TREATMENT OF CALIFORNIA STONE FRUIT WITH METHYL BROMIDE OR PHOSPHINE TO ELIMINATE PEACH TWIG BORER, *ANARSIA LINEATELLA*

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Abstract. The goal of this project is to develop postharvest chamber fumigations that ensure complete mortality of peach twig borer (PTB) in California stone fruit exports; results from preliminary toxicological and phytotoxicological research are presented.

Insects. PTB was cultured on a lima bean-based agar diet (USDA, 2009a). Eggs used in this study were deposited on ~3x1/2 cm^2 filter paper sheets over a 48 h ovipositional period, whereas fourth – sixth instar larva and pupa of mixed developmental stage were extracted from diet contents of rearing cups. The maturity of a larva was diagnosed by overall size and head capsule width.

Mortality/survivability. Mortality of non-exposed (i.e., untreated control) and fumigant-exposed larvae was assessed after a 1 day post-exposure incubation (27 ºC and 60% R.H.) and was diagnosed visually by discoloration. Survivability of larvae was diagnosed by locomotion or by prodding-induced motion. Larvae were categorized as moribund if the survivability was inconclusive. Moribund larvae were incubated for an additional two weeks as above with the agar food source; survivability was assessed by adult emergence after incubation. Mortality of non-exposed and fumigant-exposed eggs and pupae was assessed following treatment after incubation for 7 d. Using a microscope, exposed-egg mortality was diagnosed by the development of white coloration and survivability by vacated egg cases and development to neonate larva inside chorion. Pupa survivability was diagnosed by adult emergence. Dose-mortality regressions were generated using Probit analysis and control mortality was treated numerically using Abbott's method (Finney, 1971).

Laboratory Fumigations. The relative mortality of phosphine and methyl bromide to PTB life stages was established in modified Labonco® 1-ft^3 vacuum chambers in the absence of commodity (USDA 2009b). Test specimens were contained along with ~1g of agar diet in clear plastic 20 dram vials with a 8 mm diameter hole on the bottom, cap, and side (Figure 1); vials contained either eggs (n = 125-250), pupae (n ≈ 10), or larvae (n ≈ 10).

Commodity fumigations: phosphine. Fumigations were conducted in modified Labonco® 1-ft^3 vacuum chambers as described above Each chamber contained 15lbs of "O'Henry" peaches (size 36) with a the load factor ($V_{commodity}/V_{chamber}$x 100) of ~42%. Pupae, eggs, and "external feeding" larvae were treated as above. The "internal feeding" scenario was simulated by inserting larvae into slots

carved into the fruit with a cork borer. Plugs were then removed from the borer, trimmed, and then fitted to seal the cavity flush with the fruit surface. Twelve 15lbs loadings of "O'Henry" yellow peaches, "Arctic Snow" white nectarine, "September Bright" yellow nectarine, "Snow Giant" white peach, and "Angeleno" plums were exposed to 1500 ppm phosphine for 2 d and then evaluated for phytotoxicity. The effect of phosphine dose (750, 1500, and 2250 ppm) and duration (1, 2, and 3d) on phytotoxicity to "O'Henry" yellow peaches was evaluated using a multivariate design run with a total of 18 indiviual experiments.

Commodity fumigations: methyl bromide. Fumigations were conducted in triplicate 8.54 ft^3 (241.9 L) steel chambers (USDA, 2009). Each chamber contained either three boxes of volume-filled "Angeleno" plums (~28 lbs, size 40), "Autumn Flame" peaches (~25lb, size 42), or "Yellow" nectarines (~25lb, size 36). Estimates of the load factor ($V_{commodity}/V_{chamber}$x 100) were respectively 35.8, 32.7, and 35.6% for peaches, plums, and nectarines (Figure 2). Four egg-cages and seven pupa-cages were situated throughout the boxes of each fruit variety; total number of treated specimens were ~1500 and ~300 for eggs and pupae, respectively. Each of seven peaches and nectarines were sliced and carved to accommodate cages that collectively contained ~200 test specimens. The two halves of fruit were rubber-banded back together to simulate internal feeding larvae before they were situated throughout boxes of peaches and nectarines.

After tempering loaded chambers, control specimens, MB source cylinder, and gas syringes for 12 h, internal circulation fans were turned on and lids were clamp-sealed in preparation for treatment at 60°F or 50°F. A slight vacuum of ~ 5 inch Hg was established in each chamber and a 1L-syringe, filled with a volume of MB to achieve the requisite dose, was fitted to a sampling valve. The valve was manipulated so that the vacuum would steadily draw MB out of the syringe and into the chamber. The syringe was then removed and NAP was reestablished in each chamber before the valve was closed. Headspace gas samples (40mL), for MB concentration analysis with GC-FID, were removed with a syringe via the sampling valve at ~1 (start), 30, and 120 (end) minutes.

After a 2-hr exposure period, chamber valves were opened to atmosphere and vacuum was pulled for 30 min to ventilate the systems. Chambers were opened, boxes were unloaded, and cages containing the test insects were retrieved. Pupa-containing cages were inspected for adults that emerged over the tempering and/or fumigation period; the adults were counted, physically removed from the treatment cages with an aspirator, and placed in a 30-dram clear plastic vial as described above (Figure 4). Post-fumigation development of yellow color and/or the presence of blackhead stages (first larval instar) in treated eggs were recorded, as was the maturation of treated larvae into prepupae and pupae. All treated insect cages were then placed inside pull-string cloth bags and transferred to an incubator set at 27ºC and 60% R.H..

Chemical Analysis. Gas chromatography retention times were used for chemical verification during fumigation trials and residue quantifications. Doses required to achieve requisite fumigant concentrations, as well as the retention index, were determined each day in calibration studies conducted prior to fumigation. Quantification during and after fumigations was with a HP 6890 and splitless injection (150 °C) using a gas sampling port (125 °C) with a 250 µL-sample loop, a megabore® GSQ analytical column (L 30 m, i.d. 0.53 mm) held at 100 °C for 10 min, and 15 ml/min He carrier flow. A PFPD at 250 °C and a FID (30mL/min H_2, 250 mL/min air, and 5.0 mL/min N_2 make-up) at 280 °C, which were plumbed to receive only 10% of the 1.2 ml He/min column flow, were used to detect phosphine and methyl bromide, respectively. For methyl bromide residue determinations, a Varian 3800 was used with a megabore® GSQ analytical column (L = 60 m, ID = 0.53mm) at 100 °C and ECD detection at 300 °C with N_2 make-up flow. Splitless injections of 50µL were at 220°C and utilized an intital pressure pulse of 15 psi (28.2 mL/min) for 3.96 min that was reduced to 11.5 psi (20.0mL/min) until 12min. Qualitative verification of methyl bromide was with a HP 5890 system and GCD mass spectrometric detection in EI mode (1694 eV) over m/z 10-200 at 260 °C, which was otherwise outfitted as the residue system described above.

Comparative sorptions. The relative sorption of fumigant by different fruit and packing cartons was evaluated in modified Labonco® 1-ft^3 (27.97 L) chambers under NAP at 60 over 48 hr exposures. In general, the mass and the volume of materials were recorded prior to fumigation so that these factors, which are known to influence fumigant sorption, were appropriately accounted for in numerical models of equilibrium conditions. A single cardboard carton, either a new wespak "mum" (825 g, 3551 cm^3) or a 2 yr-old pretty belle "tray-pack" (872 g, 3958 cm^3), was cut into pieces and completely transferred into a single chamber prior to fumigation. For the comparison of fruit, 10 lbs of "Black" plums (size 70, 3.2 L/test chamber), "Yellow" peaches (size 64, 5.4 L/test chamber), or "Yellow" nectarines (size 36, 3.1 L/test chamber) were bundled in cheesecloth and transferred into a single chamber prior to fumigation.

Residues. Methyl bromide residues resulting from the APHIS T-104-a-1 schedule at 60°F (40 mg/L, NAP, 2h) were quantified. Fumigations were in the triplicate ~9 ft^3 chambers described above, which contained either three volume-filled wespak "mum" boxes of "black" plums (28 lbs, size 70), "yellow" peaches (25lb, size 32), "yellow" nectarines (25lb, size 36). Initial MB residue levels were determined within 5 min of a 30 min post fumigation ventilation and before treated cartons of fruit were transferred to storage at 1°C. Subsequent residue determinations were at 1, 2, 3, 4d post fumigation. Three fruit from each treatment were cut to 25 g sections, combined (75g/treatment), and homogenized in 500-mL air-tight glass blending-vessels with 200 mL of 0.01M NaHCO3 buffer at pH 7 and 0.1µ NaCl. Vessels were stored at 19 °C for 24 h and then triplicate 50 µL aliquots of headspace were withdrawn with a 250 µL-Pressure-Lok® glass

syringe and analyzed with GC-ECD as described below.

Fruit quality. After fumigation the fruit were stored for 2 weeks at 33.8 ºF followed by 2 days at 73.4 ºF and then evaluated for fruit quality. 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). Weight loss was calculated by weighing 10 fruit individually for each replication before treatment and then the same fruit following storage and determining the difference. Surface injury was visually rated on all of the fruit in a replication and fruit placed into classes ranging from 0 to 4 (0=no injury, 1=very slight, 2=slight, 3=moderate, 4=severe). The number of decays was also noted. An average rating index was calculated by multiplying the number of fruit in each class by the class number (0 to 4), summing, and dividing by the total number of fruit in each class. Average values exceeding 2.0 would be considered as questionable in their marketability. The percentage of marketable fruit was estimated by dividing the fruit in classes 0, 1 and 2 by the total number of fruit. Internal browning of the flesh was visually rated from 0 (none) to 4 (severe) for 60 fruit per replication and a browning index calculated in the same manner as was that for surface injury. From this the percentage of fruit with internal browning was calculated. The percentage of fruit that had internal mealiness was also estimated on 60 fruit per replication. Brix and acidity were determined from the juice of 5 pooled fruit for each replication using a digital refractometer and titration with NaOH. Brix and acidity were not done on the "Autumn Flame" peaches or "Yellow" nectarines due to the high level of decay present in the fruit after ripening.

References.

Finney, D.J. Probit Analysis; 3rd ed.; Cambridge Univ. Press: 1971
USDA 2009a http://www.ars.usda.gov//Main/site_main.htm?docid=18134
USDA 2009b http://www.ars.usda.gov/Main/docs.htm?docid=18577