FINAL PROJECT REPORT **YEAR: 2 of 2**
WTFRC Project Number: CH-13-104

Project Title: Novel postharvest fumigation of sweet cherries for fruit fly pests

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Total Project Funding: Year 1: $34,000 **Year 2:** $14,000

Other funding sources: None

Budget history:

Item	2013	2014
Salaries (60% GS-5)	23,950	5,500
Benefits (included above)		
Wages		
Benefits		
Equipment		
Supplies	8,550	5,500++++
Travel	1,500	
Miscellaneous (shipping)		3,000
Plot Fees		
Total	34,000	14,000

Footnotes: Supplies include 1-pallet of fruit, rearing supplies and costs related to fumigation
++++++ if more fruit quality evaluations are wanted, more fruit will be needed

Original objectives:

Specific objectives - Year 2 (2013)

This project is planned in 3 phases as indicated below. Each phase will have its own objective and these objectives will feed those of the following phase.

Phase I. Establish and maintain a colony of SWD in Parlier, CA with the throughput necessary to routinely conduct fumigation studies.

Timeline: Already accomplished.

Phase II. Determine the mortality of phosphine as well as several key phosphine mixtures to eggs, larvae, pupae and adults of SWD in $1ft^3$ chambers at 35 °F. Report dose-mortality regressions with statistical validity (Probit v. 2007 software) to establish most tolerant SWD life stage.

Timeline: April-May

Phase III. Optimize phosphine and its mixtures to control the most tolerant SWD lifestage as quickly as possible at 35 °F. With intent of decreasing stand-alone fumigation requirements, the effect of hydro-cooling on SWD mortality will be evaluated and integrated with fumigation data to be reflective of mortality expected from entire "packing system".

Timeline: Nov-Feb 2012, May-June

Phase IV. Perform a confirmatory treatment at the dose derived from Phase II in 9 $1ft^3$ chambers at 35 °F with 30,000 SWD specimens (most tolerant stage) while fruit is packed in wooden bins. To ensure adequate exposure for complete mortality, gas concentrations will be measured throughout load over the course of the fumigation. Sorption and box effects will be quantitatively analyzed and reported.

Timeline: May-June

Phase V. Document phytotoxicity (Dr. Obenland) that occurs from exposure to phosphine and its mixtures at dosages that are efficacious toward the most tolerant stage of the SWD. Three key export varieties (recommended by industry) will be investigated.

Timeline: Concurrent with Phase IV

Phase VI. Quantify residues in cherries that result from exposure to phosphine and its mixtures at dosages that are efficacious for killing the most tolerant stage of the SWD.

Timeline: Concurrent with Phase IV & V.

Specific Objectives Year 2 (2014)

Repeat Year 1 (2013) objectives with another species of fruit fly pest, such as the Western cherry fruit fly and the brown marmorated stinkbug (BMSB) maintained in the Contained Research Facility at the University of California at Davis.

Significant findings:

- Phosphine fumigation at cold-storage temp will control SWD in 36 to 48 h
- Phosphine fumigation at cold-storage temp will control BMSB in 36 to 48 h
- Fumigate max. chamber loads of packed and palletized cherries
- Maintaining a threshold concentration 750 to 1500 ppmv phosphine is required
- Residues and worker exposure with phosphine are favorable (relative to MB)
- Fruit quality evaluations following phosphine fumigation look promising
- Phosphine fumigation of fresh fruit gaining "international traction"

Results & Discussion:

Materials and 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. As described in Walse et al. (2012), larvae were reared on standard cornmeal-(dextrose or sucrose)-agar-yeast medium layered to ($\bar{x} \pm s$, 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. 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.

BMSB (*Halyomorpha halys*) eggs were obtained from the laboratory colony of Dr. Tracey Leskey (USDA-ARS-Kearneysville, WV) that originated from wild specimens captured in small fruit and orchard crops in West Virginia, USA. Upon receipt of the BMSB eggs in Oct. 2012 at the Contained Research Facility at UC Davis (Davis, CA), a BSL-III agricultural quarantine facility, all specimens were transferred to an environmentally-controlled chamber set at 26 °C, 65% RH and a 16:8 diurnal light cycle until a strong colony of mixed life stages could be established. A second shipment of eggs was received from Dr. T. Leskey in March 2013 to supplement the CA colony. All non-egg life stages are reared concurrently inside 0.34-m^3 aluminum mesh cages on live bush bean and cowpea plants, supplemented with organic raw almonds, pumpkin seeds, sunflower seeds, and walnuts. Cages are maintained inside a

greenhouse at 26 °C, 65% RH and a 16:8 diurnal light cycle. Eggs are collected every 48 h from rearing cages and allowed to hatch on green bean plants in an isolated cage to prevent egg predation. 1st instar nymphs are then transferred via a #4 (3/32") round brush back into the rearing cages 4 d after hatching to complete their life cycle and to maintain reproductive populations at ~500/cage.

Fruit infestation

To simulate a naturally occurring infestation scenario for SWD, 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, infested cherries were transferred to a pull-string cloth bag (~25 per bag) (8" x 12"; ULine, Waukegan IL), and used in laboratory-scale exploratory fumigations or buried throughout the load of commercial fruit bins in confirmatory-scale fumigations. Alternatively, cloth bags were not fumigated and held as untreated controls to estimate the number of individuals treated during a respective fumigation.

All seven life stages (eggs, 1st – 5th instar nymphs, and adults) of BMSB were evaluated in the exploratory fumigations. Egg samples were cut from oviposition substrate (usually a dried bean leaf) and counted under a dissecting microscope under low (10x) power. Nymphs (1st – 5th instars) were randomly collected from the rearing cages described above. Adults were aspirated from the walls and top of the rearing cages. Samples from each life stage were then isolated in vials with mesh-screen covered openings on the top, bottom and sides (2 locations) and placed into cloth bags (8" x 12"; ULine, Waukegan IL). Several pumpkin seeds and a wetted cotton wick were placed in to the cages to serve as food and water sources, respectively. Cloth bags containing vials of the life stages to be treated, were placed inside the environmental room, housing the fumigation chambers, for tempering.

For the exploratory fumigations, SWD-infested fruit were incubated for 0, 48, or 96 h to yield ages, respectively, of 0- to 48-h, 48- to 96-h, and 96- to 120-h old specimens at the start of a 12-h pre-fumigation period of temperature equilibration (i.e., tempering). Infestations and subsequent incubations were synchronized so that all BMSB life stages and all SWD ages concurrently entered the tempering period that preceded fumigation. For the confirmatory fumigations, infestation and incubation was planned to yield 2nd and 3rd instar BMSB as well as 12- to 60- h old SWD (age at fumigation), only the most PH3-tolerant forms (*vide infra*) .

Exploratory fumigations

Exploratory fumigations were performed in modified Labonco® 28.32-L vacuum chambers housed in a walk-in environmental incubator with programmable temperature and humidity (USDA, 2009). A series of experiments was conducted determine the treatment duration, ranging from 6 to 72 h, required to control ages comprising the egg through pupal life stages of SWD as well as all life stages of BMSB with phosphine (PH3) at headspace concentrations $\geq$ 1.5 mgL^{-1} (1000ppmv) at 1.4 ± 0.5°C ($\bar{x} \pm s$). Chambers loaded with SWD-infested fruit, fruit infested with control specimens of SWD, BMSB cages (treated and untreated control specimens), source-gas cylinders, and gas-tight syringes were tempered for at least 12 h to treatment temperatures prior to fumigation. Chamber temperature was confirmed prior to fumigation by a HOBO data logger (HOBOware version 2.7). Chamber lids were then clamp-sealed in preparation for treatment. SWD and PTB were fumigated concomitantly in a chamber.

A pressure of approximately 70 mmHg was established in each chamber. Gas-tight super-syringes (Hamilton ® 500, 1000, or 1500 mL) were filled with a volume of fumigant from a cylinder of 1.6 % (v/v) PH3 balanced with nitrogen (Cytec Canada, Inc., Niagara Falls, Ontario, Canada) to achieve the requisite applied dose of ~ 1.65 mgL^{-1} (1100ppmv) as predetermined in preliminary calibration studies. A syringe was fitted to a LuerLok ® sampling valve, which was subsequently opened so that fumigant was steadily drawn into the chamber. The syringe was then removed and normal atmospheric pressure was established in each chamber before the valve was closed; this marked the beginning of the exposure period. Gas samples (40 mL) were taken temporally at standard intervals from the chamber headspace through a LuerLok® valve using a B-D® 100 mL gas-tight syringe and quantitatively analyzed for PH3 with GC-PFPD. Fumigant concentrations were measured and exposures, expressed as concentration × time product ("CT"s) calculated by the method of Monro (1969), were tracked.

Following the final sampling to determine headspace concentration, chamber valves were opened to atmosphere, a 1-h aeration period was initiated, and chamber lids were then opened. Treated and non-treated SWD-infested cherries were retrieved from the bags, transferred in pairs into a stainless-steel mesh ball cage (5.1-cm diameter), and the mesh cage was placed back into the respective bag. SWD-infested cherries as well as caged-BMSB were transferred into a rearing incubator at 27.0 ± 1.0 °C and 80 ± 2% RH ($\bar{x} \pm s$), and placed into treatment-respective 0.03-m^3 nylon-mesh rearing cubicle.

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). On the same day that they were packaged for export, either Bing or Coral variety sweet cherries were obtained from commercial wholesale sources in California or Washington State. Uninfested fruit were removed from cartons and replaced with an equivalent volume of cloth bags containing SWD-infested cherries or caged-BMSB. Liners, if present, were not sealed but folded over atop the cherries. The chamber was first loaded with four 0.5 ft^3 sand bags each wrapped in plastic packaging that displaced ~84.9 L total of chamber volume. Chambers were loaded with six cartons (~8 kg/carton, fruit size 12 row, 43.2 l x 33.0 w x 14.0 h cm carton size, 19.9 L volume). The chamber load was estimated as a fractional percentage, 76.4 ± 0.8% ($\bar{x} \pm s$), of the volume occupied by the load relative to the chamber volume (i.e., V_L $(V_{chamber})^{-1}$ x 100) (Monro, 1969).

Chambers loaded with test specimens and uninfested fruit as well as control specimens were acclimated to fumigation temperature of ~1.4 °C (~34.3°F) for 12 h prior to treatment (i.e., tempered) within a temperature-controlled storage unit (USDA, 2009). Fruit pulp temperature was confirmed prior to fumigation by each of three probes (YSI scanning tele-thermometer) that recorded the respective pulp temperature in three uninfested fruit distributed at different locations within the load of the fruit undergoing treatment. Temperature probes were then removed and chamber lids clamp-sealed in preparation for treatment. The chamber ventilation valve was opened and chambers 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. The valve was then closed which 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, SWD were transferred to mesh cages as described above, and all specimens were placed into separate 0.03-m^3 nylon-mesh rearing cubicles maintained in an incubator at 27.0 ± 1.0 °C and 80 ± 2% RH ($\bar{x} \pm s$) as described above. 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)(*methods and results available upon request*).

Mortality evaluation

SWD mortality was assessed at 1-d intervals post-fumigation for 21 d; rearing cubicles were examined and live adult specimens were tallied and discarded before cubicles were resealed for further incubation and evaluation. The cumulative number of adults, which emerged from each piece of fruit designated as an untreated control for paired fumigation trials, was counted. An average ($\bar{x}$) emergence from each infested fruit left untreated was calculated along with a standard deviation (± *s*). The number of SWD specimens ($n \pm s$) that were treated was estimated by multiplying the number of infested fruit treated in each trial by the average emergence from each fruit that was infested and untreated ($\bar{x} \pm s$). The total number of specimens that were treated across all exploratory- or confirmatory-trials was estimated by summing the number from each respective trial and propagating the respective standard deviation.

Two days following treatment, BMSB specimens were retrieved from all cages for evaluation. Egg mortality was diagnosed visually by discoloration, while survivability of other life stages was diagnosed by locomotion or by prodding-induced motion. Post-embryonic BMSB were categorized as moribund if the survivability was inconclusive. Moribund BMSB were placed inside a labeled plastic snap-cap cage with food and water source as described above prior to further incubation until an additional evaluation the following day. Mortality of control BMSB specimens was included as a natural response in the efficacy modeling for exploratory fumigations. For the confirmatory trials, Abbott's method (Finney, 1971) was used to estimate the percentage mortality of BMSB used in Probit calculations. The total number of specimens that were treated for each exploratory- or confirmatory-trial was estimated by summing the numbers treated, while the total number of specimens treated across exploratory- or confirmatory-trials was estimated by summing the numbers from each respective trial.

Rearing and incubation conditions of 27.0 ± 1.0 °C, 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 < 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 and BMSB development, for both species we generally observed increases

in the mortality of all 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.

Chemicals and Chemical analysis

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

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

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.

Executive summary:

A new postharvest treatment option to control SWD and BMS has been developed for those Western US sweet cherry growers/packers. Packed-boxes need to be fumigated at cold-storage temperature for 48 h. A report can be drafted ad presented to Industry (and thereafter APHIS) for consideration. Currently, market options include those countries willing to fumigate with phosphine on arrival (e.g., chile). ARS is working with industry and USEPA to gain registration for PH3 so that fumigations can be done at the packinghouse.

PH3 chamber fumigations at 1.4 ± 0.5 ºC ($\bar{x} \pm s$)(~34.5°F) were evaluated for postharvest control of spotted wing drosophila, *Drosophila suzukii*, and brown marmorated stink bug (BMSB) in fresh sweet cherry exports from Western USA. The most PH3-tolerant age of SWD (0 – to 48-h old, ~95% eggs) as well as life stage of BMSB (2nd & 3rd instars) was established via a series of exploratory fumigations. In confirmatory fumigations, which simulated the commercial scenario, complete mortality of 35,265 ± 1,006 (*n* ± *SE*) SWD eggs as well as 5,149 BMSB, was achieved with an applied dose of 1000 ppm, a load factor of ~ 76.4%, and a treatment time of 48 h at 1.4 ± 0.5 ºC ($\bar{x} \pm s$)(~34.5°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.