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Mitigating trans-boundary movement of *Bemisia tabaci* (Hemiptera: Aleyrodidae) on *Mentha* sp. by pre-shipping treatments of biopesticides

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ABSTRACT

Bemisia tabaci (Gennadius) (Hemiptera: Aleyrodidae) cryptic species complex represents a major threat to several important crops including vegetables, cereals, fruits, and ornamentals worldwide. One important mode of its dispersal is through the trans-boundary (domestic, international) movement of infested plant materials. To prevent the spread of this invasive insect pest on inter-state ornamental shipments, the efficacy of biopesticides was tested through dip treatments of plant material pre-shipping. In several experiments, clean *Mentha* sp. (Lamiaceae), mint plants were exposed to adults of *B. tabaci* for 1 or 6 days. Adults were removed from plants and eggs, and immature stages were treated with one or a combination of the following: entomopathogenic fungi *Beauveria bassiana* (BotaniGard®), *Isaria fumosorosea* (Preferal®), the surfactant Natur'l Oil™, and soap (Publix®). After treatment application, plants were placed in commercial cardboard boxes and shipped from Apopka, Florida (FL) to Fort Pierce, FL. Assessments for the treatment efficacies were conducted at 3, 7, and 14 days post arrival of the shipment. Fungal entomopathogens; *B. bassiana, I. fumosorosea*, and the surfactant Natur'l Oil™, showed significant reduction in the whitefly population compared to the untreated control ($P < 0.05$). Significantly higher corrected mortality of the whitefly populations was observed when *B. bassiana* was applied alone (81%) or in combination with Natur'l Oil™ (86%), as well as *I. fumosorosea* applied in combination with Natur'l Oil™ (77%). Our results showed that pre-shipping dip treatment applications of either Natur'l Oil™ mixed with *B. bassiana* or *I. fumosorosea* or *B. bassiana* applied alone could help mitigate the inter-state spread of whitefly on ornamental shipments.

1. Introduction

In the past decade, the globalization and international trade of living plant materials such as ornamentals have played an important role in the dispersal and establishment of many invasive pests including *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) into new territories (Chu et al., 2006; Drayton et al., 2009; Kumar et al., 2013; Buitenhuis et al., 2016). *Bemisia tabaci* is one of the most destructive invasive insect pests worldwide that has been reported affecting more than 600 plant species (Oliveira et al., 2001). Apart from causing feeding damage, *B. tabaci* vectors more than 100 plant damaging viruses (Jones, 2003). According to De Barro et al. (2011), losses in crop production have increased drastically since the 1980s due to the spread of new virulent morphologically indistinguishable species (previously known as biotypes) of *B. tabaci* around the world. Perring (2001) had suggested that both Middle East-Asia Minor 1 (MEAM1) and Mediterranean (MED), also known as biotypes B and Q respectively, are the most aggressive and invasive *B. tabaci* species affecting multiple crop production systems. Since the greenhouse and nursery productions are important for the United States (US) economy, and especially in Florida which is the second largest contributor of the horticultural industry after California (USDA, 2014), an invasive pest such as *B. tabaci*, known to be resistant to synthetic insecticides (Horowitz et al., 2004; Dennehy et al., 2005; Caballero et al., 2013) is an issue that concerns producers,

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Table 1
Details of the surfactants and biopesticides used in different studies.

Product name	Company	Concentration	Description of product
Publix® dishwashing soap	Publix	1%	Dishwashing soap detergent/anionic and non-ionic surfactant
Natur'l Oil™	Stoller USA	1%	Vegetable oil/non-ionic surfactant
WetCit®	Oro Agri, Inc.	1%	Wetting agent/ionic surfactant
Vapor Gard®	Miller Chemical and Fertilizer Corporation	1%	96% di-1-p Menthene (Pinolene). Non-ionic, anti-transpirant concentrate
Barricade II® Fire Blocking Gel	Barricade International, Inc.	1%	Fire suppression gel
WashGard®	Pace International, LLC	1%	Carnauba wax adjuvant
Preferal™	SePRO Corporation	2.1 g/L	Entomopathogenic fungi *Isaria fumosorosea* Apopka strain 97 20% WDG (1×10^9 CFUs/g)
BotaniGard®	Laverlam International Corporation	2.4 g/L	Entomopathogenic fungi *Beauveria bassiana* strain GHA 22WP (2×10^{13} spores/lb),

sanitary authorities, and customers. Considering the limitations on the use of certain effective chemistries, and low tolerance of the ornamental pests on the export items, the presence of *B. tabaci* on the plant shipment can lead to rejection of the inspected plant materials, and may risk future businesses. Thus, the dispersal of this aggressive pest from Florida which has both MEAM1 and MED, to other locations through shipment of ornamental plants can have significant implications on the US economy and potentially lead to the loss of agricultural jobs.

In a recent survey of *B. tabaci* in North America, McKenzie et al. (2012) detected MED in 23 states of the US and in Bermuda, Mexico, and Canada. It was found in protected commercial horticultural greenhouse plantings of 45% of all collections of ornamental and herb plants and a single tomato transplant collection, but never detected in open-field agriculture. However, in 2016, out of the 53 positive confirmations of MED from 12 counties of Florida, about 30 detections came from retail garden centers or wholesale nurseries, and the others were detected in residential landscapes and open-field agriculture (McKenzie and Osborne, 2017; Kumar et al., 2017a). This was the first out-break report of MED whitefly outside the protected production systems in the US, presenting direct evidence of *B. tabaci* spread with the movement of infested plant materials. In the past, several researchers indicated the possible role of infested plant materials in the dispersal of *B. tabaci*, and other invasive pests in parts of Africa, Europe, North and South America (Navia et al., 2010; Cuthbertson et al., 2011; Saccaggi, and Pieterse, 2013; Kumar et al., 2014; Cuthbertson and Vänninen 2015; Buitenhuis et al., 2016). Such activities assisting invasion and establishment of a highly pestiferous species pose a significant threat to ornamentals, vegetables and other economically important hosts including cotton grown in the southern US.

Bemisia tabaci has developed resistance to different groups of synthetic insecticides in the US and elsewhere (Horowitz et al., 2004; Dennehy et al., 2005; Caballero et al., 2013). In order to address problems pertaining to their control and reduce the development of resistance to certain chemical classes of insecticides, new alternative products such as horticultural oils, surfactants, botanicals, and natural enemies were evaluated against *B. tabaci* (Osborne et al., 2008; Mascarin et al., 2014; Cuthbertson and Collins, 2015). In recent years, entomopathogenic fungi *Beauveria bassiana* (Bals.-Criv.) Vuill. (Hypocreales: Clavicipitaceae) and *Isaria fumosorosea* Wize [=*Paecilomyces fumosoroseus* (Hypocreales: Cordycipitaceae)] have received considerable attention as biological control agents of whitefly and other important invasive insect pests (Wraight et al., 2000; Lacey et al., 2008; Kumar et al., 2017b). Natural occurrence of *Isaria* sp. with high virulence against nymphs of *B. tabaci* was reported by Cabanillas and Jones (2009) in Texas. Wraight et al. (2000) reported more than 85% mortality of immature stages of *B. argentifolli* resulted after multiple applications of *I. fumosorosea* and *B. bassiana* on cucumber and melons.

Considering the passive dispersal of *B. tabaci* through movement of infested plant materials as an important mode for their invasion into new regions, and regulations on the use of certain chemical classes before shipping of the plant materials, Osborne et al. (2008) performed a pilot study and evaluated the potential use of *I. fumosorosea* for control of MEAM1 on ornamental plants under simulated shipping conditions. In the simulation study, high cumulative mortality (> 80%) of *B. tabaci* fourth instar (L4) nymphs was reported for *I. fumosorosea* 7 days post-treatment on infested poinsettia plants. These results encouraged and warranted conducting further studies under actual shipping conditions, using regimes of soft insecticides commonly used for whitefly control. Thus, in the current study, we evaluated different fungal biopesticides and surfactants, applied alone or as combination treatments against immature stages of MEAM1 under real shipping conditions. The specific objectives of this study were: 1) to evaluate the compatibility of surfactants with commercial formulations of the *B. bassiana* and *I. fumosorosea* and 2) evaluate the effectiveness of entomopathogenic fungi alone and in combination with selected surfactants against *B. tabaci* applied pre-shipment of the plant material.

2. Materials and methods

2.1. Insecticides

The entomopathogenic fungal products used in this study were Preferal™ (microbial insecticide), a blastopore formulation of *Isaria fumosorosea* Apopka 97 strain (20% WDG, 1×10^9 CFU/g) (SePRO Corporation, Carmel, IN) and BotaniGard® 22WP (2×10^{13} spores/lb), a wettable powder conidial formulation of *Beauveria bassiana* strain GHA (Laverlam International Corporation, Butte, MT). In addition, six surfactants that were hypothesized as potential synergists were tested alone or in combination with the entomopathogenic fungi. Description of the products, concentrations and their manufacturers are listed in Table 1.

2.2. Source of insects

The whitefly, *B. tabaci* used for experiments were obtained from a laboratory colony of MEAM1 maintained at the Mid-Florida Research and Education Center (MREC), University of Florida in Apopka, FL (28.63N, 81.55W). The colony was maintained and reared on mint plants, *Mentha* sp. (L) (Lamiaceae) in an insectary room at 24 ± 2 °C, 60–80% RH and 12 h light (L):12 h dark (D) photoperiod.

2.3. Compatibility assessment of entomopathogenic fungi and surfactants

Before testing the efficacy of two commercially available entomopathogenic fungal products alone or in combination with surfactants under shipping conditions, their compatibility with different surfactants were assessed under sterile laboratory conditions following the protocol of Avery et al. (2013). All surfactants were mixed at 1%