Survey of *Bemisia tabaci* (Hemiptera: Aleyrodidae) in Agricultural Ecosystems in Georgia[1]

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Abstract *Bemisia tabaci* (Gennaduis) is a large complex of cryptic species whose members are invasive pests of economically important commodities, including cotton, vegetables, and ornamental crops. A new state detection of Mediterranean (MED; biotype Q) whitefly on poinsettia from a commercial greenhouse was made in Wisconsin in July 2018, bringing the total positive MED whitefly states to 27, indicating that MED is still expanding its geographical range in the United States. Middle Eastern Asia Minor 1 (MEAM1; biotype B) and MED whiteflies were the primary targets for this survey of agricultural ecosystems from field, greenhouse, and nursery plants. Seventy samples were collected from 19 crops across 23 counties in Georgia, with the bulk of the samples taken in 2016 and 2017. Five whitefly samples were collected in both 2011 and 2012, representing nine counties and five different host plants (verbena, lantana, pepper, cucumber, and poinsettia). Overall, cotton was the most heavily sampled commodity (n = 27), followed by 7 samples of bell or ornamental pepper and 6 samples each of poinsettia, peanut, and squash. Other crops sampled included soybean, cowpea, corn, snap bean, zucchini, kale, tomato, sweet potato, eggplant, cantaloupe, and mum. MED whitefly of the *B. tabaci* cryptic species complex was detected on verbena and lantana in 2011 and poinsettia in 2012 at commercial greenhouses. Only MEAM1 whitefly was detected in all the field grown commodities sampled in Georgia regardless of the year. This survey serves as a baseline for Georgia in the event that MED whiteflies are eventually detected in the field.

Key Words whitefly, MEAM1, MED, vegetables, ornamentals

Bemisia tabaci (Gennaduis) is a cryptic species complex of more than 24 different whitefly species and is considered one of the most notorious agricultural pests throughout the tropical and subtropical regions of the world (Dinsdale et al. 2010). It is a polyphagous pest with the ability to feed on more than 900 plant taxa (Oliveira et al. 2001; Simmons et al. 2008) and transmit over 111 plant viruses (Jones 2003). The capacity of *B. tabaci* to feed on and colonize a seemingly endless number of food, fiber, and ornamental crops has been the central cause of

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its pest status. In the United States, Mediterranean (MED or Q biotype) whitefly, a highly fecund and insecticide-resistant member of the *B. tabaci* species complex, was first documented in 2004 and subsequently detected in 26 states, including Georgia (McKenzie et al. 2012). MED has established as a pest in protected culture but had never been reported in outdoor environments in the United States until 2016 when it was detected in 10 residential landscapes and 2 agricultural field environments in Florida (McKenzie and Osborne 2017).

Environmental conditions in the Southeast were apparently extremely favorable for whitefly population explosions in 2016, with exceptionally high populations and whitefly-transmitted viruses of cucurbits and green bean reported from multiple growers in Florida and Georgia (Gordon 2016; Martini et al. 2016). Severe crop losses were incurred in squash, cucumber, watermelon, and green bean from cucurbit leaf crumple virus and cucurbit yellow stunting disorder virus infections, with the latter being reported in Georgia for the first time (Gadhave et al. 2018). In 2017, whitefly and virus pressure in the Florida panhandle and southern Georgia was so intense that many vegetable growers opted to destroy their plantings and debated whether to replant (Glades Crop Care, pers. comm.). During 2016–2017, virus-like symptoms of unknown etiology were also observed in southeastern cotton fields heavily infested with whitefly and aphids, which has since been determined to be a new virus (cotton leafroll dwarf virus associated with cotton blue disease) transmitted by aphids (Avelar et al. 2019). With high whitefly populations that were difficult to control and new viruses in high-dollar agricultural crops appearing across the Southeast, growers were very concerned if MED whiteflies had also moved into the field in Georgia. To address their concerns, a statewide survey of *B. tabaci* was conducted in 2016 and 2017 across agricultural commodities in Georgia. Data are also presented from earlier collections made in Georgia that were collected after the North American survey (McKenzie et al. 2012) was published and that have not been reported elsewhere.

Materials and Methods

Adults or immature stages of whiteflies were collected directly from the field, nursery, or greenhouse and immediately placed in 95% ethanol for molecular analysis. If available, at least 12 adult whiteflies from each sample were used for species determination following the protocol developed by Shatters et al. (2009). DNA was extracted from individual whiteflies by placing a single whitefly in a 1.5-ml Eppendorf tube, adding 50 μl of DNA lysis buffer, and grinding with a pestle. The pestle was rinsed with an additional 50 μl of DNA lysis buffer and collected in the same tube. Tubes were placed in a metal boiling rack, boiled at 95°C for 5 min, and then placed directly in ice for 5 min. Tubes were then centrifuged at 8,000 × *g* for 30 sec, and the supernatant (crude DNA lysate) was transferred to another tube and stored at −80°C for future processing.

Cryptic species (biotype)-specific polymerase chain reaction (PCR) primers designed by Shatters et al. (2009) were used that amplify unique *mtCOI* gene regions within each of the MEAM1 (B), NEW WORLD (NW), and MED (Q) species and produce different sized products depending on the source of the isolated template DNA and do not require DNA sequencing. Each *mtCOI* primer pair was

chosen to amplify a species-specific fragment of a different size with the MED, NW, and MEAM1 species-amplified fragments of 303 bp, 405 bp, and 478 bp, respectively. The 30-μl final volume PCR reactions were run using a PTC-200 Peltier thermal cycler (MJ Research, Inc., Waltham, MA) under the conditions described by Shatters et al. (2009), and gel electrophoresis of samples was performed using either the Flashgel system (Cambrex, East Rutherford, NJ) for comparison of 12 or fewer samples or 2% Egel 48 (Invitrogen, Carlsbad, CA) for analysis of 48 samples.

PCR amplifications for the *mtCOI* gene were performed using the Btab-Uni primer set described by Shatters et al. (2009) for three randomly selected whiteflies (~20% of sample size) determined to be MEAM1 by the species-specific primer cocktail. If the whiteflies were identical, a consensus sequence was submitted to GenBank for each sample. If not, all sequences that were different were submitted. All individual MED whiteflies were sequenced to determine the haplotype. *mtCOI* sequence analysis was performed first by PCR amplification of an approximately 700- to 800-bp *mtCOI* DNA fragment and then sequencing the PCR amplified DNA. The 30-μl PCR reactions were run using a T100 Thermal Cycler (Bio-Rad Laboratories, Inc., Hercules, CA) under the conditions described by Shatters et al. (2009). Before being sequenced, the amplified products were cleaned using Montage PCR cleanup filters (Millipore, Billerica, MA). Fifty nanograms of total whitefly genomic DNA was used in BigDye sequencing reactions. All sequencing was performed bidirectionally with the amplification primers and BigDye Terminator v3.1 Cycle Sequencing Kits (Applied Biosystems, Foster City, CA). Sequence reactions were analyzed on an Applied Biosystems 3730XL DNA sequence analyzer and were then compared and edited using Sequencher software (Gene Codes, Ann Arbor, MI). Biotype determination was based on direct sequence comparisons using the web-based NCBI BLAST sequence comparison application (http://blast.ncbi.nlm.nih.gov/Blast.cgi), and consensus sequences were deposited in GenBank.

Results

Seventy *B. tabaci* samples were collected from 20 crops across 23 counties in Georgia since 2011, with the bulk of the samples taken in 2016 and 2017 (Fig. 1). In total, 1,098 individual whiteflies were processed (1,076 MEAM1 and 22 MED). Five samples from four counties were collected in 2011, and five poinsettia (*Euphorbia pulcherrima* Willd. ex Klotzsch) samples were collected in 2012, representing five different counties (Table 1). No samples were collected from Georgia in 2013, 2014, and 2015. Overall, cotton (*Gossypium hirsutum* L.) was the most heavily sampled commodity both in number of samples (27) and counties (10) sampled; followed by seven samples of ornamental or bell pepper, *Capsicum annuum* L. (7 counties); and six samples each of peanut, *Arachis hypogaea* L., (6 counties), poinsettia (5 counties), and crook-neck squash, *Cucurbita moschata* (Duchesne) Duchesne ex Poir. (4 counties) (Table 1). Other crops sampled included soybean (*Glycine max* (L.) Merr.), cowpea, (*Vigna unguiculata* (L.) Walp.), corn (*Zea mays* L.), snap bean (*Phaseolus vulgaris* L.), verbena (*Verbena* L. sp.), lantana (*Lantana camara* L.), cucumber (*Cucumis sativus* L.), zucchini squash (*Cucurbita pepo* L.), kale

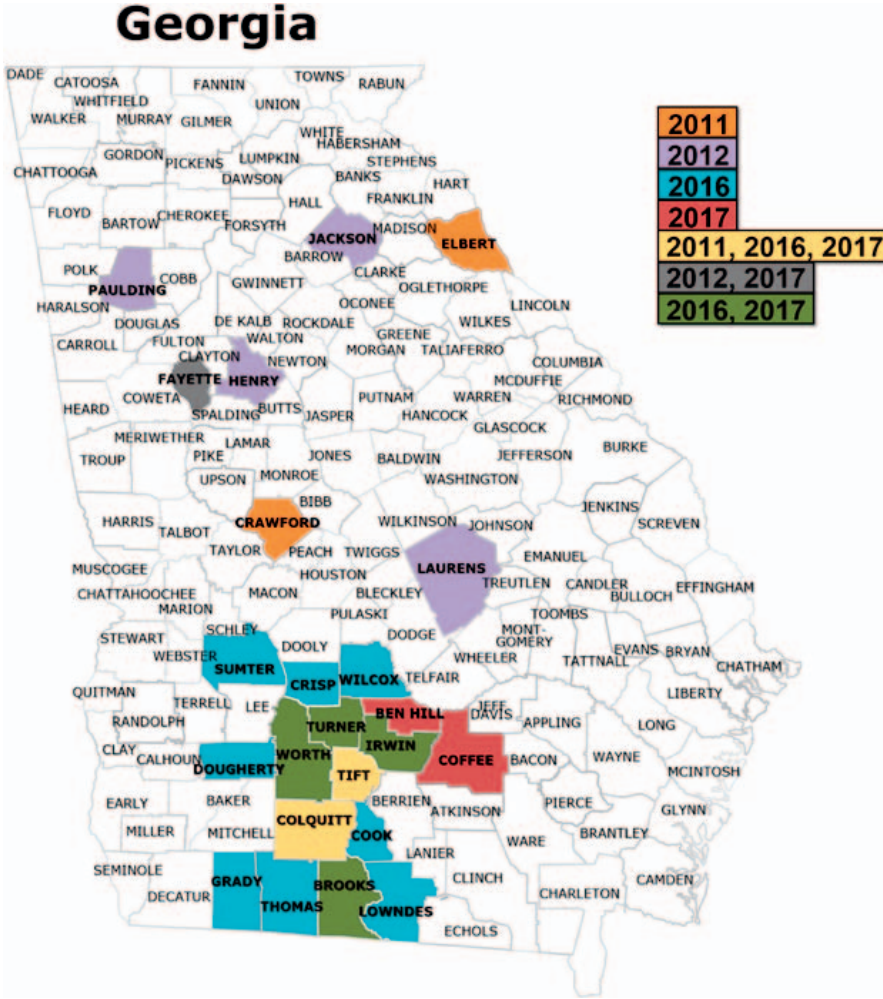

Fig. 1. Georgia counties and years surveyed for *Bemisia tabaci*.

(*Brassica oleracea* L.), tomato (*Lycopersicon esculentum* Mill.), sweet potato (*Ipomoea batatas* (L.) Lam.), eggplant (*Solanum melongena* L.), cantaloupe (*Cucumis melo* L. var. *cantalupensis* Naudin), and mum (*Chrysanthemum* x *morifolium* Ramat.) (Table 1). Colquitt was the most heavily sampled county, with 13 collections representing 6 different commodities. However, one more commodity (7) was sampled in Tift County (Co.) and fewer samples were collected (10). Colquitt and Tift counties were the most heavily sampled because that area has the most consistent and most severe whitefly infestations each fall.

MED (Q) whitefly was detected on *Verbena* L. spp. in 2011 from Elbert Co. (GenBank accession number MH613845) and on poinsettia in 2012 from Jackson Co. (GenBank accession number MH613851) in commercial greenhouses from

Table 1. *Bemisia tabaci* host plants and counties surveyed in Georgia agricultural ecosystems.

Host plant[a]		Georgia County (*n* of collection sites)
Common Name	**Scientific Name**	
Bean (snap)	*Phaseolus vulgaris* L.	Grady (1); Wilcox (1)
Cantaloupe	*Cucumis melo* L. var. *cantalupensis* Naudin	Brooks (1)
Corn	*Zea mays* L.	Worth (2)
Cotton	*Gossypium hirsutum* L.	Ben Hill (1); Colquitt (6); Crisp (2); Dougherty (2); Irwin (4); Sumter (1); Thomas (1); Tift (4); Turner (4); Worth (2)
Cowpea	*Vigna unguiculata* (L.) Walp.	Colquitt (1); Tift (1)
Cucumber	*Cucumis sativus* L.	Tift (1)
Eggplant	*Solanum melongena* L.	Colquitt (1)
Kale	*Brassica oleracea* L.	Grady (1)
Lantana	*Lantana camara* L.	Crawford (1)
Mum	*Chrysanthemum* x *morifolium* Ramat.	Coffee (1)
Peanut	*Arachis hypogaea* L.	Ben Hill (1); Brooks (1); Irwin (1); Tift (1); Turner (1); Worth (1)
Pepper (bell, ornamental)	*Capsicum annuum* L.	Brooks (1); Coffee (1); Colquitt (2); Cook (1); Lowndes (2)
Poinsettia	*Euphorbia pulcherrima* Willd. ex Klotzsch	Fayette (2); Henry (1); Jackson (1); Laurens (1); Paulding (1)
Soybean	*Glycine max* (L.) Merr.	Brooks (1); Colquitt (1); Tift (1)
Squash (crook-neck)	*Cucurbita moschata* (Duchesne) Duchesne ex Poir.	Colquitt (2); Lowndes (2); Thomas (1); Tift (1)
Squash (zucchini)	*Cucurbita pepo* L.	Lowndes (1)
Sweet potato	*Ipomoea batatas* (L.) Lam.	Tift (1)
Tomato	*Lycopersicon esculentum* Mill.	Irwin (1)
Verbena	*Verbena* L. spp.	Elbert (1)

[a] Host plant common and scientific names according to Brako et al. (1995).

northern Georgia. All MED whiteflies sequenced were identical and considered to have an Eastern Mediterranean origin (Dickey et al. 2013). Only MEAM1 (B) whiteflies were detected in all the field-grown commodities regardless of the year sampled, and sequences were identical except in one cotton sample from Colquitt Co. (GenBank accession number MH613907), which had a single nucleic polymorphism.

Nucleotide sequence accession numbers. The GenBank accession numbers for the *mtCOI* fragment amplified from MEAM1 and MED whiteflies from 23 Georgia counties and various host plants across the state are MH613844 through MH613914 and are available for use online (https://www.ncbi.nlm.nih.gov/).

Discussion

Georgia was one of the most extensively surveyed states (56 collections from 16 commodities) as part of a survey (McKenzie et al. 2012) that was conducted to determine the distribution of *Bemisia* biotypes in North America after the MED whitefly invasion into the United States in 2004. In that survey, sample collections were split about equally between ornamentals (31) and vegetable and row crops (25), with the greatest percentage taken from poinsettia (22). In Georgia, MED whitefly detections shifted from 100% Q2 or Western MED (five collections) in 2005 to 100% Q1 or Eastern MED (10 collections) beginning in the summer of 2007 (McKenzie et al. 2012; Dickey et al. 2013). Both MED collections in 2011 and 2012 in the present survey were confirmed to also be Eastern MED. To date, only MEAM1 has been detected in open agriculture in Georgia.

Earlier *B. tabaci* surveys indicated that MED whiteflies had not moved into field crops in the United States since its first detection in 2004 until 2011 (Dennehy et al. 2010; McKenzie et al. 2004, 2009, 2012) despite somewhat common coexistence on nursery plants and vegetable transplants destined for the field. However, in 2016, MED was detected in two field environments in Florida (McKenzie and Osborne 2017), but they did not become established. One collection was totally comprised of immature nymphs removed from a sweet potato leaf from an isolated open field planted 90 d prior, indicating that because whitefly immatures are not mobile after the first instar, MED had oviposited on sweet potato in the field. Still, MED was never picked up from that field again, which was sampled extensively. The second detection was from morning glory weeds on the border of a fallow field that was detected twice over time, but a crop was never planted in that field. So, although detections continue to be made, MED has not become established in agricultural fields to date in the United States. Conversely, MED was detected in 10 individual residential landscapes in Florida, primarily on hibiscus for the first time in 2016 (McKenzie and Osborne 2016), with new residential detections in 2017 (GenBank accession numbers MK703803 to MK703805) and 2018 (GenBank accession number MK703806). A new state detection of MED on poinsettia from a commercial greenhouse was made in Wisconsin in July 2018 (GenBank accession number MK599415), bringing the total positive MED states to 27, indicating that MED is still expanding its geographical range in the United States.

It is unclear why MED has not become established in open-field agriculture in the United States when in other parts of the world MED thrives in unprotected

environments. In Israel, MED and MEAM1 are present throughout the country, and field populations may consist of a mixture of the two; however, MEAM1 predominates on organic farms, whereas numerous insecticide applications have selected for MED elsewhere (Horowitz et al. 2008). In Senegal on the western coast of Africa, MEAM1 and MED (Q1) distribution on vegetable crops (okra, eggplant, pepper, and tomato) ranged from 100% MED to 85:15 MEAM1:MED on tomato at two different localities, with the distribution of cryptic species in between on other vegetables (Delatte et al. 2015). In 2007, MED was detected in most Chinese field populations with other cryptic species of *B. tabaci*, including MEAM1 (Rao et al. 2011). In two years, MED had become the dominant species in 44 sites (100%), compared to MEAM1 that was dominant in 17 locations (100%), indicating MED has rapidly displaced MEAM1 in most field locations in China (Pan et al. 2011). We speculate intensive, repeated insecticide exposure and MED's ability to develop high levels of insecticide resistance to many different chemical classes and modes of action play a crucial role in MED predominance in field and protected culture environments.

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