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Assessing the risk of containerized citrus contributing to Asian citrus psyllid (*Diaphorina citri*) spread in California: Residence times and insecticide residues at retail nursery outlets

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ABSTRACT

For phytophagous insects and plant pathogens, the unregulated movement of plant material can inadvertently promote long-distance spread, facilitating biological invasions. Such human-assisted spread has contributed to the invasion of the Asian citrus psyllid (*Diaphorina citri*), a vector of the pathogens associated with huanglongbing. Following the detection of *D. citri* in California, regulations were instituted to limit movement of *D. citri* host plants, by mandating insecticide treatments of citrus nursery stock, and limiting the amount of time host plants can reside at retail sites. We used a set of surveys and a field experiment to evaluate how well these steps mitigate the threat of containerized citrus playing a role in *D. citri* spread. A qualitative analysis of data collected by state regulators throughout Southern California found that containerized citrus may reside at retail sites for extended durations, in extreme cases upwards of 2 years post treatment. More detailed surveys at nearly 30 retail sites in Southern California showed that the majority of citrus plants were present past the 90 day regulatory limit, 33% had been treated more than 1 year prior, and 90% had imidacloprid residues below those known to be effective against *D. citri* nymphs. A field experiment confirmed that imidacloprid residues in trees grown in containers were affected by citrus species, watering level, soil mix, and time since treatment. Overall, plants had *D. citri*-effective residues for approximately 12 weeks, suggesting that imidacloprid treatments should protect the majority of containerized citrus against *D. citri* for approximately the duration of the 90 day regulatory limit. To further protect trees from infestation, nurseries should be encouraged to adopt practices that maximize the effectiveness of insecticide treatments, including ways to reduce residence times of host plants at retail sites.

1. Introduction

Invasive species impose enormous economic and environmental costs to agriculture, natural resources, and human health (CISR, 2016; Simberloff et al., 2013). The identification of pathways of introduction and spread that facilitate invasions of non-native species is critical to the successful implementation of management strategies that mitigate the threat of invasive species (Bayles et al., 2017). In the agricultural sector, the movement of plant material by individuals and through avenues of trade can be especially problematic because of the potential for the dissemination of species over a broad geographical range within a relatively short period of time, often before effective measures of containment and eradication are implemented (Halbert et al., 2010; Morse et al., 2016; Palumbo and Natwick, 2017).

The Asian citrus psyllid, *Diaphorina citri* Kuwayama (Hemiptera: Liviidae), was detected on citrus in southern California in 2008 at a residential property in San Diego County (Grafton-Cardwell, 2010), and has since become established throughout Southern California on both residential and commercial citrus (Bayles et al., 2017). Its principal form of damage is as a vector of the pathogens (*Candidatus* Liberibacter spp.) associated with huanglongbing (HLB or citrus greening) disease in citrus, for which there is currently no readily available cure (Grafton-Cardell et al., 2013). Symptoms of HLB include progressive mottling of leaves, deformed and off-flavor fruit (Dagulo et al., 2010), reductions in yield and eventual plant death (Bové, 2006). In 2012, the first HLB-positive tree in Southern California was detected in a residential

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neighborhood in Los Angeles County (Kumagai et al., 2013). Since that initial discovery, there have been further detections in Los Angeles, Orange, and Riverside counties with all of them in citrus trees grown in private residences.

The Florida citrus industry has been severely impacted by HLB, with estimated costs in excess of $4.5 billion for the five seasons between 2006/07 and 2010/11 (Hodges and Spreen, 2012). *D. citri* was first detected in Florida in 1998 and it spread quickly to all the major citrus-growing regions within the state in less than 3 years (Halbert et al., 2012). The unregulated movement of *D. citri*-infested nursery stock, both citrus and *Murraya paniculata*, is believed to have been the major contributing factor in the spread of both *D. citri* and HLB throughout Florida (Halbert et al., 2000, 2012) and in the interstate movement of *D. citri* from Florida to Texas (French et al., 2001). In 2009, over 10% of regulatory *D. citri* samples collected in Florida retail outlets tested positive for *Candidatus* Liberibacter asiaticus, and it took an average of 9 months after positive *D. citri* were detected for inspectors to find symptomatic plants that tested positive for the pathogen (Halbert et al., 2012). At many of the retail outlets where positive *D. citri*, no symptomatic plants were ever discovered, indicating that infected plants were already sold to homeowners.

Recognizing the importance of the passive dispersal of the vector on nursery stock, the detection of *D. citri* in California triggered a comprehensive effort on the part of state and federal regulators, scientists, and citrus industry stakeholders to implement measures that would limit its spread. The California Department of Food and Agriculture (CDFA) established quarantines that restricted the movement of *D. citri* host plants from areas known to be infested with *D. citri*. Production nurseries within quarantine areas are still required to treat all citrus nursery stock, and other *D. citri* host plants, with both an approved foliar insecticide and a systemic neonicotinoid insecticide in order to receive a 90-day certification, during which time plants may be shipped from the production facility to retail outlets. For shipments outside of the quarantine areas, including inter-state movement, nurseries must apply these treatments no more than 90 days, and no less than 30 days, prior to shipment (CDFA, 2017).

Currently, all existing *D. citri* quarantine control requirements apply only to production nurseries. There are no treatment requirements for retail outlets, a decision likely guided by the expectation that plants would reside at these nurseries for a short time. As a result, the residency time of citrus trees at retail nurseries may represent a critical window for *D. citri* infestation and spread, particularly if the 90-day certification period is exceeded. Furthermore, there is an increased likelihood that overwatering of trees at retail nurseries may contribute to lower neonicotinoid residues due to leaching of insecticide from pots (Cox et al., 1997; Liu et al., 2006).

In this study, we investigated the relationship between residency time at retail outlets and declining imidacloprid titers in containerized citrus trees, as well as the interaction between these parameters and the increased levels of *D. citri* infestation. To achieve this, we conducted an independent survey of citrus trees in retail outlets in Southern California and determined their imidacloprid concentrations and treatment histories from CDFA records.

To examine the effect of irrigation on insecticide titer, we conducted an outdoor trial in which potted trees treated systemically with imidacloprid were maintained under different watering schedules. We also incorporated citrus species and soil mix variables into our experimental plan. We were particularly interested in comparing citrus species with contrasting flushing patterns, and hypothesized that species with more frequent flushing habits would be limited in their capacities to maintain effective concentrations of imidacloprid due to the diluting effect of the new flush on available residues. Such trees would be at greater risk of infestation because the newer tissue is favored by *D. citri* for feeding and oviposition (Catling, 1970).

2. Materials and methods

2.1. CDFA nursery inspections

Following the establishment of the quarantine areas in California, the CDFA coordinated numerous inspections of production and retail nurseries to ensure compliance with regulations. When *D. citri* were detected at retail stores, a numerical tagging system that was implemented as part of quarantine regulations, permitted accurate traceability of infested trees to determine their origin and treatment histories. These data were documented by CDFA inspectors and represent an early record of the number of *D. citri*-infested nursery shipments as a function of the time since insecticide application. We were permitted access to this CDFA database to investigate a potential link between the incidence of *D. citri* infestations and extended residency times of trees at the retail outlets. Because no insecticide residue data were collected at the time of the inspections, the residency time would serve as a proxy for insecticide levels, with the expectation that titers would decline within the trees as the residency time lengthened.

Between May 2011 and June 2013, CDFA inspectors documented a total of 434 nursery shipments in Los Angeles (187), Orange (49), Riverside (118), San Bernardino (79), and San Diego (1) Counties, in which *D. citri* life stages were present on at least one plant in the shipment. Unfortunately, the number of *D. citri*-free nursery shipments was not recorded, so a formal analysis of the frequency of *D. citri* occurrence in nursery stock could not be determined. Nonetheless, the summary statistics for the dataset are presented as an indication of the scale of *D. citri* occurrence on nursery stock during this time period relative to the time elapsed since the trees were treated.

2.2. Detailed nursery inspections

To study the relationships among residence times, insecticide residues, and *D. citri* infestation on retail citrus trees, we conducted a set of detailed inspections at 29 randomly selected independent retail nurseries (14) and chain store garden centers (15) from Southern California. We compared these two types of retail sites because of our expectation that residence times of trees at independent nurseries might be longer due to a slower turnover of a more diverse citrus inventory, in contrast to the less specialized chain store garden centers that bulk buy more popular varieties that will provide them with a more rapid turnover of citrus stock. The inspections were conducted in Riverside, San Bernardino, and San Diego Counties between January and November 2013. Although each site was surveyed for all common *D. citri* host plants (CDFA, 2018), no non-citrus hosts were found during the surveys. At both types of retail sites, we inspected citrus nursery stock for the presence of *D. citri* with two-minute timed visual searches of each tree, noting separately the presence or absence of *D. citri* adults or any juvenile stages (i.e. eggs or nymphs). We visually inspected all citrus nursery stock at sites that had less than 50 citrus trees; at sites with 50 or greater citrus trees we randomly selected at least 20 trees for inspection. For each inspection, we recorded the predominant category of flush, the species, the trunk diameter at 5 cm above the graft union, and the shipment tag number. For flush characterization, we recorded the predominant stage of foliage present on each side of the tree, using four categories of classification ranging from new “feather flush” to mature “hardened-off” leaves: A) “feather flush”: most fragile leaves, light green (petiole) with pink hue (apical buds/leaves), ranging from 0.1 to 1 cm in length; B) less fragile leaves, light green (pink/red hue more pronounced) on apical leaves, ranging from 1.5 to 3 cm; C) leaves with much greater integrity (more firm), darker green (loss of red tint), ranging from 3.5 to 5 cm; D) “hardened-off”: largest of the leaves on the tree, full coloration (darkest green) on the entire tree (red tint absent), coarse, thick, and often brittle. Finally, for at least 20 of the trees inspected at each site, we collected a minimum of 4 leaf tissue samples that represented the youngest flush category available for imidacloprid