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Fogging loads of California fresh citrus for control of Asian citrus psyllid, *Diaphorina citri*

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DOI 10.5073/jka.2018.463.169

Abstract

Contact insecticides are commonly applied as fogs to disinfest and disinfect spaces. Recently, these fogs have been adapted to treat commodity within the spaces, and much has been learned regarding the efficacy of this process. When considering fresh citrus in California, fogs are applied to control both insects and microbes. One insect pest, the 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 fogging with 1,100-L of an aqueous mixture containing 0.2% 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 conducted to quantify, and subsequently model, insecticidal coverage as a function of temperature, surface area, droplet size, and fog volume. Results are discussed in the context of experimental variability across confirmatory trials and continued efforts to optimize the technical and economic feasibility of fogging as a postharvest control strategy.

Keywords: food security, food safety, pyrethrins, postharvest fogging

1. Introduction

Asian citrus psyllid (ACP), *Diaphorina citri*), which transmits citrus greening disease (Huanglongbing or HLB)-associated liberibacter (‘*Candidatus* Liberibacter asiaticus; Las), has the potential to devistate the production of fresh citrus in California (Grafton-Cardwell et al. 2016). Moreover, the presence of ACP in the marketing channel can create a phytosanitary barrier for exports, which are key to the industries profitability. While ACP adults are removed from fresh citrus that has been subjected to cleaning and packing procedures standard to commercial production and distribution, State and Federal quaratines often restrict movement of fruit from ACP infested orchards to packhouses (CDFA, 2018).

Accordingly, a treatment must be developed to disinfest field-run fruit prior to its exiting the grove to control any incidental transportation of ACP and potential spreading of the insect and its associated disease. This work describes the development of a high-pressure fogging system using Evergreen® to control ACP in trailer loads of field-run fruit. The proposed treatment will reduce the number of psyllids in bulk citrus and reduce the insecticides applied to the grove, which will in turn improve worker safety, reduce environmental impacts, and improve IPM of ACP and other pests.

2. Materials and Methods

2.1. 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).

2.2. Insects, rearing, and infestation

The 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 an 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 (Fig. 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.

2.3. 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 (Fig. 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 weight basis 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 and 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 an 8-mm diameter stainless-steel 100 wire mesh gas-portal, were buried throughout separate container of sourced fruit to serve as non-treated controls (Fig. 3B).

Within a de-greening 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 6^{th} and 7^{th} rows of bins (Fig. 4). The container of non-treated controls was transferred to an adjacent de-greening 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 that discharged into the airflow, ~ 6″ below from the front of the fan. The de-greening room doors were shut, which marked the start of the treatment.

After the solution was delivered, the fans were turned off, 10 minutes elapsed to let the fog settle, and the de-greening 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.

2.4. 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 (Fig. 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).

3. Results

3.1. 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 ≥ 10% of the fogging enclosure. Although the 23 October 2017 commercial-scale trails were conducted in a de-greening 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.

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

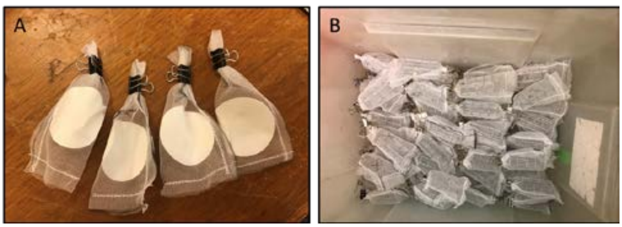
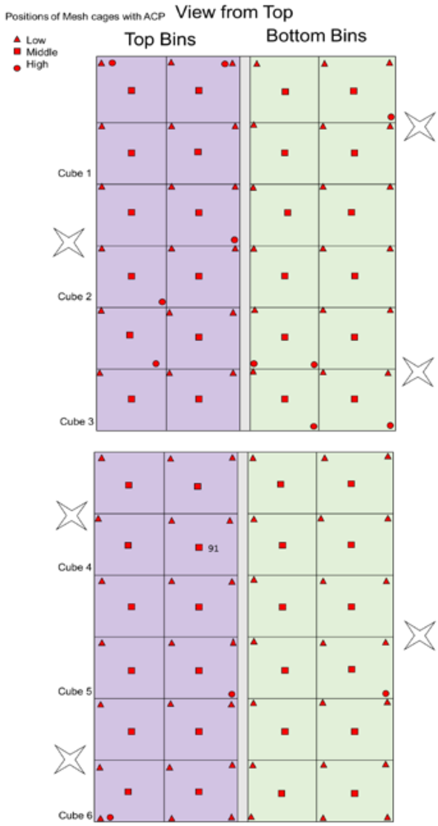


Fig. 2 Nylon mesh cages containing fiberglass filter paper (A) and containing ACP (B). Note that the open end of the nylon cage was closed using a binder clip.

Fig. 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).



Numbers within each bin are percentage mortalities. Symbols indicated in red are 100% mortalities.

Fig. 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).

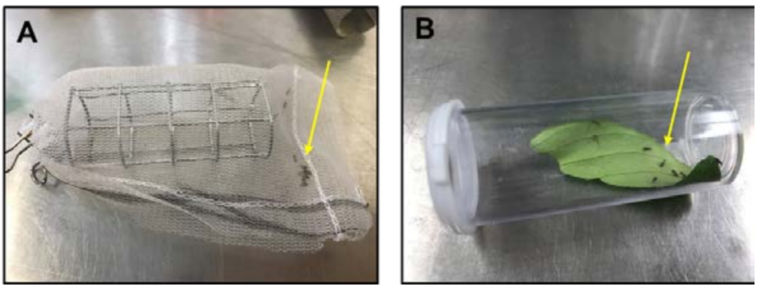


Fig. 5 Asian citrus psyllid (ACP) after treatment (A) – dead ACPs as indicated by an arrow. Live ACP feeding on lemon leaf in the non-treated controls caged in a plastic vial (B).

Fig. 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.

4. Discussion

Future research effort will focus on minimizing the amount of fogging material required for efficacy, which will likely involve a transition to low-volume applicatiors, such as ultrasonic and thermal fogging technologies. Additionaly, treatment withina curtain-sided truck will be persued to further minimize the infrastructural requirement to tent the truck, while still remaining "mobile", within the grove. From a regulatory prespective, the proposed treatment represents a shift in paradign from the familiar pre- and post-harvest designations, and this divergence must be addressed at both the State and Federal levels. Considered a preharvest treatment, the application of Evergreen® is excempt from residue tolerance (USEPA and CA), has a 0-h postharvest interval, and a 12-h re-entry period for workers (CA). It is critical to note, however, workers will not be reuired to touch treated fruit after treatment, as it is already in field bins. While postharvest space foggings and direct applications to binned fruit on trucks are permitted postharvest treatments, with corresponding Personal Protective Equipment (PPE) and re-entry requirments, the proposed treatment occurs prior to washing and packing, which remove residues, contradicting the reationale for a postharvest tolerance. Regardless of the ultimate regulatory categorization, the proposed treatment could reduce the amount of insecticides applied to the grove, which will in turn improve worker safety, reduce environmental impacts, and improve IPM of ACP and other pests.

Acknowledgement

This work was funded by the California Citrus Research Board (Project 5300-184). We would like to acknowledge substantial contributions of David Sorenson and Dirk Hartley during the trials. We would also like to thank the UC Riverside Insectary and Quarantine staff for providing ACP samples, Alan Washburn for providing fruit, Dr. Joseph Morse, UCR staff (Nastaran Tofangsazi, Imon Riabi, Brandon Skylar Rogers, Tobias Moyneur) and USDA-ARS staff (Matthew Rodriguez, Stephen Corbett, Erik Rivera) for technical assistance, and especially thank co-operating packinghouses, namely, Blue Banner Co., Corona-College Heights Orange and Lemon Association, Valley PackLine Solutions, and Harding and Leggett.

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Toxicity of fine powders, filter cake and Triplex against *Sitophilus zeamais* adults

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DOI 10.5073/jka.2018.463.170

Abstract

Filter cake and Triplex are powdered by-products of aluminum sulfate and soap factories, respectively. There is limited data about the use of these powders as grain protectants. This study was aimed at determining contact toxicity of both powders against *Sitophilus zeamais*, a common pest of stored grains. Lethal concentration of both powders to *S. zeamais* was determined by exposing 10 adults for 12 h in 9 cm diameter concrete arenas inside Petri dishes dusted with filter cake (0 - 8 g/m^2) or Triplex (0 - 9 g/m^2). Lethal time was determined by exposing adults to 3 g/m^2 filter cake and 9 g/m^2 Triplex for 1 to 24 h. Each treatment was replicated 3 times. At the intended exposure time, adults were transferred to 150-ml round plastic containers with 30 g of wheat and held at 28 degree Celsius and 65% r.h. for 14 d to determine mortality. Adult progeny production was determined after 42 d. A 50% mortality of adults was obtained at 0.61 g/m^2 of filter cake and 1.61 g/m^2 of Triplex concentrations with a 12 h exposure. The corresponding effective concentrations for 50% reduction of progeny production were 0.18 g/m^2 of filter cake and 2.66 g/m^2 of Triplex. Lethal times for 50% mortality of adults after exposure to 3 g/m^2 of filter cake and 9 g/m^2 of Triplex were 4.42 and 4.29 h, respectively. The corresponding effective times for 50% reduction of progeny production after exposure to 3 g/m^2 of filter cake and 9 g/m^2 of Triplex were 1.74 and 2.34 h respectively. The overall result indicated that filter cake was highly toxic to *S. zeamais* than Triplex. Therefore, filter cake is a potential powder to be included in the integrated pest management practice in small holder farmers' storage structures after tested under real field conditions.

Keywords: Filter cake; Triplex; *Sitophilus zeamais*; Toxicity

1. Introduction

Grain losses due to insect pests in sub-Saharan Africa are very high, and the magnitudes of losses vary from country to country and from region to region (Abate et al., 2015). In countries like Ethiopia, about 80% of all grain produced is estimated to be stored at the farm or village level (Tadesse and Eticha, 2000). Grain storage losses in Ethiopia due to insect pests were estimated to be in the range of 10 to 21% (Abraham et al., 2008). A number of chemical insecticides used by Ethiopian small holder farmers to protect their grain in storage have been reported by several researchers (Abraham et al., 2008; Girma et al., 2008a,b; Hengsdijk and De Boer, 2017; Mengistie et al, 2017; Dessalegn et al., 2017). Recently, chemical pesticides, regardless of their inherent hazards, are used extensively in the fast changing agricultural sector of Ethiopia (Nigatu et al., 2016). However, Ethiopia is confronted with a number of problems related to unsafe handling of pesticide distribution and use, such as use of unsafe storage facilities, improper training on safe use of pesticides, and inadequate infrastructure to regulate safe use of pesticides (Mengistie et al., 2017). A survey done by Nigatu et al. (2016) on knowledge, attitude, and practices of farmers and farm workers in Ethiopia reported that 85% of farm workers (pesticide mixers/loaders, sprayers, and application supervisors) (n = 601) and 100% of female re-entry farm workers (harvesters, pesticide assessors, irrigation workers, irrigation supervisors, packing and sorting workers, transport/push car workers), (n = 275) did not receive pesticide-related training. In addition, 62% of farm workers did not shower after pesticide application, and none of the small-scale farm workers (n = 258) used personal protective equipment. A considerable increment in chemical pesticide usage intensity, illegitimate usage of DDT and Endosulfan on food crops, and direct import of pesticides without the formal Ethiopian registration process were also reported by Nigatu et al. (2016).