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Residual toxicity of selected organic insecticides to *Diaphorina citri* (Hemiptera: Liviidae) and non-target effects on *Tamarixia radiata* (Hymenoptera: Eulophidae) in California

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ABSTRACT

The Asian citrus psyllid, *Diaphorina citri* Kuwayama (Hemiptera: Liviidae) threatens California's citrus industry because of its ability to transmit the bacterium, *Candidatus* Liberibacter asiaticus, associated with huanglongbing (HLB). To reduce the risk of HLB spread, an area-wide management strategy was established to control *D. citri* in both conventional and organic commercial citrus. In addition, releases of the parasitoid *Tamarixia radiata* Waterston (Hymenoptera: Eulophidae) as a classical biological control agent have been conducted in certain urban areas of California to improve control of *D. citri*. The objectives of this study were to document the residual efficacy of organic insecticides against *D. citri* nymphs, compare the efficacy of multiple applications of the organic spinosad relative to a single application of the non-organic insecticide fenpropathrin, and estimate the non-target effect of selected foliar insecticides on *T. radiata*. Spinosad + oil showed greater residual control of *D. citri* nymphs compared to pyrethrins + oil. The effects of organic insecticides were short lived; by 10 d post treatment there was no detectable effect. Fenpropathrin provided significantly longer control of *D. citri* nymphs and the efficacy of two or more applications of spinosad was comparable to one application of fenpropathrin. Of the organic insecticides, spinosad + oil resulted in the greatest non-target effects on *T. radiata* and pyrethrins, pyrethrins + oil, and oil alone had the least severe effects on *T. radiata*. This study provides important information regarding the residual control of *D. citri* by organic insecticides and their compatibility with the biological control agent *T. radiata*.

1. Introduction

Citrus is one of the most important crops in California, producing most of the nation's fresh market citrus, especially sweet oranges (*Citrus sinensis* (L.) Osbeck), lemons (*Citrus limon* (L.) Burm f.) and a variety of mandarins and their hybrids valued at more than $2 billion annually (USDA NASS, 2016). *Diaphorina citri* Kuwayama (Hemiptera: Liviidae) constitutes one of the most significant threats to California's citrus industry (Grafton-Cardwell et al., 2013), due to its role as a vector of the bacterium, *Candidatus* Liberibacter asiaticus (*C*Las), associated with the citrus disease huanglongbing (HLB) (Halbert and Manjunath, 2004; Gottwald, 2010; Bove, 2006; Grafton-Cardwell et al., 2013). Despite decades of research, HLB still has no cure and is regarded as one of the most devastating diseases of citrus worldwide (Grafton-Cardwell et al., 2013). In 2008, *D. citri* was detected in Southern California in a residential area of San Diego County (Kumagai et al., 2013). It has since become well-established throughout Southern California, and has been detected in low numbers in the major citrus growing region of the Central Valley (Civerolo, 2015). In March 2012, *C*Las was first detected in a tree in a residential area in Los Angeles County (Kumagai et al., 2013). Since then, numerous additional cases of HLB have been found in Southern California, thus far restricted to residential citrus (Hornbaker and Kumagai, 2016). Given these cases of the disease in California, suppression of *D. citri* populations is viewed as essential for slowing the spread of HLB in both residential areas and commercial orchards (Grafton-Cardwell et al., 2016a).

As part of an effort to enhance control of *D. citri* in California, a classical biological control program was implemented by University of California and California Department of Food and Agriculture (CDFA). Initially, this program consisted of releases of the ectoparasitoid *Tamarixia radiata* Waterston (Hymenoptera: Eulophidae), starting in September 2011 (Hoddle and Hoddle, 2013; Hoddle and Pandey, 2014)

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in Southern California. *Tamarixia radiata* preferentially parasitizes 3rd to 5th instars of *D. citri* and host-feeds on 1st and 2nd *D. citri* instars (Hoddle and Hoddle, 2013). To date, the CDFA has concentrated their release efforts in residential areas, releasing more than two million *T. radiata* adults in more than four thousand sites throughout Southern California from 2011 to 2015 (Kistner et al., 2016a). After 3 years, a survey of 100 residential sites throughout the area detected *T. radiata* at over 90% of those inspected sites, including 24 sites at which the parasitoid had not been released (Kistner et al., 2016a). These releases were followed later by releases of the endoparasitoid *Diaphorencyrtus aligarhensis* Shafee, Alam and Agarwal (Hymenoptera: Encyrtidae) (Bistline-East et al., 2015; Hoddle et al., 2015). Releases of both parasitoid species continue throughout much of Southern California. However, insecticides used to control *D. citri* will likely adversely affect natural enemy predation and parasitism rates (Flores and Ciomperlik, 2017). The low parasitism rates of *T. radiata* in Florida compared to Reunion Island, Guadeloupe and other release areas have been attributed, in part, to potentially disruptive effects of numerous insecticide treatments targeting *D. citri* (Pluke et al., 2008; Hall and Nguyen, 2010).

Key strategies for the management of the *D. citri*/HLB complex are reduction of the inoculum and vector control (Bassanezi et al., 2008). Reduction of inoculum is difficult to achieve, as symptom expression occurs months or years after initial infection and psyllids can transmit the disease in the interim (Coletta-Filho et al., 2014; Lee et al., 2015). Inoculum reduction is also expensive given that it requires a trained scout team to perform continuous visual surveys of the orchard, molecular confirmation of the suspected trees, and replacement of the trees, in addition to losses in production of the replaced trees (Bassanezi et al., 2008; Monzo and Stansly, 2017). Chemical control of the vector *D. citri* demands multiple insecticide applications that greatly increase the cost of production and negatively impact natural enemies (Bassanezi et al., 2008; Monzo and Stansly, 2017). At present in California, in areas where the psyllid population is low (Central Valley), coordinated insecticide treatments are applied in an 800–3000 m area around locations where *D. citri* are detected in both orchards and residential citrus in an attempt to locally eradicate the psyllid. In Southern California, where the psyllid is well-established, parasitoids are released in residential areas and citrus growers participate in area-wide psyllid management programs in which they apply insecticide treatments in a coordinated manner over large areas, targeting winter populations of adult psyllids and major leaf flushes of citrus when egg laying occurs. The commercial citrus psyllid area-wide management program utilizes both conventional and organic insecticides (Grafton-Cardwell et al., 2015, 2016b).

Organic citrus growers often benefit from premium prices, but they also face many challenges such as developing effective integrated pest management (IPM) strategies (Linares et al., 2008). The OMRI (Organic Materials Review Institute) approved insecticides available for *D. citri* control are limited to a small number of choices (spinosad, neem, pyrethrins, various botanicals and horticultural oils). Although organic insecticides caused high mortality (80–95%) of adult and nymph stages of *D. citri* upon direct contact, the residual impact declined rapidly for adults during the week after treatment (Bethke et al., 2014; Khan et al., 2014). Therefore, organic insecticides may need to be applied more frequently to provide comparable effectiveness to conventional insecticides. However, organic insecticides may be more compatible with conservation and augmentation biological control programs (Qureshi et al., 2013).

There is an urgent need to develop effective *D. citri* management strategies for organic citrus production, which constitutes nearly 4% of all citrus production in California (USDA ERS, 2013; Klonsky and Healy, 2013). Most insecticides are fairly effective in killing *D. citri* adults but nymphs are often tucked inside developing leaves and may not be directly contacted by the insecticides (Halbert and Manjunath, 2004). While Bethke et al. (2014) examined residual toxicity of organic insecticides for adults, additional information is needed for nymphs. The present study examines the residual impact of fenpropathrin, pyrethrins, spinosad and horticultural mineral oil on *D. citri* nymphs and larvae, and adults of one the most important biological control agents, *T. radiata*. In addition, the impact of multiple applications of spinosad on all *D. citri* stages were compared to a single application of fenpropathrin under California field conditions.

2. Material and methods

2.1. Insects and insecticides

Diaphorina citri and *T. radiata* used in the laboratory and field bioassays were obtained from the mass-rearing program at the University of California Riverside Insectary and Quarantine Facility (UCR- I & Q). The *D. citri* colony was established in 2012 from 200 field-collected adults in a residential area of Los Angeles County (N34.12, W117.9). All *D. citri* were reared on curry leaf *Murraya koenigii* (L) Sprengel (Rutaceae) as described by Soper et al. (2014) without exposure to insecticides. Pots of *M. koenigii* were placed inside rearing cages (60 cm × 60 cm × 60 cm) covered with nylon mesh fitted with sleeves for access. Approximately 20 adult *D. citri* were introduced into each cage and allowed to oviposit for 4 d, after which they were removed and transferred to a new cage for oviposition. Approximately 100 newly emerged adult *D. citri* were randomly collected from between 12 and 33 rearing cages and tested approximately every 3 months, via a molecular assay, to verify they were free of *C*Las (Bistline-East et al., 2015). The *T. radiata* colony was established in 2012 with parasitoids originally collected from the Punjab of Pakistan and maintained in separate rooms from *D. citri* at UCR- I & Q (Hoddle and Hoddle, 2013). For parasitoid rearing, potted *Murraya koenigii* infested with second instar *D. citri* are transferred periodically to the parasitoid rooms, and 3–5 day old *T. radiata* adults are added to the cages at a ratio of 1–50 (*T. radiata*: *D. citri*). Additional details on parasitoid rearing are described by Simmons et al. (2013).

Throughout this study, we define first and second *D. citri* instars as “early instars” and third to fifth instars as “late instars”. In order to obtain the appropriate instars for our experiments, a cohort of curry leaf plants were exposed to *D. citri* adults from the colony for 48 h. Afterward, adults were removed and plants were kept in insect rearing cages (BugDorm® BD2120F MegaView Science, Taiwan) until the desired instars were obtained. Early and late instars were distinguished visually by their wing pads using a stereomicroscope (Hall, 2008).

For all experiments, insecticides were applied at the highest recommended label rate. The conventional insecticide used was fenpropathrin, at a rate of 0.61 l of active ingredient (AI)/ha (Danitol 2.4EC, Valent USA Corp. Walnut Creek, CA). The OMRI approved organic insecticides included pyrethrins 0.018 l AI/ha (PyGanic Crop Protection EC 1.4 II, McLaughlin Gormley King Company, Minneapolis, MN) and spinosad 0.16 l AI/ha (Entrust SC, Dow AgroSciences LLC, Indianapolis, IN). OMRI approved horticultural mineral oil at a rate of 0.25% was applied in combination with the pyrethrins and spinosad or applied alone. Three types of oils [Omni Supreme Spray 14.03 l AI/ha (Mineral Oil, Helena Chemical Company, Collierville, TN), Purespray Green 14.03 l AI/ha (Mineral Oil, Petro-Canada Lubricants Inc., Mississauga, ON), and Organic JMS Stylet Oil 14.03 l AI/ha (Paraffinic oil, Flower Farms Inc., Vero Beach, FL)] were used in laboratory, field, and non-target effects studies, respectively. The use of different oils in separate experiments is simply a reflection of the product on hand at the time these separate, non-concurrent, experiments were conducted. The use of different oils confounds somewhat direct comparisons among the experiments. Therefore, we largely restrict our comparisons to different products within a given experiment. All treatments were diluted at the rate of 1869.3 l of water/ha.