Development of Bemisia tabaci MEAM1 and MED on tomato (Solanum lycopersicum) alone and in a mixed population

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Abstract

The invasive whitefly species Bemisia tabaci MED (Gennadius) (Hemiptera: Aleyrodidae) has demonstrated the ability to develop higher levels of insecticide resistance than B. tabaci MEAM1, leading MED to displace MEAM1 in some regions where insecticide resistance management is not practiced. Displacement of MEAM1 by MED is influenced also by host plant. MED established recently in the Florida landscape, making it necessary to evaluate the risk that MED will displace MEAM1 on tomato and other economically important crops. The development of MEAM1 and MED was observed on tomato (cv. 'Florida 91') 30, 45, and 70 d after inoculation separately and in the presence of the other species. MEAM1 were more abundant than MED on plants where both were combined 30, 45, and 70 d after inoculation. MEAM1 reached higher numbers than MED on plants where they were established separately 30 and 70 d after inoculation. At 70 d after inoculation, there were significantly more MED on tomato plants where MEAM1 was not present than on plants infested with both species. Our results indicate that MEAM1 has a competitive advantage over MED on tomato in the absence of insecticide applications. In addition, we tested 13 populations of MEAM1 from commercial vegetable fields and 2 populations of MED from residential hibiscus for tolerance to imidacloprid and thiamethoxam. The 2 MED populations did not demonstrate high levels of tolerance to these insecticides relative to the MEAM1 populations. Our results suggest that the displacement of MEAM1 by MED in Florida tomato fields is unlikely at the present time.

Key Words: sweetpotato whitefly; invasive species; competition; resistance monitoring

Resumen

La especie invasora de mosca blanca Bemisia tabaci MED (Gennadius) (Hemiptera: Aleyrodidae) ha demostrado la capacidad de desarrollar niveles más altos de resistencia a los insecticidas que B. tabaci MEAM1, lo que lleva a MED a desplazar a MEAM1 en algunas regiones cuando no se practica el manejo de la resistencia a los insecticidas. El desplazamiento de MEAM1 por MED está influenciado también por la planta hospedera. MED se estableció recientemente en los campos de la Florida, por lo que es necesario evaluar el riesgo de que MED desplace a MEAM1 en el tomate y otros cultivos económicamente importantes. El desarrollo de MEAM1 y MED se observó en tomate (cv. 'Florida 91') 30, 45 y 70 días después de la inoculación por separado y en presencia de las otras especies. MEAM1 fue más abundante que MED en plantas donde ambas se combinaron 30, 45 y 70 días después de la inoculación. MEAM1 alcanzó números más altos que MED en las plantas donde se establecieron por separado 30 y 70 días después de la inoculación. A los 70 días después de la inoculación, hubo significativamente más MED en las plantas de tomate donde MEAM1 no estaba presente que en las plantas infestadas con ambas especies. Nuestros resultados indican que MEAM1 tiene una ventaja competitiva sobre MED en tomate en ausencia de aplicaciones de insecticidas. Además, probamos 13 poblaciones de MEAM1 de campos de hortalizas comerciales y 2 poblaciones de MED de hibisco residencial para determinar la tolerancia al imidacloprid y al thiamethoxam. Las 2 poblaciones MED no demostraron altos niveles de tolerancia a estos insecticidas en relación con las poblaciones MEAM1. Nuestros resultados sugieren que el desplazamiento de MEAM1 por MED en los campos de tomate de Florida en las condiciones actuales es poco probable.

Palabras Clave: mosca blanca del camote; especies invasivas; competencia; monitoreo de resistencia
significant losses in tomato each yr by transmitting tomato yellow leaf curl virus, a begomovirus, and by inducing irregular ripening, a plant disorder associated with feeding by *B. tabaci* nymphs (Schuster et al. 2008).

*Bemisia tabaci* Mediterranean (MED) has been detected in Florida since 2005, initially on potted plants in the nursery section of box stores (McKenzie et al. 2009). In 2016, populations of *B. tabaci* MED were detected in multiple counties in Florida established on landscape plants in residential areas (McKenzie & Osborne 2017). MEAM1 and MED are characterized by the ability to develop high levels of resistance to a broad range of key insecticides (Castle et al. 2010). MED has demonstrated the ability to develop significantly higher levels of resistance to many modes of action than MEAM1, and expresses higher levels of detoxification compounds including glutathione s-transferases (Ye et al. 2014; He et al. 2018). The tendency of MED to develop higher levels of insecticide resistance than MEAM1 has contributed to the displacement of MEAM1 by MED in China, where mixed populations of MEAM1 and MED have been subjected to intensive insecticide selection pressure (Pan et al. 2015; Yao et al. 2017). Over-reliance on pyriproxyfen led MED to replace MEAM1 in Israel on tomato, a situation that has been reversed through improved insecticide resistance management (Horovitz et al. 2005; Kotsedalov et al. 2012). In addition to the tendency of MED to develop higher levels of resistance than MEAM1, some populations of MED have demonstrated the ability to retain resistance for multiple generations in the absence of insecticide exposure (Nauen et al. 2002).

Whereas MED tends to displace MEAM1 under intensive insecticide application regimes, MEAM1 demonstrates the ability to outcompete MED on certain host plants in the absence of insecticide selection pressure (Sun et al. 2013). In mixed populations, MEAM1 males are more adept at finding and mating with females of their own type than MED males, and so reproduce more efficiently (Crowder et al. 2010). MEAM1 males interfere with mating by males of other *Bemisia* species, contributing to the dominance of MEAM1 in mixed populations (Liu et al. 2007). In addition to intrinsic differences in reproductive behavior, life history parameters of MEAM1 and MED can vary according to host plant (Lida et al. 2009; Jiao et al. 2012). For example, MEAM1 exhibits a higher intrinsic rate of population increase on tomato than MED at 30°C (Tsueda & Tsuchida 2011), but developmental times on pepper (*Capsicum annuum* L.; Solanaceae) are shorter for MED than MEAM1 (Muniz & Nombela 2001). Thus, the tendency of either MEAM1 or MED to predominate is determined in part by human activity in the form of insecticide applications, and in part by intrinsic biological differences related to reproductive behavior and host plant.

Florida is the foremost producer of fresh market tomatoes in the USA, harvesting over $260 million in 2017 (USDA NASS 2018). Ongoing surveys indicate that MED has not established in field-grown tomatoes in Florida (Smith et al. 2016; McKenzie & Osborne 2017). However, insecticide use is intensive in this crop and insecticide resistant populations of MEAM1 have been detected in Florida tomato fields (Schuster et al. 2010; Smith et al. 2016).

The establishment of MED populations in residential areas on the fringes of Florida agricultural areas makes it necessary to assess the risk that these populations pose to field-produced tomato and other vegetables. MED is not known to cause irregular ripening of tomato; however, Pan et al. (2012) demonstrated that MED was a more efficient vector of tomato yellow leaf curl virus than MEAM1. Lida et al. (2009) found hatching rates for MEAM1 and MED did not differ on tomato (cv. ‘House Momotaro’), but egg-to-adult development was faster on tomato for MEAM1 and nymphal survival was higher. Sun et al. (2013) determined that MEAM1 was able to displace MED on tomato (cv. ‘He-Zou 903’) in 7 generations in the absence of insecticide applications. These reports indicate that MEAM1 has an advantage over MED on tomato. MED is grouped by some researchers into distinct subclades, or cytotypes, and the subclade of MED involved in these studies was not indicated. Subclades vary according to their mitochondrial cytochrome oxidase I (mtCOI) sequences and endosymbiont fauna (Chu et al. 2012; Fujiwara et al. 2015; Hadjistylli et al. 2016). The subclade of MED most commonly recovered in Florida is the Q2 or the Eastern MED (McKenzie & Osborne 2017).

In early 2017, the vegetable entomology program at the University of Florida, Gulf Coast Research and Education Center, Wimauma, Florida, USA, began establishing colonies of MED from infested sites in Palm Beach County to assess the threat that they pose to Florida agriculture. The objectives of the risk assessment are to determine the likelihood that MED populations would displace MEAM1 populations on common vegetables in Florida, either because of higher levels of insecticide resistance or competitive advantage due to host plant. This paper describes the results of a growth room study in which tomato plants of a commonly grown commercial field variety, ‘Florida 91’ (Seminis Vegetable Seeds, St. Louis, Missouri, USA), were inoculated with equal numbers of MEAM1 and MED, either alone or in combination, then analyzed over 3 generations to determine how each species developed on tomato alone and in the presence of the other whitefly species. We hypothesized that our test population of Eastern MED (Q2), collected from a residence in Palm Beach County, would demonstrate a competitive disadvantage on tomato in relation to MEAM1 as MED had in China. However, given that the pest status of MED populations can be influenced by endosymbiotic fauna, host plant, and history of exposure to insecticides, it is difficult to predict how MED populations in Florida will behave based on information derived from countries where growing conditions are in many ways distinct from Florida (Fang et al. 2014; Mouton et al. 2014; Fujiwara et al. 2015; Su et al. 2016). In addition, we present the results of probit analysis for imidacloprid and thiamethoxam tolerance from 2 MED populations collected from hibiscus (*Hibiscus rosasinensis* L.; Malvaceae) in Palm Beach County, and 13 MEAM1 populations collected from commercial vegetable fields in south Florida between late 2016 and mid-2017. The purpose of the resistance monitoring tests was to determine if populations of MED established in Florida demonstrated higher levels of tolerance to common neonicotinoids than populations of MEAM1 found in vegetable fields. Information from other countries, including China and Israel, indicates that highly resistant populations of MED will increase the likelihood that MED will displace MEAM1 (Horowitz et al. 2005; Pan et al. 2015).

**Materials and Methods**

**COMPETITION STUDY**

**Whitefly colonies**

The colony of MEAM1 used in this study was established from whiteflies collected near Bradenton, Florida, USA, in the early 1990s. It has not been exposed to insecticides since then, and is used as the susceptible strain for resistance monitoring studies (Smith et al. 2016). It was maintained on cotton (*Gossypium hirsutum* L.; Malvaceae) in a growth room at Gulf Coast Research and Education Center at 27°C (± 2°C), 50 to 75% RH, and 14:10 (L:D) photoperiod. The cotton was grown in an insect-free growth room in 13-cm pots with Fafard potting soil (Fafard No. 2 Sunshine Mix, BWI Inc., Plymouth, Florida, USA) and fertilized with 15:9:12 Osmocote®Plus, (Everris NA, Inc., Dublin, Ohio, USA). Whiteflies and cotton plants were maintained in 60 × 60 × 60 cm PVC frame cages with organyc covers on growth room benches.

The colony of MED, code named MED2-17, was established from whiteflies collected from hibiscus at a residence in West Palm Beach.
in Jul 2017, and maintained under the same conditions as the MEAM1 colony in a separate growth room. The colony was determined to be MED using the methods described by Shatters et al. (2009), and the subclone of the colony was determined using methods described below. Every 2 wk, 2 new cotton plants were added to each cage of whiteflies to provide fresh oviposition substrate for newly emerged females. Every 30 d, 20 adults from each colony were tested for species to confirm the purity of the colony.

Test plants and cages
Tomato ‘Florida 91’ seeds were grown in Fafard No. 2 Sunshine Mix treated with 15:9:12 Osmocote® plus in 128-cell seedling trays (4 x 5.5 cm). Plants were grown in an insect-free growth room at Gulf Coast Research and Education Center under the same conditions used to maintain whitefly colonies. Three weeks after planting, 36 tomato plants were transplanted into 25.4-cm-diam plastic pots and maintained in organdy-covered cages for 1 wk. One wk after transplanting, 12 tomato plants were placed individually inside either a small (34 x 34 x 61 cm), medium (36 x 36 x 122 cm), or large (46 x 46 x 183 cm) cage. Small cages (BioQuip no. 1466BW, Rancho Dominguez, California, USA) were used for plants that were sampled 30 d after inoculation with whiteflies; plants in medium-sized cages were sampled 45 d after inoculation; and plants in large cages were sampled 70 d after inoculation. Cages of different sizes were used to accommodate the growth of tomato plants over time. Sampling or break down of the cage involved collection of all whitefly adults in the cage and destruction of the plant.

Medium and large cages were custom built from 2 cm PVC pipe and organdy cloth with 2 vertical Velcro-sealed openings to allow access for placing whiteflies inside the cage and for tying the tomato stem to a supporting bamboo rod as the plant grew. Potted plants were placed on fiberglass trays measuring 51 x 38 x 2 cm that acted as a water reservoir for the plants. Trays were located outside the small cages and inside the medium and large cages, which in each case allowed for watering the plants without opening cages. Water was applied directly into the tray for small cages and into the tray through the cage mesh for medium and large cages.

Plant inoculation
On the day of inoculation with whiteflies, the tomato plants used were about 4 wk old and possessed 5 to 7 true leaves. Whitefly adults were aspirated from colony cages in groups of 6 males or females into glass eye droppers attached to a Gast vacuum pump (model DOAP704-AA; Fisher Scientific, Pittsburgh, Pennsylvania, USA). A foam plug (Jaee Identi-plug plastic foam stoppers, Fisher Scientific, Pittsburgh, Pennsylvania, USA) positioned at the wide end of the eye dropper allowed air suction, but prevented whiteflies from being drawn into the pump. The narrow end of the eye dropper was sealed with parafilm after 6 adult male or female whiteflies were aspirated into the eye dropper. To confirm that all 6 whiteflies in a given eye dropper were the same gender, whiteflies were checked under a stereomicroscope. Males were distinguished from females by their smaller size and pointed abdomens (Gill 1990).

After confirming the gender and number of whiteflies in each eye dropper, whiteflies were released into the cages according to treatment. A circular piece of construction paper had been placed over the surface of the potting soil to facilitate collection of whitefly adults on the break-down date (30, 45, or 70 d after inoculation). Eye droppers containing whiteflies were placed in a small paper cup with the foam plug end up, and the cup placed on the construction paper at the base of the plant. Then the foam plugs were removed from the eye droppers, allowing the whiteflies to enter the cage. Twelve tomato plants (4 plants from each of the 3 cage sizes) were inoculated with 1 of 3 whitefly treatments: (1) 6 male and 6 female MEAM1, (2) 6 male and 6 female MED, and (3) 6 males and 6 females of both MEAM1 and MED. Each treatment by break-down date combination was replicated 4 times in a randomized complete block design for a total of 36 cages. Only 18 cages fit in a growth room; therefore, 2 growth rooms were used for the experiment.

Whitefly data collection
Thirty d after inoculation, small cages were transferred one at a time from growth rooms to a walk-in cooler set at 3.3 °C to allow the low temperature to immobilize whitefly adults prior to removing the cage covers. A large piece of black plastic was placed underneath the fiberglass trays supporting each cage to facilitate recovery of adults falling away from the cage. The cage cover was then removed and all whitefly adults aspirated from the interior of the cage, plant surface, construction paper at the base of the plant, and from fiberglass trays underneath the pot. Whiteflies were aspirated into eye droppers using the Gast vacuum pump and labeled according to treatment. After aspirating, whiteflies were placed in 95% alcohol and stored at −20 °C. This process was repeated 45 d after inoculation with the medium sized cages, and 70 d after inoculation with the large cages. After collection, whiteflies from each cage were counted and sexed using a stereomicroscope. The species of each whitefly (MEAM1 or MED) was then determined using the DNA extraction and PCR methodology as described by Shatters et al. (2009).

Statistical analysis for competition study
The experimental design was a randomized complete block (r = 4) within each d after inoculation. Adult count data were analyzed using a generalized linear mixed model with a negative binomial distribution, and the default canonical link function was fit to the adult count data using the procedures implemented in SAS PROC GLIMMIX (SAS 2015) (SAS/STAT vers. 14.1, SAS Institute, Cary, North Carolina, USA). Fixed effect factors were D after Inoculation, Treatment (combination of Species and Competition), Sex, and all possible interactions. Random effects were Block (d after inoculation) and Treatment x Block (d after inoculation). Contrast of interest were calculated using the LSMEANS statement of the above named PROC using 3-way interaction estimates.

LC_{50} bioassays
Thirteen MEAM1 and 2 MED populations collected from the field Jan to Jul 2017 were tested for tolerance to neonicotinoid insecticides using the method described in Smith et al. 2016. Whitefly populations were collected from 13 commercial tomato fields in 6 Florida counties by aspirating adults with a backpack aspirator (product #2846, BioQuip Products, Rancho Dominguez, California, USA), and leaving potted tomato plants (cv. ‘Lanai’) on the edges of the field for 3 to 5 d to allow females to oviposit. Aspirated adults and infested tomato plants were placed in organdy cages containing cotton plants in a growth room at Gulf Coast Research and Education Center to allow populations to establish. Populations were collected from hibiscus plants at 2 locations in West Palm Beach by aspirating adults from the plants and bringing foliage infested with whitefly eggs, nymphs, and adults back to Gulf Coast Research and Education Center for establishment on cotton in growth rooms. Growth room temperature, humidity, and light as well as colony maintenance procedures were as described above. Populations were tested using the methods described by Shatters et al. (2009)
to confirm whether they were MEAM1 or MED. Populations confirmed to be MED were analyzed using the procedures described below to determine subclade.

Imidacloprid (Admire Pro, Bayer Crop Science, Raleigh, North Carolina, USA) and thiamethoxam (Platinum 75SG, Syngenta Corporation, Greensboro, North Carolina, USA) were prepared at concentrations of 0.00, 0.16, 0.80, 4.00, 20.00, 100.00, and 200.00 ppm in 50 mL Erlenmeyer flasks. Cotton leaves of similar age were placed with the petiole inserted into the flask and allowed to take up the solution for 72 h. The petiole was trimmed, and the cotton leaf was placed into a glass 100 × 15 mm Petri dish (KIMAX no. 53062/53064-10015, Fisher Scientific, Waltham, Massachusetts, USA) with the abaxial side facing up. Twelve to 14 adult whiteflies were aspirated from colony cages and, after being briefly cooled in a refrigerator to reduce movement, were placed on the leaf inside the Petri dish. Treatments consisted of the 7 concentrations of insecticide, and each treatment was replicated 4 times. Petri dishes were organized in an Office Depot brand 39 × 27 × 14 cm clear plastic storage box with saturated cotton rolls placed around the bottom to maintain humidity. After 72 h, whiteflies were recorded as ‘live’ if they appeared normal, ‘moribund’ if they appeared abnormal and did not respond to prodding with a fine brush, and ‘dead’ if no movement was observed. ‘Moribund’ responses were included with ‘dead’ for analysis. Dose response data were subjected to probit analysis using IBM SPSS (vers. 22) software (IBM Corporation, Armonk, New York, USA) (SPSS 2013).

Determination of MED subclade

Whitefly genomic DNA was extracted using a precipitation protocol from Cenis et al. (1993). The DNA samples were used as templates for PCR to amplify a 820-bp fragment of the mtCOI gene, using the primer pair C1-J-2195 (5'-TTGATTTTTTGTGCATCCAGAAGT-3') and L2-N-3014 (5'-TCAATGCAACTTGCATATA-3') (Simon et al. 1994). The PCR reactions (30 μl) contained 2 μl template DNA, 1 unit Taq polymerase, 3 μl dNTPs (2 mmol per L), 1 μl of 20 μmol per L of each primer, and 3 μl 10 × PCR buffer (Apex, Genesee Scientific, Research Triangle Park, North Carolina, USA). PCR amplifications began with 94 °C denaturation for 3 min, followed by 35 cycles of 94 °C denaturation for 1 min, 52 °C annealing for 1 min, and 72 °C extension for 2 min, and a final 72 °C extension for 10 min. Sanger sequencing of the PCR products was pursued through Genewiz, Inc. (South Plainfield, New Jersey, USA). The mtCOI sequence was aligned with the sequences in the GenBank database by BLASTN program (Altschul et al. 1997) to determine the sequence homology with closely related organisms.

DNA samples also were used as templates to PCR-amplify a 623-bp fragment of the mtCOI gene for PCR-RFLP by using the primer pair C1-J-2195 (5'-TTGATTTTTTGTGCATCCAGAAGT-3') and R-BQ-2819 (5'-CTGAAATTCGGCGAGGCGATTCC-3') (Chu et al. 2012). PCR products were then digested at 37 °C for 2 h with 2 U of Alul, a restriction endonuclease that cleaves DNA at AGCT sites. The Alul-digested PCR products were electrophoresed on a 1.0% agarose gel and visualized by ethidium bromide staining. Based on the size of bands produced by Alul digestion, the subclade (Q1 or Q2) of MED individual was determined (Chu et al. 2012).

Results

COMPETITION STUDY

Treatment, Sex, D after Inoculation, and the interaction between Treatment and D after Inoculation had a significant effect on the number of whiteflies found on tomato plants (Table 1). MEAM1 was more abundant than MED on plants where each species was inoculated separately 30 and 70 d after inoculation and all dates when they were combined (Table 2). There was no difference in numbers of MED on plants where MED was inoculated alone or in the presence of MEAM1 at 30 and 45 d after inoculation. However, at 70 d after inoculation, there were significantly more MED on plants where MEAM1 was not present than on plants that were inoculated with both species. The fact that MEAM1 and MED numbers were not significantly different 45 d after inoculation presumably explains the significant interaction of Treatment and D after Inoculation.

There were significantly more female MEAM1 than female MED on each d after inoculation, both when the 2 species were inoculated on separate plants, and when they were combined except for separate inoculations 45 d after inoculation (Table 3). At 70 d after inoculation, there were significantly more MED females on plants where MED was inoculated alone than on plants where MED was established with MEAM1. There were significantly more MEAM1 males than MED males on each d after inoculation on plants where both species were present (Table 4). On plants where species were inoculated separately, there were significantly more MEAM1 males than MED males at 70 d after inoculation. At 70 d after inoculation, there were significantly more MED males on plants where MED was inoculated alone than on plants where MED was established with MEAM1.

LC50 BIOASSAYS

The LC50 and fiducial limits for imidacloprid and thiamethoxam for the susceptible laboratory MEAM1 population were 0.32 (0.05–1.01) and 0.13 (0.01–0.47) (Table 5). The LC50s and fiducial limits for imidacloprid in the field populations of MEAM1 ranged from 1.56 (0.36–4.48) to 24.12 (0.80–64.34) ppm. Imidacloprid (Admire Pro, Bayer Crop Science, Raleigh, North Carolina, USA) were prepared at concentrations of 0.00, 0.16, 0.80, 4.00, 20.00, 100.00, and 200.00 ppm in 50 mL Erlenmeyer flasks. Cotton leaves of similar age were placed with the petiole inserted into the flask and allowed to take up the solution for 72 h. The petiole was trimmed, and the cotton leaf was placed into a glass 100 × 15 mm Petri dish (KIMAX no. 53062/53064-10015, Fisher Scientific, Waltham, Massachusetts, USA) with the abaxial side facing up. Twelve to 14 adult whiteflies were aspirated from colony cages and, after being briefly cooled in a refrigerator to reduce movement, were placed on the leaf inside the Petri dish. Treatments consisted of the 7 concentrations of insecticide, and each treatment was replicated 4 times. Petri dishes were organized in an Office Depot brand 39 × 27 × 14 cm clear plastic storage box with saturated cotton rolls placed around the bottom to maintain humidity. After 72 h, whiteflies were recorded as ‘live’ if they appeared normal, ‘moribund’ if they appeared abnormal and did not respond to prodding with a fine brush, and ‘dead’ if no movement was observed. ‘Moribund’ responses were included with ‘dead’ for analysis. Dose response data were subjected to probit analysis using IBM SPSS (vers. 22) software (IBM Corporation, Armonk, New York, USA) (SPSS 2013).

| Table 1. | Effect of treatment (MEAM1 and MED alone or in the presence of other species), sex and d after inoculation on numbers of Bemisia tabaci adults on ‘Florida 91’ tomato. |
|---|---|---|
| **Effect** | **Den DF** | **F value** | **P value** |
| Treatment | 3 | 30 | 42.29 | <0.0001 |
| Sex | 1 | 3 | 31.02 | <0.01 |
| Treatment*S | 3 | 30 | 0.84 | 0.482 |
| D after inoculation | 2 | 6 | 148.71 | <0.0001 |
| Treatment*D after inoculation | 6 | 30 | 4.47 | <0.002 |
| Sex*D after inoculation | 2 | 6 | 1.93 | 0.226 |
| Treatment*Sex*D after inoculation | 6 | 30 | 0.79 | 0.583 |

| Table 2. Mean number of MEAM1 and MED adults ± SE (males + females) from tomato plants when inoculated alone or together 30, 45, and 70 d after inoculation. |
|---|---|---|---|
| **D after inoculation** | **Species** | **Alone** | **Other species present** |
| 30 | MEAM1 | 46.0 ± 13.5 a | 44.3 ± 5.69 a |
| 30 | MED | 8.8 ± 5.1 b | 8.8 ± 3.7 b |
| 45 | MEAM1 | 53.8 ± 12.3 a | 144.8 ± 31.7 a |
| 45 | MED | 21.2 ± 3.6 a | 31.0 ± 16.03 b |
| 70 | MEAM1 | 1731.5 ± 563.1 a | 1227.8 ± 423.2 a |
| 70 | MED | 412.8 ± 151.5 B | 90.5 ± 19.9 B |

*Lowercase letters are used to compare means within the same column and d after inoculation group; uppercase letters are used to compare means in the same row. Means within the same cell (D after Inoculation × Species) followed by different lowercase letters are significantly different at P = 0.05; means within the same row followed by a different uppercase letter are significantly different at P = 0.05.*
Table 3. Mean number of MEAM1 and MED females ± SE from tomato plants when inoculated alone or together 30, 45, and 70 d after inoculation.

| D after inoculation | Species       | Alone          | Other species present |
|---------------------|---------------|----------------|-----------------------|
| 30                  | MEAM1         | 32.3 ± 8.6 a*  | 30.0 ± 1.3 a          |
| 30                  | MED           | 3.0 ± 1.7 b    | 6.0 ± 2.8 b           |
| 45                  | MEAM1         | 34.5 ± 9.6 aA  | 102.3 ± 24.6 aB       |
| 45                  | MED           | 14.5 ± 4.6 a   | 24.5 ± 14.2 b         |
| 70                  | MEAM1         | 1332.3 ± 422.4 a | 857.5 ± 300.5 a     |
| 70                  | MED           | 322.5 ± 118.1 bA | 71.8 ± 14.8 bB   |

*Lowercase letters are used to compare means within the same column and d after inoculation group; uppercase letters are used to compare means in the same row. Means within the same cell (D after Inoculation × Species) followed by different lowercase letters are significantly different at \( P = 0.05 \); means within the same row followed by a different uppercase letter are significantly different at \( P = 0.05 \).

Table 4. Mean number of MEAM1 and MED males ± SE from tomato plants when inoculated alone or together 30, 45, and 70 d after inoculation.

| d after inoculation | Species | Alone          | Other species present |
|---------------------|---------|----------------|-----------------------|
| 30                  | MEAM1   | 13.8 ± 5.0 a*  | 14.3 ± 4.7 a          |
| 30                  | MED     | 5.8 ± 3.4 a    | 2.8 ± 0.9 b           |
| 45                  | MEAM1   | 19.3 ± 3.7 a   | 42.5 ± 8.1 a          |
| 45                  | MED     | 6.8 ± 2.3 a    | 6.5 ± 2.1 b           |
| 70                  | MEAM1   | 399.3 ± 170.4 a | 370.3 ± 137.1 a      |
| 70                  | MED     | 90.3 ± 34.4 bA | 18.8 ± 5.5 bB        |

*Lowercase letters are used to compare means within the same column and d after inoculation group; uppercase letters are used to compare means in the same row. Means within the same cell (D after Inoculation × Species) followed by different lowercase letters are significantly different at \( P = 0.05 \); means within the same row followed by a different uppercase letter are significantly different at \( P = 0.05 \).

to 57.09 (36.6–97.2). Only 4 field populations of MEAM1 were tested for tolerance to thiamethoxam. The LC₅₅ and fiducial limits ranged from 3.27 (1.58–6.44) to 23.39 (8.41–77.21). The responses for the 2 MED populations fell within these ranges. Based on non-overlapping fiducial limits, the LC₅₅ for imidacloprid for MED population MED2-17 was higher than for MED1-17, the other MED population, while the LC₅₅ for thiamethoxam was lower. MED2-17 was the MED population used in the competition study. The 2 populations of MED collected from hibiscus in West Palm Beach were determined to belong to the subclade referred to as Q2 or Eastern Q.

Discussion

The average temperature in growth rooms where trials were performed was 26.6 ± 1 °C. A new generation of MEAM1 would begin about every 21 d at 27 °C on tomato, and a new generation of MED would begin about every 22 d (Yang & Chi 2006; Tsueda & Tsuchida 2011). Whiteflies collected 30 d after inoculation comprised the first generation, developing from individuals used to infest the plants on the d of inoculation. Whiteflies collected 45 d after inoculation were the first generation, developing from individuals used to infest the plants from commercial vegetable fields during the same time period underscores the need to routinely test MED populations when they are encountered.

The risk posed by MED is its ability to develop high levels of insecticide resistance. Resistance management guidelines for whiteflies in Florida vegetables emphasize using the treatment window approach to reduce application of the same modes of action to successive gen-
Table 5. LC₅₀s for imidacloprid and thiamethoxam for a susceptible laboratory colony of *Bemisia tabaci* MEAM1, 13 populations of MEAM1, designated by county, collected from vegetables in Florida, and 2 populations of MED collected from hibiscus in West Palm Beach.

| Population       | Imidacloprid | Thiamethoxam |
|------------------|---------------|---------------|
|                  | n  | Slope ± SE | LC₅₀ (ppm) | 95% Fiducial limits | χ² (df) | n  | Slope ± SE | LC₅₀ (ppm) | 95% Fiducial limits | χ² (df) |
| Lab-Susceptible  | 377 | 1.144 ± 0.12 | 0.32 | 0.05–1.01 | 191.97* (27) | 656 | 0.71 ± 0.10 | 0.13 | 0.01–0.47 | 37.12 (28) |
| Manatee2-17      | 378 | 0.71 ± 0.06 | 1.56 | 0.36–4.48 | 121.88* (28) | 373 | 0.96 ± 0.07 | 3.27 | 1.58–6.44 | 68.13* (27) |
| MED1-17*         | 394 | 1.37 ± 0.13 | 1.74 | 0.92–2.78 | 41.10* (22) | 572 | 0.96 ± 0.08 | 11.88 | 7.55–17.30 | 43.78* (28) |
| Collier3-17      | 391 | 0.75 ± 0.09 | 3.33 | 1.54–5.66 | 15.73 (22) | –   | –             | –   | –            | –         |
| Manatee5-17      | 404 | 0.54 ± 0.09 | 3.91 | 0.50–9.91 | 43.07* (22) | –   | –             | –   | –            | –         |
| Manatee3-17      | 390 | 0.70 ± 0.09 | 13.40 | 7.87–20.72 | 28.69 (22) | –   | –             | –   | –            | –         |
| MED2-17*         | 528 | 0.93 ± 0.08 | 13.87 | 9.04–20.11 | 42.51* (28) | 518 | 0.70 ± 0.07 | 4.27 | 2.18–6.98 | 29.54 (28) |
| Manatee4-17      | 381 | 0.94 ± 0.10 | 14.05 | 9.49–19.74 | 16.30 (22) | –   | –             | –   | –            | –         |
| St Johns1-17     | 386 | 0.85 ± 0.09 | 14.01 | 7.91–22.34 | 32.08 (22) | –   | –             | –   | –            | –         |
| Miami-Dade2-17   | 386 | 0.63 ± 0.09 | 15.26 | 8.63–24.46 | 27.65 (22) | –   | –             | –   | –            | –         |
| Hillsborough1-16 | 424 | 0.83 ± 0.06 | 21.80 | 10.62–47.61 | 76.12* (28) | 419 | 0.57 ± 0.05 | 23.39 | 8.41–77.21 | 88.30* (28) |
| Collier2-17      | 367 | 0.71 ± 0.09 | 31.18 | 17.03–57.45 | 33.77 (22) | –   | –             | –   | –            | –         |
| Hendry1-17       | 336 | 0.84 ± 0.07 | 32.50 | 17.11–63.78 | 48.01* (28) | 326 | 1.33 ± 0.12 | 11.40 | 5.85–21.66 | 72.84* (28) |
| Manatee1-16      | 349 | 0.61 ± 0.06 | 33.89 | 9.92–147.47 | 115.83* (28) | 351 | 0.86 ± 0.07 | 16.85 | 7.59–37.49 | 76.39* (28) |
| Hendry2-17       | 397 | 0.61 ± 0.09 | 53.05 | 32.66–95.57 | 16.40 (22) | –   | –             | –   | –            | –         |
| Miami-Dade1-17   | 365 | 0.70 ± 0.10 | 57.09 | 36.62–97.22 | 24.77 (22) | –   | –             | –   | –            | –         |

*Significant at $P < 0.05$.

*MED population collected from hibiscus in West Palm Beach.
erations of the pest (Smith 2013; Stansly et al. 2015a, b). While MED repeatedly has demonstrated the ability to develop high levels of resistance to key insecticides, including imidacloprid and pyriproxyfen, several newer insecticides have demonstrated considerable effectiveness against MED, including dinotefuran (Tokumaru & Hayashida 2010; Higuchi et al. 2016), flupyridafurone (Roditakis et al. 2017), spiriotetramat (Chen et al. 2018), cyantraniliprole (Chen et al. 2018), and pyrifluquinoxazon (Tokumaru & Hayashida 2010; Higuchi et al. 2016). The availability of multiple modes of action to manage MEAM1 and MED reduces the likelihood that 1 mode of action will be overused, and that resistance will develop.

MED is primarily associated with ornamental plants in Florida, and the risk remains that resistant MED populations may develop on ornamental and landscape plants, and may have an advantage over MEAM1 if they migrate into agricultural fields. Unlike vegetable crops, which are harvested 3 to 4 months after planting, perennial plants managed as part of a residential landscape may receive multiple insecticide applications per yr over a period of several yr. This increases the likelihood that whitefly populations established on them will develop resistance, and underlines the importance of developing biocontrol and biopesticide-based programs for managing whiteflies on landscape and ornamental plants (Buss et al. 2017). The greatest risk that MED populations will establish in Florida vegetable fields stems from the possibility that resistant populations of MED will migrate from intensively sprayed landscape plants in residential areas to nearby agricultural fields, where proper resistance management may be lacking. In Florida, the proximity of high-value, intensively managed residential landscapes to field agriculture makes it crucial that horticultural and ornamental entomologists collaborate closely to address the threat presented by MED.

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References Cited

Altschul SF, Madden TL, Schaffer AA, Zhang JH, Zhang Z, Miller W, Lipman DJ. 1997. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Research 25: 3389–3402.

Boykin LM, De Barro PJ. 2014. A practical guide to identifying members of the Bemisia tabaci species complex and the morphologically identical species. Frontiers in Ecology and Evolution 2: 1–5.

Buss EA, Mannion C, Osborne L, Dale A. 2017. Managing whiteflies on landscape ornamentals. Publication ENY 317. Florida Cooperative Extension Service, Institute of Food and Agricultural Sciences, University of Florida, Gainesville, Florida, USA. http://edis.ifas.ufl.edu/pdffiles/MG/MG25400.pdf (last accessed 2 Nov 2019).

Castle SJ, Palumbo JC, Prabhaker N, Horowitz AR, Denhom I, Stansly PA, Naranjo SE. 2010. Ecological determinants of Bemisia tabaci resistance to insecticides, pp. 423–465 In Stansly PA, Naranjo SE [eds.], Bemisia: Bionomics and Management of a Global Pest. Springer, Dordrecht, The Netherlands.

Cenis JL, Perez F, Fereres A. 1993. Identification of aphid (Homoptera: Aphididae) species and clones by random amplified polymorphic DNA. Annals of the Entomological Society of America 86: 545–550.

Chen JC, Wang Z-H, Cao L-J, Gong Y-J, Hoffman AA, Wei SJ. 2018. Toxicity of seven insecticides to different developmental stages of the whitefly Bemisia tabaci MED in multiple field populations of China. Ecotoxicology 27: 742–751.

Chu D, Hu X, Gao C, Zhao H, Nichols RL, Li X. 2012. Use of mitochondrial cytochrome oxidase I polymerase chain reaction-restriction fragment length polymorphism for identifying subclades of Bemisia tabaci Mediterranean group. Journal of Economic Entomology 105: 242–251.

Crowder DW, Sitvarin MJ, Carrière Y. 2010. Mite discrimination in invasive whitefly species. Journal of Insect Behavior 23: 364–380.

Dennehy TJ, Degain BA, Harpold VS, Zaborac M, Morin S, Fabrick JA, Nichols RL, Brown JK, Byrne FJ, Li J. 2010. Extraordinary resistance to insecticides reveals exotic Q biotype of Bemisia tabaci in the New World. Journal of Economic Entomology 103: 2174–2186.

Fang Y-W, Liu LY, Zhang H-L, Jiang D-F, Chu D. 2014. Competitive ability and fitness differences between two introduced populations of the invasive whitefly Bemisia tabaci Q in China. PLoS ONE 9: e100423. doi:10.1371/journal.pone.0100423

Fujishara A, Maekawa K, Tsuchida T. 2015. Genetic groups and endosymbiotic microbiota of the Bemisia tabaci species complex in Japanese agricultural environments. Journal of Applied Entomology 139: 55–66.

Gill RJ. 1990. The morphology of whiteflies, pp. 13–46 In Gerling D [ed.], Whiteflies: Their Bionomics, Pest Status and Management, vol. I. Intercept, Andover, United Kingdom.

Hadjistylli M, Roderick GK, Brown JK. 2016. Global population structure of a worldwide pest and virus vector: genetic diversity and population history of Bemisia tabaci and sibling species. PLoS ONE 11: e0156105. doi:10.1371/journal.pone.0156105

He C, Xie W, Yang X, Wang S-L, Wu Q-J, Wang Y-J. 2018. Identification of glutathione-S-transferase in Bemisia tabaci and evidence that GSTg7 helps explain the difference in insecticide susceptibility between B. tabaci Middle East Asian 1 and Mediterranean. Insect Molecular Biology 27: 22–35.

Higuchi S, Furui T, Goto C, Sakami Y. 2016. Inhibitory effect of two insecticides on tomato yellow leaf curl transmission by Bemisia tabaci Q-biotype. Japanese Journal of Applied Entomology and Zoology 60: 93–96.

Horowitz AR, Kontsedalov S, Khasdan V, Ishaaya I. 2005. Biotypes B and Q of Bemisia tabaci and their relevance to neonicotinoid and pyriproxyfen resistance. Archives of Insect Biochemistry and Physiology 58: 216–225.

Iida H, Kitamura T, Honda K-I. 2009. Comparison of egg-hatching rate, survival rate and development time of the immature stage between B- and Q-biotypes of Bemisia tabaci on various agricultural crops. Applied Entomology and Zoology 44: 267–273.

Jiao X, Xie W, Wang S, Wu Q, Zhou L, Pan H, Liu B, Zhang L. 2012. Host preference and nymph performance of B and Q putative species of Bemisia tabaci on three host plants. Journal of Pest Science 85: 423–430.

Kontsedalov S, Abu-Moch F, Lebedev G, Coskun H, Horowitz AR, Ghanim M. 2012. Bemisia tabaci biotype dynamics and resistance to insecticides in Israel during the years 2008-2010. Journal of Integrative Agriculture 11: 312–320.

Liu SS, De Barro PJ, Xu J, Luan JB, Zang LS, Ruan YM, Wan FH. 2007. Asymmetric mating interactions drive widespread invasion and displacement in a whitefly, Science 318: 1769–1772.

Mckenzie CI, Hodges G, Osborne LS, Byrne FJ, Shatters Jr RG. 2009. Distribution of Bemisia tabaci biotypes in Florida — investigating the Q invasion. Journal of Economic Entomology 102: 670–676.

Mckenzie CI, Osborne LS. 2017. Bemisia tabaci MED (Q biotype) in Florida is on the move to residential landscapes and may impact open-field agriculture. Florida Entomologist 100: 481–484.

Mouton L, Grispou M, Achimastou A, van Waetermeulen X, Roditakis E, Stavrakaki M, Preissner EL, Chu D, Wang S, Wu Q, Carrière Y, Zhou X, Zhang Y. 2015. Characterization of neonicotinoid and pymetrozine resistance in strains of the worldwide pest and virus vector: genetic diversity and population history of Bemisia tabaci and sibling species. PLoS ONE 11: e0156105. doi:10.1371/journal.pone.0156105

Nauen R, Stumpf N, Elbert A. 2002. Toxicological and mechanistic studies on neonicotinoid and pymetrozine resistance in strains of the wide pest. Pest Management Science 71: 452–458.

Nauen R, Tsagkaris A, Pan H, Chu D, Yan W, Su Q, Liu B, Wang S, Wu Q, Xie W, Jiao X, Li R, Yang N, Yang X, Xu B, Brown JK, Zhou X, Zhou Y. 2012. Rapid spread of Tomato yellow leaf curl virus in China is aided differentially by two invasive whitefly species. PLoS ONE 7: e34817. doi:10.1371/journal.pone.0034817

Pan H, Heissner EL, Chu D, Wang S, Wu Q, Carrière Y, Zhou X, Zhang Y. 2015. Insecticides promote viral outbreaks by altering herbivore competition. Ecological Applications 25: 1585–1595.

Qiong R, Chen L, Hong-Yu Z, Jones CM, Devine GJ, Gorman K, Denhom I. 2012. Characterization of neonicotinoid and pymetrozine resistance in strains of Bemisia tabaci from China. Journal of Integrative Agriculture 11: 321–326.

Roditakis E, Stavrakaki M, Grispou M, Achimastou A, van Waetermeulen X, Nauen R, Tsagkarikou A. 2017. Flupyradifurone effectively manages whitefly Bemisia tabaci MED and Tomato yellow leaf curl virus in tomato. Pest Management Science 73: 1574–1584.

SAS. 2015. SAS/STAT, vers. 14.1 SAS Institute, Cary, North Carolina, USA.
Smith et al.: *Bemisia tabaci* on tomato

Schuster DJ, Funderburk JE, Stansly PA. 1996. IPM in tomatoes, pp. 387–411 In Rosen D, Capinera JL [eds.]. Pest Management in the Subtropics: Integrated Pest Management – A Florida Perspective. Intercept Ltd., Andover, United Kingdom.

Schuster DJ, Stansly PA, Gilreath PR, Polston JE. 2008. Management of *Bemisia tabaci*, TYLCV and insecticide resistance in Florida vegetables. Journal of Insect Science 8: 43–44.

Schuster DJ, Mann RS, Toapanta M, Cordero R, Thompson S, Cyman S, Shurtleff A, Morriss RF. 2010. Monitoring neonicotinoid resistance in biotype B of *Bemisia tabaci* in Florida. Pest Management Science 66: 186–195.

Shatters Jr RG, Powell CA, Boykin LM, Liansheng H, McKenzie CL. 2009. Improved DNA barcoding method for *Bemisia tabaci* and related Aleyrodidae: development of universal and *Bemisia tabaci* biotype-specific mitochondrial cytochrome c oxidase I polymerase chain reaction primers. Journal of Economic Entomology 102: 750–758.

Simon C, Frati F, Beckenbach A, Crespi B, Liu H, Flook P. 1994. Evolution, weighting, and phylogenetic utility of mitochondrial gene sequences and a compilation of conserved polymerase chain reaction primers. Annals of the Entomological Society of America 87: 651–701.

Smith HA. 2013. Managing diamide resistance in Florida tomato. Publication ENY 867. Florida Cooperative Extension Service, Institute of Food and Agricultural Sciences, University of Florida, Gainesville, Florida, USA. http://edis.ifas.ufl.edu/in978 (last accessed 2 Nov 2019).

Smith HA, Nagle CA, MacVean CA, McKenzie CL. 2016. Susceptibility of *Bemisia tabaci* MEAM1 (Hemiptera: Aleyrodidae) to imidacloprid, thiamethoxam, dinofuran and flupyradifurone in South Florida. Insects 7: 57. doi: http://10.3390/insects7040057

SPSS. 2013. SPSS Statistics for Windows. IBM Corp., Armonk, New York, USA.

Stansly PA, Smith HA, Kostyk B, McAvoy G, Snodgrass C. 2015a. Managing pests and insecticide resistance in Florida tomato, pp. 31–32 In Proceedings of the Florida Tomato Institute. Naples, Florida, USA, 8–12 Sep 2015.

Stansly PA, Smith HA, Seal DR, McAvoy E, Polston JE, Gilreath PR, Schuster DJ. 2015b. Management of whiteflies, whitefly-transmitted plant virus, and insecticide resistance for vegetable production in southern Florida. Publication ENY 735. Florida Cooperative Extension Service, Institute of Food and Agricultural Sciences, University of Florida, Gainesville, Florida, USA. http://edis.ifas.ufl.edu/in695 (last accessed 2 Nov 2019).

Su M-M, Guo L, Tao Y-L, Zhang Y-J, Wan F-H, Chu D. 2016. Effects of host plant factors on the bacterial communities associated with two whitefly sibling species. PLOS ONE 11: e0152183. doi:10.1371/journal.pone.0152183

Sun D-B, Liu Y-Q, Qin L, Xu J, Li F-F, Liu S-S. 2013. Competitive displacement between two invasive whiteflies: insecticide application and host plant effects. Bulletin of Entomological Research 103: 344–353.

Thompson WMO. 2011. Introduction: whiteflies, geminiviruses and recent events, pp. 1–13 In Thompson WMO [ed.], The Whitefly, *Bemisia tabaci* (Homoptera: Aleyrodidae) Interaction with Gemini-Virus Infected Host Plants. Springer, Berlin, Germany.

Tokumaru S, Hayashida Y. 2010. Pesticide susceptibility of Q-biotype *Bemisia tabaci*. Japanese Journal of Applied Entomology and Zoology 54: 13–21.

Tsueda H, Tsuchida K. 2011. Reproductive differences between Q and B whiteflies, *Bemisia tabaci*, on three host plants and negative interactions in mixed cohorts. Entomologia Experimentalis et Applicata 141: 197–207.

USDA NASS — USDA National Agricultural Statistics Service. 2018. Vegetables 2017 Summary. https://downloads.usda.library.cornell.edu/usda-esmis/fil es/02870v86p/5425kd81z/9019s517t/VegeSumm-02-13-2018.pdf (last accessed 5 Nov 2019).

Yang T-C, Chi H. 2006. Life tables and development of *Bemisia argentifolii* at different temperatures. Journal of Economic Entomology 99: 691–698.

Yao FL, Zheng Y, Huang X-Y, Ding X-L, Zhao J-W, Desneux N, He Y-X, Weng Q-Y. 2017. Dynamics of *Bemisia tabaci* biotypes and insecticide resistance in Fujian province in China during 2005–2014. Scientific Reports 7: 40803. doi: 10.1038/srep40803

Ye X-D, Su Y-L, Su Q-Y, Xia W-Q, Liu S-S, Wang X-W. 2014. Transcriptomic analyses reveal the adaptive features and biological differences of guts from two invasive species. BMC Genomics 15: 370. doi: 10.1186/1471-2164-15-370.