the monitoring of SWD. The side wire traps increased SWD catch, do not require a rain shields, and are easier to check in the field. It also recommended to coat the walls of the trap with Fluon or other liquid Telfon material. However, the addition of Fluon to the bait is not recommended. A small amount of unscented and dye free dish soap remains the recommended surfactant for the bait.

Spotted Wing Drosophila Monitoring: Monterey Insect Bait Attractant Development Trial II

Materials and Methods: This trial was conducted in a commercial cherry orchard near Brentwood, CA. Eight treatments were replicated five times in a RCB design. Each replicate was a single standard trap. There was at least one buffer tree and row between each trap tree. The treatments (Table 9) contained commercially available ACV, MIB, Merlot, baking soda, sugar and yeast. Traps were placed on 27 April and monitored at two-day intervals until 5 May. Traps baits were replaced and trap locations rotated at each inspection.

Results and Discussion: On 29 April, treatment 7 and treatment 8 captured significantly more female SWD than all treatments except treatment 5 and treatment 6 (Table 10). Treatments 5 and 6 captured significantly more than treatment 1, treatment 2 and treatment 4, but not treatment 3. Treatment 3 captured significantly more than treatments 1 or 4. On 1 May, treatment 7 captured significantly more than all other treatments. Treatments 1 and 4 captured significantly fewer female SWD than all other treatments, which did not differ significantly. On 3 May, treatment 6 captured significantly more female SWD than treatments 1, 2, and 4. Treatments 8, 7, 5, and 3 captured significantly more females than treatments 1 and 4, but not than treatment 2. On 5 May, treatment 5 captured significantly more female SWD than treatments 1, 2, and 4. Treatments 6 and 8 captured significantly more female SWD than treatments 1 and 4, but not treatment 2. Treatment 7 captured significantly more female SWD than treatment 1. There were no other significant differences between treatments. In the trial average, treatment 7 captured significantly more female SWD than treatments 1 to 4, but was not significantly different from treatments 5, 6, and 8. Treatments 5, 6, and 8 captured significantly more female SWD than

treatments 1, 2 and 4, but were not significantly different from treatment 3. Treatment 3 captured significantly more female SWD than treatments 1 and 4, but not treatment 2.

On 29 April, treatment 7 captured significantly more male SWD than treatments 1 to 4 (Table 11). Treatments 5, 6, and 8 were not significantly different from treatment 2. Treatment 2 captured significantly more than treatments 1 and 4, but not treatment 3. On 1 May, treatment 7 captured significantly more male SWD than all treatments except treatments 2, 3, and 8. Treatments 2 and 3 captured significantly more than treatments 1 and 4, while treatment 8 only captured significantly more than treatment 4. On 3 May, treatments 3, 6, and 8 captured significantly more male SWD than treatments 1 and 4, but not treatments 2, 5, or 7. Treatment 5 captured significantly more than treatment 4, but not treatment 1. On 5 May, treatments 6 and 8 captured significantly more than treatments 1 and 4. Treatments 2, 5 and 7 captured significantly more than treatment 4. In the trial average all treatments captured significantly more male SWD than treatments 1 and 4. There were no significant differences between other treatments.

On 29 April, treatments 7 and 8 more captured significantly more total SWD than treatments 1 through 5, but not treatment 6 (Table 12). Treatment 6 captured significantly more than treatments 1, 2 and 4, but not treatments 3 or 5. Treatments 3 and 5 captured significantly more total SWD than 1 and 4, but not treatment 2. On 1 May, treatment 7 captured significantly more all other treatments. Treatments 1 and 4 captured significantly fewer than all other treatments. There was no significant difference among the remaining treatments. On 3 May, treatments 3 and 5 to 8 captured significantly more total SWD than treatments 1 and 4, but not treatment 2. Treatment 2 captured significantly more total SWD that treatment 4, but not than treatment 1. On 5 May, treatments 5-8 captured significantly more total SWD than treatments 1 and 4, but not treatments 2 and 3. In the trial averages, treatment 7 captured significantly more total SWD than treatments 1 and 4. Treatment 4 captured significantly fewer than all treatments except treatment 1.

On 29 April, treatment 5 captured significantly more other *Drosophila spp*. than treatments 1, 3, 4 and 6, but not treatments 2, 7 or 8 (Table 13). Treatment 4 captured significantly fewer other *Drosophila* spp. than treatment 2 and 7, but was not significantly different from any other treatments. There were no significant differences among treatments on 1 May, 3 May, or 5 May. In the trial averages, treatment 8 captured significantly more other *Drosophila* spp. than treatments 1, 4 and 6. Treatment 6 captured significantly fewer other *Drosophila* spp. than treatments 2, 3 and 5, but was not significantly different from treatments 1, 4 or 7.

On 29 April, treatment 6 captured a higher percent SWD than all other treatments (Table 14). Treatments 3, 7 and 8 captured a significantly higher percent SWD than treatments 1 and 2. Treatments 4 and 5 were not statistically different. On 1 May, treatment 6 captured a higher percent SWD than treatments 5 and 7, which captured a significantly higher percent SWD than treatments 2 and 3. Treatments 2 and 3, in turn, captured a significantly higher percent SWD than treatments 1 and 4. On 3 May, treatment 6 captured a higher percent SWD than all other treatments. Treatments 3, 5 and 7 captured a significantly higher percent than treatment 4, but not than treatments 1, 2, or 8. On 5 May, treatment 6 captured a higher percent SWD than all other treatments except treatment 7. Treatment 7 captured a significantly higher percentage than all treatments, except treatment 5, which was significantly higher that treatments 1 through 4, and 8. In the trial average, treatment 6 captured a higher percent SWD than all other treatments.

Treatments 3, 5 and 7 were significantly higher than treatments 1, 2 and 4 which did not difference significantly from one another.

Treatment 7 captured numerically more female, male and total SWD than other treatments and consistently captured significantly more than treatment 1 and treatment 4. However, treatment 7 was not significantly better than treatments 5, 6, and 8 on a consistent basis. Treatment 2 captured significantly more female, male and total SWD than treatment 4, indicating that wine is a more attractive ingredient than MIB. Treatments 1 and 4 captured the fewest female, male and total SWD and were the only baits that did not contain Merlot or SY. This combination likely increased attractiveness in the absence of wine in the bait through active fermentation. Treatment 6 was also significantly more selective for SWD than all other baits, at an average of 48.5% SWD. It is likely that this is due to the lowered pH of the bait as compared to treatment 7. Treatment 7, which had the highest total SWD catches, was second most selective, at 33.4% SWD, followed by treatment 5 at 31.4%. The current standard, ACV, was least selective for SWD, at 14.7%.

Conclusion: Baits containing wine performed better than those with MIB in the absence of SY. Baits with Merlot and vinegar performed better than vinegar alone. However, baits that contained ACV, MIB, Merlot and SY captured similar numbers of SWD as water, MIB, Merlot and SY. Thus, the use of ACV as compared to water did have any impact on total capture of SWD in the presence of other ingredients that ferment.

Foliage Mortality: of Low Volume Bait Applications in SWD

Materials and Methods: This trial was conducted near Stockton, CA in a 'Bing' cherry orchard. Treatments were replicated six times in a RCB design. Each replicate was individual tree. There was an untreated buffer tree between each replicate. Experimental treatments consisting of MIB and ACV based attractants in conjunction with a toxicant or alone (detailed in Table 15) were applied with a pipette to 25 individual leaves at the rate of 5 drops (10 µl drop) per leaf on one limb in an untreated orchard. Treated limbs were covered with exclusion cages to prevent feeding by wild *Drosophila* spp. or other insects.

Standard foliage exposure techniques were employed. A bouquet of 5 leaves was placed in a one-gallon plastic container. The leaf petioles were placed in a micro centrifuge vial containing water to maintain leaf viability. The vials were mounted in a sponge base and secured to the center of the container with double sticky-backed tape so that the leaf and floral vials were in the center of the container. The containers contained a small amount of food and water soaked sponges in small Petri dishes to provide food and moisture. Ten adult laboratory reared female SWD were then placed in the container through a small slit in the organdy top, which was sealed with a cotton plug after use. The tops of the cages were covered with Saran Wrap to hold the moisture. After 24 hrs of exposure, the flies were removed from the containers and mortality recorded. The experiments were conducted at 23˚C in a constant temperature cabinet with 16:8 (L:D).

Results and Discussion: At 1 DAT, all treatments containing Danitol, Malathion or Entrust (treatments 1, 2, 3 and 5) had significantly higher mortality than both of the treated checks (treatments 4 and 6) and the untreated check (treatment 7) (Table 15). At 3 DAT, bait and Malathion (treatment 2) had significantly higher mortality then all treatments except bait and

Entrust (treatment 3). There was no significant difference among bait and Entrust (treatment 3) and both baits and Danitol (treatments 1 or 5). The treated and untreated checks all had significantly lower mortality than all treatments containing toxicant. At 7 DAT, all treatments containing toxicant had significantly greater mortality than both of the treated checks, which in turn had higher mortality than the untreated check. No significant difference was observed between the attractants tested.

Conclusion: With the exception of 3 DAT, when Malathion had significantly greater mortality than the treatments containing Danitol, there were no consistent significant differences among the insecticides. All had significantly greater mortality than the check. At 7 DAT, the treated checks had significantly greater mortality than the untreated check. This may have been due to the relatively low nutritional quality or high acidity of the baits in comparison to the SWD rearing medium placed in the untreated check. Due to the equivalent efficacy of the tested toxicants, both Malathion and Danitol are recommended for further field testing against both treated and untreated checks.

Field Efficacy of Low Volume Bait Sprays for SWD Management

Materials and Methods: This study was conducted in two commercial cherry orchards near Farmington and Bryon, CA. The cultivar in the Farmington orchard was 'Bing' and the tree spacing was 24 ft. by 24 ft., while the cultivar in the Bryon orchard was 'Coral' and the tree spacing was 14 ft. by 18 ft. Treatments were replicated 4 times in a RCB design. Each replicate was 6 rows x 10 trees in Bryon and 3 adjacent trees in Farmington. Experimental treatments were applied with a squirt gun at the rate of 2 gpa. Treatments were applied weekly from 3 May to 24 May in Bryon and 6 May to 28 May in Farmington. Treatments consisted of an attractant solution (1:0.75:0.75 MIB:ACV:Merlot), combined with either Danitol 2.4EC or Malathion 57%, or alone, as well as an untreated check.

Fruit was collected weekly, beginning the morning preceding the initial application. In the block replicates, fruit was collected from the center trees of each replicate. In the tri-tree replicates fruit was collected evenly from each of the three trees. All fruit was transported back to UC Berkeley in an ice chest. The number of larvae per 100 fruit was determined by the sugar solution floatation method.

Results and Discussion: No larvae were found in pre-treatment sample of 3 May in Bryon and 6 May in Farmington(Tables 16 and 17). In the Bryon orchard, the number of larvae remained low throughout the trial and there were no significant difference among treatments except on 10 May. However, the untreated check had numerically higher number of larvae per 100 fruit in the season total than the treated fruit.

In the Farmington orchard on 28 May, attractant combined with Danitol had significantly fewer larvae than either the attractant alone or untreated check (Table 17). Attractant combined with Malathion had significantly fewer larvae than attractant alone, but was not significantly different than the untreated check. On 3 June, attractant combined with Danitol had significantly fewer larvae than the untreated check, but not attractant alone nor attractant combined with Malathion. On 10 June, mean total larvae in the attractant combined with Danitol had significantly fewer larvae than untreated check and attractant alone but not attractant combined with Malathion.

Due to the low number of larvae in the Bryon orchard, significant differences among treatments were identical to those found in the Farmington orchard until the final sample collection of 6-8 June (Table 18). On 6-8 June, attractant combined with Danitol and attractant combined with Malathion had significantly fewer larvae than the untreated check. However neither was significantly different from one another or the attractant alone.

Conclusion: Trees treated with attractant and Danitol had significantly fewer larvae than either of the untreated checks throughout the trial. Malathion had significantly fewer larvae than the untreated check, but was only numerically lower than the treated check. This preliminary study demonstrated the viability of "Attract and Kill" method of SWD control. Danitol is the recommended toxicant. Severe phytotoxicity was observed where the attractant contacted the foliage. It is assumed that the acidity of the ACV portion of the attractant is the most probable source. The attractant will be neutralized in future research using baking soda.

Spotted Wing Drosophila Infestation in Relation to Canopy Height and Cultivar in Cherry

Materials and Methods: This study was conducted in the cherry block ('Bing', 'Brooks', and 'Coral' trees) of the UC Davis Wolfskill Experimental Orchards, near Winters, CA. A monitoring trap was placed in the orchard in mid-April to establish the presence of SWD in the orchard. Cultivars were evaluated at pink, red, burgundy, and deep burgundy as the individual cultivars ripened. Fruit infestation was determined at three heights. Low canopy was defined as 5 ft. or less, mid canopy as 6 to 10 ft. and the high canopy as 11 to 15 ft. There were 4 replicates in Bing, 5 replicates in Brooks, and 3 replicates in Coral. Each replicate consisted of 1 tree, with the exception of two neighboring Coral trees with low fruit set. Samples of 100 fruit were collected from each height in each replicate when cultivars attained pink color and sampling continued for 4 weeks. Fruit were transported in ice chests to UC Berkeley for evaluation. Larval infestation was determined by the sugar solution floatation method. All larvae were placed on diet and reared to adult for species identification.

Results and Discussion: Coral, the earliest ripening cultivar, was sampled weekly beginning on 24 April (Table 19). However there was no significant difference among heights, with the exception of 1 May, when high Coral fruit contained significantly more larvae than low fruit. Mid canopy fruit was not significantly different from low or high fruit. Brooks were first collected on 1 May, when low fruit had significantly more larvae than mid or high fruit (Table 20). There were no further significant differences in larval counts in Brooks for the remainder of the trial or in total larvae. Bing fruit was first collected on 7 May. There was no significant difference in infestation by height throughout the trial, nor in total larvae (Table 21). Larvae collected early in the season were nearly exclusively SWD, switching to other *Drosophila* spp. over the course of the trial as the fruit matured (Table 22). Percent of larvae reared to be SWD shifted from 100% to 0.06% from 1 to 7 May.

The results show no consistent pattern between canopy height and rate of infestation in any cultivar. This is inconsistent with previous years' findings that showed significantly higher rates of infestation in the lower canopy as compared to the upper canopy. This study was conducted at Wolfskill Experimental Orchard, which is a non-commercial orchard with very tightly spaced trees (5 to 10 ft. x 14 ft.). Previous studies were conducted in commercial orchards, with tree

spacing of 22 ft. x 18 ft. (2011) and 24 ft. x 24 ft. (2012). The tight tree spacing at Wolfskill resulted in shading from both sides of the tree; in addition the canopies were inter-grown with neighboring canopies. In well-spaced commercial orchards, only the lower portion of the canopy is shaded (by the upper canopy). The cool, moist microclimate, seen only in the lower portion of the tree in commercial orchards was present throughout the majority of the tree canopy at the Wolfskill Experimental Orchard. Since SWD prefer the cooler, moister conditions of the under canopy, the lower canopy is more highly susceptible to SWD infestation than the upper canopy. SWD damage was therefore consistent and significant throughout the canopy in this unusual planting system. The percentage of larvae reared to adults was very consistent with previous findings. As the majority of fruit ripens or over ripens the percent of infestation due to SWD significantly declines (Figure 4).

Conclusion: SWD infestation in relation to canopy height appears to be greatly affected by tree density and tree growth characteristics. Research next season will investigate tree density and SWD infestation by canopy height.

Foliage Mortality: Spotted Wing Drosophila Insecticide Efficacy

Materials and Methods: This study was conducted near Tracy, CA in a commercial Bing cherry orchard. Treatments were replicated six times in a RCB design in two distinct trials. Each replicate was an individual tree. There was an untreated buffer between each replicate. Standard foliage exposure techniques were employed for both trials

Trial One:
Results and Discussion: At 1 DAT the mid and high rates of CHA-062 had significantly higher mortality than all other treatments (Table 23). At 3 DAT the high rate of CHA-062 had significantly higher mortality than all other treatments except Belay +Danitol. Belay + Danitol had significantly higher mortality than Malathion 57% and the untreated check, but did not have significantly higher mortality than the mid or low rates of CHA-062 or Danitol. At 7 DAT the low rate of CHA-062 had significantly higher mortality than the check, Malathion 57% and the mid rate of CHA-062. Danitol had significantly higher mortality than the untreated check. At 14 DAT, there were no significant differences among the treatments.

Conclusion: The higher rates of CHA-062 initially performed better than all other treatments, and at 3 DAT the highest rate was still significantly better than all other treatments except Belay + Danitol. Malathion 57% had the lowest performance overall, and was never significantly different than the untreated check. The performance of Belay + Danitol in comparison to Danitol at 1 DAT appears to be an aberration. The low mortality of Danitol in this trial as compared to previous trials may be due to decreased susceptibility in the laboratory-reared flies. Research is underway to evaluate the resistance of the laboratory colony.

Trial Two:
Results and Discussion: At 1 DAT Mustang and Danitol had significantly higher mortality than all treatments except Gladiator and Venom + Danitol, which had significantly higher mortality than the check and Movento, but not than Agrimek (Table 24). At 3 DAT Danitol had significantly higher mortality than Agrimek, Movento, and the check, but was not significantly different from any other treatments. At 7 DAT Danitol had significantly higher mortality than Movento, but neither was significantly different from any other treatments.

Conclusion: Danitol, Mustang, Venom + Danitol and Gladiator provided similar control with Danitol slightly outperforming the other materials. Movento had the lowest mortality and was not significantly different from the check at any evaluation. The low mortality of Mustang and Danitol in this as compared to previous trials may be due to decreased susceptibility in the laboratory-reared flies. Research is underway to evaluate the resistance of the laboratory colony.

Spotted Wing Drosophila Diel Periodicity

Materials and Methods: Three trials were conducted to examine diel periodicity of SWD under various temperature regimes. Winter trials were conducted in a Riparian zone at Brentwood, CA and the spring trial was conducted in a cherry orchard at the UC Davis Wolfskill Experimental Orchards, CA. In all, six standard traps baited with a 4 fl. oz of a soaped 2:3 Merlot: ACV bait. In the winter trials temperature data was obtained from weather station KCABRENT7 (www.wunderground.com), located 1.4 mi northwest of the field site. In the spring trial, temperature data was obtained via direct measurements at the field site at sample collection times. Samples examined under magnification (20X) in the field or at UCB. All SWD were counted and sexed, and all other *Drosophila spp.* were counted, but not sexed.

Winter Trials:
19-20 February: The trial was conducted at a creek-side location with ivy ground cover in Brentwood, CA. Traps were placed on hangers positioned 6-12 inches above the ground at one hour after sunset at 6:50 pm on 19 February. Traps were monitored at 4:50 am on 20 February, which was 2 hours prior to sunrise and then every 2 hours until 4:50 pm and then hourly between 4:50 and 6:50 pm. The bait was changed at each trap inspection.
26-27 February: The trial was conducted at the same location as the first study. Traps were placed one hour after sunset at 6:50 pm on 26 February 2013. Traps were monitored at 4:50 am on 27 February, which was 2 hours prior to sunrise and then every 2 hours until 5:00 pm and then hourly between 5:00 and 8:00 pm, which was two hours after sunset. The bait was changed at each trap inspection.

Spring Trial:
23-24 April: The trial was conducted at the UC Davis Wolfskill Experimental Orchards in Davis, CA. Traps were hung in the trees at 6-12 inches above the ground. Traps were placed one hour prior to sunset at 7:40 pm on 23 April and monitored hourly until 9:40 pm. The traps were then monitored at 4:20 am on 24 April, which was 2 hours prior to sunrise and at 2 hours intervals until 7:50 pm (sunset) and then hourly until 9:50 pm.

Results and Discussion: In both of the winter trials, the number of SWD captured was low (≤ 2 SWD/hour). In the 19-20 February trial, there was a single peak between 2:50-5:50 pm that corresponded with the temperatures between 50-60°F (Fig. 5). In the 26-27 February trial, there were two distinct peaks (Fig. 6). The first peak occurred between 6:20-10:20 am, which corresponded with the temperatures between 45-60°F. During mid-day temperatures increased to 60-70°F and the number of SWD captured decreased to zero. A second, smaller peak was observed in the evening between 6 and 7 pm, when the temperatures are in the low 60s, and the sun was setting.

In the spring trial, SWD captures were significantly higher (peak of = 37.7 SWD/hour) than the winter populations (Fig. 7). There were two distinct peaks of fight activity. The first peak was larger than the second peak and occurred in the morning. The morning peak occurred between 6:20-8:20 am when temperatures were between 55-65°F. Between 8:20-10:20 am, temperatures climbed to 75°F and SWD capture at 10:20 am had decreased to 27.5 SWD/hour. By 12:20, the temperature had reached 86 °F and SWD catch was greatly diminished to 2.4 SWD/hour. The capture rate remained at less than 1 SWD/hour, until peaking again at 10.8 SWD/hour between 6:20 and sunset at 7:50pm. During 6:20 and 7:50pm the temperature dropped from 80°F to 70°F. Thus, in the spring trial, SWD capture rates were not significantly diminished until temperatures exceeded approximately 75°F.

The mid-day drop in SWD catch cannot be attributed solely to temperature. Other factors such as crepuscularity or wind may have an impact on SWD flight activity. A consistent drop in SWD capture rates was observed in the early afternoon in both trials that exhibited a dual peak of flight activity.

Conclusions: From these very preliminary trials, it appears that when temperatures are favorable SWD flight activity is crepuscular. If temperatures are above around 50°F, then SWD flight can be expected to occur from sunrise to mid-morning and again in late afternoon to sunset. There does not appear to any flight activity during the night. This has implications for timing of insecticide applications.

Table 1. Mean total *D. suzukii* captured per trap per week near Newman, CA - 2013

Bait	Mean[a] total *D. suzukii* captured per trap per week															
	11 Dec		18 Dec		27 Dec		4 Jan		11 Jan		18 Jan		24 Jan		31 Jan		Study	
ACV	20.7	ef	38.2	a	33.3	a	25.8	a	13.0	a	8.5	bc	9.3	abc	2.2	a	18.9	ab
MIB	61.2	abc	21.3	ab	7.0	cd	3.0	c	1.2	b	20.7	a	14.2	a	3.0	a	16.4	ab
1:1.5 MIB:ACV	63.0	ab	35.8	a	24.7	ab	19.7	ab	11.8	a	6.0	b	9.3	abc	0.7	a	21.4	a
1:4 MIB:ACV	40.2	bcde	29.2	a	35.3	a	28.8	a	13.5	a	5.8	b	10.3	ab	2.8	a	20.8	a
1:1.5 MIB:water	38.0	cdef	3.3	b	5.2	d	2.5	c	1.3	b	0.7	b	1.0	d	0.8	a	6.6	c
1:4 MIB:water	14.7	f	3.7	b	6.2	d	0.8	c	1.5	b	4.2	b	2.3	cd	2.7	a	4.4	c
1:0.75:0.75 MIB:ACV:water	68.7	a	26.8	a	18.3	bc	11.3	bc	7.7	ab	15.5	ab	13.0	a	3.5	a	20.6	ab
1:2:2 MIB:ACV:water	46.7	abcd	34.0	a	18.2	bc	17.5	ab	7.5	ab	5.5	b	11.0	ab	2.7	a	17.9	ab
1:2:2 MIB:ACV:water +Yeast	35.7	def	32.7	a	13.5	bcd	8.5	c	11.2	a	3.2	b	3.8	bcd	2.8	a	13.9	b

[a] Means followed by the same letter within a column are not significantly different (Fisher's LSD $P \leq 0.05$)

Figure 1. Mean total *D. suzukii* captured per trap per week near Newman, CA -2013.

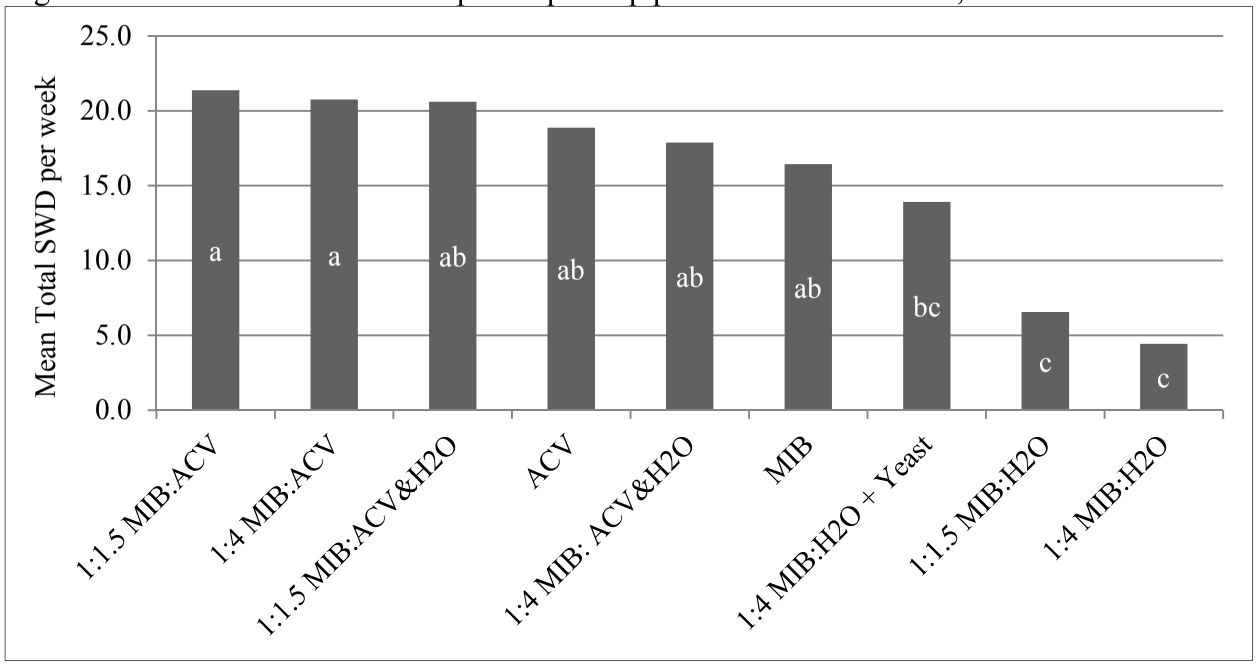


Table 2. Mean percent female of *D. suzukii* captured per trap per week near Newman, CA - 2013

Bait	Mean[a] percent female of *D. suzukii* captured per trap per week															
	11 Dec		18 Dec		27 Dec		4 Jan		11 Jan		18 Jan		24 Jan		31 Jan		Study	
ACV	38.1	c	48.2	bc	53.1	ab	50.5	a	51.6	a	69.4	ab	60.4	ab	45.8	ab	52.4	b
MIB	43.4	bc	67.3	a	35.6	b	63.3	a	70.0	a	48.6	b	51.0	ab	38.3	ab	52.4	b
1:1.5 MIB:ACV	47.7	abc	55.1	ab	48.7	ab	50.8	a	69.7	a	54.0	ab	68.1	a	0.0	b	54.2	b
1:4 MIB:ACV	51.5	ab	55.8	ab	53.8	ab	54.4	a	68.6	a	71.5	ab	68.9	a	51.7	ab	59.4	ab
1:1.5 MIB:H2O	40.8	bc	68.0	a	68.8	a	70.0	a	77.8	a	66.7	ab	75.0	a	100.0	a	68.5	a
1:4 MIB:H2O	45.6	bc	32.1	c	44.8	ab	83.3	a	41.7	a	78.3	a	55.0	ab	68.3	a	53.1	b
1:0.75:0.75 MIB:ACV:H2O	43.7	bc	50.5	abc	46.2	ab	54.7	a	48.3	a	65.5	ab	61.3	ab	67.5	a	54.7	b
1:2:2 MIB:ACV:H2O	47.6	abc	47.6	bc	49.9	ab	61.7	a	64.4	a	55.6	ab	38.8	b	64.3	ab	53.3	b
1:2:2 MIB:ACV:H2O+Yeast	59.6	a	64.4	ab	55.7	ab	79.2	a	70.1	a	64.6	ab	69.4	a	79.3	a	67.8	a

[a] Means followed by the same letter in a column are not significantly different (Fisher's LSD P ≤ 0.05)

Figure 2. Mean percent female of *D. suzukii* captured per trap per week near Newman, CA - 2013.

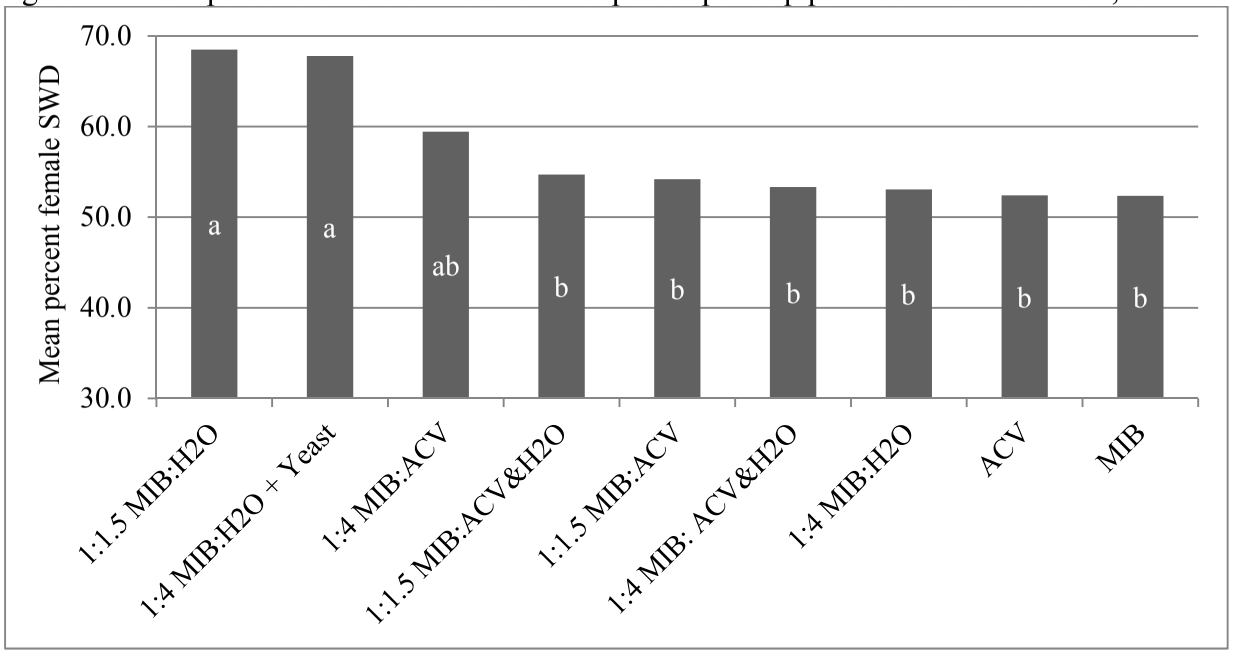


Table 3. Mean percent *D. suzukii* of all *Drosophila* spp. captured per trap per week near Newman, CA - 2013

Bait	Mean[a] percent *D. suzukii* captured per trap per week															
	11 Dec		18 Dec		27 Dec		4 Jan		11 Jan		18 Jan		24 Jan		31 Jan		Study	
ACV	30.5	d	59.5	a	77.3	a	79.4	a	56.9	a	88.6	ab	39.1	ab	20.0	cd	57.0	b
MIB	60.1	ab	70.9	a	75.5	a	86.1	a	52.8	a	73.3	abc	37.8	ab	36.3	bc	61.6	b
1:1.5 MIB:ACV	48.1	bc	70.4	a	71.5	a	83.3	a	43.5	a	73.0	abc	37.8	ab	7.7	d	54.4	b
1:4 MIB:ACV	45.6	c	70.2	a	79.6	a	89.8	a	50.5	a	66.6	bc	38.1	ab	30.5	bcd	58.9	b
1:1.5 MIB:H2O	70.2	a	66.5	a	75.1	a	86.1	a	48.3	a	100.0	a	66.7	a	69.4	a	72.0	a
1:4 MIB:H2O	60.0	ab	63.9	a	93.7	a	70.0	a	35.4	a	74.0	abc	51.0	ab	51.1	ab	62.9	ab
1:0.75:0.75 MIB:ACV:H2O	59.3	ab	61.7	a	76.9	a	96.3	a	62.0	a	61.1	bc	32.7	ab	30.5	bcd	60.1	b
1:2:2 MIB:ACV:H2O	54.8	bc	68.2	a	69.9	a	85.5	a	56.5	a	62.4	bc	27.3	b	29.8	bcd	56.8	b
1:2:2 MIB:ACV:H2O+Yeast	56.5	bc	84.4	a	73.3	a	85.1	a	66.0	a	53.5	c	46.0	ab	43.8	bc	63.4	ab

[a] Means followed by the same letter in a column are not significantly different (Fisher's LSD $P \leq 0.05$)

Table 4. Mean relative attractiveness of various baits to ACV in total *D. suzukii* caught near Newman, CA-2013

Bait	Mean[a] relative attractiveness[b]															
	11 Dec		18 Dec		27 Dec		4 Jan		11 Jan		18 Jan		24 Jan		31 Jan		Study	
Apple Cider Vinegar (ACV)	1.0	cd	1.0	bc	1.0	c	1.0	b	1.0	d	1.0	c	1.0	b	1.0	b	1.0	d
2:3 ACV: Merlot	2.1	abc	1.9	a	2.2	ab	2.2	a	5.4	ab	2.7	a	2.4	ab	3.5	a	2.9	a
3:2 ACV: Merlot	2.8	a	2.4	a	1.9	a	1.9	a	6.1	a	2.0	abc	2.0	ab	3.5	a	3.1	a
2:3 ACV: Chardonnay	0.5	d	0.7	c	1.4	bc	1.4	ab	2.6	cd	1.5	bc	2.5	ab	3.0	ab	1.7	cd
2:3 ACV: Sake	2.2	ab	2.4	a	1.5	a	1.5	ab	4.2	abc	2.1	abc	2.4	ab	2.7	ab	2.6	ab
2:3 RV: Sake	2.3	ab	2.1	a	1.7	ab	1.7	ab	4.0	abc	2.5	abc	3.1	a	4.3	a	2.8	ab
2:3 RV: Chardonnay	1.3	bcd	1.7	ab	2.3	a	2.3	a	3.1	bcd	1.3	bc	2.1	ab	2.3	ab	2.2	bc

[a] Means followed by the same letter in a column are not significantly different (Fisher's LSD $P \leq 0.05$)

[b] Relative attractiveness = number of flies captured by a bait/ number of flies captured by ACV.

Table 5. Mean number of total *D. suzukii* captured per trap per week near Newman, CA - 2013

Bait	Mean[a] total *D. suzukii* caught per trap per week															
	11 Dec		18 Dec		27 Dec		4 Jan		11 Jan		18 Jan		24 Jan		31 Jan		Study	
Apple Cider Vinegar (ACV)	65.3	cd	75.2	bc	58.0	c	93.5	b	8.5	b	19.7	b	17.8	b	3.8	b	42.7	c
2:3 ACV: Merlot	121.3	abc	134.7	a	130.8	abc	175.5	a	29.3	ab	39.5	ab	26.3	ab	14.0	a	83.9	ab
3:2 ACV: Merlot	151.0	a	152.8	a	193.3	a	135.7	ab	34.0	a	35.0	ab	37.8	ab	12.8	a	94.1	a
2:3 ACV: Chardonnay	30.3	d	45.8	c	80.8	bc	95.2	b	17.2	bc	23.5	ab	23.8	ab	13.3	a	41.3	c
2:3 ACV: Sake	129.3	ab	165.5	a	175.8	a	115.2	b	20.7	abc	39.2	ab	27.2	ab	11.8	a	85.6	ab
2:3 RV: Sake	135.0	ab	138.3	a	137.2	ab	146.8	ab	25.0	ab	42.0	a	45.0	a	16.5	a	85.7	ab
2:3 RV: Chardonnay	80.5	bcd	120.2	ab	155.5	ab	150.5	ab	15.3	bc	19.7	b	33.5	ab	9.5	ab	73.1	b

[a] Means followed by the same letter within a column are not significantly different (Fisher's LSD $P \leq 0.05$)

Table 6. Mean percent female of *D. suzukii* captured per trap per week near Newman, CA - 2013

Bait	Mean[a] percent female of *D. suzukii*															
	11 Dec		18 Dec		27 Dec		4 Jan		11 Jan		18 Jan		24 Jan		31 Jan		Study	
Apple Cider Vinegar (ACV)	44.8	a	52.0	a	44.7	a	50.6	a	49.0	a	62.3	a	50.1	a	66.7	a	52.5	a
2:3 ACV: Merlot	44.4	a	49.4	a	47.7	a	53.3	a	57.5	a	62.6	a	63.5	a	62.9	a	55.2	a
3:2 ACV: Merlot	47.8	a	53.0	a	49.2	a	56.0	a	57.8	a	47.1	a	55.7	a	64.0	a	53.8	a
2:3 ACV: Chardonnay	44.6	a	53.8	a	53.0	a	53.5	a	50.7	a	59.7	a	61.0	a	64.7	a	55.1	a
2:3 ACV: Sake	46.3	a	53.9	a	52.9	a	50.3	a	54.2	a	56.3	a	65.5	a	56.8	a	54.5	a
2:3 RV: Sake	42.6	a	49.2	a	51.0	a	52.2	a	62.0	a	62.8	a	58.5	a	62.6	a	55.1	a
2:3 RV: Chardonnay	52.0	a	54.8	a	51.5	a	55.3	a	59.9	a	49.9	a	58.5	a	48.1	a	53.7	a
Mean per date	46.1		52.3		50.0		53.0		55.8		57.3		59.0		60.8		54.3	

[a] Means followed by the same letter in a column are not significantly different (Fisher's LSD $P \leq 0.05$)

Table 7. Mean percent *D. suzukii* of all *Drosophila* spp. captured per trap per week near Newman, CA - 2013

Bait	Mean[a] percent *D. suzukii* of total Drosophila								
	11 Dec	18 Dec	27 Dec	4 Jan	11 Jan	18 Jan	24 Jan	31 Jan	Study
Apple Cider Vinegar (ACV)	52.9 a	74.3 a	87.7 a	87.5 a	49.0 a	84.5 a	51.3 a	46.1 c	66.7 c
2:3 ACV: Merlot	63.2 a	78.7 a	82.9 ab	84.0 a	59.8 a	83.9 a	51.4 a	64.3 abc	71.0 abc
3:2 ACV: Merlot	58.4 a	75.5 a	82.5 ab	87.9 a	56.4 a	80.9 a	51.1 a	54.2 bc	68.4 c
2:3 ACV: Chardonnay	38.2 b	56.3 b	76.7 bc	83.5 a	65.9 a	90.3 a	61.2 a	61.1 abc	66.6 c
2:3 ACV: Sake	63.2 a	81.2 a	88.4 a	93.0 a	56.1 a	90.9 a	56.1 a	72.6 ab	75.2 a
2:3 RV: Sake	66.5 a	77.7 a	82.7 ab	87.3 a	56.2 a	87.1 a	57.3 a	79.1 a	74.2 ab
2:3 RV: Chardonnay	52.8 a	75.3 a	73.3 c	88.6 a	63.9 a	85.1 a	55.5 a	60.5 abc	69.4 bc

[a] Means followed by the same letter in a column are not significantly different (Fisher's LSD $P \leq 0.05$)

Table 8. Mean number of *D. suzukii* captured per trap per week near Newman, CA - 2013

Trap type	Mean [a] number of total *D. suzukii* captured per trap per week							
	11 Dec	18 Dec	27 Dec	4 Jan	11 Jan	18 Jan	24 Jan	Season
Top wire trap	19.3 a	22.2 bc	19.5 b	30.5 b	21.5 b	13.5 c	4.7 b	18.7 c
Side wire trap	40.7 a	43.7 ab	51.5 a	32.7 b	32.0 ab	29.7 b	6.8 ab	33.9 ab
Side wire trap + Fluon on walls	22.8 a	57.8 a	40.5 a	52.5 a	45.2 a	42.3 a	6.8 ab	38.3 a
Side wire trap + Fluon on walls & in bait	37.0 a	20.0 c	34.8 ab	56.3 a	43.3 a	27.2 b	10.2 a	32.9 bc

[a] Means followed by the same letter in a column are not significantly different (Fisher's LSD $P \leq 0.05$)

Figure 3. Mean total *D. suzukii* captured by each trap type near Newman, CA - 2013.

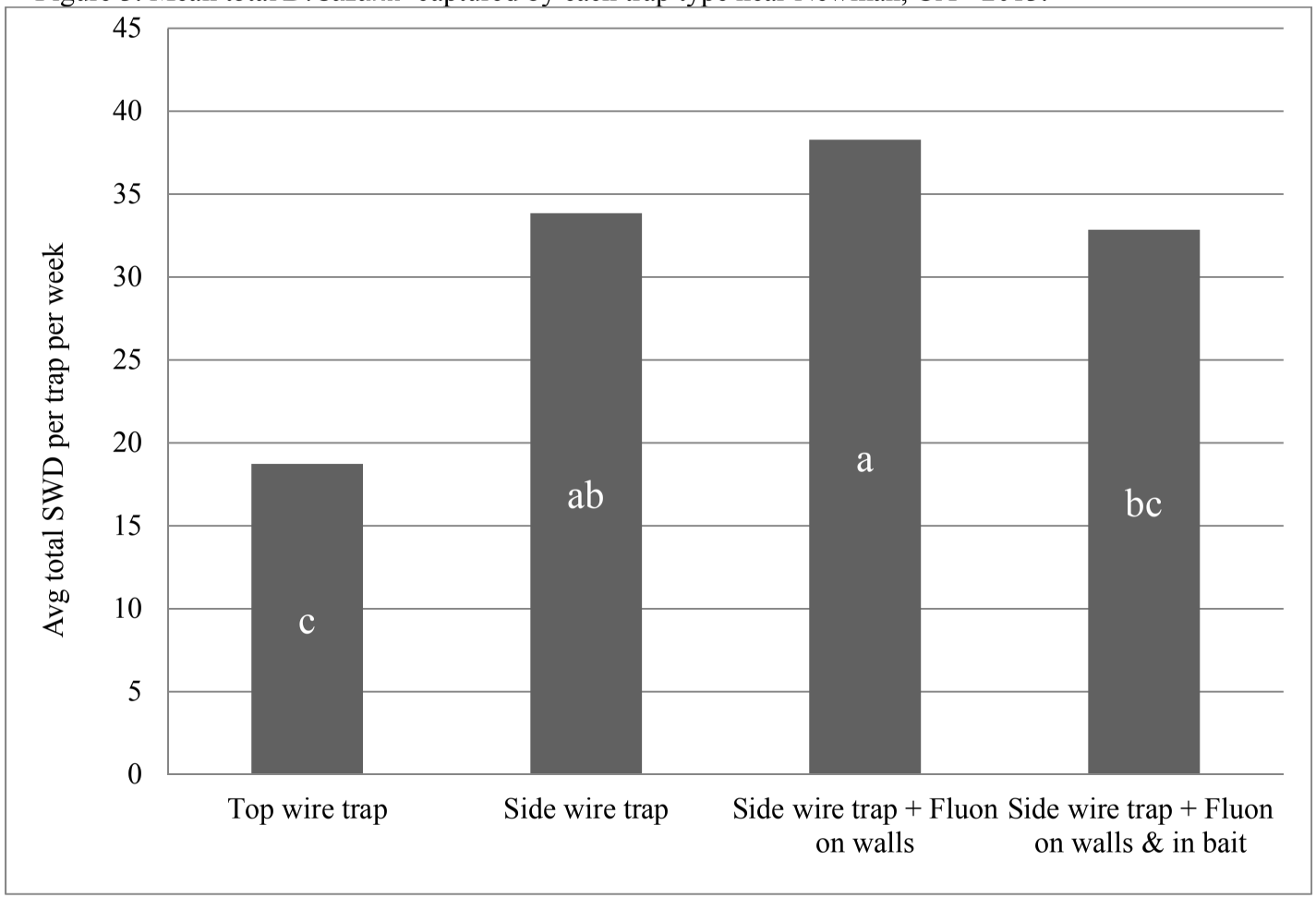


Table 9. Treatments evaluated for ***D. suzukii*** attractiveness in Brentwood, CA - 2013

Treatment	Solution ratio	Solution components
1	---	ACV
2	1:0.75:0.75	ACV + Merlot + water
3	1:0.75:0.75	ACV + MIB + Merlot
4	1:0.75:0.75	ACV + MIB + water
5	1:0.75:0.75	ACV + MIB + water + Sugar[a] Yeast (SY)[b] + Baking Soda (BS)[c]
6	1:0.75:0.75	Water + MIB + Merlot + SY [ab]
7	1:0.75:0.75	ACV + MIB + Merlot + SY [ab] + BS[c]
8	1:0.75:0.75	ACV + MIB + Merlot + SY [ab]

[a] 1 cup sugar per 1 gallon warm bait solution
[b] 2.5 oz Baker's Yeast per 1 gallon warm bait solution
[c] 0.7 teaspoons of baking soda per 1 oz bait solution

Table 10. Mean number of female *D. suzukii* captured per trap per day near Brentwood, CA - 2013

		Mean[a] female *D. suzukii*									
	Treatment	29 Apr		1 May		3 May		5 May		Study	
1	ACV	1.9	d	3.7	c	2.7	c	2.1	d	2.6	d
2	ACV + Merlot + water	4.3	cd	12.2	b	7.2	bc	4.8	bcd	7.1	c
3	ACV + MIB + Merlot	6.6	bc	12.3	b	9.2	ab	5.2	abcd	8.3	bc
4	ACV + MIB + water	1.7	d	2.5	c	2.4	c	2.6	cd	2.3	d
5	ACV + MIB + water + SY + BS	7.7	ab	13.5	b	11.7	ab	8.8	a	10.4	ab
6	H_20 + MIB + Merlot + SY	9.4	ab	11.3	b	12.2	a	8.0	ab	10.2	ab
7	ACV + MIB + Merlot + SY + BS	10.8	a	20.9	a	9.5	ab	6.4	abc	11.9	a
8	ACV + MIB + Merlot + SY	10.3	a	10.7	b	10.6	ab	8.4	ab	10.0	ab

[a] Means followed by the same letter in a column are not significantly different (Fisher's LSD $P \leq 0.05$)

Table 11. Mean number of male *D. suzukii* captured per trap per day near Brentwood, CA - 2013

	Treatment	Mean[a] male *D. suzukii* 29 Apr	1 May	3 May	5 May	Study
1	ACV	0.6 d	2.0 cd	2.3 bc	2.2 bc	1.8 b
2	ACV + Merlot + water	3.0 bc	6.5 ab	4.2 abc	4.4 ab	4.5 a
3	ACV + MIB + Merlot	2.9 c	9.5 ab	6.1 a	3.7 abc	5.6 a
4	ACV + MIB + water	1.0 d	1.2 d	0.9 c	0.8 c	1.0 b
5	ACV + MIB + water + SY + BS	3.3 ab	5.7 bc	5.6 ab	5.0 ab	4.9 a
6	H_20 + MIB + Merlot + SY	4.0 ab	5.9 bc	6.2 a	5.6 a	5.4 a
7	ACV + MIB + Merlot + SY + BS	5.1 a	10.6 a	4.1 abc	5.0 ab	6.2 a
8	ACV + MIB + Merlot + SY	5.0 ab	6.3 abc	6.4 a	5.6 a	5.8 a

[a] Means followed by the same letter in a column are not significantly different (Fisher's LSD $P \leq 0.05$)

Table 12. Mean number of total *D. suzukii* captured per trap per day in Brentwood, CA-2013

	Treatment	Mean[a] total *D. suzukii* 29 Apr	1 May	3 May	5 May	Study
1	ACV	2.5 d	5.7 c	5.0 bc	4.3 b	4.4 bc
2	ACV + Merlot + H_20	7.3 c	18.7 b	11.4 ab	9.2 ab	11.7 ab
3	ACV + MIB + Merlot	9.5 bc	21.8 b	15.3 a	8.9 ab	13.9 ab
4	ACV + MIB + H_20	2.7 d	3.7 c	3.3 c	3.4 b	3.3 c
5	ACV + MIB + H_20 + SY + BS	11.0 bc	19.2 b	17.3 a	13.8 a	15.3 ab
6	H_20 + MIB + Merlot + SY	13.4 ab	17.2 b	18.4 a	13.6 a	15.7 ab
7	ACV + MIB + Merlot + SY + BS	15.9 a	31.5 a	13.6 a	11.4 a	18.1 a
8	ACV + MIB + Merlot + SY	15.3 a	17.0 b	17.0 a	14.0 a	15.8 ab

[a] Means followed by the same letter in a column are not significantly different (Fisher's LSD $P \leq 0.05$)

Table 13. Mean total other *Drosophila* spp. captured per trap per day near Brentwood, CA - 2013

	Treatment	Mean[a] other *Drosophila* spp. 29 Apr		1 May		3 May		5 May		Study	
1	ACV	15.3	bc	36.4	a	29.0	a	30.8	a	27.9	bcd
2	ACV + Merlot + H_20	28.5	ab	53.0	a	58.2	a	48.9	a	47.2	ab
3	ACV + MIB + Merlot	16.9	bc	56.8	a	55.2	a	53.2	a	45.5	abc
4	ACV + MIB + H_20	9.0	c	28.1	a	38.5	a	30.4	a	26.5	cd
5	ACV + MIB + H_20 + SY + BS	36.2	a	41.3	a	58.4	a	45.2	a	45.3	abc
6	H_20 + MIB + Merlot + SY	16.4	bc	17.7	a	26.2	a	24.2	a	21.1	d
7	ACV + MIB + Merlot + SY + BS	25.1	ab	45.2	a	55.5	a	28.6	a	38.6	abcd
8	ACV + MIB + Merlot + SY	23.3	abc	41.6	a	79.2	a	61.3	a	51.4	a

[a] Means followed by the same letter within a column are not significantly different (Fisher's LSD $P \leq 0.05$)

Table 14. Mean percent *D. suzukii* per trap per day near Brentwood, CA-2013

	Treatment	Mean[a] percent *D. suzukii* 29 Apr		1 May		3 May		5 May		Study	
1	ACV	17.2	c	13.1	d	15.3	bc	13.1	c	14.7	d
2	ACV + Merlot + H_20	20.7	c	27.4	c	16.4	bc	16.8	c	20.3	cd
3	ACV + MIB + Merlot	37.8	b	28.7	c	25.5	b	18.4	c	27.6	b
4	ACV + MIB + H_20	27.4	bc	13.5	d	8.5	c	10.6	c	15.0	d
5	ACV + MIB + H_20 + SY + BS	30.5	bc	41.3	b	26.2	b	27.6	b	31.4	b
6	H_20 + MIB + Merlot + SY	58.1	a	55.4	a	42.1	a	38.4	a	48.5	a
7	ACV + MIB + Merlot + SY + BS	40.8	b	41.9	b	20.9	b	29.9	ab	33.4	b
8	ACV + MIB + Merlot + SY	41.5	b	29.5	c	19.0	bc	18.4	c	27.1	bc

[a] Means followed by the same letter in a column are not significantly different (Fisher's LSD $P \leq 0.05$)

Table 15. Mean percent mortality of 10 female *D. suzukii* during 24 hours of exposure to treated foliage near Stockton, CA - 2013

No.	Treatment	Rate form/gal	Attractant solution	Mean[a] percent mortality 1 DAT	3 DAT	7 DAT
1	MIB:ACV:water + Danitol	4.27 fl. oz	1:0.75:0.75	79.9 a	76.6 b	86.3 a
2	MIB:ACV:water + Malathion	8.0 fl. oz	1:0.75:0.75	100.0 a	97.7 a	100.0 a
3	MIB:ACV: water + Entrust[b]	0.5 oz	1:0.75:0.75	86.7 a	87.3 ab	100.0 a
4	MIB:ACV: water		1:0.75:0.75	15.7 b	10.8 c	36.8 b
5	MIB:ACV:Merlot + Danitol	4.27 fl. oz	1:0.75:0.75	82.3 a	82.0 b	87.3 a
6	MIB:ACV:Merlot		1:0.75:0.75	21.4 b	7.8 c	27.1 b
7	Untreated check		----	24.3 b	12.8 c	8.1 c

[a] Means followed by the same letter in a column are not significantly different (Fisher's LSD P ≤ 0.05)

[b] Baking soda added to adjust pH to 8

Table 16. Mean total number of larvae per 100 fruit collected weekly near Brentwood, CA - 2013

Bait	Fl. oz/ac	Mean[a] number of larvae per 100 fruit 3 May	10 May	18 May	24 May	29 May	6 Jun	Trial Total
Attractant + Danitol 2.4 EC	10.66		0.0 b	0.0 a	0.3 a	0.0 a	0.0 a	0.3 a
Attractant + Malathion 57%	14.93		0.3 b	0.0 a	0.0 a	0.0 a	0.0 a	0.3 a
Attractant	----		1.3 a	0.3 a	0.0 a	0.0 a	0.0 a	1.5 a
Untreated check	----	0	0.0 b	1.8 a	1.3 a	0.0 a	0.8 a	3.8 a

[a] Means followed by the same letter in a column are not significantly different (Fisher's LSD P ≤ 0.05)

Table 17. Mean number of larvae per 100 fruit collected weekly near Stockton, CA - 2013

		Mean[a] number of larvae per 100 fruit						
Bait	Fl. oz/ac	6 May	13 May	20 May	28 May	3 Jun	10 Jun	Trial Total
Attractant + Danitol 2.4 EC	10.66		0.5 a	0.0 a	0.3 c	0.5 b	0.0 b	1.3 b
Attractant + Malathion 57%	14.93		1.0 a	1.8 a	3.5 bc	2.5 ab	0.5 ab	9.3 ab
Attractant	----		2.0 a	2.3 a	12.8 a	4.0 ab	3.5 a	24.5 a
Untreated check	----	0 ---	1.3 a	1.0 a	8.5 ab	10.8 a	3.8 a	25.3 a

[a] Means followed by the same letter in a column are not significantly different (Fisher's LSD P ≤ 0.05)

Table 18. Mean number of larvae per 100 fruit collected weekly in both Brentwood and Stockton, CA - 2013

		Mean[a] number of larvae per 100 fruit						
Treatment	Fl. oz/ac	3-6 May	10-13 May	18-20 May	24-28 May	29 May-3 Jun	6-8 Jun	Trial Total
Attractant + Danitol 2.4 EC	10.66		0.3 a	0.0 a	0.3 c	0.3 b	0.0 b	0.8 c
Attractant + Malathion 57%	14.93		0.6 a	0.9 a	1.8 bc	1.3 ab	0.3 b	4.8 bc
Attractant	----		1.6 a	1.3 a	6.4 a	2.0 ab	1.8 ab	13.0 ab
Untreated check	----	0 ---	0.6 a	1.4 a	4.9 ab	5.4 a	2.3 a	14.5 a

[a] Means followed by the same letter in a column are not significantly different (Fisher's LSD P ≤ 0.05)

Table 19. Mean number of *Drosophila* larvae collected per 100 Coral fruit near Winters, CA - 2013

	Mean[a] larvae per 100 Coral fruit				
Height	24 Apr	1 May	7 May	14 May	Total
low	51.3 a	8.7 a	4.0 a	26.0 a	90.0 a
mid	51.0 a	9.3 ab	7.3 a	29.7 a	97.3 a
high	47.0 a	12.7 a	1.0 a	21.7 a	82.4 a

[a] Means followed by the same letter in a column are not significantly different (Fisher's LSD $P \leq 0.05$)

Table 20. Mean number of *Drosophila* larvae collected per 100 Brook fruit near Winters, CA - 2013

	Mean[a] larvae per 100 Brook fruit				
Height	1 May	7 May	14 May	21 May	Total
low	4.6 a	1.4 a	20.4 a	23.4 a	49.8 a
mid	2.6 b	0.8 a	23.6 a	15.2 a	42.2 a
high	2.0 b	1.6 a	27.8 a	20.6 a	52.0 a

[a] Means followed by the same letter within a column are not significantly different (Fisher's LSD $P \leq 0.05$)

Table 21. Mean number of *Drosophila* larvae collected per 100 Bing fruit near Winters, CA - 2013

	Mean[a] larvae per 100 fruit				
Height	7 May	14 May	21 May	28 May	Total
low	0.0 a	0.8 a	11.8 a	36.5 a	49.0 a
mid	0.3 a	2.3 a	5.8 a	32.3 a	40.5 a
high	0.3 a	2.8 a	11.0 a	15.3 a	29.3 a

[a] Means followed by the same letter in a column are not significantly different (Fisher's LSD $P \leq 0.05$)

Table 22. Percent adult *D. suzukii* or other *Drosophila* spp. reared from collected larvae near Winters, CA - 2013

	24 Apr	1 May	7 May	14 May	21 May
SWD	91.5%	100.0%	0.6%	1.8%	19.3%
Others	8.5%	0.0%	99.4%	98.2%	80.7%
Total *Drosophila* reared	235	28	335	1034	88

Figure 4. Percent adult *D. suzukii* or other *Drosophila* spp. reared from collected larvae in Winters, CA, 2013.

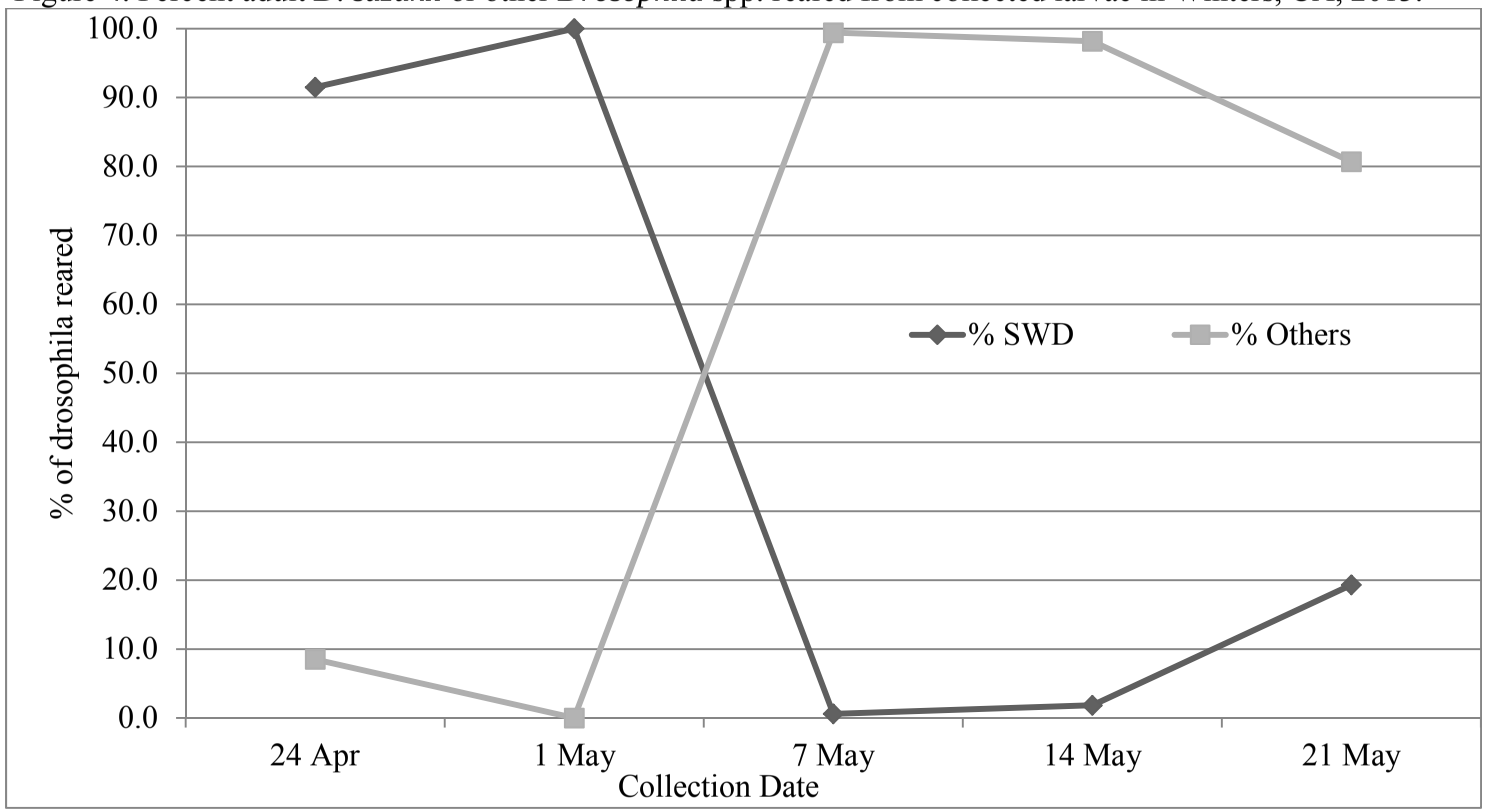


Table 23. Mean female *D. suzukii* mortality at 1, 3, 7 and 14 DAT in trial one near Tracy, CA - 2013

Treatment	Rate form/acre	Mean[a] female mortality 1DAT	3 DAT	7 DAT	14 DAT
CHA-062	14.0 fl. oz	41.1 b	20.6 bc	27.0 a	12.0 a
CHA-062	28.0 fl. oz	67.2 a	22.5 bc	6.5 bc	13.1 a
CHA-062	56.0 fl. oz	70.2 a	48.7 a	19.4 abc	7.9 a
Malathion 57%	45.0 fl. oz	34.2 b	5.9 c	6.2 bc	16.2 a
Belay 2.13SC[b]+ Danitol 2.4EC	6.0 fl. oz + 21.3 fl. oz	21.9 b	31.7 ab	16.5 abc	6.0 a
Danitol 2.4EC	21.3 fl. oz	41.9 b	16.6 bc	25.0 ab	15.1 a
Untreated check		20.9 b	6.5 c	3.7 c	8.9 a

[a] Means followed by the same letter in a column are not significantly different (Fisher's LSD P ≤ 0.05)
[b]Latron B-1956 was applied at 0.125%v/v.

Table 24. Mean percent female *D. suzukii* mortality at 1, 3 and 7 DAT in trial two near Tracy, CA - 2013

Treatment	Rate form/acre	Mean[a] female mortality 1DAT	3 DAT	7 DAT
Agri-Mek 0.75SC[b]	4.3 fl. oz	12.9 bc	3.1 b	9.3 ab
Movento 2SC[b]	9.0 fl. oz	0.0 c	5.2 b	1.3 b
Gladiator	19.0 fl. oz	29.0 ab	15.0 ab	5.0 ab
Mustang	4.3 fl. oz	40.6 a	15.5 ab	11.5 ab
Venom 70SG [c] + Danitol 2.4EC	3.0 oz + 21.3 fl. oz	30.2 ab	18.0 ab	9.3 ab
Danitol 2.4EC	21.3 fl. oz	46.3 a	22.2 a	21.4 a
Untreated check		1.9 c	3.5 b	8.4 ab

[a] Means followed by the same letter in a column are not significantly different (Fisher's LSD P ≤ 0.05)
[b] PureSpray Green horticultural oil was applied at 0.25%v/v.
[c] Latron B-1956 was applied at 0.125%v/v.

Figure 5. Mean number of total *D. suzukii* captured per preceding hour near Brentwood, CA

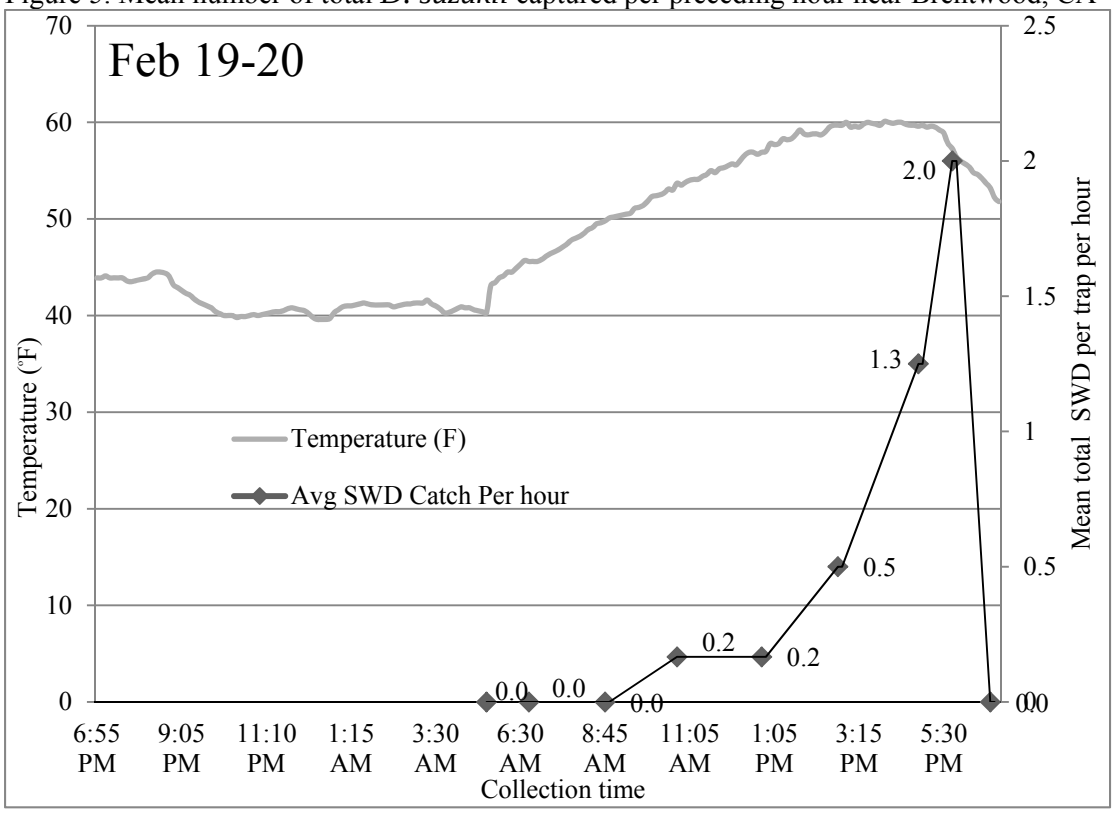


Figure 6. Mean number of total *D. suzukii* captured per preceding hour near Brentwood, CA

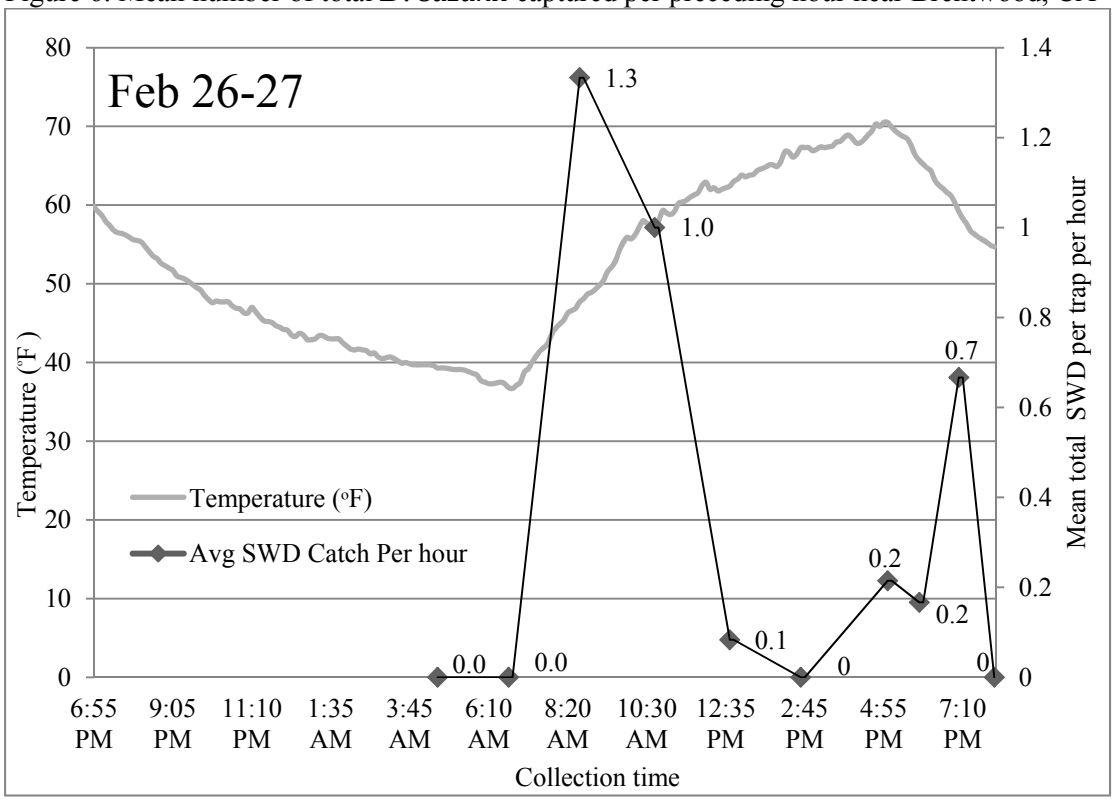
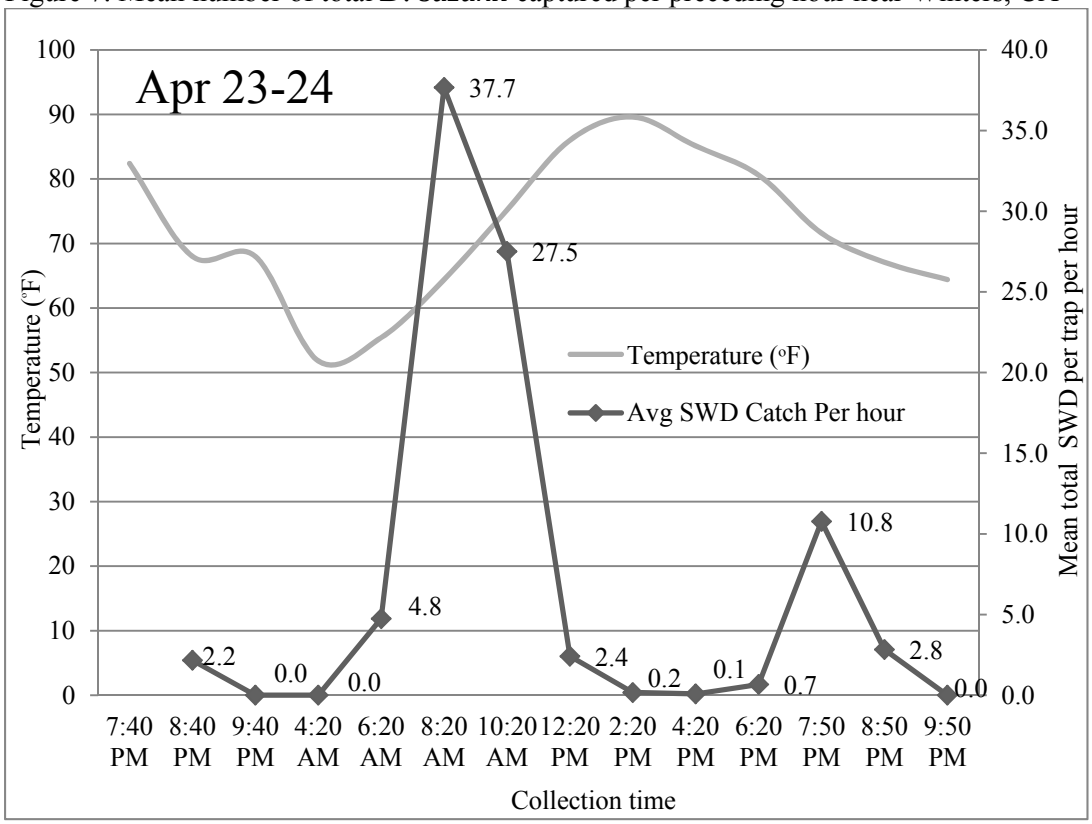


Figure 7. Mean number of total *D. suzukii* captured per preceding hour near Winters, CA



Project Title: Evaluation of Spirotetramat as a Post-Plant Nematicide in Cherries (Year 1 of 3)

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

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 in a variety of ways. Root lesion nematodes, *Pratylenchus* spp., penetrate the root surface and tunnel through root tissues. Dagger nematode, *Xiphinema americanum*, feed from outside the roots but can reduce vigor and yield when their long styles access and feed on vascular tissues. Dagger nematode is also a vector of cherry rasp leaf virus that causes raspleaf disease, as well as certain strains of tomato ringspot virus that cause cherry mottle leaf, yellow bud mosaic, and Prunus stem pitting diseases. Root knot nematode, *Meloidogyne* spp., damages roots by causing swellings of the entire root. Feeding by other nematode species such as pin nematode, *Paratylenchus* spp., and ring nematode, *Criconemoides xenoplax*, can also result in tree stress.

Nematode management is primarily accomplished through the use of pre-plant fumigation and rootstock selection. This is a concern due to increased regulations and decreased availability of fumigants such as methyl bromide, metam sodium and 1,3-dichloropropene. Resistant rootstocks are also problematic due to varied levels of resistance within each variety. For example, the Mazzard rootstock is immune to *Meloidogyne incognita* and resistant to *Meloidogyne javanica*, but is susceptible to dagger and root lesion nematodes. Mahaleb is considered resistant to *Meloidogyne incognita*, but is susceptible to *Meloidogyne javanica*, dagger and root lesion nematodes.

During the past few years there have been a series of studies by Dr. Mike McKenry of the University of California, Riverside with regards to the use of a new foliar-applied pesticide called Movento that contains the active ingredient spirotetramat. This active ingredient was initially tested, registered, and marketed by Bayer CropScience as an insecticide against sucking insects like mealybugs and aphids. However, work done in both grapes and walnuts has shown

that spirotetramat is capable of moving systemically to tree and vine roots and that it also has nematicidal properties. A supplemental 2(ee) label now exists for stone fruits (including cherries) that allows for the use of Movento as a post-harvest nematicide in California.

The goal of this project was to help understand how spirotetramat moves within cherry trees, and to determine the effects it has on nematodes. This includes information on where it is located, for how long, in what concentration, and the subsequent effects on resident nematode populations.

Materials and Methods

Experimental design- During 2013 we began a multi-year study to evaluate the uptake and distribution of spirotetramat (Movento) in two commercial cherry orchards in the lower San Joaquin Valley. Sites are located near Wasco and Arvin in Kern County using trees of the varieties Tulare and Sequoia, respectively. Each site is organized as a randomized complete block design with four blocks of two treatments; four plots were sprayed with Movento and four plots were not sprayed and were kept as untreated checks. Plot sizes for Wasco and Arvin are 15 and 20 trees, respectively.

Application- Plots were sprayed with Movento (or left untreated) at a rate of 9 fl oz per acre in 100 GPA of water on 11 July, 2013 using an Air-blast sprayer at 2 mph with 4 fl oz of Dyne-Amic per 100 gal of water as a surfactant.

Nematode sampling- 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. 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 nematodes. Data were summarized and analyzed using analysis of variance.

Spirotetramat sampling- 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)). As of the time of writing this report in December 2013 all 320 tissue samples have been collected and are currently located in a freezer.

During the last three months of the current funding cycle (Jan to Mar 2014) the samples will be processed. Analysis will be done through a multistep process whereby the spirotetramat and its derivative spirotetramat-enol are extracted from the plant tissues, isolated, and evaluated through Mass Spectometry (MS) and High Performance Liquid Cromatography (HPLC) to determine the parts per billion (ppb) of spirotetramat and spirotetramat-enol in the solvents used to extract these chemicals from plant tissues. Once data are collected they will be analyzed to help determine where spirotetramat is located in the cherry tree, for how long, at what concentrations, and in what ratios of the parent and -enol derivative. These data will help determine characteristics of movement in the plant that can help determine the best way to use this product for nematodes, as well as help researchers in the future to know where to focus their efforts when evaluating nematode research trials. Comparisons will also be made to test for correlations between the spirotetramat concentrations found in root tissues and counts of nematodes found over a 5-month period in treated and untreated plots.

Results

Nematode samples- The effects of Movento applications on nematode density are shown in Table 1 and are represented graphically in Figure 1. 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 7 to 114 nematodes per 500 cc of soil with an average of 42 ± 20 across all dates. Nematode densities in plots treated with Movento ranged from 10 to 141 nematodes per 500 cc of soil with an average of 68 ± 34. There were no significant differences in nematode density between the treated and untreated plots prior to treatment or during any of the monthly evaluations through 5 weeks after treatment ($P > 0.32$).

Densities of pin nematode in untreated plots ranged from 410 to 1,861 nematodes per 500 cc of soil with an average of 954 ± 214 across all dates. Nematode densities in plots treated with Movento ranged from 320 to 1,412 nematodes per 500 cc of soil with an average of 636 ± 129. There were no significant differences in nematode density between the treated and untreated plots prior to treatment or during any of the monthly evaluations through 5 weeks after treatment ($P > 0.43$).

Densities of lesion nematode in untreated plots ranged from 41 to 300 nematodes per 500 cc of soil with an average of 194 ± 14 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 322 ± 83. There were no significant differences in nematode density between the treated and untreated plots prior to treatment or during any of the monthly evaluations through 5 weeks after treatment ($P > 0.26$).

Spirotetramat samples- Analysis of data from the 320 tissue samples collected to evaluate spirotetramat titer in cherry leaves and roots will be completed during the last three months of the current funding cycle from January to March 2014. At that time we will use the data to characterize how the active ingredient and its -enol derivative move in the cherry tree and try to determine why there were no significant differences in the density of any species of nematode at any evaluation date at either trial location through 5 months after treatment in 2013.

Conclusions

The first year of this research project is progressing according to our research timeline. At this point field sites have been identified, treatments were made, nematode data were collected and processed, and tissue samples have been collected. Tissue samples will be processed during the final three months of the current research cycle that ends in March 2014.

As of December 2013 we consider it preliminary to try to draw conclusions from this research project, other than to say that we were unable to detect any affects of Movento treatments on the density of dagger, pin or lesion nematodes on any evaluation date at either site through five months after treatment. However, perhaps the most important part of this project is to understand how spirotetramat moves within the cherry tree with the hopes of explaining the mechanism and timeline by which an effect on nematodes might occur. Conclusions related to this objective, and the implications it may have for the mechanisms and timeline with which Movento might affect nematodes, will be available after March when the current research cycle is concluded.

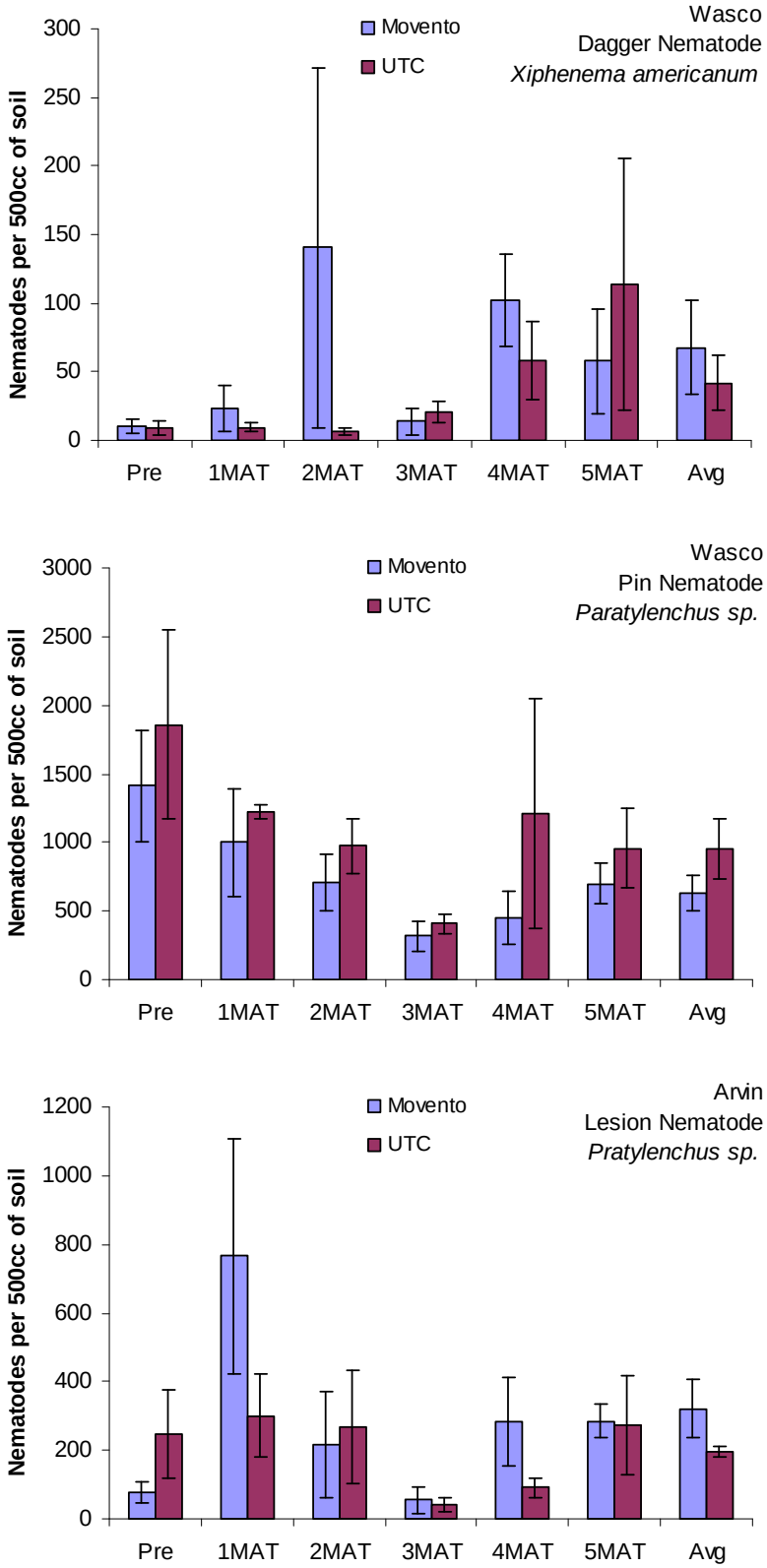
Table 1. Effects of a July application of Movento (spirotetramat) on the principal nematode species in two commercial cherry orchards in Kern Co, CA. through five months after application in 2013

	Dagger nematode (*Xiphenema americanum*) per 500 cc of soil, Wasco						
	Precounts	1 MAT	2 MAT	3 MAT	4 MAT	5 MAT	Average
Movento	10 ± 6	24 ± 10	141 ± 131	14 ± 10	102 ± 34	58 ± 38	68 ± 34
Untreated	9 ± 5	94 ± 3	7 ± 3	21 ± 8	58 ± 29	114 ± 92	42 ± 20
F	0.04	0.60	1.02	0.23	0.53	0.29	0.30
P	0.8418	0.4513	0.3286	0.6388	0.4796	0.5994	0.5955

	Pin nematode (*Paratylenchus* sp.) per 500 cc of soil, Wasco						
	Precounts	1 MAT	2 MAT	3 MAT	4 MAT	5 MAT	Average
Movento	1412 ± 408	1000 ± 391	707 ± 205	320 ± 108	453 ± 190	700 ± 144	636 ± 129
Untreated	1861 ± 683	1224 ± 49	973 ± 196	410 ± 73	1206 ± 836	959 ± 290	954 ± 214
F	0.15	0.10	0.26	0.15	0.65	0.23	0.42
P	0.7025	0.7533	0.6210	0.7023	0.4326	0.6388	0.5276

	Lesion nematode (*Pratylenchus vulnus*) per 500 cc of soil, Arvin						
	Precounts	1 MAT	2 MAT	3 MAT	4 MAT	5 MAT	Average
Movento	77 ± 30	765 ± 343	218 ± 155	55 ± 38	285 ± 128	285 ± 50	322 ± 83
Untreated	248 ± 129	300 ± 121	268 ± 164	41 ± 22	91 ± 28	272 ± 145	194 ± 14
F	1.18	1.03	0.04	0.08	1.36	0.00	0.62
P	0.2964	0.3274	0.8426	0.770	0.2622	0.9504	0.4453

Figure 1. Effects of a July application of Movento (spirotetramat) on nematode density in two commercial cherry orchards in Kern Co., CA. in 2013



Project Title: Management of Spotted Wing Drosophila in the lower San Joaquin Valley

Principal Investigators

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Objectives of Proposed Research (Year 1 of 3)

Improve Management of Spotted Wing Drosophila (SWD) in the lower San Joaquin Valley by

1) Determining seasonal patterns in adult SWD activity
2) Evaluating the phenology of fly movement between overwintering hosts and cherries
3) Conducting an exploratory survey for parasitoids of drosophilans in the lower San Joaquin Valley.

Justification and Importance of Proposed Research

Spotted wing drosophila (SWD) is a significant new pest of cherries throughout the western United States. Since 2008 damage from this pest, coupled with added expenses for management programs, have resulted in significant economic losses to cherry growers throughout California.

Due to the significance of this pest several researchers have begun studies that will lead to sustainable management programs for cherry growers. These researchers have made significant progress on several aspects of integrated pest management programs such as trapping, defining relative susceptibilities of different crop stages, evaluating chemical controls, and determining developmental fly biology. The purpose of this project is to fill some of the current voids in this research as they pertain to understanding the field biology and phenology of SWD, particularly in the lower San Joaquin Valley.

Experimental Procedures to Accomplish Objectives

1) Determine seasonal patterns in adult SWD activity in the lower San Joaquin Valley.

During 2013 we conducted our third and final year of a SWD trapping program in citrus, cherries and blueberries in the General Beale and Edison regions of Kern County. During this final year we did weekly monitoring of 22 traps in 11 different citrus or cherry orchards from 17 Oct 2012 to 18 Jun 2013. In each orchard we placed two bucket traps on opposite ends of the orchard. The trap consists of a 26 fl oz. plastic container with a transparent lid with a 3.1 inch diameter opening on the lid covered with 1/8 inch hardware cloth. Each trap was baited with approximately 5 fl oz of apple cider vinegar and hung from a tree scaffold approximately four feet from the ground. On a weekly basis traps were collected, returned to the laboratory and

evaluated for the number of male and female SWD. Data were analyzed by plotting trends in trap catches over time in a chart to visualize changes in pest density throughout the trapping period.

From fall 2010 to June 2012 we determined that SWD has two main periods of activity in cherries. The first period is in the fall from October to December and the second period is from April through mid-June. In citrus there was just one prolonged period of activity that started in October and continued through April. During the final year of trapping in 2013 data from all commodities were pooled together and a similar trend was seen as in previous years whereby adult fly activity began in late October, increased through December, maintained itself through late winter and early spring, and then decreased in late April (Fig.1). However, it is important to note that all monitoring sites were within commercial orchards and received insecticide treatments for SWD (cherries in April) or for glassy winged sharpshooter and citrus thrips (citrus in April). Therefore it is logical to interpret that the reductions in April were due to pesticide applications across all of our trapping locations, and not because of environmental conditions. In previous years of data we showed that if SWD is not sprayed, it survives very well through May and into June, and by the first week in July environmental conditions are too hot and dry for adult flies to be active.

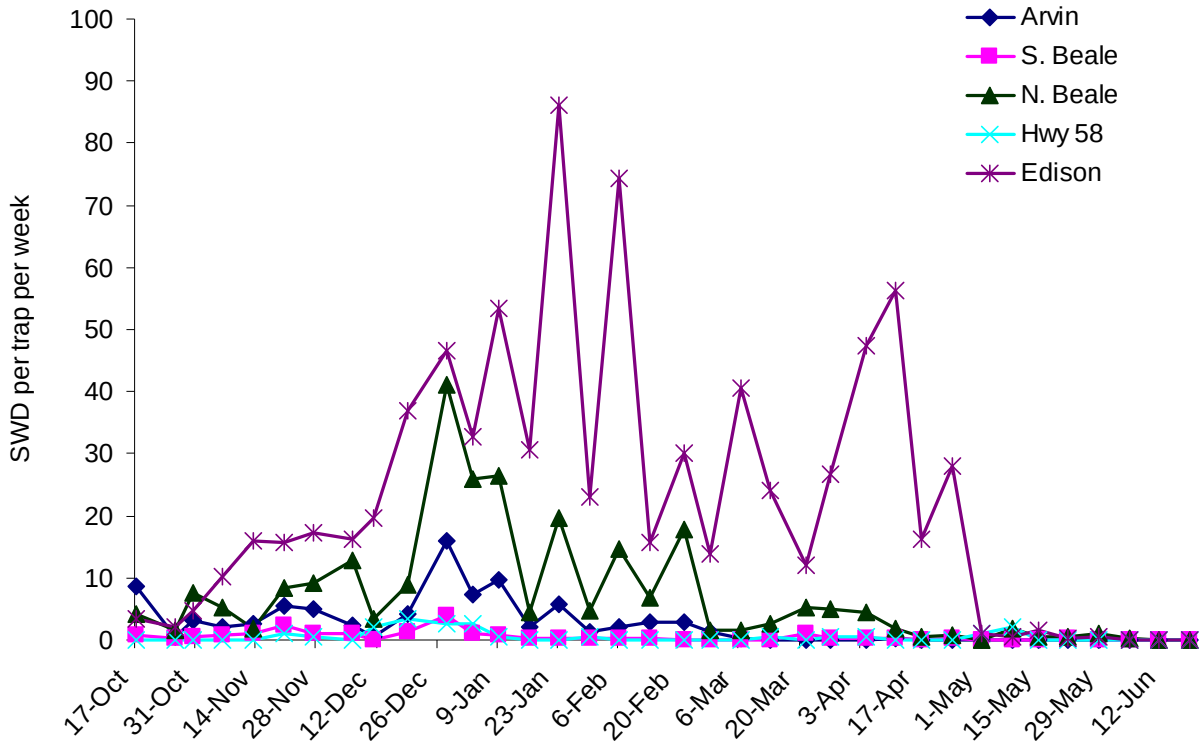

Fig. 1. Average SWD adults collected from 22 bucket traps placed in 11 citrus and cherry orchards placed in the Arvin, General Beale and Edison regions of Kern County from October 2012 to June 2013.

2) Evaluate the phenology of fly movement between overwintering hosts and cherries
Kern County offers a unique opportunity to research the regional movement of SWD among crops due to the presence of a known overwintering host for SWD (citrus) immediately next to cherries. This allows us to do transect studies to evaluate fly behavior with regards to movement from one crop to the other. During the past two years we have conducted transect studies extending 1/10 of a mile into citrus and 1/10 of a mile into cherries. Studies have allowed us to learn that flies are almost exclusively in citrus prior to 5 weeks to harvest, that fly movement to cherries begins 3-5 weeks prior to harvest of cherries, and that the first flies to migrate to cherries are predominantly female. This has led to recommendations regarding the use of trapping and guidelines for how to interpret the results of trap catches as they relate to management programs.

During 2013 we collected our final set of transect data. Two transects of bucket traps (18 traps per transect) were placed in a line perpendicular to the interface between citrus and cherries. Traps were placed at 85 foot (5 row) intervals to 510 feet (.08 mile) into the citrus orchard and 1105 feet (0.21 mile) into the cherry orchard. If the transect had been extended in the citrus it would have continued into more citrus; if the transect had been extended in the cherries it would have gone into several miles of rangeland. Traps were placed into the field on 20 Feb and were evaluated weekly for the number of adult SWD males and females through 14 May.

During the first four weeks of evaluation (20 Feb to 21 Mar) SWD adults were almost exclusively in the citrus (Fig. 2a). During this period of time the ten traps in citrus averaged 43 SWD per trap per week whereas the 26 traps in cherries averaged 0.5 SWD per trap per week.

Approximately five weeks prior to harvest SWD began migrating into the cherries (Fig. 2b). During the three weeks from 21 Mar to 11 Apr the average number of flies in citrus was 27 per trap per week compared to 30 per trap per week in the cherries. Further analysis of the cherry data shows an edge effect whereby traps that were within 510 feet of citrus (= 30 rows of trees at 17' spacing) averaged 48 SWD per trap per week compared to 14 SWD per trap per week in traps greater than 510 ft from the citrus-cherry interface. Analysis of data from week to week on 21 Mar, 27 Mar, 4 Apr and 11 Apr showed that almost no flies were present in the cherries prior to 21 Mar, that they had moved approximately 600ft into the cherries by 27 Mar, approximately 900 feet into the orchard by 4 Apr, and throughout the full 1105 feet of the orchard by 11 Apr.

Approximately three weeks until harvest the cooperating cherry grower began a weekly insecticide treatment program to control SWD prior to harvest (Warrior, followed by Delegate, followed by Malathion). As a result, the number of SWD in the cherry orchard became significantly reduced during the three weeks prior to harvest (Fig. 2c). Additionally, around the 20th of April the citrus orchard was sprayed with Danitol as part of a USDA areawide treatment program targeting glassy-winged sharpshooter. As a result of these treatments there were almost no SWD collected in either the citrus or the cherries during the 3-week harvest period from 23 Apr to 14 May (Fig. 2d).

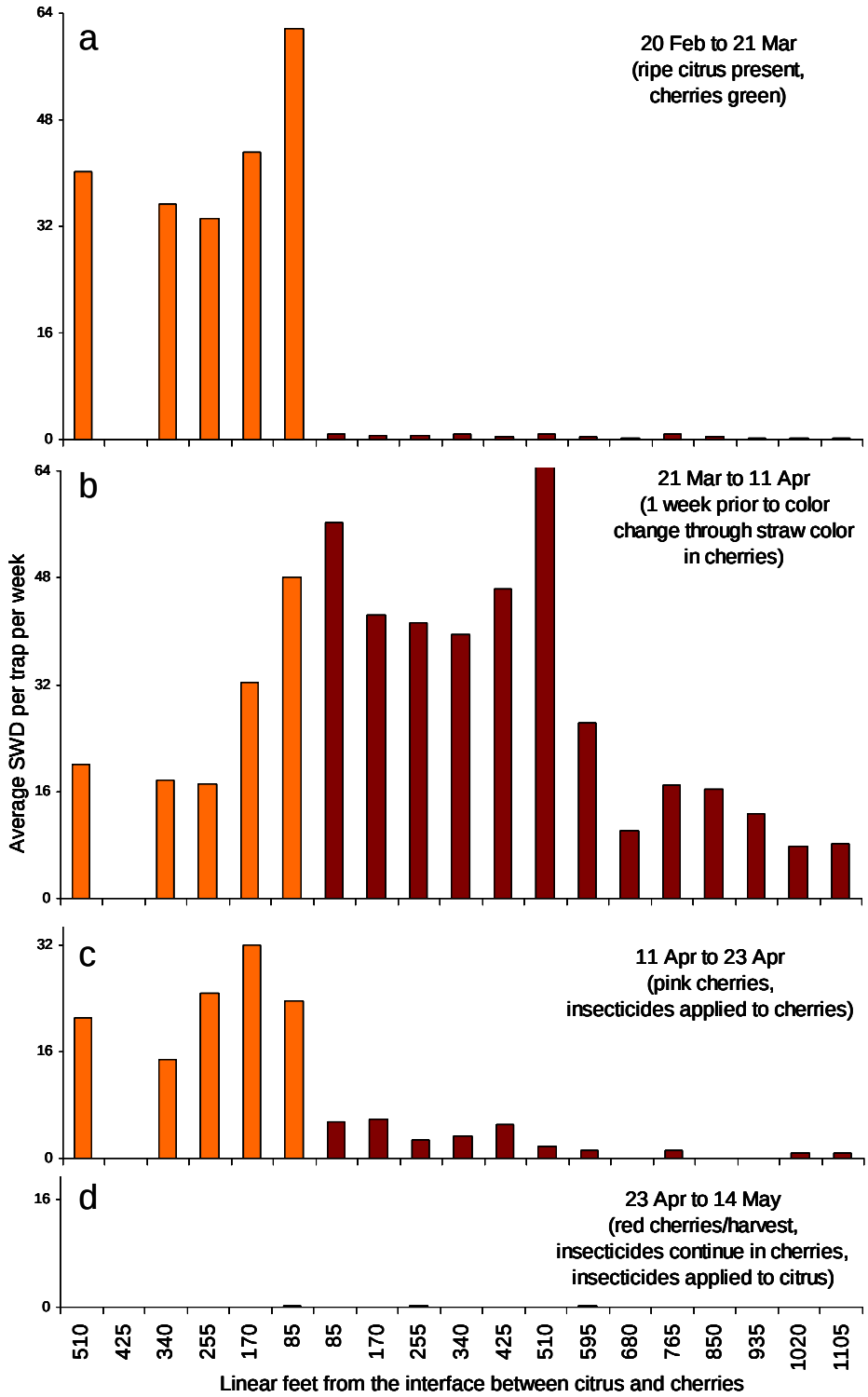

Fig. 2. Captures of SWD in a transect of bucket traps placed perpendicular to the interface between citrus and citrus orchards during four time periods prior to cherry harvest. Data show a) that SWD is primarily in citrus while cherry fruit are green from 20 Feb to 21 Mar, b) that SWD moves to cherries during the period of early color development in late March to early April, c) SWD populations once insecticide treatments begin in cherries, and d) SWD populations when insecticide treatments when both cherries and citrus have been treated with insecticides.

Gender-biased movement
One of the purposes of SWD traps is to monitor prior to harvest to determine the need for treatment. However, data collected in 2013 corroborates previous concerns from our research in 2011 and 2012 that this may be difficult due to a gender bias in SWD captures in cherries.

Analysis of data from evaluation dates of 6 Mar, 14 Mar, 21 Mar and 27 Mar during the period of time of early migrating of SWD into the cherry orchards shows that the SWD population is approximately 50% male and 50% female in the citrus (Fig. 3a). However, the initial invasion of SWD into cherries is primarily done by females (Fig. 2b). In total the 26 SWD traps in cherries evaluated from 6 Mar to 21 Mar captured 45 SWD, of which only 6 (13.3%) were males. This means that 87% of the SWD population, comprised of the part of the population that damages the crop, is going undetected during this period of time. This can easily lead growers and pest control advisors to a false conclusion that SWD is not present in the orchard at the same time as female SWD have begun to sting fruit and damage the cherry crop. It is also important to remember that during the same three-week period of time that 26 SWD traps in the cherries only caught 6 males, a set of 10 SWD traps in the adjacent citrus caught a total of 560 SWD. This means that a cherry grower or PCA, if only using traps in their cherry orchard, and only evaluating traps for drosophila with spots on their wings (males), is likely to be completely oblivious to the SWD threat that has built up in the neighboring citrus orchard, and is likely going to be unable to detect the presence of SWD in his or her own orchard until after damage has already begun to occur.

3) Conduct an exploratory survey for parasitoids of drosophilans in the lower San Joaquin Valley.
Biological control of SWD is a topic that has not been widely explored in California. However, it is relatively safe to assume that biological control organisms are already present in California due to the long-time presence of other drosophila species. During 2013 we did some exploratory work to determine which species of parasitoids were already present in California, and to determine if they could attack SWD.

During 2013 we conducted field evaluations for SWD parasitoids in the spring (completed) and the fall (in progress from Oct-Dec 2013). For the spring surveys we placed five sentinel traps underneath random citrus trees in each of two citrus orchards from Feb through May. Each trap consisted of a 591 ml flat-bottomed square plastic container with a snap-on lid. Approximately 200 ml of artificial drosophila diet (Jazz-mix drosophila diet, Fischer Scientific) was placed into the bottom of each container and allowed to cool. After cooling, a total of 40 mixed gender adult SWD were put into each container for a period of 12 days to lay eggs. Eggs were allowed to hatch and grow under ambient conditions on an indoor laboratory countertop. After the 12 days the lid was removed and the traps were placed in the orchard. At this time traps had mixed-stage SWD maggots and new pupae. Traps were left in the field for 7 days, at which time they were collected and stored in the laboratory for a period of 5 weeks with the lid replaced. It was determined that a period of 5 weeks was long enough for all parasitoids to emerge, but not long enough for a second generation of parasitism to occur. At the end of 5 weeks each trap was opened and adult parasitoids were counted and identified.

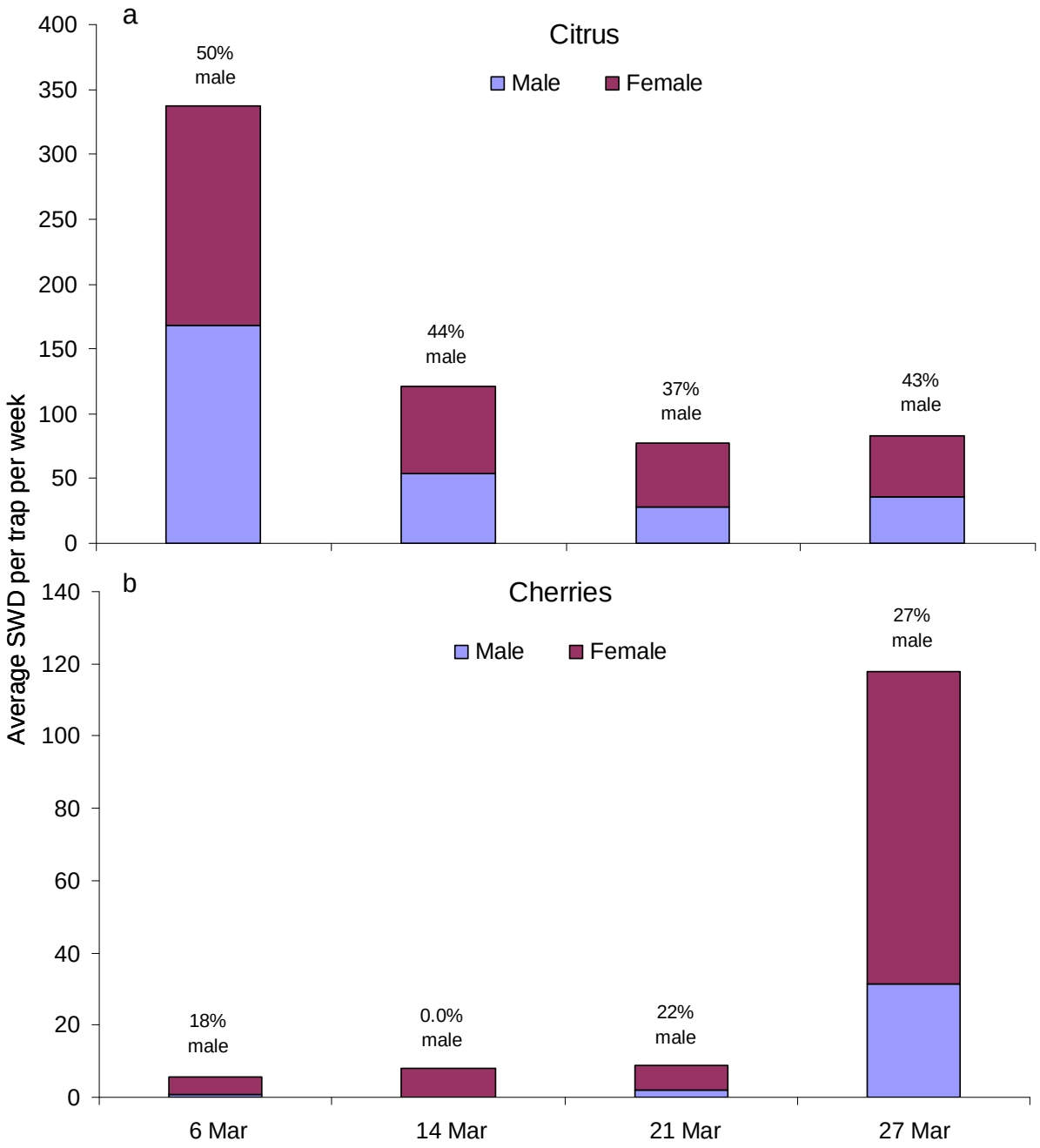

Fig 3. Male to female ratios of SWD collected in a) citrus and b) cherries from late February through late March during the period of time that SWD begins migrating from citrus to cherries. During this period of time growers and pest control advisors often monitor for SWD to determine the need for treatment. Data show that counting only males during the period of early migration could lead to false negatives (thinking SWD is absent when really it is present).

Sentinel traps were evaluated weekly for 12 weeks from Feb through May, with a three-week gap from mid-April to early May (Fig. 4). In total we collected 1,671 parasitoids, of which 100% were in the genus *Pachycrepoideus*, likely *P. vindemmiae* (Rondani). This species is known as an ectoparasitic idiobiont parasitoid that attacks puparia of many different groups of flies, including drosophilans, as well as insects in several other insect orders. During the first three weeks of evaluation prior to 27 Feb we collected very few parasitoids. Most of the parasitoids were collected from early March to early May. Parasitoid captures decreased again during the last two weeks of May, though we are uncertain if this was due to decreases in the density of SWD hosts, parasitoid biology, pesticide use, or due to hotter climatic conditions that caused the artificial diet in our sentinel traps to dry up and become unsuitable for SWD. It is probably that all four factors played a role in the decrease.

After parasitoid emergence we collected live parasitoids from sentinel cages and placed them in a pure colony of SWD to confirm host status. Parasitoids were able to oviposit on SWD and consistently completed their life cycles under colony conditions. Once parastioid colonies were established they were provided to the laboratory of Dr. Kent Daane for future studies.

Fall surveys were completed from October through December. At the time of writing this report the data are still being collected. At this time parasitoids have emerged from the earliest weeks of trapping, other traps are in the lab while we wait for parasitoids to emerge, and the last traps are being prepared to be put into the field. Results from the fall trapping will be available in the first quarter of 2014 during the last three months of the 2013-14 funding cycle of this project.

Data from this project suggest that parasitism can play a role in the management of SWD on a landscape scale in alternate hosts like citrus. However, the biological profile of these parasitoids that states that they parasitize pupae of SWD suggests that they are unlikely to play a major role in biological control in cherries. For example, under Kern County conditions SWD adults migrate into cherries about five weeks prior to harvest, but parasitism could only occur after flies have already laid eggs, larvae have hatched, developed within fruit, and exited the fruit to pupate, at which time parasitism could occur. For this reason it is unlikely that this parasitoid will have practical value each spring within cherry orchards prior to harvest, but may have long-term benefits on a landscape scale at different times of the year.

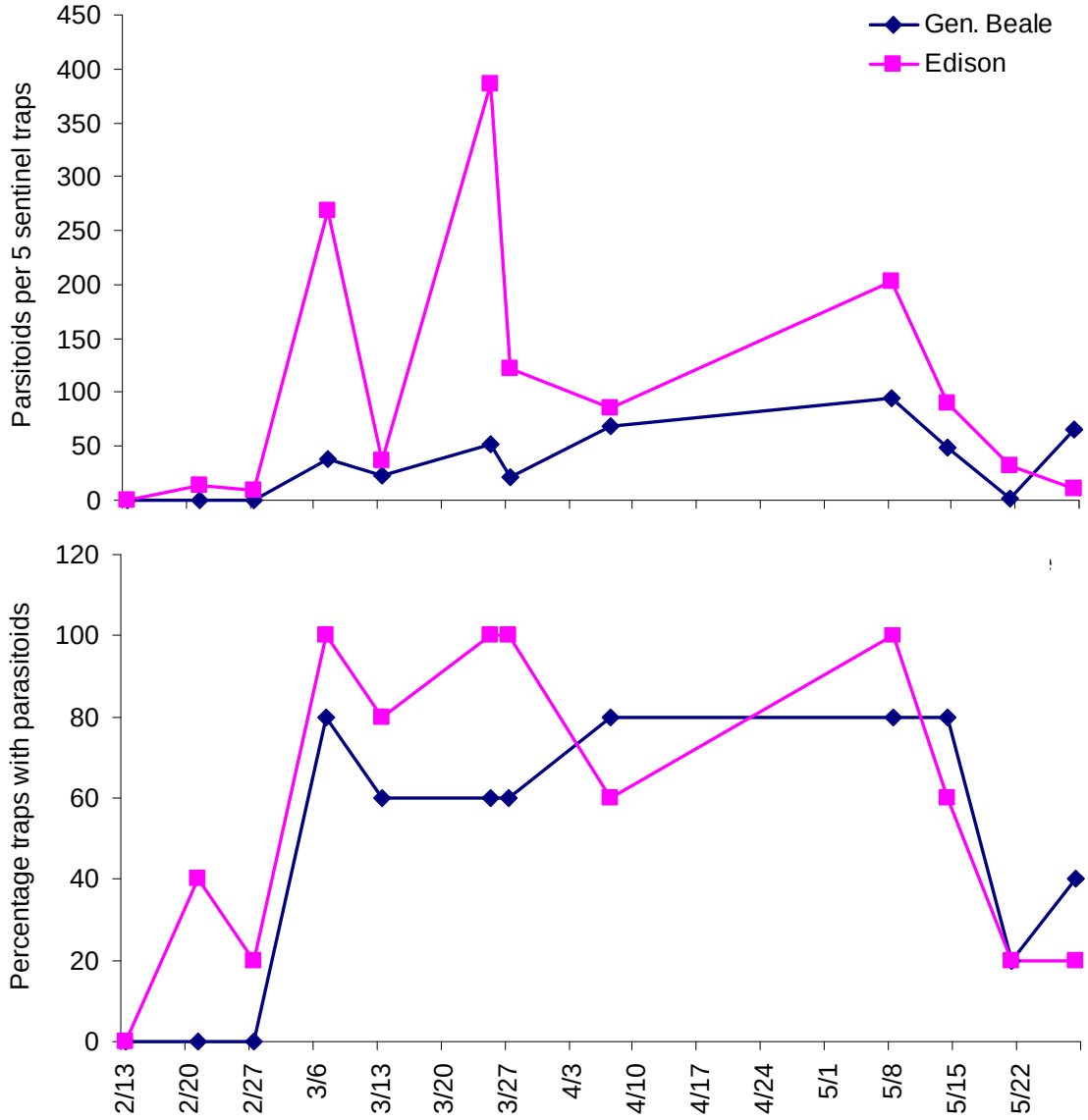

Fig. 4. Collections of the parasitoid Pachycrepoideus vindemmiae (Rondani) using sentinel traps in two citrus orchards in Kern County showing a) the total number of flies collected and b) the percentage of traps containing parasitoids.

Investigating Biological Controls to Suppress Spotted Wing Drosophila Populations
(2013 Annual Crop Report, California Cherry Board)

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Abstract. Spotted wing drosophila (SWD), *Drosophila suzukii* Matsumura is a newly invasive pest that attacks cherries and other various 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 the field to search for susceptible host fruit and to lay eggs. Because a wide range of crop and landscape plant species serve as SWD refuges for overwintering or 'off-fruit' seasonal habitats, there will always be untreated SWD populations nearby to re-infest the cash crop and thus repeating the need for future insecticide sprays. It is therefore crucial to suppress source populations on non-crop hosts and post-harvest cash crops at the landscape level in order to reduce pest pressure in susceptible crops (e.g., cherries). Natural enemies may track the movement of the pest, and target the source populations in unmanaged habitats. Any reduction in the sizes of source populations surrounding the crop fields would greatly improve the efficiency of other control strategies. We first plan to investigate SWD populations' selection of host plant species and movement among host plant species, and the impact of natural controls by resident parasitoids on different host plant species, particularly OUTSIDE of the cash crop (2013-2014). We will also begin a foreign exploration program to import (if needed) SWD parasitoids from Asia or other regions (2014-2017). The project is currently funded for one year (starting April 2013). Here we report some preliminary results from field monitoring of SWD populations dynamics, survey of resident drosophila parasitoids, evaluation of parasitoids on SWD, as well as various aspects of SWD biology and ecology.

(1) Field trap monitoring of SWD population dynamics

To monitor seasonal occurrence and abundance of adult SWD, and other fruit fly species, in different habitats (sites) and geographical locations, apple cider vinegar (ACV) traps were placed in nine different sites in cherry orchards and non-cherry habitats in Brentwood (Contra Costa County), three different orchards (two organic cherry, one mixed peach / nectarine) in Stockton (San Joaquin County), four different fruit orchards (two cherry, one Kiwi, and one pear) in Courtland (Sacramento County), and 15 different fruit orchards at the UC Kearney Agricultural Research and Extension Center, Parlier (Fresno County). Traps were checked weekly, collecting the flies and at the same time refilling the bait-traps with fresh ACV in the field. We have a large collection of flies (60 traps per week and many locations equal hundreds of vials) and not all have been sorted to identify the trapped flies (this will occur primarily in fall and winter). Here we report early traps counts (April to June), which are most important for cherry fruit damage (Table 1).

Table 1. Total number (female) of adult SWD caught in apple cider vinegar traps in different fields and geographical locations (April to June 2013)

Brentwood area, Contra Costa County (1 trap per site):								
	05/22	5/30	06/05	06/14				
Cherries	1(0)	1(1)	2 (2)	2(1)				
Cherries near peaches and apricots	5(5)	15(10)	7(3)	16(6)				
Fig tree	22(3)	19(1)	39(12)	10(3)				
Lemon tree	10(3)	22(12)	16(6)	0				
Peaches near cherries	0	1(1)	0	3(1)				
Pears	26(6)	15(9)	14(7)	4(2)				
Riparian	1(0)	4(0)	17(5)	1(1)				
Riparian	0	12(4)	0	3(2)				
Riparian near plums	15(5)	32(14)	9(1)	1(0)				
Stockton, San Joaquin County (3 traps per site)								
	05/02	05/16	05/22	06/05	06/14			
Cherry (Baker Avenue)	N/A	1(1)	0	0	0			
Peach / nectarine (Ketcham Avenue)	9(3)	4(1)	5(2)	0	0			
Cherry near (Murphy Road)	N/A	41(24)	87(42)	45(32)	11(8)			
Courtland, Sacramento County								
	04/11	04/18	04/24	04/30	05/09	05/14	06/07	06/12
Cherry 1 (3 traps)	46(38)	4(3)	20(9)	34(22)	6(4)	2(2)	10(6)	0
Cherry 2 (3 traps)	6(4)	11(10)	36(33)	44(21)	6(4)	5(1)	3(1)	0
Kiwis near cherries (3 traps)	8(6)	0	4(3)	2(2)	5(5)	0	9(8)	0
Pears near cherries (1 trap)	0	0	20(12)	9(7)	9(5)	0	1(0)	0
UC Kearney Agricultural Research and Education Center, Fresno County (3 traps per site)								
	04/09	04/16	04/23	04/30	05/07	05/14	05/21	05/28
Apple	1(0)	2(1)	1 (0)	1 (0)	4(3)	4(1)	4(2)	3(0)
Apricot	1(0)	1(0)	0	1(0)	4(2)	1(0)	0	1(0)
Blackberry	1(0)	2(2)	3(3)	5(1)	1(1)	9(5)	8(6)	14(13)
Blueberry	0	1(1)	0	0	0	2(1)	0	0
Cherry	0	1(1)	1(1)	6(5)	7(5)	30(18)	18(9)	16(4)
Cherry mixed with peach	0	1(0)	1(0)	3(1)	7(3)	3(2)	27(8)	5(3)
Citrus	2(2)	0	3(1)	1(0)	0	1(0)	0	1(0)
Fig	0	1(1)	0	0	1(0)	0	0	2(2)
Grape	0	0	1(0)	0	0	0	0	0
Kiwi	0	1(0)	0	1(0)	1(0)	0	0	1(0)
Nectarine	1(1)	0	0	0	0	0	0	0
Peach	1(1)	0	0	3(1)	1(0)	0	0	0
Persimmon	1(1)	1(1)	2(1)	5(2)	1(0)	2(0)	0	3(1)
Plum	0	0	1(0)	0	3(1)	1(1)	3(2)	0
Pomegranate	0	0	0	0	0	0	0	0

(a) Bentwood Area: The Brentwood site is a unique, organic mix-fruit farm. We are using these results as baseline data for future studies in more conventional farming systems. SWD were found in all 9 traps in different habitats from mid-May to mid-June (Table 1). Based on the total trap counts during this period, the traps on fig, peach, and in a riparian site near ornamental

plums had the highest numbers, followed by traps on lemon and cherry – both near peach and apricot, and two riparian sites. The traps in the commercial cherry orchard had the lowest number (which was expected because of the insecticide treatments). This suggests that whereas the commercial cherry orchard was treated to lower SWD, other non-commercial fruit crops in the area could sever as alternative hosts (or refuge) for SWD to re-infest treated orchards. In particular, these riparian sites could be ideal habitats for biological control of SWD (prior to the adult SWDs' entrance into the cherry orchards).

(b) Stockton Area: SWD adults were found in traps in all three sites, but the numbers at the organic cherry orchard (near Murphy Road) were much higher than the other mixed fruit sites (organic cherry and peach/nectarine) in Linden. Based on the trap counts from mid-May to mid-June, a total of 184 SWD (106 females) were trapped a the organic cherry (as high as 67 SWD in one trap were found on 30 May), but only one female SWD was collected at the nearby conventional cherry orchard and only 19 SWD (6 females) were trapped at the peach/nectarine orchard.

(c) Courtland Area: SWD were trapped in all four orchard sites in the Courtland collections. Trap counts from mid- to late-April revealed rather large populations of vinegar flies (*D. melanogaster*), ranging from 195 to 3408 flies per trap. The numbers of trapped SWD in the two cherry orchards were generally larger than the nearby kiwi or pear orchard in the early fruit season. Adult flies consisted of a high ratio of sexually matured females, as dissections found most females contained mature eggs (8.8 ± 1.2 eggs, $n = 33$). This suggests that these SWD had likely moved from other habitats rather than from a resident population in the cherry orchard at the time of trapping.

The highest SWD count was found in early-April (about 20 SWD per trap); with rapidly decreasing trap counts thereafter. From June 7 forward (from samples we have processed), not a single SWD was found in the cherries, and all other drosophila numbers were low. Low numbers of SWD in traps in the adjacent kiwi field suggests SWD were concentrated in the cherries at this time at this site, which exemplifies their mobility to find preferred oviposition sites. The numbers of other vinegar flies remained high through April and early May, but then decreased rapidly in mid- to late-May; numbers in late-May were generally under 300 total vinegar flies per trap. In early June, total vinegar fly counts dropped dramatically to >100 flies per trap, with only 1 or 2 female SWD found. After June 7 (through the samples currently processed), not a single SWD was found in the cherries, and all other drosophila numbers were low. These low numbers could have been the result of increased temperatures during this period) or the use of artificially flavored apple cider vinegar as bait, or both.

(d) UC Kearney Agricultural Research and Education Center: We are using the diversity of fruit crops at the Kearney station to investigate the occurrence and abundance of SWD at a landscape level. SWD were trapped in all 13 different crop fields, except the pomegranate orchard. Numbers of SWD were generally low in all crop fields, except cherries and blackberries in which trap counts significantly increased in early summer and then decline dramatically in June. Overall, the numbers of vinegar flies caught in this location were low and especially in June, possibly due to the summer heat.

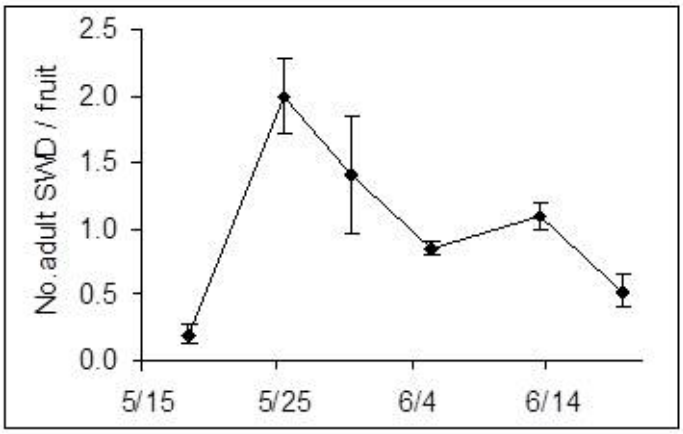
Dissections found that trapped female flies from the cherry orchard had only 3.3 ± 0.9 mature eggs (n = 59) while those from other crops combined had 11.4 ± 1.5 mature eggs (n = 59). During this period, obviously all other crops bear no fruit or immature fruit, and only cherry fruits were available. This suggests that the fly population was concentrated in the cherry orchard.


Photo. Collected spotted winged drosophila adults using a vinegar trap (left) and then sorting through the samples (right). For this project, the importance is to document the presence of SWD and different fruit fly species present in each different habitat sampled.

(2) Survey of frugivorous *drosophilid* species and their parasitoids

It is prudent to understand the impact of resident natural enemies (mainly parasitoids) before considering the introduction of any exotic natural enemies for the control of SWD in USA. Therefore, we conducted surveys of resident drosophila parasitoids using banana-baited fruit trap at UC Kearney and UCB Berkeley areas. Parasitoids were also surveyed by collecting various fruits in different commercial cherry locations (described previously). The field sampling of fruit will also help understand host plant use by SWD.

(a) Field sampling of fruit: We conducted field collections of cherry fruit during the season in an unsprayed orchard at the Kearney site. SWD-infested (with SWD eggs or feeding holes) and damaged cherry fruit (e.g., bird eaten or rotten) were collected from trees. The cherry fruit were seriously infested by SWD (Fig. 1). For example, a mean of 1.68 ± 0.20 (n = 50) adult SWD emerged per fruit from SWD-infested fruit, but no other drosophila fly and parasitoid emerged from these fruit.



From a collection of 106 damaged cherries, we recovered 1.79 ± 0.13 adult SWD flies per fruit. There were a few parasitoids (three individual *Leptopilina* spp. (there is no common name)), and

other drosophila flies (mainly *D. melanogaster*). We note that it is likely that these *Leptopilina* parasitoids may not have been attacking SWD, but were reared from other drosophila flies in the same damaged fruit that the SWD were reared from. This parasitoid species (*Leptopilina* spp.) was also reared from damaged cherry fruit collected from ground and from damaged peach fruit collected both from the trees and on ground (the peaches were nearby the cherry orchard). In this case, the majority of the flies that emerged from damaged peaches were *D. melanogaster*. Field parasitism of *D. melanogaster* by *Leptopilina* spp. ranged from 0 to 26.7% in our collections where we could isolate the fruit fly species.

We also collected fruit from ornamentals in Brentwood, and held the fruit for fruit fly or parasitoid emergence and also tested some fruits in the laboratory to confirm their suitability as a host for SWD. As expected, adult SWD emerged from field-collected cherries (which we used as our control to get a standard level of mortality per fruit species tested – or better stated host suitability). We found that damaged loquats, older peaches and apricot (without signs of damage) could host SWD. The cynipid parasitoid (*Leptopilina* spp.) emerged from damaged loquats that were infested by SWD (3 flies) as well as *D. melanogaster* (85 flies), suggesting again that the parasitoid may be attacking the vinegar fly rather than SWD. SWD were able to develop from two different ornamental plums and cactus species (the flies can lay eggs into cracked stem-end area of the fruit). Brix values of the large and small ornamental plums were 10.5 ± 0.25 and 17.4 ± 0.65 (n =10) and percentage of fly eggs successfully developed into adults were 58.4 ± 9.0 % and 82.1± 6.3% (n = 25), respectively.

(b) Banana-baited fruit trap: At least five different parasitoids have been collected from field-placed fruit traps, including three cynipid parasitoids (*Ganaspis* sp., *Leptopilina heterotoma*, and *L. boulardi* all Figitidae), *Trichopria* sp. (Diapriidae), and *Pachycrepoideus vindemmiae* (Pteromalidae) (none of these have common names). All these parasitoids were known to attack *D. melanogaster* and, in our samples, most of these probably emerged from *D. melanogaster* as no SWD has been found from the same trap where the parasitoids have emerged. Most of these indigenous parasitoids appear not to attack SWD, although formal tests are still in progress.

(3) Evaluation of major parasitoid species

(a) Evaluation of *P. vindemmiae*: *P. vindemmiae* is a generalist pupal parasitoid. We found it was effective against SWD, parasitizing as many as 19 hosts per female per 24 hours and producing as many as 117 offspring over the female's life-time under suitable laboratory conditions (24 °C). The parasitoid is known to attack other *Drosophila* species and many other cyclorrhaphous flies. For example, it can also attack and develop from *D. melanogaster* and olive fruit fly *Bactrocera oleae* (an invasive olive pest in California). These hosts are obviously different in their body size (Fig. 2).

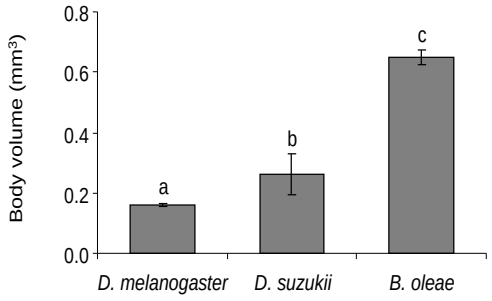


Fig. 2. Body size of three different fruit fly species: the volume V of a prolate ellipsoid fly puparium with maximum body length l and width w was estimated on the formula: $V = (4/3\pi)((l / 2)(w / 2)^2)$; ($F_{2,57} = 687.2$, $P < 0.001$).

When *P. vindemmiae* were reared on the three different-sized host species, there was a positive correlation between the size of emerged parasitoids and the size of their host fly species (Fig. 3).

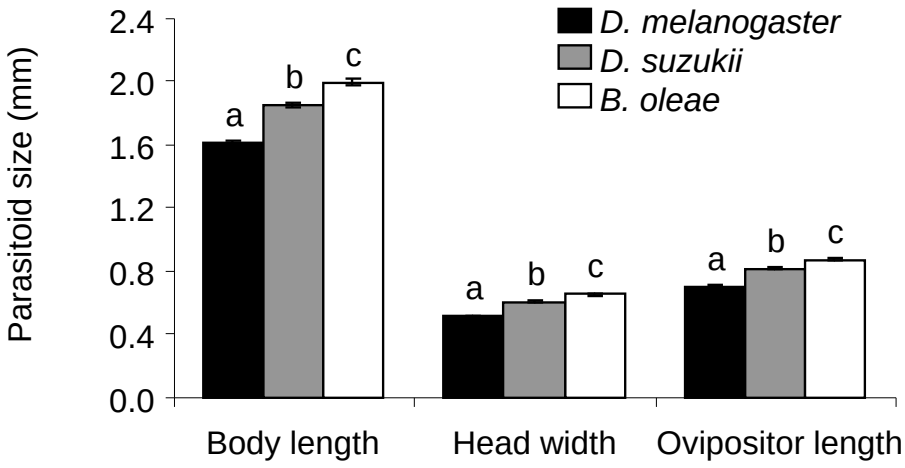


Fig. 3. The size of the parasitoid offspring, reared from three different fruit fly species (statistics for body length are $F_{2,63} = 147.6$, $P < 0.001$; head width are $F_{2,63} = 93.6$, $P < 0.001$; and ovipositor length are $F_{2,63} = 159.9$, $P < 0.001$).

Regardless of its rearing host species, the resulting adult *P. vindemmiae* preferred to attack the larger (SWD) than 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. 4). Large wasps parasitized more hosts than did small ones ($F_{2,59} = 30.6$, $P < 0.001$) (Fig. 4).

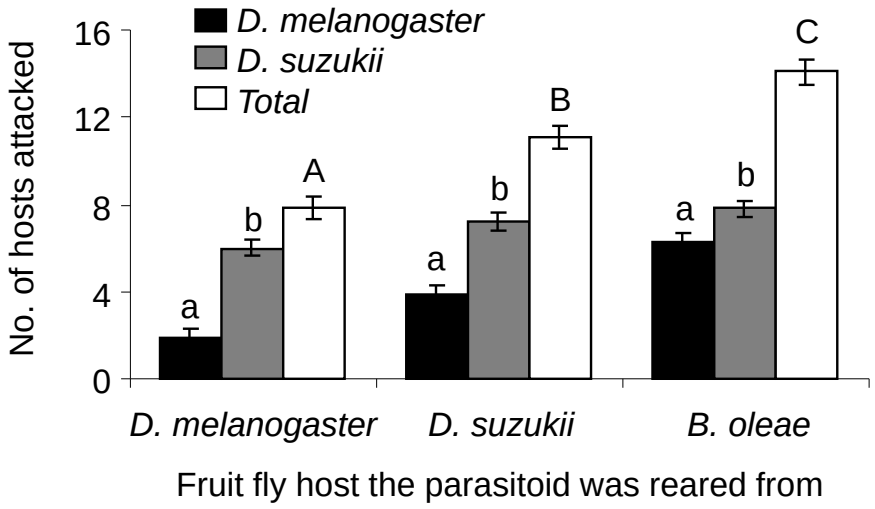


Fig. 4. Effects of host body size on the *P. vindemmiae* attack rate shows that larger wasps (e.g., those reared from *B. oleae* or *D. suzukii*) attacked more fruit flies, regardless of the fruit fly species.

We also found the parasitoid successfully developed without apparent costs to offspring development or survival in large host species such as SWD, and with a fitness advantage deriving from the larger body size of the parasitoid. Our results also suggest that *P. vindemmiae* grows faster on the larger SWD than on the smaller *D. melanogaster*, as there was no pattern of development time that was dependent on host size within each of the host species. These results reflect the plasticity of body growth in this generalist parasitoid; it is plausible that *P. vindemmiae*, and similar generalist parasitoid species that lack any apparent costs in growing to a larger size and still gain a fitness advantage by being larger, should selectively attack the larger host species (such as SWD over the vinegar fly).

There is a diverse array of *Drosophila* species (most of them are smaller than SWD) that could be attacked by this parasitoid, invasion of SWD could increase the wild population size of this parasitoid, and this may eventually lead to an increased impact on SWD through sharing host species. However, the field abundance and distribution of *P. vindemmiae* is unknown in California and elsewhere. In the near future, we intend to document the potential of *P. vindemmiae* on SWD in the field. We are also conducting studies to investigate the parasitoid host stage preference, fecundity and behavioral mechanisms of size-dependent host species selection.

(b) Evaluation of exotic *drosophila* parasitoids against SWD. In cooperation with colleagues at Oregon State University (Drs. Vaughn Walton, Jeff Miller, and Peter Shearer), we have begun foreign exploration to evaluate (in Quarantine) novel parasitoids that may attack SWD and may be used for introduction into the USA to improve natural regulation of SWD in California.

In August and September, Drs. Jeff Miller, Peter Shearer and Betsey Miller made a 3 week trip to South Korea to collect material (details of this report can be provided, but this was not funded by the California Cherry Board). Field-collected materials from South Korea were sent to UC Berkeley's quarantine in August and September 2013, and more collections from South Korea and other Asian countries are planned in near future. Our goal is to explore, import and select most specialized and effective parasitoids on SWD from the pest's native range (East Asia) for future field release in the USA.

From the OSU collection, a total of 3266 individual fly pupae were reared from six different regions in South Korea using sentinel fruit traps or direct samplings of infested fruits, and brought to UC Berkeley's quarantine facility. After arrival at the quarantine, all emerged flies were collected into 95% alcohol bottles for later identification. All emerging parasitoids were used for tests against SWD (dead parasitoids were also preserved in alcohol for later identification). After all flies and parasitoids had emerged, all dead pupae were dissected by first reconstituting the pupa and contents in a water bath for 1-2 days and then dissected the pupae to determine the presence or absence of a recognizable fly or parasitoid cadaver (pharate adults or larvae).

At least four different parasitoid species, including two larval parasitoids *Asobara* spp. (Braconidae) and *Ganaspis* spp. (Figitidae), and two pupal parasitoids, *Pachycrepoideus* sp. (Pteromalidae), *Trichopria* sp. (Diapriidae) (Fig. 3) emerged from these collections in South

Korea (Fig. 5). 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*.

Asobara spp. (Braconidae)

Ganaspis spp. (Figitidae)

Trichopria sp. (Diapriidae)

Pachycrepoideus sp. (Pteromalidae)

Fig. 5. Parasitoid species collected in August-September 2013 from South Korea, these are photos of the actual species, taken using a quarantine microscope.

Each of the four parasitoid species was tested to determine if they can attack and develop from SWD and *D. melanogaster* (the dominant fly species from the collections). Parasitoids were exposed to either host species in artificial diet or blue berries (cherries were not available in the fall and winter in California) for a 2-3 day periods. After exposure, all vials were monitored to record the number of emerged flies and parasitoids. Preliminary results showed that all the four parasitoids can attack and develop from SWD maggots (Table 2), either presented in the artificial diet or host fruit.

We are currently conducting further detailed evaluations of these parasitoids on (1) their relative effectiveness against SWD (i.e. how many hosts can they attack per female per unit time day); (2) host species preference and its consequence of host species selection (which host do they prefer to attack); and (3) non-target risk (can they attack other non-pest drosophila species).

Table 2. Individual numbers of flies and parasitoids that emerged in parasitism experiments

Parasitoid species	Host species	Host medium	Wasps / vial	No. of replicates	Flies	Parasitoids
Asobara spp.	*D. melanogaster*	Diet	2♀1♂	6	390	80
	D. suzukii	Blueberry	2♀1♂	3	5	1
		Diet	2♀1♂	5	14	31
Ganaspis spp.	*D. melanogaster*	Diet	1♀1♂	2	0	6
	D. suzukii	Blueberry	1♀1♂	2	2	2
		Diet	1♀1♂	2	2	7
Pachycrepoideus sp.	*D. melanogaster*	Diet	1♀	3	58	19
	D. suzukii	Blueberry	1♀	2	0	1
		Diet	1♀	2	3	2
Trichopria sp.	*D. melanogaster*	Diet	1♀1♂	7	205	90
	D. suzukii	Blueberry	1♀1♂	4	14	3
		Diet	1♀1♂	4	1	22

(4) Laboratory studies on SWD biology

Since cherry fruits come in different size, color, sugar concentration, and fruit firmness at maturity (Table 3), we have been conducting a series of laboratory experiments to evaluate the effects of cherry varieties, host density, and fruit size on host preference by and suitability for SWD. This may directly or indirectly affect the parasitoids, as well.

In choice test with two different size fruit (small: 4.4 ± 0.08 g and large: 8.7± 0.09 g, n = 35), adult female SWD preferred to oviposit on larger than small fruit ($F_{1,68}$ = 5.89, P = 0.018) (Fig. 6). But the percentage of eggs that developed into adults was not affected by the fruit size (56.9 ± 6.5% and 62.2 ± 9.1% from large and small fruit, $F_{1,68}$ = 0.001, P = 0.973).

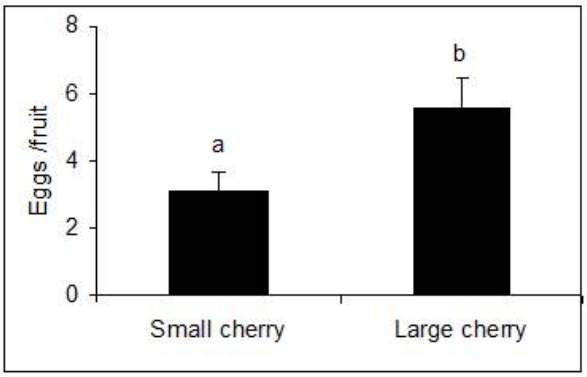


Fig. 6. Fruit size preference by adult female SWD. Different letters over the bars indicate significant difference (One-way ANOVA and Tukey's HSD, $P < 0.05$).

These results are fairly obvious – the larger the fruit the more maggots it will produce. Attack rate will obviously depend on other factors, such as the fruit surface condition.

The percentage survival from fly egg to adult was measured on 10 different cherry varieties (Table 3). Generalized Linear Model (with binomial distribution and a logit link function) analysis showed that immature survival rate decreased with increasing relative density (i.e., number of eggs per g fruit, χ^2 = 28.04, df = 1, 251, $P < 0.001$) but was not significantly affected

by the fruit variety (i.e., brix which was coded from high to low based on difference, $\chi^2 = 2.88$, df = 1, 251, $P = 0.089$).

The percentage of eggs that successfully developed into adults (y) decreased with increasing egg density (x) per cherry fruit (cv. 'Bing') ($y = 0.882 - 0.015x$, $n = 94$, density ranged 1-43 eggs per fruit, $r^2 = 0.296$, $P < 0.001$). Individual female SWD laid more eggs per fruit on large (5.57 ± 0.88 eggs) than small (3.09 ± 0.58 eggs) cherries.

Data on other fitness parameter (developmental time and body size) have not been analyzed yet. Studies on the effect of fruit color on host preference are still in progress.

Table 3. Fruit size (weight), sugar, and surface penetration force of different cherry fruit

Variety *	*n*	Color	Weight	Brix	*n*	Surface penetration force (g mm^{-1})
R2T9	11	Black	4.36 ± 0.13 e	24.2 ± 0.6 bc	15	52 ± 4 e
R1T35	10	Black	3.64 ± 0.28 e	15.6 ± 0.8 d	15	N/A
R1T7	10	Purple	7.44 ± 0.44 b	23.9 ± 0.8 bc	15	100 ± 3 c
CH2	10	Purple	8.65 ± 0.37 a	21.5 ± 0.6 c	15	77 ± 8 d
R1T48	10	Yellow	2.52 ± 0.10 f	15.9 ± 0.9 d	15	114 ± 4 bc
R1T13	10	Yellow	4.79 ± 0.24 de	22.5 ± 0.4 bc	15	133 ± 2 b
R2T2	10	Yellow	5.64 ± 0.34 cd	18.3 ± 0.9 d	15	97 ± 4 c
R1T19	10	Pink	4.35 ± 0.18 e	27.3 ± 0.5 a	15	129 ± 4 b
R1T40	10	Pink	6.50 ± 0.14 bc	24.6 ± 0.3 ab	15	100 ± 4 c
R1T66	10	Red	4.25 ± 0.14 e	22.6 ± 0.7 bc	15	157 ± 4 a

*Cherry variety needs to be verified and we are presenting only our coding here. Values are mean ± SE and different letters within the column indicate significant difference (One-way ANOVA and Tukey's HSD, $P < 0.05$).

Many other major fruits in the San Joaquin Valley could serve as alternative or overwintering hosts for SWD when cherry seasons are over. We have evaluated the potential of peach as a host for the fly through examining the effects of indument or peach fuzz, and various existing damages on the ovipositional success by the fly. Existing damages included feeding damage by other two pests (the peach twig borer, *Prunus persica* and katydid, *Scudderia furcata*), needle punctures to stimulate damage by sucking insects, and harvest damage to the stem-end area of the hand-picked fruit (e.g., skin was ripped off).

The results showed that adult female *D. suzukii* did not lay eggs into packable healthy fruit, but could occasionally lay eggs into less fuzzy stem-end area of the fruit. When the fuzz of the fruit was removed ("shaved") the fly readily laid eggs into the shaved area. Existing damage by *P. persica* or *S. furcata* facilitated the fly's ovipositional success and the number of eggs per damaged area generally increased with the relative size of the damage. Harvest damage received the highest numbers of eggs per damaged spot. The fly did not lay eggs into small punctures (0.3 or 0.5 mm), but did lay eggs into large puncture (1 mm). Fruit firmness and sugar content

appeared not affect the fly's oviposition on treated fruit. Direct observations on the fly's ovipositional behavior further confirmed that presence of fuzz discouraged the fly's oviposition, while removal of the peach skin (i.e., existing surface damage) facilitated the fly's ovipositional success. Mean ovipositional duration was shorter in softer than tougher surface substrates. A manuscript from this study has been drafted. We have also been taking movies of SWD oviposition, which we will make available to all cooperators.

We are also evaluating the suitability of grapes, pomegranate seeds and oranges as potential developing host for SWD using a standard protocol. Although intact pomegranate and orange fruit are unlikely attacked by SWD due to their thick skin, cracked pomegranate or damaged orange could potentially sever as food or developing hosts for SWD.

Table 4. Suitability of some fruit crops as potential developing hosts for *D. suzukii*

Fruit	*n*	Brix	Fruit surface firmness (g mm^{-1})	*n*	Eggs /fruit unit	Eggs /per mg fruit	Eggs developed to adults (%)
Wine grape	20	23.5 ± 0.5a	124 ± 6b	22	2.8 ± 0.5	4.8 ± 0.5a	4.5 ± 3.1d
Raisin grape	20	17.9 ± 0.2c	60 ± 7c	52	3.6 ± 0.4	2.2 ± 0.3b	26.5 ± 4.5c
Table grape	20	21.2 ± 0.3b	166 ± 5a	33	4.0 ± 1.0	1.1 ± 0.4b	31.4 ± 6.1bc
Pomegranate	20	16.9 ± 0.2c	46 ± 3c	49	1.9 ± 0.1	4.1 ± 0.3a	70.7 ± 5.9a
Orange	20	11.5 ± 0.1d	N/A	53	3.9 ± 0.3	1.8 ± 0.3b	53.9 ± 6.6ab

* Values are mean ± SE and different letters within the column indicate significant difference (One-way ANOVA and Tukey's HSD, $P < 0.05$).

We found that percentages of eggs successfully developed into adults were lower in grapes, particularly in wine grape, despite of its higher sugar concentration than pomegranate or orange ($F_{3,76} = 91.7$, $P < 0.001$) (Table 4). Fly can lay eggs into various types of grapes and pomegranate seeds as surface firmness of these tested fruit units (Table 4) were within the range of mature cherry fruits (see Table 3). Because cut fruit pieces were used for orange (as if the fruit was damaged by other animals), the firmness data for orange was unavailable. The fly developed well in pomegranate seeds; one small seeds (0.4-0.6 mg) could support the successful development of up to 4 individual flies. SWD could also develop from orange, despite of its low sugar concentration. The results suggest that some compounds in grapes may affect the fly growth and development. We are currently conducting further studies to determine the mechanisms (e.g. organ acids, PH value) that may affect the fly's development in grapes. We stress here that these are laboratory studies and there is strong circumstantial and observational evidence that table grapes are not utilized as SWD hosts in California's SJV.

We have shown above that SWD can develop from different commercial fruit and we suggest that the post-harvest presence of these fruits may also provide food sources for adult flies and affect adult fly's survival and reproduction in the field, particularly when other fruit sources are unavailable in winter or early spring. We are currently conducting experiments to determine potential effects of various fruit juices (orange juice, pomegranate juice, grape juice, and apple juice) on adult fly's survival and reproduction, when compared to honey water and water only.

We are also evaluating the potential of other fruit crops serving as host for SWD. Currently, we have evaluated peach as a host for the fly through examining the effects of "indumenta" or peach fuzz, and various existing types of damage on the ovipositional success by the fly.

(5) SWD overwintering biology in San Joaquin Valley

Several field experiments are currently being conducted to determine the survival rates of various developmental stages (egg, larva, pupa and adult) of SWD over the winter seasons in the field. Beginning once every two weeks from November 2013 to May 2014, laboratory reared flies of each stage are placed in drosophila vials and moved to the field cages. The cages are hung inside the canopies of citrus trees. Additionally, fly pupal are also placed in drosophila vials and the vials are buried 1–2 cm below the soil surface under the tree canopies. The adult survival tested is consisted of four different food provision treatments: (1) no food or water; (2) water only; (3) 50% honey water only; and (4) honey water + food and /or ovipositional media (a piece of sliced orange). Direct sampling of any susceptible fruits (e.g. grapes, figs, apples, plums, pomegranates, oranges, and kiwi) that are left over or fallen to ground are collected at the UC Kearney Agricultural Research and Education Center and hold in cages under the lab conditions to record the numbers and species of emerged flies or parasitoids.

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Postharvest treatment of sweet cherries with methyl bromide to control spotted wing drosophila, *Drosophila suzukii*

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Executive Summary. Methyl bromide (MB) chamber fumigations were evaluated for postharvest control of spotted wing drosophila, *Drosophila suzukii*, in fresh sweet cherry exports from Western USA. Sweet cherries were infested with SWD, infested cherries containing the most MB-tolerant SWD life stage (3rd instar larvae) were buried amongst uninfested fruit in fruit bins (wood or plastic) at 30 to 50% load factors, and then the fruit bins were fumigated for 2 to 3 h. Treatment efficacy was diagnosed by the percentage of survivors emerging as adults from fumigated cherries relative to that from non-fumigated controls. Designation of "Probit 9" efficacy was based on ≤ 1 survivor out of > 30,000 treated. Residue evaluations were based on methods described in the June 2012 report to APHIS for the Korea market. Quality evaluations are detailed below.

Applied (mg/L)	Time (h)	Temp. (±0.5 °C)	Temp. (±0.8 °F)	Load (%)	Bin type	CxT Exposure ±5.8 (mgL⁻¹h)	% mortality ("Probit 9")	quality work (complete)	residue work (complete)
current treatment options (availiable 2013 season to AUS, JPN, KOR)									
72.0	2.0	8.3	47.0	30.0	wood	~120 needed	Yes	Yes	Yes
64.0	2.0	10.5	51.0	30.0	wood	~110 needed	Yes	Yes	Yes
options moving forward at T = 47 °F (2013 research)									
72.0	2.0	8.3	47.0	35.0	plastic	~120 observed	Yes	Yes	Yes
64.0	2.5	8.3	47.0	40.0	wood	~130 observed	Yes	Yes	No
64.0	2.5	8.3	47.0	45.0	plastic	~125 observed	Yes	Yes	Yes
64.0	3.0	8.3	47.0	50.0	w or p	~125 observed	Yes	No	No
options moving forward at T = 51 °F (2013 research)									
64.0	2.0	10.5	51.0	35.0	plastic	~110 observed	Yes	Yes	Yes
56.0	2.5	10.5	51.0	40.0	wood	~118 observed	Yes	Yes	Yes
56.0	2.5	10.5	51.0	45.0	plastic	~115 observed	Yes	Yes	Yes
56.0	3.0	10.5	51.0	50.0	w or p	~115 observed	Yes	No	No
work in progress at T < 47 °F (2014 research)									
80.0	2.5	6.1	43.0	30	wood	>140 needed	No	No	No
72.0	3.0	6.1	43.0	30	wood	>140 needed	No	No	No
64.0	3.5	6.1	43.0	30	wood	>140 needed	No	No	No

Fruit quality. The effects of fumigation on fruit quality were quantified by methods reported in Obenland et al. (2011) and Mitcham et al (2003) by evaluating characteristics of non–fumigated cherries relative to those fumigated in confirmatory SWD fumigations. Quality parameters were evaluated after storage for 2 days at 1.1 ± 0.6 °C ($\bar{x} \pm s$) (~34.0°F) plus 16 hours at 22.2 ± 0.6 °C ($\bar{x} \pm s$) (~72.0°F) to simulate air shipment and marketing. Surface browning, stem browning, pitting, cracking, shrivel, decay and overall acceptability were subjectively evaluated as listed in Table 1. Ratings that would likely be unacceptable to a consumer are indicated. Ratings are presented as calculated indices or in terms of acceptability. Skin color was evaluated using a Minolta colorimeter by measuring the same spot on the skin of 10 fruit for each replication before treatment and after storage and expressed in the L*C*h scale as amount of color difference (poststorage - pretreatment). Acidity was determined from the juice of 5 pooled fruit for each replication by titration with NaOH. Soluble solids were measured from the same juice using a digital refractometer as in Obenland et al. (2005). Firmness (g-1mm deflection) was measured with a Bioworks Firm Tech 2 instrument. Percent considered marketable. Pitting was a subjective rating with scores of 0, 1, 2 or 3, where a score of 2 indicates that the fruit would likely not be acceptable to a consumer. Pitting ratings were calculated indexes: (i.e. number of cherries with score 0*0) + (number of cherries with score 1*1)/total number of cherries, where a lower value indicates better quality. Statistical significance of fumigation treatments from controls were based on paired t-tests ($P < 0.05$).

Table 1. Subjecting rating scores for cherries.

Quality Attribute	Score	Description
Surface browning	0	No browning, full red color
	1	Slight browning, 1 - 25% of the fruit surface
	2	Moderate browning, 26 - 50% of the fruit surface
	3	Severe browning, >50% of the fruit surface
		Scores > 1 considered unacceptable
Stem browning	0	None
	1	1 - 25% brown
	2	26- 50% brown
	3	51 - 75% brown
	4	76 - 100% brown
		Scores > 2 considered unacceptable
Surface pitting	0	None
	1	Slight
	2	Moderate
	3	Severe
		Scores > 1 considered unacceptable
Surface cracking	0	None or insignificant cracking
	1	Slight, 1 - 2 mm long, shallow crack
	2	Moderate, 2 - 4 mm long, deep crack
	3	Severe (>5mm long, deep crack)
		Scores > 1 considered unacceptable
Surface shrivel	0	None
	1	Obvious shrivel
		Scores > 0 considered unacceptable
Decay	0	None
	1	Decay
		Scores > 0 considered unacceptable
Overall acceptability	0	Very good
	1	Some damage but still good and marketable
	2	Obvious damage but still marketable
	3	Unacceptable

"Bing' cherry quality following methyl bromide fumigation at a range of temperatures, doses and fumigation times. Subsequent storage was for 2 days at 34 °F plus 16 hours at 68 °F to simulate air shipment and marketing.

Treatment	Surface browning (% acceptable)[a]	Stem browning (% acceptable)[a]	Pitting (rating)[b]	Decay (%)	Overall acceptability (%)[a]	Color L (Lightness)	Color C (Chroma)	Color h (Hue)	Firmness (g)[c]	Soluble Solids (%)	Acidity (%)
Control, 47 °F, 2h	100a	90a	0.09a	1a	89a	30.93a	15.98b	15.38a	273.42a	23.48a	0.99a
72 mg/L, 47 °F, 2h	100a	98a	0.08a	0a	92a	31.59a	17.73a	16.76a	260.75ab	23.63a	0.98a
64 mg/L, 47 °F, 2.5h	100a	91a	0.05a	0a	89a	31.60a	17.80a	16.94a	246.81b	22.78a	0.98a
Fumigation effect[d]	NS	NS	NS	NS	NS	NS	*	NS	*	NS	NS
Control, 51 °F, 2.5h	100a	95a	0.03a	2a	93a	30.86a	16.68a	15.32a	262.26a	22.30a	0.91a
64 mg/L, 51 °F, 2h	100a	94a	0.08a	0a	91a	31.18a	18.01a	16.59a	265.79a	22.15a	0.94a
56 mg/L, 51 °F, 2.5h	100a	96a	0.08a	0a	94a	31.22a	18.07A	16.62a	259.90a	22.35a	0.98a
Fumigation effect[d]	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Control, 54 °F, 2h	100a	100a	0.04a	0a	100a	30.97a	17.28a	15.86b	270.95a	23.18a	0.95a
56 mg/L, 54 °F, 2h	100a	98a	0.09a	0a	98a	31.12a	17.71a	17.28a	263.97a	22.78a	0.94a
Fumigation effect[d]	NS	NS	NS	NS	NS	NS	NS	*	NS	NS	NS
Control, 57 °F, 2h	100a	93a	0.05a	2a	89a	31.09a	17.94a	15.82a	283.36a	22.95a	0.94a
48 mg/L, 57 °F, 2h	100a	100a	0.00a	1a	96a	31.29a	18.04a	16.57a	266.42a	23.18a	0.98a
Fumigation effect[d]	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

[a]Percent considered marketable.
[b]Pitting was a subjective rating. Pitting: 0, 1, 2 or 3 with 2 indicating that the fruit would likely not be acceptable to a consumer. Ratings are calculated indexes: (i.e. number of cherries with score 0*0) + (number of cherries with score 1*1)/total number of cherries. Lower value = better quality.
[c]Grams required to cause a 1 mm deflection of the fruit surface.
[d]Statistical significance of fumigation treatments from controls. NS = not significant, * = significant. Red numbers show significance.

"Bing" conclusions: Overall not much effect. Some fairly subtle alterations in color as a result of fumigation may be occurring but these were not major.

'Coral' cherry quality following methyl bromide fumigation at a range of temperatures, doses and fumigation times. Subsequent storage was for 2 days at 34 °F plus 16 hours at 68 °F to simulate air shipment and marketing.

Treatment	Surface browning (% acceptable)[a]	Stem browning (% acceptable)[a]	Pitting (rating)[b]	Decay (%)	Overall acceptability (%)[a]	Color L (Lightness)	Color C (Chroma)	Color h (Hue)	Firmness (g)[c]	Soluble Solids (%)	Acidity (%)
Control, 47 °F, 2h	0.01a	100a	0.09a	0	96a	31.68a	20.38a	16.04	310.01a	18.03a	1.07a
72 mg/L, 47 °F, 2h	0.08a	99a	0.08a	0	95a	32.42a	22.56a	16.76	321.78a	17.30a	1.04a
64 mg/L, 47 °F, 2.5h	0.04a	99a	0.18a	0	89b	32.06a	22.58a	16.90	324.66a	17.88a	1.01a
Fumigation effect[d]	NS	NS	NS	NS	*	NS	NS	NS	NS	NS	NS
Control, 51 °F, 2.5h	0.05a	99a	0.10a	0	89a	31.90a	21.91a	16.03b	322.27a	17.48a	0.97a
64 mg/L, 51 °F, 2h	0.06a	99a	0.08a	0	98a	32.19a	22.29a	17.27a	335.94a	17.33a	1.00a
56 mg/L, 51 °F, 2.5h	0.00a	100a	0.05a	0	90a	32.02a	22.07a	16.61ab	324.52a	18.45a	1.02a
Fumigation effect[d]	NS	NS	NS	NS	NS	NS	NS	*	NS	NS	NS
Control, 54 °F, 2h	0.00a	100a	0.09a	0	99a	31.75a	21.65a	15.90a	329.37a	17.75a	1.02a
56 mg/L, 54 °F, 2h	0.03a	100a	0.09a	0	99a	32.29a	22.10a	17.51a	334.29a	17.93a	1.07a
Fumigation effect[d]	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Control, 57 °F, 2h	0.04a	99a	0.11a	0	100a	31.81a	20.96a	15.77b	333.49a	17.38a	0.93b
48 mg/L, 57 °F, 2h	0.08a	98a	0.11a	0	100a	31.93a	21.81a	16.72a	329.36a	17.23a	1.02a
Fumigation effect[d]	NS	NS	NS	NS	NS	NS	NS	*	NS	NS	*

[a]Percent considered marketable.
[b]Pitting was a subjective rating. Pitting: 0, 1, 2 or 3 with 2 indicating that the fruit would likely not be acceptable to a consumer. Ratings are calculated indexes: (i.e. number of cherries with score 0*0) + (number of cherries with score 1*1)/total number of cherries. Lower value = better quality.
[c]Grams required to cause a 1 mm deflection of the fruit surface.
[d]Statistical significance of fumigation treatments from controls. NS = not significant, * = significant. Red numbers show significance.

"Coral" conclusions: Fruit was dark red in color, making it difficult to see surface injury if any were present. Little or no evidence of any impact of fumigation with the exception of a small decline in overall acceptability in one of the fumigation treatments at 47 ºF and a small increase in hue angle in two of the fumigation treatments that was not readily visible to the eye.

"Brooks' cherry quality following methyl bromide fumigation at a range of temperatures, doses and fumigation times. Subsequent storage was either for 2 days at 34 °F plus 16 hours at 68 °F to simulate air shipment and marketing.

Treatment	Surface browning (% acceptable)[a]	Stem browning (% acceptable)[a]	Pitting (rating)[b]	Decay (%)	Overall acceptability (%)[a]	Color L (Lightness)	Color C (Chroma)	Color h (Hue)	Soluble Solids (%)	Acidity (%)
Control, 47 °F, 2h	100a	69.64b	0.04a	0.00	91.96a	40.21a	33.02a	28.38a	19.80	1.08a
72 mg/L, 47 °F, 2h	86b	88.10a	0.00a	0.00	80.95a	38.25b	30.37a	27.76a	20.50	0.98b
64 mg/L, 47 °F, 2.5h	81b	85.71a	0.04a	0.00	78.57a	37.38b	27.39b	26.64a	20.70	0.94b
Fumigation effect[c]	*	*	NS	NS	NS	*	*	NS	NS	*
Control, 51 °F, 2.5h	92a	65.48a	0.01a	0.00	72.62a	39.11a	30.81a	28.48a	20.73a	1.09a
64 mg/L, 51 °F, 2h	86a	71.43a	0.00a	0.00	67.86ab	39.19a	30.29a	28.59a	20.53a	1.01ab
56 mg/L, 51 °F, 2.5h	84a	49.11a	0.04a	0.00	57.14b	39.85a	31.09a	29.65a	19.70a	0.98b
Fumigation effect[c]	*	NS	NS	NS	*	NS	NS	NS	NS	*
Control, 54 °F, 2h	92a	48.81a	0.02a	0.00	51.19a	39.09a	30.63a	28.38a	19.80a	0.97a
56 mg/L, 54 °F, 2h	89a	32.14a	0.01a	0.00	46.43a	40.51a	32.59a	29.84a	20.30a	1.03a
Fumigation effect	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Control, 57 °F, 2h	92a	50.00a	0.00a	0.00	47.62a	39.28a	31.06a	28.17a	20.30a	1.04a
48 mg/L, 57 °F, 2h	87a	34.52b	0.01a	0.00	34.52a	39.59a	30.09a	29.05a	20.23a	0.99a
Fumigation effect[c]	NS	*	NS	NS	NS	NS	NS	NS	NS	NS

[a]Percent considered marketable.
[b]Pitting was a subjective rating. Pitting: 0, 1, 2 or 3 with 2 indicating that the fruit would likely not be acceptable to a consumer. Ratings are calculated indexes: (i.e. number of cherries with score 0*0) + (number of cherries with score 1*1)/total number of cherries. Lower value = better quality.
[c]Statistical significance of fumigation treatments from controls. NS = not significant, * = significant. Red numbers show significance.

"Brooks Conclusions": Fruit having the least amount of visible bruising were selected for this study from a field bin that had a pack-out of ~30% due to wind-damage in the orchard. The most noticeable effect of fumigation was that it tended to make stems browner, the effect being significant at 47 °F and 57 °F. The influence of fumigation on browning was difficult to determine because there was so much bruising present. No pitting, shrivel or cracking were noted. Little or no decay was observed. The biggest influence on quality was the temperature of fumigation. As fumigation temperature increased the quality declined. This must have been largely a result of the conditioning (tempering) time as it doesn't seem likely that 2 or 2.5 hours could make such a difference. Some loss in acidity was observed as a result of fumigation at 47 °F and 51 °F.

Novel postharvest fumigation with cylinderized phosphine to control fruit fly pests of sweet cherries: quality evaluations of key export varieties

by

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Significant findings:

- Cylinderized phosphine (PH3) fumigation at cold-storage temp controls SWD in 36 to 48 h.
- Residues and worker exposure with PH3 are favorable (relative to MB)
- Fruit quality evaluations look promising; more varieties recommended

Methods:

Insects. SWD pupae were obtained from the laboratory colonies of Drs. Arytom Kopp (University of California at Davis) and Robert Van Steenwyk (University of California at Berkeley; both colonies originated from wild specimens captured in cherry orchards of coastal California USA. SWD pupae were also obtained from a laboratory colony of Dr. Jana Lee (USDA-ARS), which originated from wild specimens captured in raspberry fields of Marion County, Oregon USA. Pupae from these three sources were integrated into a single colony that was maintained in several (6-8 ct.) nylon mesh enclosures (Bug Dorm-2®, BioQuip Products, Rancho Dominguez, CA, US) housed in an 22.65-m^3 incubation unit (24-27 °C, 80% RH, 16:8 [L:D] h) at the USDA-ARS-SJVASC (Parlier, California USA). Approximately twice a year, SWD adults were captured in raspberry fields located in the Salinas Valley of California and introduced into the SJVASC colony along with new pupae from each of the original sources. Plastic vials (20-dram) containing saturated aqueous solutions of sucrose were capped with cotton wicks to serve as a food and water source for adults. Larvae were reared on standard cornmeal-(dextrose or sucrose)-agar-yeast medium layered to ($\bar{x}$ ± *s*, AVE. ± STDEV) 4.0 ± 0.6 mm on the bottom of 8.7 ± 0.1-cm diameter Petri dishes, which also served as ovipositional substrate (Figure 1). Formalin ® (2 mL), a fungistat, was added to each 4-L batch of diet. Four diet-containing Petri dishes were placed in each enclosure, replaced after 2-d ovipositional periods, and transferred to a separate communal rearing enclosure for the duration of development. When adults began to emerge from a particular dish, it was transferred back into a community of reproductively-active adults maintained at ~ 2000 individuals per enclosure.

Fruit infestation. To simulate a naturally occurring infestation scenario, ovipositional/diet substrate was removed from an enclosure and replaced with stainless-steel trays (30 × 30 × 2 cm) that were filled with a monolayer of fresh sweet cherries. The stainless-steel trays containing infested sweet cherries were removed after ovipositional periods that varied by test type and then infested cherries were transferred in pairs into a stainless-steel mesh ball cage (5.1-cm diameter). Mesh ball cages containing infested cherries were randomly selected, placed inside a pull-string cloth bag (~25 per bag), and used in laboratory-scale exploratory fumigations or buried throughout the load of commercial fruit bins in confirmatory-scale fumigations. Alternatively, mesh ball cages were not fumigated and held as untreated controls to estimate the number of individuals treated during a respective fumigation. For the exploratory fumigations, removal of cherries from rearing cages was synchronized to yield profiles of discrete development across all SWD life stages (less adults). For the confirmatory fumigations, cherries were removed from an enclosure after a 24-h ovipositional period so that only 0-24 h old eggs, the most PH3-tolerant age of SWD (*vide infra*), were present at the start of a pre-fumigation period of temperature equilibration (i.e., tempering).

Exploratory fumigations. To determine the treatment duration required to control the life stages of SWD with 1.6 mgL^{-1} (1000ppmv) and 3.7 mgL^{-1} (2500ppmv) phosphine (PH3) at 1.7 ± 0.5°C ($\bar{x} \pm s$) (~35°F), a series of exploratory fumigations were conducted in modified Labonco® 28.32-L vacuum chambers. Chambers were housed in a walk-in environmental incubator with tunable temperature, humidity, and pressure (USDA, 2010). Test specimens, non-fumigated control specimens, source-gas cylinders, and gas-tight syringes were acclimated, or tempered, to fumigation temperature of 1.7 ± 0.5 °C ($\bar{x} \pm s$)(~35°F) for 12 h prior to treatment. Sweet cherries infested with the various life stages of SWD were fumigated concomitantly within a chamber for a particular fumigation trial.

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 dose 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 the pressure needed for the respective trials 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.

Following the final sampling for fumigant concentration, chamber valves were opened to atmosphere and a 1-h aeration period was initiated. Chamber lids were then opened and the treated and non-treated infested sweet cherries were collected and transferred to an incubator at 27.0 ± 1.0 °C (~80°F) and 80 ± 2% RH ($\bar{x} \pm s$) prior to mortality evaluation.

Confirmatory export fumigations. To simulate a commercial scenario, fumigations were conducted using 241.9-L steel chambers housed in a walk-in environmental incubator with programmable temperature and humidity (USDA, 2010) set to treatment temperature of at 1.3 ± 0.5°C ($\bar{x} \pm s$)(~34.3°F). On the same day that they were packaged for export, either Bing or Coral variety sweet cherries were obtained from commercial wholesale sources. Cloth bags containing infested cherries were buried amongst noninfested cherries in wooden fruit bins (45.72l × 45.72w × 30.48h cm), which were constructed out of 1.3 cm –thick plywood as scaled-down replicates of those used in industry, to a level of ~75% capacity (Figure 2). The chamber was loaded with two fruit bins, bringing the chamber load to ~ 50 %($V_{commodity}/V_{chamber}$x 100), as calculated by the method of Monro (1969).

Chambers loaded with infested and uninfested 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 cherries distributed at different locations within bins of the infested cherries undergoing treatment. 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 76-127 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 dose as predetermined in preliminary calibration studies. A syringe was fitted to a LuerLok ® sampling valve, which was subsequently opened so that PH3 was steadily drawn into the chamber. The syringe was then removed and normal atmospheric pressure (NAP) was reestablished in each chamber before the valve was closed; this marked 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 quantitatively analyzed for PH3 with GC-PFPD at standard intervals corresponding to 5 (initial), 60, 480, 1440 (1-d end), or 2880 (2-d end) min. Fumigant exposures were expressed as concentration × time cross products, "CTs", and calculated by the method of Monro (1969).

After completion of the exposure, chamber valves were opened to atmosphere and vacuum was pulled to aerate the chamber until headspace concentration of the fumigant was below the mandated ventilation requirements of 0.3 ppm (0.45μg/L) phosphine. Chamber lids were opened and the treated and non-treated specimens were collected, placed into respective pull-string cloth bags, and transferred into separate 0.03-m^3 nylon-mesh rearing cubicles maintained in an incubator at 27.0 ± 1.0 °C and 80 ± 2% RH ($\bar{x} \pm s$). noninfested fruit was retrieved and used for residue determination and fruit quality evaluation. Samples of noninfested fumigated fruit (75 g each), selected from 3 different locations within the load, were placed into a cooler filled with dry ice within 5 minutes of the end of aeration and were used to estimate initial residue levels. The remaining noninfested fumigated fruit transferred into cold storage at 1.1 ± 0.6 °C ($\bar{x} \pm s$) (~34.0°F) and temporally retrieved from storage and used for residue determination(s)(*not discussed*).

Mortality evaluation. Mortality of treated specimens was assessed at 1-d intervals post-fumigation for 21 d; cages were removed from the cloth bags, opened, and live adult specimens were tallied and discarded. The cages were then resealed, and placed back into the cloth bags for further incubation and evaluation. Quartered pieces of an uninfested cherry were added to the mesh ball cages approximately every other day to keep the test fruit and insects hydrated. The number of treated specimens was estimated by the cumulative number of adults that emerged from untreated controls.

Rearing and incubation conditions of 27.0 ± 1.0 °C (~80°F), 80 ± 2% RH, and 16:8 [L:D] h photoperiod were fixed to maintain a consistent progression of development between trials and controls; resulting mortality in control specimens was assumed to be equal to that in fumigation trials. Insects were more likely to survive and there was greater certainty in diagnosing survivorship after the treatment if incubated under conditions described above rather than if refrigerated post-fumigation at 2-5 °C under simulated commercial transport conditions, which confound the effect of a fumigation event on mortality. To be detailed in a forthcoming publication on the effect of refrigeration on SWD, we generally observed increases in the mortality of all SWD life-stages, the length of the developmental periods of each life-stage, and heterogeneity in the times required to complete development within each life-stage.

Chemical analysis. Fumigant levels in headspace of fumigation chambers were measured using gas chromatography; retention time were 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 (Walse et al 2012a & b, Walse et a., 2013). Detector response and retention indices were determined each day in calibration studies by diluting known volumes of gaseous 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.

Fruit quality. The effects of fumigation on fruit quality were quantified by methods reported in Obenland et al. (2011) and Mitcham et al (2003) by evaluating characteristics of non–fumigated cherries relative to those fumigated in confirmatory SWD fumigations with 1000 ppm PH3 and treatment durations of either 24 or 48 h. Quality parameters were evaluated after storage for 2 days at 1.1 ± 0.6 °C ($\bar{x} \pm s$) (~34.0°F) plus 16 hours at 22.2 ± 0.6 °C ($\bar{x} \pm s$) (~72.0°F) to simulate air shipment and marketing. Surface browning, stem browning, pitting, cracking, shrivel, decay and overall acceptability were subjectively evaluated as listed in Table 1. Ratings that would likely be unacceptable to a consumer are indicated. Ratings are presented as calculated indices or in terms of acceptability. Skin color was evaluated using a Minolta colorimeter by measuring the same spot on the skin of 10 fruit for each replication before treatment and after storage and expressed in the L*C*h scale as amount of color difference (poststorage - pretreatment). Acidity was determined from the juice of 5 pooled fruit for each replication by titration with NaOH. Soluble solids were measured from the same juice using a digital

refractometer as in Obenland et al. (2005). Firmness (g-1mm deflection) was measured with a Bioworks Firm Tech 2 instrument.

Results & Discussion:

Executive summary. Phosphine chamber fumigations were evaluated for postharvest control of spotted wing drosophila, *Drosophila suzukii*, in fresh sweet cherry exports from Western USA. A series of exploratory fumigations were conducted to establish a toxicological response for pupae, larvae, and egg life stages. Fruit were infested with the various life stages and fumigated with 1.6 mgL^{-1} (1000ppmv) or 3.7 mgL^{-1} (2500ppmv) phosphine for 12, 24, 36, and 48 h at 1.7 ± 0.5 ºC ($\bar{x} \pm s$)(~35.0°F). The applied dose of cylinderized phosphine (1,000 or 2,500 ppm) did not affect the efficacy of fumigation, suggesting that the load factor and the load geometry are inconsequential, as long as the minimum headspace concentration at the end of fumigation is ca. 1000 ppm phosphine. In confirmatory fumigations, which simulated the commercial scenario, complete mortality of 35,265 ± 1,006 (*n* ± *SE*) eggs (ca. 12 to 36-h old at fumigation), the most tolerant SWD life stage, was achieved with an applied dose of 1000 ppm, a load factor of ~ 50%, and a treatment time of 48 h at 1.7 ± 0.5 ºC ($\bar{x} \pm s$)(~35.0°F). Sorption, off-gassing (i.e., depuration), and residue data were obtained. Results can be used by industry in the context of quantifying fumigant inputs to ingestion exposure and worker inhalation exposure that are respectively derived from the consumption of fruit residues and off-gassing of palletized fruit in cold-storage. Relative to methyl bromide, ~10-fold less mass of phosphine is sorbed by palletized loads of fruit during fumigation, phosphine respectively off-gasses ~15-fold faster from loads in cold-storage, and ~15-fold shorter amount of time is required for phosphine residues in sweet cherries to meet USEPA food tolerances.

Results from fruit quality evaluations following confirmatory SWD fumigations with 1000 ppm PH3 and treatment durations of either 24 or 48 h are detailed below.

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Table 1. Subjecting rating scores for cherries.

Quality Attribute	Score	Description
Surface browning	0	No browning, full red color
	1	Slight browning, 1 - 25% of the fruit surface
	2	Moderate browning, 26 - 50% of the fruit surface
	3	Severe browning, >50% of the fruit surface
		Scores > 1 considered unacceptable
Stem browning	0	None
	1	1 - 25% brown
	2	26- 50% brown
	3	51 - 75% brown
	4	76 - 100% brown
		Scores > 2 considered unacceptable
Surface pitting	0	None
	1	Slight
	2	Moderate
	3	Severe
		Scores > 1 considered unacceptable
Surface cracking	0	None or insignificant cracking
	1	Slight, 1 - 2 mm long, shallow crack
	2	Moderate, 2 - 4 mm long, deep crack
	3	Severe (>5mm long, deep crack)
		Scores > 1 considered unacceptable
Surface shrivel	0	None
	1	Obvious shrivel
		Scores > 0 considered unacceptable
Decay	0	None
	1	Decay
		Scores > 0 considered unacceptable
Overall acceptability	0	Very good
	1	Some damage but still good and marketable
	2	Obvious damage but still marketable
	3	Unacceptable

'Bing' cherry quality following phosphine fumigation at fumigation times of 24 h and 48 h.
Subsequent storage was for 2 days at 34 °F plus 16 hours at 68 °F to simulate air shipment and marketing.

Treatment	Surface browning (% acceptable)[a]	Stem browning (% acceptable)[a]	Pitting (rating)[b]	Decay (%)	Overall acceptability (%)[a]	Color L (Lightness)	Color C (Chroma)	Color h (Hue)	Firmness (g)[c]	Soluble Solids (%)	Acidity (%)
Control, 24 h 35 °F	100a	33a	0.09a	0a	33a	33.35a	22.77a	18.16a	312.11b	18.50a	0.73a
PH3, 24 h 35 °F	100a	19a	0.09a	0a	19a	31.40b	17.74b	15.65b	365.53a	22.00a	0.87a
Fumigation effect[d]	NS	NS	NS	NS	NS	*	*	*	*	NS	NS
Control, 48 h 35 °F	100a	35a	0.09a	0a	35a	31.19a	16.10a	14.75a	338.68a	22.50a	0.88a
PH3, 48h 35 °F	99a	43a	0.09a	0a	45a	32.81a	20.46a	16.98a	297.79a	21.78a	0.81a
Fumigation effect[d]	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

aPercent considered marketable.
bPitting was a subjective rating. Lower value = better quality. Pitting: 0, 1, 2 or 3 with 2 indicating that the fruit would likely not be acceptable to a consumer.
Ratings are calculated indexes: (i.e. number of cherries with score 0*0) + (number of cherries with score 1*1)/total number of cherries.
cGrams required to cause a 1 mm deflection of the fruit surface.
dStatistical significance of fumigation treatments from controls. NS = not significant, * = significant. Red numbers show significance.

"Bing" conclusion: Some change in color was noted for the fumigated fruit but this was only the 24 h treatment and not the 48 h and is probably not an important factor. Firmness was enhanced in the fumigated fruit, but again only for the 24 h treatment. No change in any of the other quality attributes as a result of fumigation. Stems for both control and fumigated fruit were markedly browner in the fruit used for the phosphine tests as compared to simultaneous MB testing (for Korea export). The high amounts of stem browning are what caused the low levels of overall acceptability but there was no difference due to fumigation.

'Coral' cherry quality following phosphine fumigation at fumigation times of 24 h and 48 h.
Subsequent storage was for 2 days at 34 °F plus 16 hours at 68 °F to simulate air shipment and marketing.

Treatment	Surface browning (% acceptable)[a]	Stem browning (% acceptable)[a]	Pitting (rating)[b]	Decay (%)	Overall acceptability (%)[a]	Color L (Lightness)	Color C (Chroma)	Color h (Hue)	Firmness (g)[c]	Soluble Solids (%)	Acidity (%)
Control, 35 °F	100a	84b	0.10ab	0.00	83.75c	31.78a	20.64a	15.64a	307.66a	17.75a	1.04a
"7a", 24 h, 35 °F	100a	99a	0.13ab	0.00	98.75a	31.64a	20.36a	15.99a	301.62a	18.08a	1.01a
"7b", 24 h, 35 °F	100a	95ab	0.05b	0.00	98.75a	32.19a	21.85a	16.62a	309.84a	17.10a	1.04a
"8a", 48 h, 35 °F	100a	95ab	0.10ab	0.00	96.25ab	32.45a	22.69a	16.87a	299.34a	17.45a	1.09a
"8b", 48 h, 35 °F	100a	98a	0.21a	0.00	91.25bc	32.09a	21.91a	16.59	292.17a	17.73a	1.03a
Fumigation effect[d]	NS	*	NS	NS	*	NS	NS	NS	NS	NS	NS

aPercent considered marketable.
bPitting was a subjective rating. Lower value = better quality. Pitting: 0, 1, 2 or 3 with 2 indicating that the fruit would likely not be acceptable to a consumer.
Ratings are calculated indexes: (i.e. number of cherries with score 0*0) + (number of cherries with score 1*1)/total number of cherries.
cGrams required to cause a 1 mm deflection of the fruit surface.
dStatistical significance of fumigation treatments from controls. NS = not significant, * = significant. Red numbers show significance.

"Coral" conclusion: Virtually no negative effect of fumigation with the exception of a very small increase in pitting in 8b.

Annual Report - 2013

Prepared for the California Cherry Advisory Board

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

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, powdery mildew, brown rot blossom blight and fruit decays, and postharvest decays including brown rot, gray mold, and Rhizopus rot. We also report a new bacterial disease of cherry in California. Accomplishments are outlined below as:

1) A new bacterial disease known as bacterial spot caused by *Xanthomonas arboricola* pv. *pruni* was found at a low incidence on cherry cvs. Garnett and Ruby in 2013. Disease symptoms developed on leaves, green stems, and fruit. The pathogen also infects buds of other *Prunus* spp. No significant economic damage was found in cherry orchards; however, economic damage occurred in almond orchards especially on cv. Fritz. In general, bacterial spot is a major disease of *Prunus* spp. in high rainfall areas.

2) In studies on bacterial canker (branch) and bacterial blast (flower) caused by *Pseudomonas syringae* pv. *syringae*, the new antibiotic kasugamycin (Kasumin), as well as the biologicals Actinovate and Blossom Protect were compared to copper treatments.

   a. In two small-scale field studies on cv. Coral, leaf scars and branch wounds were treated and inoculated. Inoculated leaf scars were not susceptible and cankers did not develop. In branch puncture wound studies, 100 and 200 ppm Kasumin and Kasumin + 4% oil significantly reduced the incidence of cankers to zero or near zero levels. Blossom Protect and Actinovate reduced the incidence of cankers by approximately 50%. Copper was ineffective and the incidence of cankers was similar to the untreated control. In large-scale air-blast trials on cvs. Coral and Bing, only Kasumin treatments were highly effective and reduced canker incidence by > 95%.

   b. In blossom studies, two applications of the treatments described above, kasugamycin with and without oxytetracycline (e.g., Fireline) significantly reduced bacterial blast after inoculation. Kasumin, Fireline, Actinovate, or mixtures of Kasumin and Actinovate or Fireline alone were highly effective. Copper treatments were ineffective.

   c. Based on data from the last several years, oxytetracycline was nominated for registration on cherry in the IR-4 program with support from the registrants, the California Cherry Board, and other researchers in the North Central and North Eastern regions of the US.

3) In powdery mildew epidemiological studies, the disease developed on leaves of water sprouts and then on new shoots on terminal branches prior to any other tissue. Green fruit stems were colonized before fruit symptoms occurred. Results of fungicide trials were as follows:

   a. In a trial in Lodi (San Joaquin Co.), nineteen fungicide treatments were evaluated with a wide range of effectiveness. New chemistries such as FRAC Group (FG) 7 (e.g., Fontelis) or FG U8 (e.g., Vivando) and pre-mixtures were highly effective in a three-spray program (full bloom, petal fall, and early fruit development) with efficacy similar or higher to that of Quintec (FG 13). The most effective treatments included FG 7/11 fungicides (e.g., Luna Sensation, Merivon) and FG 3/11 fungicides

(e.g., Quadris Top). Overall the FG 3-DMI and FG 7-SDHI fungicides were the most effective, whereas FG 13 and U8 were also very effective.

b. The organic fungicide Serenade Optimum was only moderately effective but performed better in rotations with Luna Sensation.

c. Selected fungicides (FG 19 and FG 7/11) were also evaluated in post-infection studies and were shown to suppress the further development of the disease. Development of fungicides with unique modes of action (such as FG 7 and U2) is important to prevent overuse of FG 13 (quinoline), FG 3 (DMI), FG 11 (QoI), and potentially FG 7/11 fungicides.

4) In laboratory brown rot and Botrytis blossom blight studies, new and registered fungicides were very effective as protective treatments. Highly effective fungicides with excellent pre- and post-infection activity against brown rot and Botrytis blossom blight included Luna Sensation, Merivon, Quadris Top, and Custodia (FG 3/11). Fenpyrazamine (FG 17). Organic products (e.g., CX-10440 and Fracture) were moderately effective as protectants.

5) In preharvest brown rot efficacy trials using non-wound inoculated, treated fruit, most fungicides evaluated provided excellent disease control at both preharvest application intervals (7 or 0 days PHI) evaluated. When fruit were wound-inoculated with *M. fructicola* after treatment, only FG 3, 3/11, and 3/17 fungicides reduced the incidence of decay to low levels. For gray mold, only FG 17 or FG 3/17 and 17/19 mixtures were effective. Organic formulations of several products (e.g., Serenade Optimum, Fracture, and polyoxin-D) were less effective as protectants.

6) A comparative evaluation of new and registered postharvest fungicides was done in laboratory studies where fruit were inoculated-treated or treated-inoculated. Among newer fungicides evaluated, polyoxin-D (exempt from tolerance and petitioned as an organic) was effective against brown rot and gray mold, but not as effective as Scholar or Elite. Mentor was similarly effective as Elite against brown rot and Rhizopus rot, but showed reduced efficacy against gray mold decay in inoculated-treated studies. A generic fludioxonil was also evaluated and was comparable to Scholar. A new fermentation product that is exempt from tolerance and may be considered an organic postharvest fungicide treatment was also tested and showed excellent results against all three major decay pathogens.

INTRODUCTION

Overview. The goals of this project focus on the pre- and postharvest management of fungal and bacterial pathogens causing flower, foliar, fruit, and branch diseases of sweet cherry. For immediate benefits to the industry, we evaluated new fungicides, bactericides, natural products, and biological materials. In the last few years, numerous new fungicides were registered and additional ones are being developed. Compounds used in our 2013 studies, including their trade names, active ingredients, and FRAC groups (FG) are summarized in Table 1. Most of the newer fungicides (picoxystrobin and other QoIs, fluopyram – Luna Privilege, fluxapyroxad - Xemium, penthiopyrad - Fontelis, metrafenone - Vivando, metconazole - Quash, polyoxin-D - Ph-D, etc.) 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. One of these strategies is the use of pre-mixtures with at least two ingredients of different mode of action that are both active against the pathogen(s). Following the introduction of the first pre-mixture Pristine, others such as Adament (tebuconazole + trifloxystrobin), Luna Sensation (fluopyram + trifloxystrobin), Quilt Xcel (azoxystrobin + propiconazole), Quadris Top (azoxystrobin + difenoconazole), and Merivon (fluxapyroxad + pyraclostrobin) have been developed and are continued to be evaluated in our studies under different environmental conditions that occur each year. Goals are to identify and develop treatments to: 1) Prevent overreliance on any one fungicide class and develop treatments that allow for rotations and high levels of control of brown rot; 2) Develop new treatments for managing blossom and fruit diseases caused by

Monilinia spp. and *Botrytis cinerea*; and 3) Identify additional modes of action against powdery mildew. Natural products/biocontrols are also being 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 has been documented in California. The antibiotics oxytetracycline (Mycoshield, Fireline) and kasugamycin (Kasumin) that are or are currently being registered in the United States for management of other bacterial diseases of agricultural crops were evaluated, as well as the biological Actinovate that is already registered on a number of crops, have been the most promising treatments in our studies and these experiments were continued in our current research.

For postharvest management, fungicides with mostly unique modes of action registered on sweet cherry include: tebuconazole (Tebuzol), fludioxonil (Scholar), fenhexamid (Judge), pyrimethanil (Penbotec), and propiconazole (Mentor). These products could be used alone or in mixtures to manage decays of sweet cherry. In 2013, we continued to evaluate Mentor that was recently registered on stone fruit crops in California. The fungicide has excellent activity against brown rot, Rhizopus rot, and sour rot. This latter "yeast-like" decay is an occasional problem on cherry in wet years or when fruit are bruised during handling. An organic formulation of polyoxin-D (Ph-D) is also being evaluated and is proving to be a promising treatment and the most effective organic compound ever evaluated in our program.

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, Serenade Opitmum, Blossom Protect, Fracture).
   b. Antibiotics – Kasugamycin – large-scale trials once federally registered.
   c. Sanitizers - AgriTitan and Citrox
   d. 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, Q8Y78), as well as Scholar and polyoxin-D compared to registered fungicides 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, and SDHI compounds (fluopyram, fluxapyroxad, penthiopyrad, and premixtures using these fungicides) using different rates and timings and develop a powdery mildew fungicide program that integrates new materials with single- and multi-site mildew fungicides.
   b. Evaluate biologicals and OMRI approved organic treatments such as polyoxin-D (Ph-D).

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 Tebuzol-Elevate mixtures with an emphasis on Scholar due to its 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 and OMRI approved organic treatments (Ph-D).

Table 1: Fungicides, bactericides, and biologicals used in 2013 studies*.

Pesticide	FRAC group	Trade name	Active ingredient
Fungicides	***Single***		
	2	Rovral, Iprodione	iprodione
	3	Bumper, Tilt, Mentor	propiconazole
	3	Elite, Tebuzol	tebuconazole
	3	Quash	metconazole
	3	TopGuard, Rhyme	flutriafol
	7	Fontelis	penthiopyrad
	7	Xemium	fluxapyroxad
	12	Scholar	fludioxonil
	13	Quintec	quinoxyfen
	17	Elevate, Judge	fenhexamid
	17	Protexio	fenpyrazamine
	19	Ph-D, Oso, CX-10440	polyoxin-D
	U8	Vivando	metrafenone
	Double (Premixtures)		
	7 + 11	Luna Sensation	fluopyram + trifloxystrobin
	7 + 11	Merivon	fluxapyroxad + pyraclostobin
	7 + 11	Pristine	boscalid + pyraclostrobin
	3 + 11	Quadris Top	difenoconazole + azoxystrobin
	Multiple		
	M1	Kocide 3000	copper hydroxide
	M1+others	ReZist	copper, zinc, manganese
Bactericides	Aminoglycoside	Kasumin	kasugamycin
	Tetracyclines	Fireline/Mycoshield	oxytetracycline
Biologicals	Bacterium	Actinovate	*Streptomyces lydicus* WYEC108
	Bacterium	Serenade Optimum	*Bacillus subtilis* QST713
	Plant Extract	Fracture	protein from *Lupinus alba*
	Yeast	Botector, Blossom Protect	*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 (DA).

MATERIALS AND METHODS

Evaluation of treatments for control of bacterial blossom blast and canker. Treatments for the management of bacterial canker were done to inoculated branches by hand-spraying or by commercial applications in mid-December of 2012. For inoculation, the bark of 2-year-old twigs was puncture-wounded using a 12-gauge needle (3 wounds per twig). In these 'treated-inoculated' studies, wounds were sprayed to run-off using a hand sprayer and spray-inoculated after 2 h with *Pseudomonas syringae* pv. *syringae* (10^7 cfu/ml). Wounds were either wrapped with Parafilm or left unwrapped. Treatments included Kocide 3000, Actinovate, Blossom Protect, and Kasumin with or without 4% oil in hand-sprayer and in the commercial application trials. In Feb. 2013, inoculated branches were evaluated for the incidence of cankers and then sampled. The bark was removed and canker lengths were measured.

Several trials on bacterial blossom blast were done in a cvs. Coral and Rainier cherry in San Joaquin Co. 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 (Kocide 3000, Kasumin, Fireline, Actinovate, and Botector) were made using a hand sprayer. After air-drying for 2 h, blossoms were inoculated with *P. syringae* (10^7 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 treatments to control the natural incidence of blossom blast, applications to trees were done at 50% bloom using a backpack air-blast sprayer at 100 gal/A on 3-8 or 3-21-13. The same treatments as in the hand-sprayer trial above were used. For each single-tree replication, 150 spurs were evaluated for disease after 5 to 18 days, and the incidence of blast was determined based on the number of diseased spurs of the total number of spurs evaluated. Data were analyzed using analysis of variance and LSD mean separation procedures of SAS 9.1.

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 (protection from primary inoculum or ascospores from overwintering chasmothecia), and were followed by two additional treatments (protection from secondary infection from conidia) with selected fungicides (see Fig. 4) at petal fall and early fruit development to shift the disease progress curve to later in the growing season. Single fungicides, pre-mixtures, and four rotation programs were evaluated. The incidence of powdery mildew was evaluated on forty leaves from five shoots from inside the tree and on five shoots from the outer tree perimeter for each of the four single-tree replications on May 14, 2013. Additionally, fungicide treatments were evaluated to arrest the further development of disease after the disease was detected on leaves. For this, trees with powdery mildew were evaluated and treated with fungicides on 5-2 and 5-14-2013 (no fungicides were applied prior to this date). Twelve inside (water sprouts) and twelve outside terminal shoots were evaluated per single tree replication on 5-14-2013. Fruit stems were evaluated on 5-21-2013. Leaves and stems were evaluated using the following rating: 0=healthy, 1 = 1-3 lesions, 2 = <25%, 3 = up to 50%, 4 = >50% of leaf or stem area affected. Data were analyzed using analysis of variance and LSD mean separation procedures of SAS 9.1.

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* (15,000 conidia/ml) until water droplets formed on anther filaments. To evaluate the post-infection ("kick-back') activity, blossoms were collected, inoculated, and treated after 24 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 9.1).

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 7 or 0 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* (40,000 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 applied 7 or 1 day PHI, also 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.

Evaluation of preharvest treatments for postharvest decay control. To evaluate preharvest fruit treatments for postharvest decay management and the persistence of the fungicides on the fruit that were treated in San Joaquin and Solano Co. orchards, fruit were washed in water for 5 min. prior to wound and non-wound inoculations of harvested fruit. Fruit were inoculated with *M. fructicola* or *B. cinerea* as described above. Percent incidence of decay was determined as the number of fruit infected of the total number of fruit evaluated. Data were analyzed as described above.

Efficacy of new and registered postharvest treatments for managing powdery mildew infections on fruit as well as brown rot, gray mold, and Rhizopus rot fruit rots of sweet cherry. One laboratory study evaluated the effectiveness of postharvest fungicides in preventing the development of powdery mildew lesions on fruit under storage and transport conditions. For this, mildew infected fruit were treated using an air-nozzle sprayer, incubated for 5 days at 20C, and evaluated for continued development of powdery mildew based on the scale: 0 = no mycelium in lesions; 2 = <50% of lesion with mycelium; 3 = >50% of lesion with mycelium; and 3 = extensive mycelium inside and outside of original lesion. Four laboratory studies focused on the efficacy of two formulations of polyoxin-D and a new numbered compound, both exempt from tolerance, against brown rot, gray mold, and Rhizopus rot. A fifth study was done to compare generic fludioxonil with Scholar. The dry flowable formulation of polyoxin-D was evaluated at 2 rates (6.2 and 12.4 oz); whereas the SC formulation was evaluated at 3.5, 7, and 12 fl oz. The first two rates of each formulation contained the same amount of active ingredient. The efficacy of these treatments was compared to that of Scholar. In another trial, the experimental N-1 was compared to polyoxin-D, and to mixtures of Scholar with Mentor, Tebuzol (an Elite replacement), or polyoxin-D (Ph-D). Lastly, the efficacy of Mentor was evaluated against the three major decays. Fungicides were applied as aqueous solutions using an air-nozzle sprayer either 11-14 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) unless otherwise stated. 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 9.1.

RESULTS AND DISCUSSION

Evaluation of treatments for control of bacterial canker and blossom blast. In studies on bacterial canker, among the treatments tested on freshly wound-inoculated branches, Kasumin and Kasumin-oil treatments at 100 or 200 ppm had the highest efficacy and were effective as protective pre-infection treatments of Coral cherry on Mahaleb or Mazzard rootstocks (Fig. 1). The biologicals Actinovate and Blossom Protect also reduced canker formation in both trials. The 12-oz rate of Actinovate was more consistently effective than the 24-oz rate. In both trials copper resistant strains of the pathogen were used and thus, copper (Kocide 3000) was the least effective treatment.

In commercial air-blast trials on Bing and Coral cultivars, Kocide Actinovate, and Kasumin were evaluated. Kasumin applied at 100 ppm with or without oil were the most effective treatments (Fig. 2). Actinovate was also effective and reduced the incidence of canker on both cultivars. Copper (Kocide 3000) reduced bacterial canker similar to Actinovate on cv. Coral but was ineffective on cv. Bing.

Fig. 1. Evaluation of antibacterial treatments for protection of cv. Coral cherry on Mahaleb or Mazzard rootstock from bacterial canker in 2012-2013

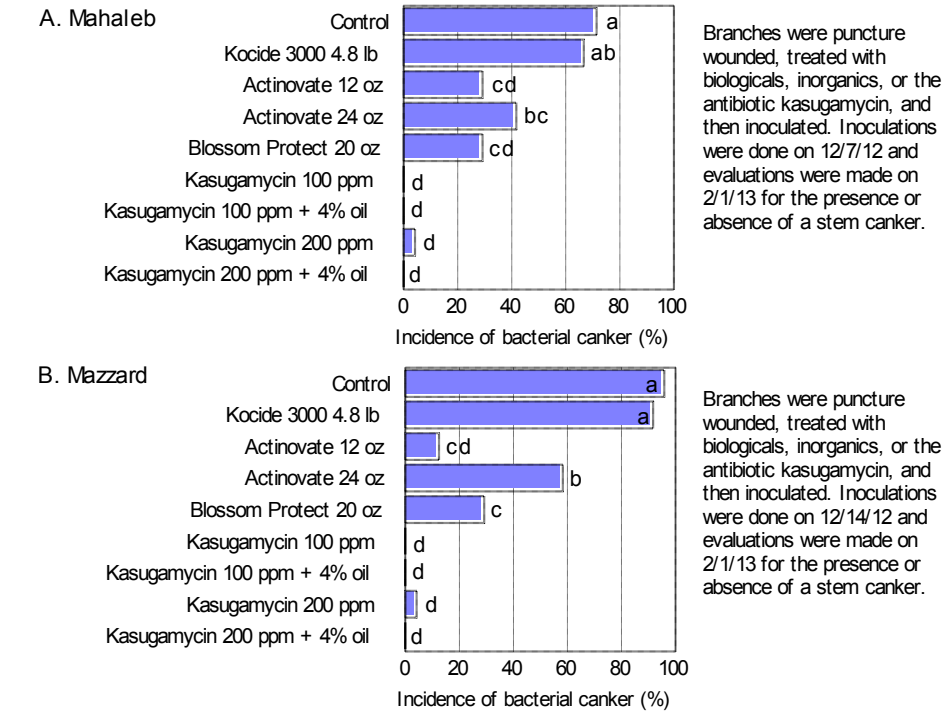
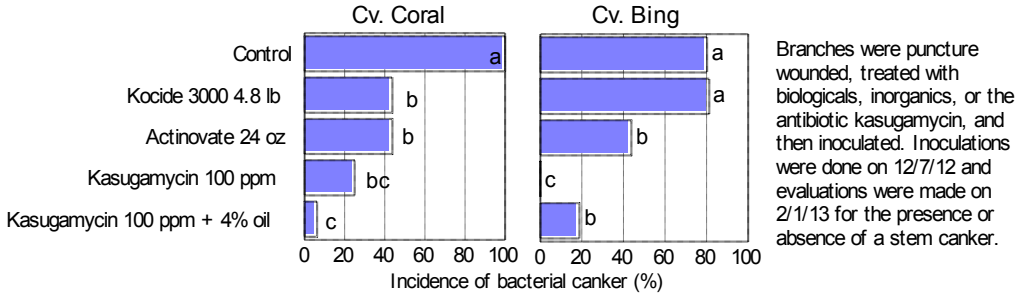


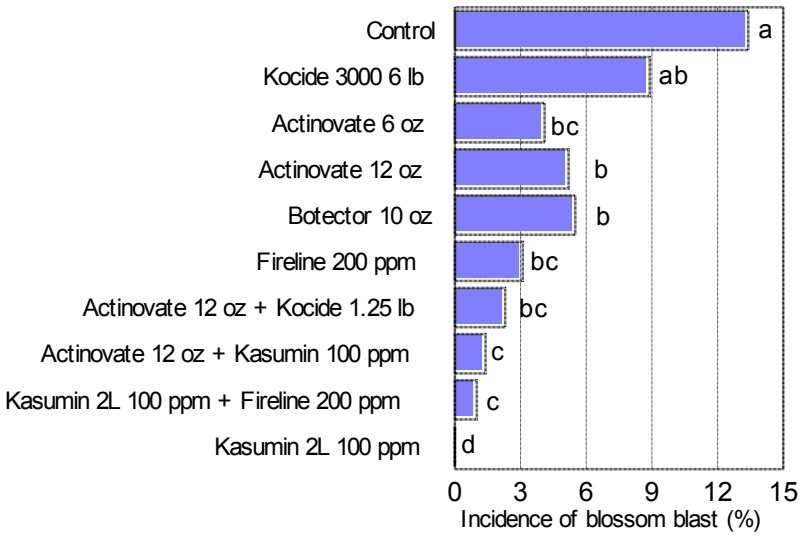
Fig. 2. Commercial air-blast treatments of antibacterials for protection of cv. Coral and Bing cherry on Mahaleb rootstock from bacterial canker in 2012-2013



Blossom inoculation studies were done on cv. Coral on Mahaleb rootstock in the spring of 2013. Kasumin at 100 ppm was the most effective treatment and mixtures of products such as Kasumin-Fireline and Kasumin-Actinovate were also highly effective (Fig. 3). Fireline by itself and a mixture of Actinovate and Kocide 3000 (low rate of copper) showed an intermediate efficacy; whereas Actinovate at 12 oz and Botector were less effective but still significantly reduced bacterial blast. Kocide by itself was least effective and the treatment was not significantly different from the untreated control. No natural incidence of bacterial blast developed in trials on cv. Rainier. The spring of 2013 was generally warm, and cold periods during bloom were not observed.

In summary, in four years of research on the management of bacterial blossom blast, we identified Kasumin as a highly effective treatment and two biologicals (Actinovate at 12 oz and Blossom Protect/Botector) as effective treatments to reduce canker and blast in inoculation trials. This is important progress because rest-breaking treatments are being used widely by the cherry industry to achieve an early harvest, shifting 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.

Fig. 3. Evaluation of antibacterial treatments for protection of inoculated blossoms of cv. Coral cherry on Mahaleb rootstock against bacterial blast in 2013



Blossoms were partially emasculated by cutting off pistils, stamens, and part of the petals. Applications were made using a hand sprayer. After 2 h, blossoms were inoculated with *Pseudomonas syringae* (10^7 cfu/ml) by hand-spraying. Inoculated branches were covered with white plastic bags for 18 h. Disease was evaluated after ca. 2 weeks.

In a two-spray application program, Fireline/Mycoshield and Kasugamycin likely can be used in a rotation or in mixtures. Oxytetracycline was included in our research because it is known to be effective against bacterial diseases. Both registrants of the antibiotic support the registration of oxytetracycline for managing bacterial blast and canker on cherry. 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. The development of kasugamycin and the acceptance of oxytetracycline into the IR-4 program in Sept. 2013 are also very important for developing multiple products for improved efficacy and resistance management. Additionally, Actinovate can be used with Kasumin or low rates of copper. Currently, Actinovate is registered on a number of crops against several diseases and the label can be amended. Treatments with copper had little or no effect on the incidence of blossom blast in all experiments where it was included. This reflects the widespread occurrence of copper resistance in the pathogen *P. syringae*.

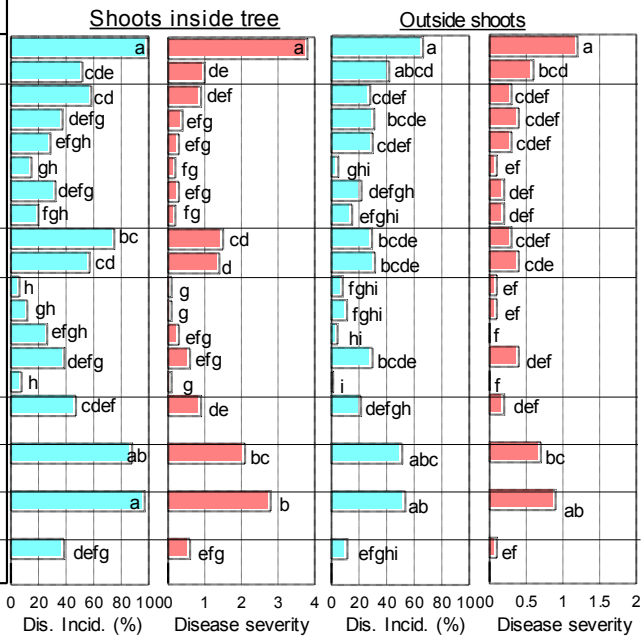
Progress is also being made on the management of bacterial canker. Our trials, however, are based on inoculations at a specific time after treatment. Epidemiological trials are needed to determine conditions that are most favorable for canker development. In general terms, cold and wet winter weather 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 will likely provide a longer residual efficacy as compared to organo-chemical treatments such as oxytetracycline and kasugamycin that are metabolized. Thus, a long-term goal is to integrate newly identified tools for managing bacterial canker.

Evaluation of new fungicides for control of powdery mildew of sweet cherry. The efficacy of new fungicides and new pre-mixtures was evaluated in our research plot 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, leaves on trunk shoots (water sprouts) and the older outside canopy showed symptoms of powdery mildew in the untreated control. The most effective treatments included the SDHI-

Fig. 4. Efficacy of preharvest fungicide applications for management of powdery mildew of Bing sweet cherries in San Joaquin Co. - 2013

	Treatment*	Product rate /100 gal	FB 3-29	PF 4/16	PF 5/2
	Control	-----	-----	-----	-----
	Serenade Opti.	16 fl oz	@	@	@
	Quash	4 oz	@	@	@
	Topguard	5 fl oz	@	@	@
	Topguard	7 fl oz	@	@	@
	Fontelis/NIS	14 + 8 fl oz	@	@	@
	Vivando/Silwet	10.3 fl oz + 0.03%	@	@	@
	Vivando/Silwet	15.4 fl oz + 0.03%	@	@	@
	Ph-D + Elevate	6 oz + 24 oz	@	@	@
	Ph-D + Elevate	12 oz + 24 oz	@	@	@
	Luna Sensation	5 fl oz	@	@	@
	Quadris Top + DA	14 + 16 fl oz	@	@	@
	A13703N + DA	14 + 16 fl oz	@	@	@
	Pristine	14.5 oz	@	@	@
	Merivon	5.5 fl oz	@	@	@
Rotations	Iprodione	32 fl oz	@	---	---
	Quintec	7 fl oz	---	@	@
	Iprodione	32 fl oz	@	---	---
	Fracture	30 fl oz	---	@	@
	Iprodione	32 fl oz	@	---	---
	Rezist	7 fl oz	---	@	@
	Luna Sensation	5 fl oz	@	---	@
	Serenade Opti.	16 fl oz	---	@	---

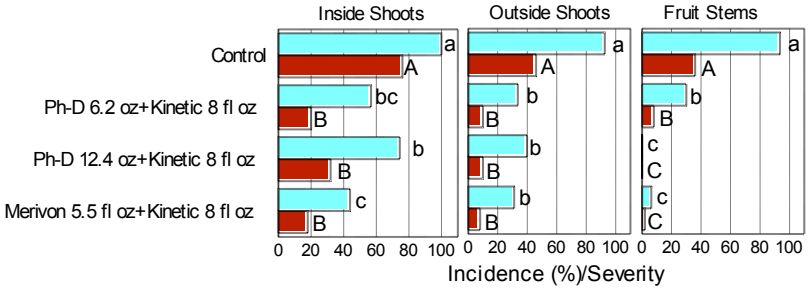


Treatments were applied in the field using an air-blast sprayer (100 gals/A). Evaluation was done on 5-14-13. For this, 40 leaves from 5 random shoots from inside or outside of the tree were sampled. Disease was evaluated using the following rating: 0=healthy, 1 = 1-3 lesions, 2 = <25%, 3 = up to 50%, 4 = >50% of leaf area affected.

containing pre-mixture fungicides (FG 7/11) Luna Sensation and Merivon, as well as the SDHI Fontelis, and selected DMI (FG 3) such as TopGuard, or DMI-containing fungicides such as Quadris Top (Fig. 4). The high rate of Vivando (FG U8) mixed with a surfactant (e.g., Silwet) was also very effective. Serenade Optimum that was applied in rotation following a bloom application of Luna Sensation reduced the incidence and severity of disease to moderate levels but was less effective than Luna Sensation (Fig. 4). Quintec (FG 13) performed well, reducing the incidence of the disease on both inside and outside shoots (and the severity of disease on the outside shoots). Overall, there was a higher severity of disease on inside shoots than on outside shoots, and most treatments performed better on the outside shoots.

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 were 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. Although not recommended as a general practice over preventative treatments, fungicide treatments for arresting development of mildew on leaves and fruit stems after the disease was already detected on leaves were also evaluated in our 2013 trials (Fig. 5). Initial incidence of disease was 50%, 35%, and 0% on inside and outside shoots and fruit stems, respectively. Applications of Merivon or Ph-D significantly reduced the continued development of disease as shown by significantly lower incidence and severity of mildew on inside and outside shoots prior to harvest. Incidence and severity of disease was dramatically reduced on fruit stems (Fig. 5).

Fig. 5. Evaluation of fungicide treatments for arresting powdery mildew development after disease detection



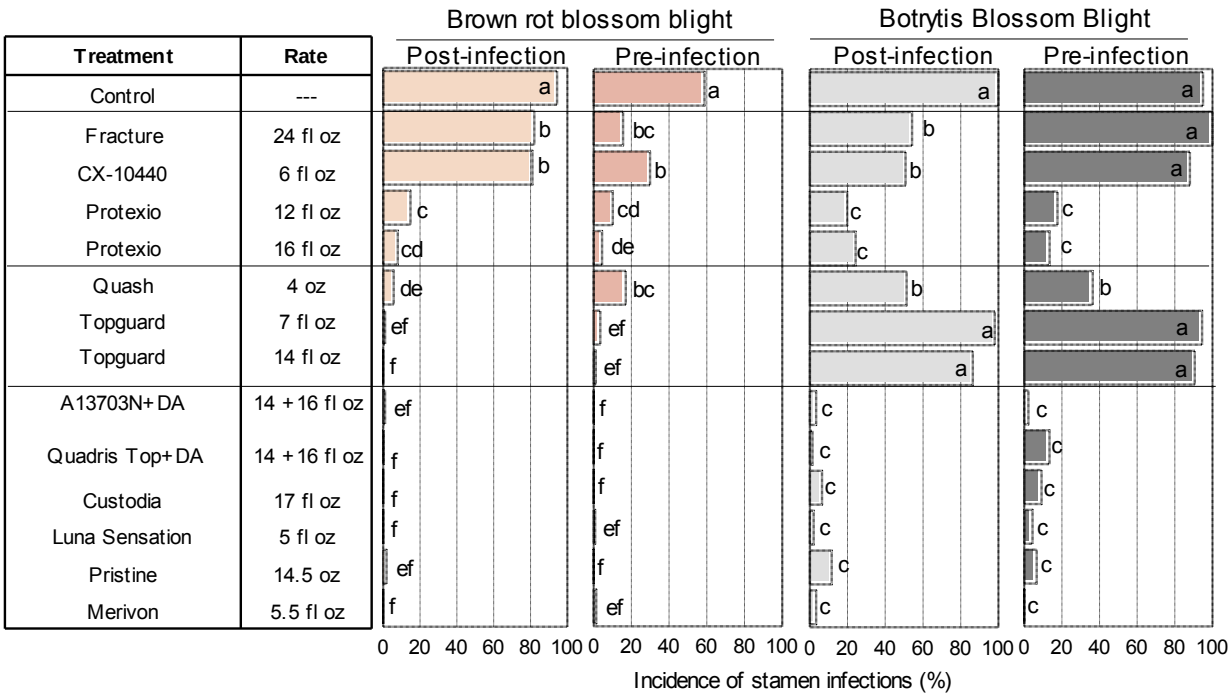
Trees were treated for powdery mildew on 5-2 and 5-14-2013 (no fungicides were applied prior to this date). Twelve inside (water sprouts) and twelve outside terminal shoots were evaluated on 5-14-2013. Fruit stems were evaluated on 5-21-2013. Initial incidenc of disease was 50%, 35%, and 0% on inside and outside shoots and fruit stems, respectively. Leaves and stems were evaluated using the following rating: 0=healthy, 1 = 1-3 lesions, 2 = <25%, 3 = up to 50%, 4 = >50% of leaf or stem area affected. Severity ratings are 20x on the 0-4 scale. Statistics for the incidence data are shown in lower case letters; whereas upper case letters are f severity data (bars followed by the same letter are not significant different based on ANOVA and least significant mean separation procedures..

This ongoing research has demonstrated the sequential development of powdery mildew on developing leaves in the inside and outside of the tree canopy, on fruit stems, and on fruit. Additionally 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.

Development of fungicides with unique modes of action (such as SDHI and U8 fungicides) needs to be continued to provide options in rotation programs and to prevent overuse of selected fungicides including quinoline (i.e., Quintec), DMI, and QoI fungicides. 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.

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. 6). Highly effective fungicides with excellent pre- and post-infection activity against both blossom diseases included: FG 7/11 fungicides (e.g., Pristine, Luna Sensation, Merivon), FG 3/11 fungicides (e.g., Quadris Top, A13703N, Custodia), and the FG17 Protexio. The FG 3 (DMI) fungicides Quash and TopGuard were very effective against brown rot but less effective against gray mold (Fig. 6). The natural products Fracture and CX-10440 were effective in reducing brown rot and Botrytis blossom blight infections of stamens as pre-infection (protective) and post-infection treatments, respectively. 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.

Fig. 6. Efficacy of pre- and post-infection treatments with selected fungicides for management of brown rot and Botrytis blossom blight of Bing sweet cherry

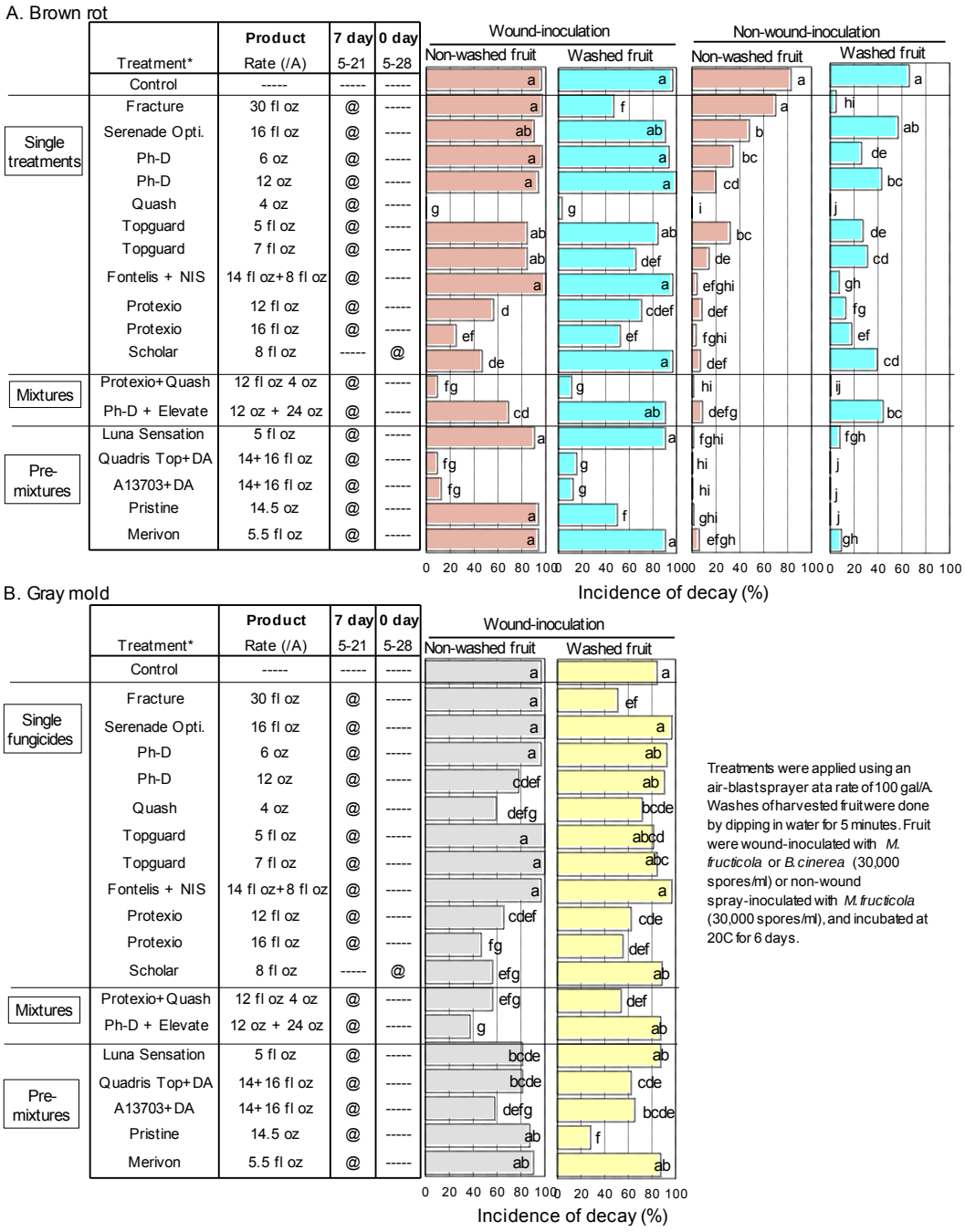


For evaluation of the pre-infection activity, closed blossoms were collected in the field, allowed to open, and treated in the laboratory using a hand sprayer. After 12 h blossoms were inoculated with a spore suspension of *M. fructicola* (15K/ml). For post-infection activity, blossoms were inoculated and treated after 24 h. Blossoms were evaluated for stamen infections after 4-5 days of incubation at 20 C.

Evaluation of preharvest treatments for fruit decay control without postharvest washes and for postharvest decay control after postharvest washes. Two preharvest efficacy trials were done in 2013. In wound inoculation studies, most fungicides performed poorly on washed and non-washed fruit. The DMI fungicide (FG 3) Quash and mixtures that included DMI fungicides such as Quadris Top and A13703, as well as the hydroxyanilide (HA) (FG17) Protexio and Protexio mixed with Quash had the highest efficacy against brown rot of non-washed and washed fruit when applications were made seven days before harvest (Fig. 7). Scholar applied as a preharvest treatment 1 day before harvest did well on non-washed fruit and poorly on washed fruit, demonstrating non-systemic activity when applied in the field to dry fruit. In a second trial (Fig. 8A,B), Quash, Protexio, Quadris Top, and A13703N had the highest efficacy against brown rot on wounded and non-wounded fruit. Topguard, Ph-D, Luna Sensation, Pristine, and Merivon performed very well on non-wounded fruit but were ineffective on wound-inoculated fruit (Fig. 8A, B). This indicates the non-systemic, contact properties of these fungicides. Fracture and Serenade Optimum were not effective. Thus, the DMI (FG 3) fungicides with their locally systemic action are still unrivaled for management of brown rot decay in wounded and non-wounded fruit. All of the conventional fungicides were very effective against brown rot when non-wounded fruit were not washed or washed and inoculated (Fig. 7). Most DMI (FG 3), HA (FG 17), DMI/QoI (FG 3/11), DMI/HA (FG 3/17), and SDHI/QoI (FG 7/11) fungicides performed the best in our trials this year (Figs. 7, 8). Ph-D was consistent in all trials over the last several years in reducing brown rot of non-wounded fruit. This is an important finding because of the fungicide's potential to be formulated as an organic treatment and it is currently registered as a pesticide exempt from tolerance.

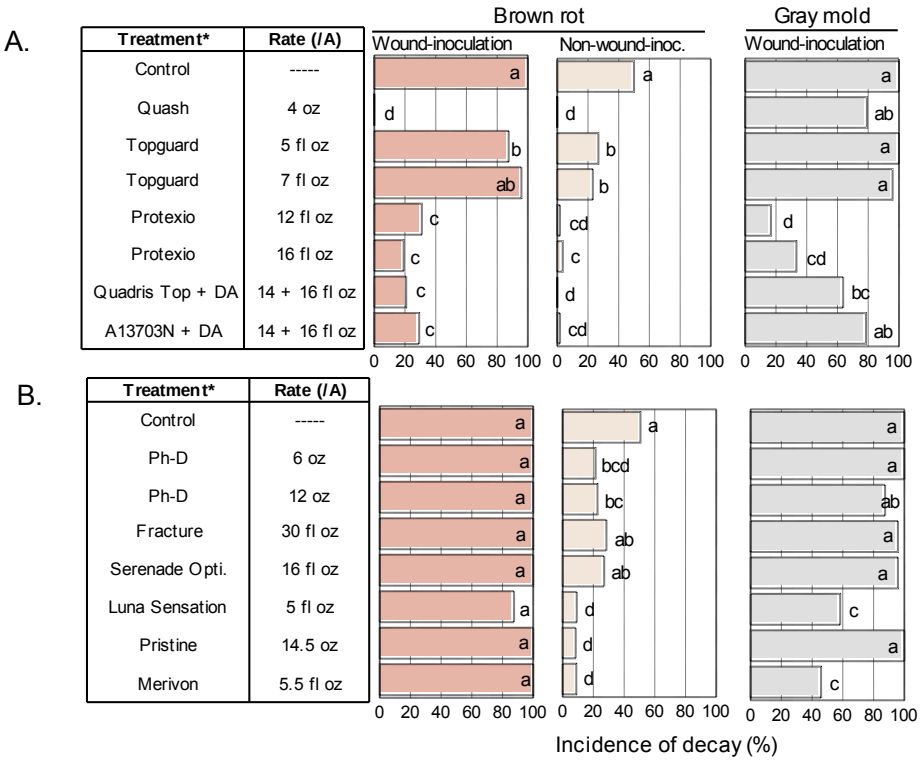
For gray mold, fruit had to be wounded to obtain consistent results. Therefore, fungicides need local systemic action to provide some penetration into the fruit surface or high residues on the fruit surface that can be re-distributed into the wounds. In these studies, the best treatments were the FG17

Fig. 7. Efficacy of 7- and 0-day preharvest fungicide treatments for management of postharvest brown rot and gray mold of Bing cherries - Orchard 1, San Joaquin Co.



Protexio, mixtures that included a FG 17 (Protexio+Quash, Elevate+Ph-D), and the FG 12 Scholar. Luna Sensation and Merivon (FG 7/11) resulted in some reduction of gray mold on non-washed fruit (Figs. 7B, 8B) but were consistently ineffective when fruit were washed. This is probably due to their low usage rates and mostly protective properties. Pristine and the plant extract product Fracture did poorly on non-washed fruit but reduced decay from that of the control on washed fruit perhaps because the treatments were re-distributed during washing. In previous trials, however, Pristine performed poorly on washed fruit. Thus, limited washing may not have removed the residues but re-distributed them. Serenade Optimum generally performed poorly against gray mold fruit decay.

Fig. 8. Efficacy of 6-day preharvest fungicide treatments for management of postharvest brown rot and gray mold of Bing cherries - Orchard 2

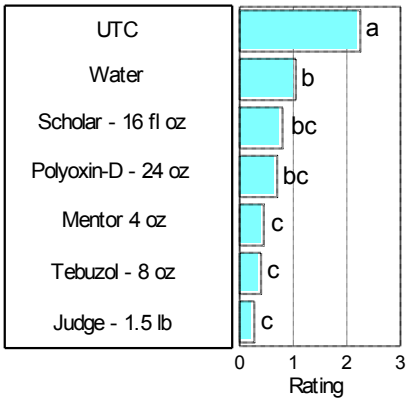


Treatments were applied on 5-15-13 using an air-blast sprayer at a rate of 100 gal/A. Fruit were wound-inoculated with *M. fructicola* or *B. cinerea* (30,000 spores/ml) or non-wound-inoculated with *M. fructicola* (200,000 spores/ml) and incubated at 20C for 6 days.

Efficacy of new and registered postharvest treatments for managing powdery mildew infections on fruit as well as brown rot, gray mold, and Rhizopus rot fruit rots of sweet cherry. In postharvest decay management in 2013, several studies were done for optimizing performance of currently registered products and for the possible development of new postharvest fungicides with unique modes of action that potentially could have exempt registration status. Additionally, we evaluated currently registered materials for suppressing powdery mildew infections on fruit that continue to develop during storage, transportation, and marketing of sweet cherries. For this, fruit with powdery mildew lesions were treated and incubated for 5 days at 20C. Postharvest washing of fruit with water reduced the continued development of powdery mildew on fruit lesions from a rating of 2.3 on non-washed fruit (UTC) to a rating of 1. Mentor, Tebuzol, and Judge were the best treatments with ratings of less than a 0.45 on a 0 to 3 scale with 3 having extensive mycelial growth over and extending beyond the lesion (Fig. 9). Scholar and polyoxin-D were intermediate in their performance between water and the other postharvest fungicides.

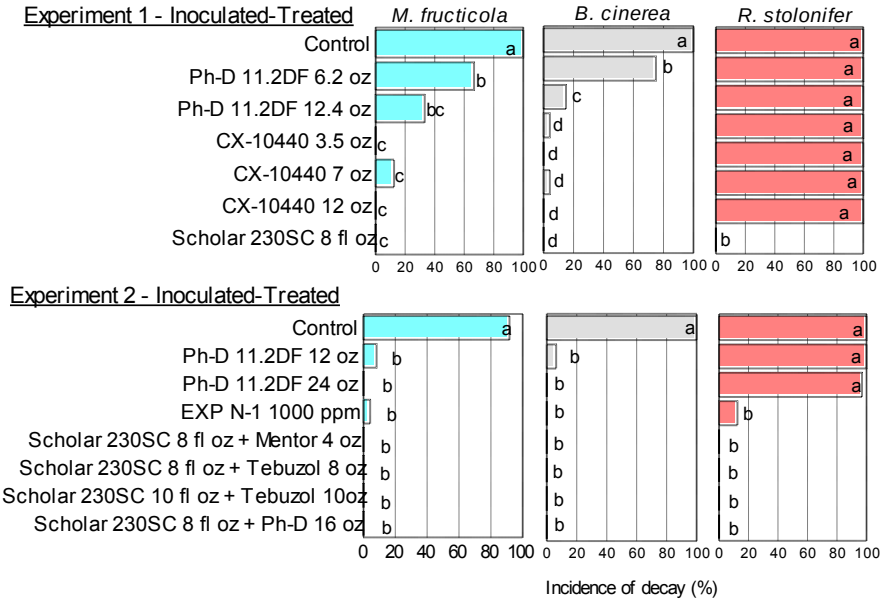
We evaluated several rates and two formulations of polyoxin-D (Ph-D and CX-10440) as postharvest treatments of cherry (Fig. 10). These products are currently exempt from tolerance and efficacy data will allow registration on sweet cherry. The CX-10440 SC formulation showed excellent performance over a rage of rates against brown rot and gray mold in our inoculation studies. Higher rates of the Ph-D formulation were required (e.g., 12 and 24 oz) to obtain similar performance data (Fig. 10). Interestingly, the same amount of active ingredient is in the 3.5-fl oz rate of the SC formulation as the 6.2-oz rate of the WG formulation. Particle size and formulation adjuvants probably contribute to the SC formulation's improved activity. Currently, polyoxin-D is being considered for postharvest registration by the registrant.

Fig. 9. Efficacy of postharvest fungicide treatments for preventing continued development of powdery mildew lesions of Bing cherry fruit



Postharvest fungicide treatments were prepared at rates per 100 gal of water and were applied using an air-nozzle sprayer to fruit with powdery mildew lesions. After treatment, fruit were incubated for 5 days at 20C and evaluated for continued development of powdery mildew based on the scale: 0 = no mycelium in lesions; 2 = <50% of lesion with mycelium; 3 = >50% of lesion with mycelium; and 3 = extensive mycelium inside and outside of original lesion. UTC = untreated control.

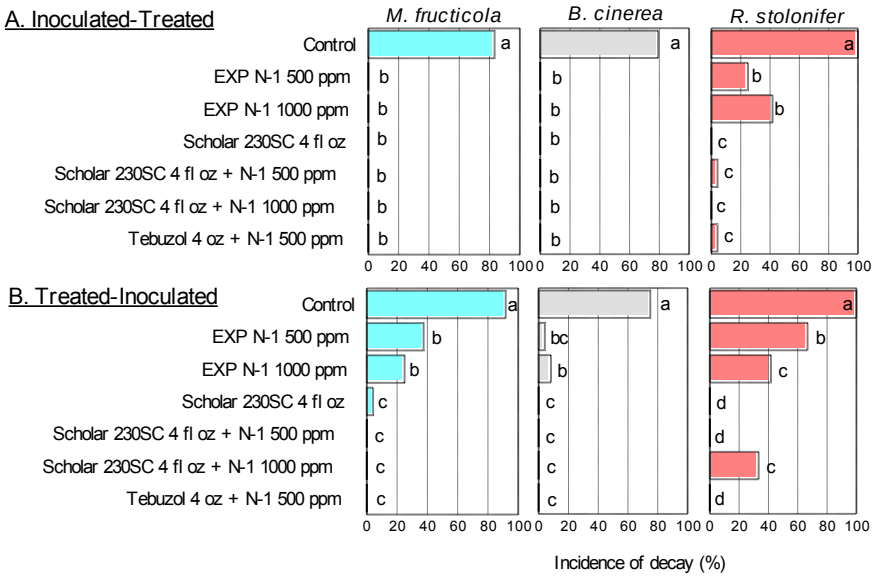
Fig. 10. Postharvest treatments with registered and new fungicides for decay control of sweet cherry fruit in laboratory studies



Fruit were wounded and inoculated with spores of *M. fructicola*, *B. cinerea*, or *R. stolonifer* (30K spores/ml) and then treated after 14 h with aqueous fungicide solutions using an air-nozzle sprayer. Fruit were then incubated at 20C.

Combinations of postharvest fungicides such as Scholar mixed with Mentor, Tebuzol, or polyoxin-D (Ph-D WG formulation) all proved excellent against brown rot, gray mold, and Rhizopus rot (Fig. 10 – Experiment 2). These trials were done to lower rates of Scholar and several of the other fungicides used in combination. Thus, Scholar at 8 fl oz is only 150 ppm of fludioxonil which is typically used at 300 ppm when used alone. These types of trials demonstrate high performance in decay control (e.g., 100% control) with the lower rates and may allow different application strategies to be employed during the postharvest handling of cherry fruit to prevent decays. Additionally, mixtures of Scholar with polyoxin-D, a fungicide exempt from tolerance, will allow immediate usage in many international markets.

Fig. 11. Postharvest treatments with registered and new fungicides for decay control of sweet cherry fruit in laboratory studies

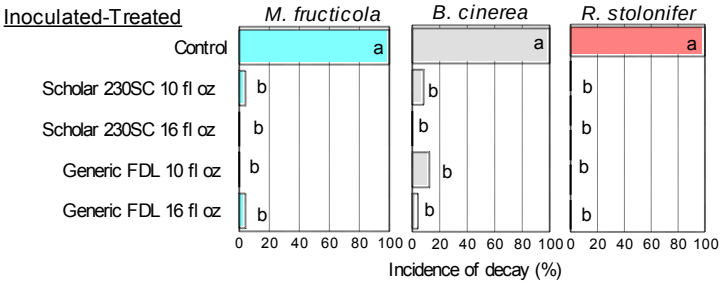


Inoculated-Treated: Fruit were wounded and inoculated with spores of *M. fructicola*, *B. cinerea*, or *R. stolonifer* (30K spores/ml) and then treated after 14 h with aqueous fungicide solutions using an air-nozzle sprayer. Treated-Inoculated: Fruit were first treated and air-dried and then inoculated. Fruit were then incubated at 20C.

A numbered compound (EXP N-1), also with potential exempt registration status, was evaluated for its potential use as a postharvest treatment of sweet cherry (Fig. 10 – Experiment 2; Fig. 11). This fungicide was effective against all three major pathogens of cherry – brown rot, gray mold, and Rhizopus rot. Lower rates in mixtures with Scholar or Tebuzol and EXP N-1 were also extremely effective in both 'inoculated and treated' (post-infection activity) and 'treated and inoculated' (pre-infection activity) trials (Fig. 11).

In trials evaluating Scholar and a generic formulation of fludioxonil, both products showed similar performance against brown rot, gray mold, and Rhizopus rot (Fig. 12). Thus, two sources of this product are now available and every effort should be made to prevent resistance to fludioxonil from developing by using it properly and in combination with other fungicides. Fludioxonil is an important postharvest fungicide for the cherry and other fruit industries with most countries around the world accepting its usage and safety. 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 the 'exempt from tolerance' materials for preventing decays of sweet cherry in international markets.

Fig. 12. Postharvest treatments with registered and new fungicides for decay control of sweet cherry fruit in laboratory studies



Fruit were wound-inoculated with spores of *M. fructicola*, *B. cinerea*, or *R. stolonifer* (30K spores/ml) and treated after 13-14 h with aqueous fungicide solutions using an air-nozzle sprayer. Fruit were then incubated at 20C.