Fogging trailer loads of California fresh citrus with a high-pressure sprayer for control of Asian citrus psyllid, *Diaphorina citri*

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Executive Summary. Asian citrus psyllid (ACP), *Diaphorina citri*, is a quarantine pest in California and limiting its geographic distribution is a major goal of the California citrus industry. While a variety of phytosanitary measures can be used to control adult ACP once fruit is at a packing house, ultimately, a treatment must be developed to disinfest field-run fruit prior to its exiting the grove. High-pressure spraying with 1,100-L of an aqueous mixture containing 0.1% Evergreen® (6% pyrethrins & 60% piperonyl butoxide) and 0.5% (v/v) BreakThru® (polysiloxane surfactant) was explored in laboratory-, pilot-, and commercial-scale trials as an approach to disinfest a 48-bin trailer load of fresh citrus. Laboratory-scale studies were used to confirm the complete mortality of 2,124 adult ACP following exposure to the lowest surface coverage quantified (ca. 500 ng m^{-2} pyrethrins) during pilot- and commercial-scale trials. During a commercial-scale fogging trial conducted at Blue Banner Citrus in Riverside, California on 24 October 2017, in which 1,986 adult specimens were caged and distributed throughout a 48-bin trailer load of fresh citrus, only two specimens survived, resulting in 99.778 % mortality (probit 7.85 at the 95 confidence level (CL)) via several statistical metrics common to quarantine entomology. These results provide evidence to support the conclusion that adult ACP will be controlled in 48-bin trailer loads of fresh citrus subject to the high-pressure spray treatment. Results are discussed in the context of experimental variability across commercial-scale confirmatory trials and continued efforts to optimize the technical and economic feasibility of this ACP control strategy.

Materials and Methods

Chemicals. Evergreen® Pro 60-6 (McLaughlin Gormley King (MGK) Company, Minneapolis, MN), an aqueous mixture of 6% pyrethrins & 60% piperonyl butoxide, was sourced from Fruit Growers Supply (Exeter, CA) (EPA Reg. No. 1021-1770). BreakThru® S240, a polysiloxane surfactant (CA REGISTRATION #1051059-50001-AA), was sourced from Evonik Corporation (Hopewell, VA). Prior to dilution of the active and the surfactant, water was deionized using a Portable Deionizing System (ion exchange resin).

Insects, rearing, and infestation. Asian citrus psyllid (ACP), *Diaphorina citri*, were reared on potted *Murraya koenigii* (L.) plants contained within ca. 0.5-m^3 rearing enclosures housed in a environmental room at the UC Riverside Insectary & Quarantine Facility set to 85 ± 2°C, 65% RH, and 16: 8 (L:D). Movement of the psyllids from the quarantine facility was permitted by CDFA (Permit # 3280)

To obtain an aliquot of adult ACP for efficacy studies, 10 specimens were consecutively aspirated into mesh cages using a customized arrangement of the aspirator and cage (Figure 1). With respect to the commercial-scale trial conducted on 23 October 2017, two cage types were used. Cylindrical ~8-mL stainless-steel cages (30-mesh), as shown in Figure 1, were fabricated and following aspiration of the specimens, these cages were capped with a cork. Nylon mesh cages (3/4" diameter and 2.5" height) were fabricated by shrouding (Fig. 2A & 2B) – a wire cylinder

(1/2” diameter and 2” height) with a square of nylon hardware cloth. After aspiration, the open end of the nylon cage was closed using a binder clip.

Commercial-scale fogging. At 07:00 PST on 23 October 2017 a 48-bin trailer load of field-run fresh navel oranges (ca. 56 to 88 size), sourced from Gless Ranch (Riverside, CA) arrived at Blue Banner Citrus in Riverside, California. Bins were off-loaded, numbered, and ACP specimens, which were caged ca. 0.5- to 1.5-h earlier, were buried throughout the bins at locations that were previously shown to have the relatively lowest piperonyl butoxide residues following a treatment (Figure 3A). A total of 140 nylon cages were buried, 78 and 14 at low- and high-corner positions, respectively, and, 48 at the center of respective bins. To quantify residues throughout the load, and particularly those in proximity to nylon-caged specimens, nylon cages containing glass microfiber filter papers (1.6μm, 95 ± 1 mg, ~20 m^2/g, 4.7-cm diameter, 53 g/m^2 basis weight, Whatman GF/A) were place next to nylon-caged specimens at each location. A stainless-steel cage containing ACP (used in previous experiments) was also placed at the center of respective bins. A total of 22 aliquots of ACP specimens, 12 nylon mesh cages as well as 10 “cage-less” 7-dram clear plastic aspirator vials, each of which contained a host leaf and a snap cap with a 8-mm diameter stainless-steel 100 wire mesh gas-portal, were buried throughout separate container of sourced fruit to serve as non-treated controls (Figure 3B).

Within a degreening room (V = 753.6 m^3; 21.3 l x 5.8 w x 6.1 h meters) at ca. 70°F, infested bins of fruit were re-oriented into the geometry of the truck load, two bins wide by two bins stacked, with a ca. 3-ft span between the 6th and 7th rows of bins (Figure 4). The container of non-treated controls were transferred to an adjacent degreening room, also set to ca. 70°F. The high-pressure

spray system, designed and fabricated by Valley PackLine Solutions (Reedley, CA) was then situated around the load. Industrial Air Circulating Fans (34"- Fan Blade Dia, 17000 cfm Max Air Flow) were alternately arranged, directed laterally toward the center of each of the six 8-bin cubes comprising the load. An aqueous mixture (1,100-L) of ca. 0.1% Evergreen® (6% pyrethrins & 60% piperonyl butoxide) and 0.5% (v/v) BreakThru® (polysiloxane surfactant) was prepared in the reservoir of the spray system (note: Max label is 914mL per 290 gallons). The fans were turned on, and the aqueous solution was directed at 1000 psi to each of the six fans, outfitted respectively with a ¾"- steel manifold and a 45° fan nozzle (xxxx) that discharged into the airflow, ~ 6" below from the front of the fan. The degreening room doors were shut, which marked the start of the treatment.

After the solution was delivered, the fans were turned off, 10 minutes were allowed to elapse to let the fog settle, and the degreening room door was opened. The treated specimens were retrieved from the bins, along with the caged filter papers. Treated as well as non-treated control specimens were placed in bin- and location-specific Ziploc bags (separate from the caged filter papers that were organized similarly). Bagged-specimens were placed in a cooler and returned to the UC Riverside Insectary & Quarantine Facility for mortality evaluations (vide infra), which occurred at ca. 3-h following treatment.

Mortality evaluation. After returning the specimens, both treated and non-treated, to UC Riverside Insectary & Quarantine Facility the treatment, preparations were immediately made to evaluate mortality that resulted from the treatment. All specimens, less the non-treated controls specimens already in 7-dram snap cap vials, were transferred from nylon and stainless-steel cages into7-dram clear plastic "snap cap" cage modified with 8-mm diameter stainless-steel 100 wire mesh gas-

portals on the cap (Figure 5). A fresh lemon leaf was introduced into all plastic cages. Approximately 3 h following the treatment, all cages were visually inspected. Mortality was diagnosed by lack of motion and was calculated by subtracting the number of survivors from the number of treated specimens. Mortality of non-treated control specimens was treated numerically using Abbott's method (Abbott, 1925). Mortality, calculated as a percentage of the response per treatment, was expressed as a function of the number of specimens treated via probit analysis of Finney (1944 & 1977) at the 95% confidence level (CL), as further derived in Couey and Chew (1986) as well as Liquido and Griffin (2010).

Results and Discussion

Commercial-scale fogging. The fogging process commenced at 10:30 AM and terminated at 12:10 PM. All but five non-treated control specimens survived, a single specimen did not survive in the plastic vial, while four did not survive in the nylon mesh. Only 2 specimens survived from 1,968 total treated, one specimen each from a stainless-steel and nylon mesh cage, both situated in the middle of the same bin on the top row, opposite the nearest fan. Using the statistical methods described above, the treatment resulted in 99.778% mortality (probit 7.85 at the 95% CL).

These results provide evidence to support the conclusion that adult ACP will be controlled in 48-bin trailer loads of fresh citrus subject to the high-pressure fogging treatment, at least when the volume of the load is $\geq$ 10% of the fogging enclosure. Although the 23 October 2017 commercial-scale trails was conducted in a degreening room, analogues trials have been conducted on 48-bin trailer loads that were driven into a tent structure, as shown in Figure 6, methodology that is consistent with the need to disinfest field-run fruit prior to its exiting the grove.

Figure 1. Method of collecting adult Asian citrus psyllid (ACP), *Diaphorina citri*, into 30-mesh stainless-steel cages using a standard mouth aspirator apparatus.

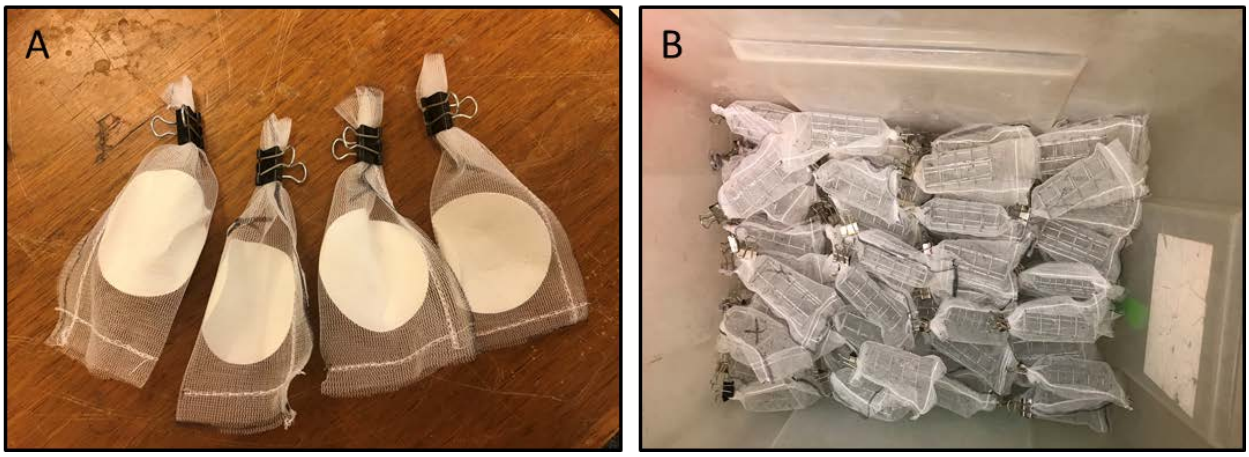

Figure 2. Nylon mesh cages containing fiberglass filter paper (A) and containing ACP (B). Note that the open end of the nylon cage was closed using a binder clip.

Figure 3. A bin filled with field run fruit showing placement of caged specimens in the middle position (A), a container filled with the same fruit that was used to analogously burry the non-treated, control ACP in the nylon mesh cages (B), the fruit were carefully positioned back atop the caged specimens (C), and the geometry of the trailer load was reconfigured within the degreening room (D).

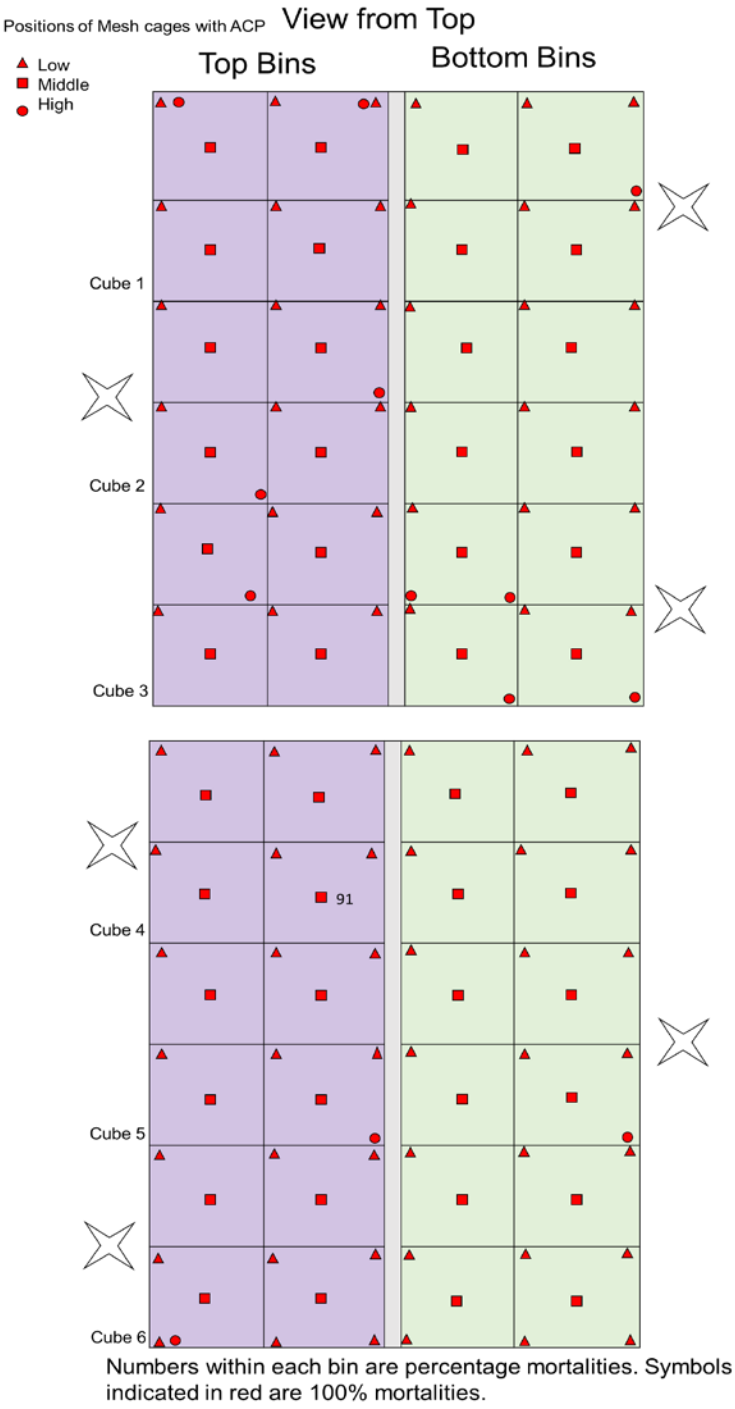

Figure 4. Layout of 48-bin trailer loads of fresh citrus. Purple and green color indicate top- and bottom-stack positioning, respectively. Different shapes show location of cages within the bin (middle positon had both nylon and stainless-steel mesh cages). Note that the two survivors were found in the 8th row (top to bottom), one specimen each from a stainless-steel and nylon mesh cage, both situated in the middle of the same bin on the top row, opposite the nearest fan (denoted by stars).

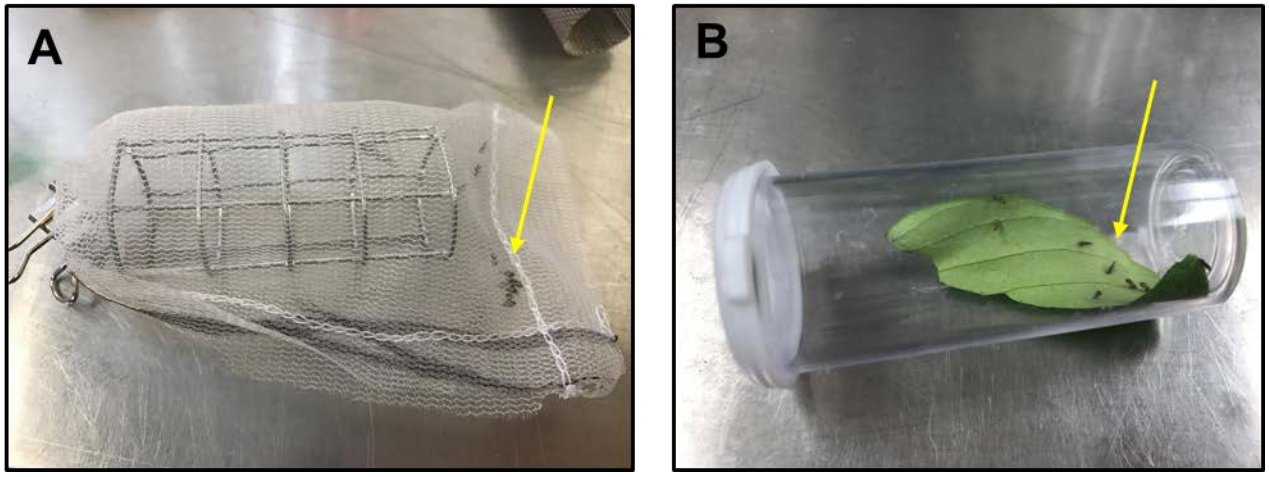

Figure 5. Asian citrus psyllid (ACP) after treatment (A) – dead ACPs as indicated by an arrow. Live ACP feeding on lemon leaf in the non-treated controls caged in a plastic vial (B).

Figure 6. The high-pressure fogging with an aqueous mixture containing 0.1% Evergreen® (6% pyrethrins & 60% piperonyl butoxide) and 0.5% (v/v) BreakThru® (polysiloxane surfactant) can be conducted on a 48-bin trailer load driven into a tent structure, methodology that is consistent with the need to disinfest field-run fruit prior to its exiting the grove.