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Efficacy of an autodisseminator of an entomopathogenic fungus, *Isaria fumosorosea*, to suppress Asian citrus psyllid, *Diaphorina citri*, under greenhouse conditions

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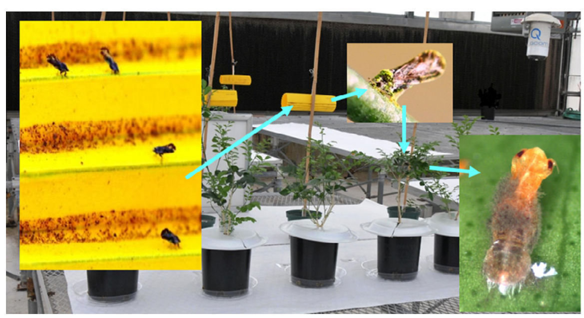
HIGHLIGHTS

- We tested an autodisseminator for inoculating Asian citrus psyllid with an entomopathogen.
- In greenhouse tests, 55% of the adults became infected and died.
- Adult psyllids acquired fungal spores and spread them to immatures.
- Nymphal mortality increased and adult production decreased.
- *Isaria fumosorosea* blastospores are sensitive to direct sunlight.

GRAPHICAL ABSTRACT



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ABSTRACT

We are developing an autodisseminator ('dispenser') to inoculate Asian citrus psyllid with entomopathogens for the purpose of inducing epizootics in residential trees and abandoned groves, areas where chemical control is problematic. The dispenser prototype consisted of a bright yellow pleated tube coated with a spore formulation made from pulverized cotton burrs and blastospores of *Isaria fumosorosea*. Adult psyllids were released within an array of dispensers and potted citrus saplings in a greenhouse. After 24 h, they were collected from the foliage, surface sterilized, and placed in an incubator. A mean of 55% of the adults developed mycosis ($n = 3$ tests), demonstrating that the dispensers could cause primary infection. The potted plants used in the horizontal transmission tests were infested with immature psyllids; 27–35% of which became infected following contact with adults that had visited the dispensers ($n = 2$ tests). When mycosed adult cadavers with mature conidia were placed near immatures on the potted plants, over 90% of the immatures mycosed, indicating that conidia from the cadavers were highly contagious. On dispensers left in the greenhouse for three weeks, the infectivity of blastospores exposed to direct sunlight decreased by 46% after 7 d and by 60% after 20 d, while infectivity levels remained high in blastospores that were shaded. These results confirmed that that the basic dispenser design was sound with respect to attracting and infecting psyllids. It requires further modification to work effectively under ambient conditions, since exposure to direct sunlight decreased blastospore infectiveness over time.

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1. Introduction

The Asian citrus psyllid (ACP), *Diaphorina citri* Kuwayama (Hemiptera: Liviidae), transmits *Candidatus* Liberibacter asiaticus and *Candidatus* Liberibacter americanus, the bacteria that are the causative agents of citrus greening disease or huanglongbing (HLB). This is the most devastating citrus disease in the world today and has resulted in the loss of hundreds of thousands of hectares of orchards and billions of dollars in productivity (Halbert and Manjunath, 2004; Bové, 2006; Gottwald et al., 2007; Grafton-Cardwell et al., 2013). The adults are highly mobile and readily move between residential areas and commercial groves (Tiwari et al., 2010). It is generally accepted by researchers, growers, and regulators that control of ACP outside of commercial orchards will rely heavily on biological control by parasitoids, predators, and pathogens (Grafton-Cardwell et al., 2013; Hall et al., 2012).

ACP is susceptible to a number of native pathogens in the U.S. (Meyer et al., 2007, 2008), some of which show potential as biopesticides for ACP (Zimmermann, 2008; Avery et al., 2009, 2011; Hoy et al., 2010; Hunter et al., 2011; Stauderman et al., 2012) and can be formulated as blastospores for inexpensive mass production (Jackson et al., 2003). For example, in a laboratory study, 94% of ACP adults and immatures (nymphs) were killed within four days of infection by a strain of *Isaria fumosorosea* Wize (=*Paecilomyces fumosorosea*) (Hypocreales: Cordycipitaceae) (*Ifr*) (USDA-Agricultural Research Service Entomopathogenic Fungi Collection (ARSEF), strain 3581) (Moran et al., 2011), originally isolated from sweet potato whitefly biotype B, *Bemisia tabaci* (Gennadius), in southern Texas in 1992 (Wraight et al., 1998; Poprawski and Jackson, 1999; Jackson et al., 2003). Two other *Ifr* strains are commercially available as biopesticides and have demonstrated efficacy against psyllids: PFR 97 20% WDG™ (Certis Inc.) and Ifr 9901 (NoFly™) (Natural Products) (Meyer et al., 2008; Avery et al., 2009, 2011; Hunter et al., 2011). Trials conducted in potato fields using spray applications of PFR 97™, *Metarhizium anisopliae* (F 52™, Novozymes Biologicals) and abamectin (Agri-Mek™, Syngenta) showed reductions in potato psyllid (*Bactericera cockerelli* (Sulc) (=*Paratrioza cockerelli* (Sulc))) eggs and nymphs along with decreased plant damage and symptoms of zebra chip disease caused by *Candidatus* Liberibacter solanacearum (Lacey et al., 2011).

Unfortunately, in residential citrus, abandoned orchards, and similar situations, spray application of biopesticides to control ACP is problematic for a variety of reasons. The challenge then is to develop a means of efficiently inoculating psyllids with pathogen spores for the purpose of inducing epizootics in these situations. A potential mechanism for this undertaking is an autodisseminator of pathogen spores. Conceptually, these devices would be hung from tree branches and would inoculate psyllids when they landed or crawled on them. Precedence for the notion of using an autodisseminator comes from a study by Avery et al. (2009) and who showed that ACP can auto-inoculate while crawling on *Ifr* blastospore-coated cards and then disseminate the spores to leaves. However, autodisseminators have not been used before to control psyllids, so their potential practicality or likely impacts are unknown.

The present study reports on proof-of-concept tests of a prototype autodisseminator used in conjunction with a blastospore formulation of *Ifr* ARSEF strain 3581. The tests were conducted in a greenhouse to ameliorate the extremes in ambient conditions (i.e., temperature, relative humidity, exposure to sunlight, wind, dust, precipitation, etc.) that would have been encountered in field tests. The objectives of this study were to evaluate: (1) the capabilities of this dispenser design and blastospore formulation to elicit primary infection, horizontal transmission, and secondary infection in a greenhouse population of ACP; and, (2) spore durability after exposure over time to ambient conditions within the greenhouse. The results of these studies will be used to design dispensers suitable for use in residential and unmanaged citrus.

2. Methods and materials

2.1. Study site

The tests were conducted in a greenhouse to minimize abiotic effects on the fungal spores and psyllids. During these tests psyllids were released from a cage and could fly freely among an array of dispensers and potted citrus arranged on the greenhouse benches. The greenhouse (25 mL × 12 mW × 6 mH) was located at the former USDA-ARS laboratory in Weslaco, TX. The greenhouse had a system of sliding benches with wire mesh decks that served as a platform for the test materials. The tests were conducted from 2009 to 2011. Environmental parameters in the greenhouse were automatically recorded and controlled by a computer-directed system (HortiMaX, B.V., Pijnacker, The Netherlands) and by data loggers (Onset Computer Corporation, Bourne, MA) hung from plants used in the tests. Greenhouse conditions mirrored summer field conditions in south Texas, with daily average temperatures of (mean ± SEM) 28.5 ± 0.3 °C in the greenhouse and 29.0 °C in the field, respectively. In the greenhouse, daytime high temperatures reached 39.6 °C (±0.6 °C) and relative humidity (RH) declined to 52% (±1.5%). Nighttime (2100–0800 h) minimum daily temperature averaged 22.2 ± 1.1 °C, and relative humidity ranged from 90% to 92%.

2.2. Psyllids

ACP for the primary infection tests were collected from the research orchard at the Texas A&M University-Kingsville's Citrus Center in Weslaco, TX. Colonies of ACP for all other experiments were supplied from the USDA-APHIS-CPHST laboratory in Mission, TX. These ACP colonies were maintained on either orange jasmine, *Murraya paniculata* (L.) grown in greenhouses, or on mature Mexican lime trees, *Citrus aurantifolia* (Christm.) enclosed by screen-cages (366 × 366 × 304 cm) at the laboratory's research grove.

2.3. Plants

Two-to three-year old sour orange (*Citrus aurantium* L.) saplings were used for the primary infection study. They were grown in 2 L plastic pots with a sand-peat-perlite potting mixture containing recommended amounts of time-release macronutrient and micronutrient fertilizers. Plants were pruned to a height of 45–60 cm. To facilitate visual inspection, tracking and sampling of psyllids, the number of stems was reduced by pruning. For all other tests, orange jasmine plants were used. The plants were 1–2 years old, grown in 4 L pots, and pruned to facilitate performing the experiments. Insecticidal soap was used as needed to control pest insects.

2.4. Autodisseminator

The prototype autodisseminator (hereafter referred to as 'dispenser') had several features which potentially enhanced ACP attraction and retention on the device and promoted inoculation (Fig. 1): (1) a bright yellow color, which is highly attractive to ACP (Hall, 2009; Hall et al., 2010; Godfrey et al., 2013); (2) a pleated surface, providing multiple edges, to take advantage of

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