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An evaluation of mosquito repellents and essential plant oils as deterrents of Asian citrus psyllid

David G. Hall[a,*], Dov Borovsky[a], Kamlesh R. Chauhan[b], Robert G. Shatters[a]

[a] USDA-ARS, US Horticultural Research Laboratory, 2001 South Rock Road, Fort Pierce, FL 34945, USA
[b] USDA-ARS, Invasive Insects Biocontrol & Behavior Laboratory, 10300 Baltimore Avenue, Beltsville, MD 20705, USA

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ABSTRACT

A study was conducted to evaluate mosquito repellents against Asian citrus psyllid (ACP), *Diaphorina citri* Kuwayama (Hemiptera: Liviidae) and to expand our knowledge of the potential of essential plant oils as deterrents of ACP infestations. The psyllid vectors a bacterium, '*Candidatus* Liberibacter asiaticus,' presumed causal agent of a serious disease of citrus, huanglongbing (also known as citrus greening disease). Twenty-two candidate deterrents were tested as 20% solutions in methanol using a laboratory assay. Adults were free to enter and settle in one of three vials containing orange jasmine flush, one vial treated with a candidate deterrent, one vial treated with water or one treated with methanol. Seven mosquito repellents (CisPMD, DEET, DHS220, Icaridin, IR-3535, S-220, and TransPMD) and four essential oils (citronella, lavender, lemon eucalyptus, and a commercial mixture of oils from orange, clove, cinnamon, eucalyptus and rosemary) reduced ACP infestations on flush in the vials. Following a 24-h assay period, an average of 75% (range 58–88%) fewer psyllids were found in vials treated with these deterrents as compared to the combined number found in the control vials. Greenhouse evaluations were conducted on phytotoxicity of 5 or 25% solutions of eight deterrents and on ACP colonization of citrus seedlings sprayed with these deterrents. Few ACP colonized seedlings sprayed with 25% solutions, but this was attributed to phytotoxicity. Infestations were reduced on seedlings treated with 5% solutions, but these reductions also were largely attributed to phytotoxicity. Although unsprayed seedlings were in close proximity, adults found these plants and laid relatively large numbers of eggs. While some of the mosquito repellents and essential plant oils deterred infestations, none was completely effective and 5–25% solutions were too phytotoxic to be feasible as direct plant sprays. These results indicated the deterrents as tested hold little potential as a major tactic for reducing ACP infestations in citrus. Alternative application approaches to avoid phytotoxicity could be explored, and individual constituents of the more promising essential plant oils could be investigated.

1. Introduction

Asiatic huanglongbing (HLB) is the most serious citrus disease worldwide. Also known as citrus greening disease, HLB is presumed to be caused by a bacterium '*Candidatus* Liberibacter asiaticus' (CLas) vectored by the Asian citrus psyllid (ACP) *Diaphorina citri* Kuwayama (Hemiptera: Liviidae) (Bové, 2006; Hall et al., 2013a). In the United States of America, HLB has devastated the Florida citrus industry since its discovery in 2005. Hodges and Spreen (2012) estimated a production loss to HLB of $1.7 billion in Florida during the 2006–2007 to 2010–2011 harvest seasons. The citrus acreage in Florida estimated at 290,575 ha (718 thousand acres) in 2001 declined to 198,303 ha (490 thousand acres) by the 2012–2013 harvest season, largely due to HLB (Alvarez et al., 2016). Many growers established intensive insecticide programs against ACP in hopes of managing HLB, however, losses to the disease continue at an alarming rate. Furthermore, the high costs associated with the intensive insecticide programs in conjunction with their adverse environmental effects are not sustainable, thus growers need alternative ACP management tactics. This need is increased given concerns and observations on insecticide resistance in some ACP populations (Tiwari et al., 2011).

Considerable research has been conducted on mosquito repellents, and a number of essential plant oils and their constituents have been identified as repellent to mosquitos (Maia and Moore, 2011). The most well-known mosquito repellent is DEET (Fradin and Day, 2002; Cilek et al., 2004; Leal, 2014). Other mosquito repellents include IR-3535

* Corresponding author.
E-mail addresses: David.Hall@ars.usda.gov (D.G. Hall), dovborovsky@gmail.com (D. Borovsky), Kamal.Chauhan@ars.usda.gov (K.R. Chauhan), Robert.Shatters@ars.usda.gov (R.G. Shatters).

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Table 1
Mosquito repellents evaluated for deterrents to Asian citrus psyllid. All liquids were initially tested as 20% solutions of product in methanol. Two solids (CisPMD and TransPMD) were tested using the equivalent of 20 mg of product in 100 μL methanol. DHS220 and ILFP were not completely compatible with methanol; continual agitation was required to keep these materials in solution.

Chemical	Purity, Source
CisPMD	100%, p-menthane-3,8-diol [(+)-Cis-P-Menthane-3,8-Diol]; product R751898, Sigma-Aldrich, St. Louis, MO, US$125/25 mg
DEET	97% *N,N*-diethyl-*m*-toluamide (=diethyl toluamide); product D100951, Sigma-Aldrich, St. Louis, MO, US$55.44/500 g
DHILeF	100%, provided by K. R. Chauhan, USDA-ARS, Invasive Insects Biocontrol & Behavior Laboratory, Beltsville, MD, Zhang et al. (2009)
ILFP	100%, provided by K. R. Chauhan, USDA-ARS, Invasive Insects Biocontrol & Behavior Laboratory, Beltsville, MD, Zhang et al. (2009)
DHS220	100%, provided by K. R. Chauhan, USDA-ARS, Invasive Insects Biocontrol & Behavior Laboratory, Beltsville, MD, patent pending, Frances et al. (2009)
Icaridin	100%, hydroxyethyl isobutyl piperidine carboxylate [nepetalactone (catnip oil)]; provided by K. R. Chauhan, USDA-ARS, Invasive Insects Biocontrol & Behavior Laboratory, Beltsville, MD, commercially available
IR-3535	100%, 3-[n-N-butyl-N-acetyl] aminopropionic acid ethyl ester; product CDS003177, Sigma-Aldrich, St. Louis, MO, US$50/g
S-220	100%, (1S, 29S)-2-methylpiperindinyl-3-cyclohexen-1-carboxamide; provided by K. R. Chauhan, USDA-ARS, Invasive Insects Biocontrol & Behavior Laboratory, Beltsville, MD, patent pending, Frances et al. (2009)
TransPMD	100%, p-menthane-3,8-diol [(−)-Trans-P-Menthane −3,8-Diol]; product R426601, Sigma-Aldrich, St. Louis, MO, US$125/250 mg

(Fradin and Day, 2002; Cilek et al., 2004; Collins et al., 1993); PMD isomers, (cis- and trans-) (Collins et al., 1993); icaridin, also known as picaridin (Klun et al., 2006); and S-220, a commercially viable version of SS220 (Frances et al., 2009). USDA-ARS (Invasive Insects Biocontrol & Behavior Laboratory, Beltsville, MD) has developed three synthetic compounds that repel mosquitos: DHS220 (dihydro S-220) (Frances et al., 2009), DHILeF (dihydroisolongifolenone) and ILFP (cycloacetal of dihydroisolongifolenone) (Zhang et al., 2009).

In addition to repelling mosquitos, essential plant oils are known to repel other types of arthropods including certain species of ants (Wagan et al., 2016a); beetles (Agarwal et al., 2001; Chaubey, 2012; Obeng-Ofori and Reichmuth, 1997); moths (Landolt et al., 1999; Zhang et al., 2004); sandflies (Valerio and Maroli, 2005); spider mites (Miresmailli and Isman, 2006); and ticks (Schreck et al., 1995) as well as two hemipteran groups, aphids (Assis et al., 2007) and whiteflies (Baldin et al., 2013; Salas, 2001). Some essential plant oils not only repel insects, they may suppress oviposition (Chaubey, 2012; Zhang et al., 2004; Baldin et al., 2013; Wagan et al., 2016b), some may discourage feeding, and many essential oils are toxic to insects (Cloyd et al., 2009).

A number of essential plant oils have been investigated as ACP repellents/infestation deterrents, primarily in laboratory olfaction experiments. Working with a two-port divided T-olfactometer, Mann et al. (2011) reported that garlic chive essential oil was repellent to ACP and that four constituents of the oil were significantly repellent at 0.5 and 1.0% concentrations. Similar olfaction experiments demonstrated repellency to ACP of the following essential oils evaluated as 0.5% solutions in ethylene glycol: coriander, lavender, rose, thyme, and tea tree, as well as the major constituent of rue oil (Mann et al., 2012). In addition to being repellent to ACP, some essential oils including coriander and lavender were found to be toxic to adult ACP (Mann et al., 2012). Silva et al. (2016) reported that guava oil extracts repelled ACP using a laboratory vial assay. Onagbola et al. (2011) reported that guava leaf volatiles and notably the constituent dimethyl disulfide inhibited ACP behavior in laboratory olfactometers, and that dimethyl disulfide deployed in polyethylene vials hung in citrus trees reduced populations of ACP. Kuhns et al. (2016) reported that 5 and 15% solutions of Canadian fir needle oil repelled ACP females using olfactometer assays, whereas *Litsea* and citronella oils did not. ACP avoided plants treated with fir oil in laboratory choice studies, and in a field study depending on release rates, fewer numbers of ACP were observed in plots of citrus trees where dispensers of fir oil were deployed (Kuhns et al., 2016). Three repellent compounds associated with essential oils from 'Murcott' tangor (*Citrus reticulata* x *C. sinensis*) were hypothesized to be responsible for reduced ACP attraction to this genotype (Andrade et al., 2016).

The objectives of research presented here were to evaluate mosquito repellents against ACP and to expand our knowledge of a number of essential plant oils as potential ACP deterrents. A laboratory vial assay was used to screen 22 candidate deterrents (10 specific chemicals and 12 essential plant oils) at a concentration of 20% because this is the concentration at which mosquito repellents are typically studied. Three potential deterrents were studied across a wide range of concentrations using the same assay. These assays were followed by greenhouse evaluations on ACP colonization of citrus seedlings directly sprayed with 5 or 25% solutions of eight potential deterrents including five mosquito repellents and three essential plant oils.

2. Materials and methods

ACP adults were obtained from a colony established in 2000 at the USDA-ARS U.S. Horticultural Research Laboratory (Fort Pierce, FL). The psyllids were originally collected from citrus in the field during 2000 and subsequently reared in an air-conditioned greenhouse in cages containing orange jasmine (*Murraya exotica* L. = *Murraya paniculata* auct. non.) until March 2010, when *Citrus macrophylla* Wester was substituted as the rearing plant. The colony is maintained using procedures similar to those described by Skelley and Hoy (2004), with no infusion of wild types. The colony is checked quarterly by qPRC (Li et al., 2006) to confirm that it is not infected by CLas. The colony is not exposed to insecticides.

2.1. Laboratory assays on 20% solutions of candidate deterrents

A vial assay (Hall et al., 2015) was used to evaluate 22 candidate infestation deterrents (Tables 1 and 2). Briefly, fresh young orange jasmine flush shoots with two leaves were excised from greenhouse plants and inserted into 25 dram clear-plastic tubes (#8925, BioQuip Products, Inc., Rancho Dominguez, CA) as previously described (Hall et al., 2015). The exposed portion of each leaf averaged 5.9 ± 0.1 cm (± S.E.M) in length, and each leaf had leaflets appropriate for ACP oviposition. A white plastic lid with a hole (6 mm) was then snapped onto each vial. A light grey paper tube was slipped over the vial to hide the contents. To evaluate each candidate deterrent, three vials with jasmine shoots were placed together onto the floor of a cage (30 × 30 × 30 cm, Bugdorm-1 insect cage, DP1000, Megaview Science Co., Ltd., Taichung, Taiwan). A 20% solution (5 μL) of a candidate deterrent in methanol was pipetted onto a small piece of filter paper surrounding the entrance hole of a vial's lid (Hall et al., 2015). A second vial was similarly treated with methanol (5 μL) and the third vial was treated with tap water (5 μL). The three vials were placed on the cage floor positioned ~14 cm from each other emulating the 3 points of a triangle with equal sides. The cage was kept in an environmental chamber at 28 °C, 14 h daily illumination, ambient humidity. Fifty adult ACP (no regard to sex) were aspirated into a small glass vial, sealed with a lid, the vial was placed on the floor at the center of the cage, the lid was then removed, and the location of the adults in the cage was determined 24 h later.

The assay vials were used multiple times. After each 24 h assay, all