FORUM

Distribution of *Bemisia tabaci* (Hemiptera: Aleyrodidae) Biotypes in North America After the Q Invasion

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ABSTRACT After the 2004 discovery of the *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) Q biotype in the United States, there was a vital need to determine the geographical and host distribution as well as its interaction with the resident B biotype because of its innate ability to rapidly develop high-level insecticide resistance that persists in the absence of exposure. As part of a coordinated country-wide effort, an extensive survey of *B. tabaci* biotypes was conducted in North America, with the cooperation of growers, industry, local, state, and federal agencies, to monitor the introduction and distribution of the Q biotype. The biotype status of submitted *B. tabaci* samples was determined either by polymerase chain reaction amplification and sequencing of a mitochondrial cytochrome oxidase I small subunit gene fragment and characterization of two biotype discriminating nuclear microsatellite markers or esterase zymogram analysis. Two hundred and eighty collections were sampled from the United States, Bermuda, Canada, and Mexico during January 2005 through December 2011. Host plants were split between ornamental plant and culinary herb (67%) and vegetable and field crop (33%) commodities. The New World biotype was detected on field-grown tomatoes (*Solanum lycopersicum* L.) in Mexico (two) and in commercial greenhouses in Texas (three) and represented 100% of these five collections. To our knowledge, the latter identification represents the first report of the New World biotype in the United States since its rapid displacement in the late 1980s after the introduction of biotype B. Seventy-one percent of all collections contained at least one biotype B individual, and 53% of all collections contained only biotype B whiteflies. Biotype Q was detected in 23 states in the United States, Canada (British Columbia and Ontario territories), Bermuda, and Mexico. Forty-five percent of all collections were found to contain biotype Q in samples from ornamentals, herbs and a single collection from tomato transplants located in protected commercial horticultural greenhouses, but there were no Q detections in outdoor agriculture (vegetable or field crops). Ten of the 15 collections (67%) from Canada and a single collection from Bermuda contained biotype Q, representing the first reports of biotype Q for both countries. Three distinct mitochondrial haplotypes of *B. tabaci* biotype Q whiteflies were detected in North America. Our data are consistent with the inference of independent invasions from at least three different locations. Of the 4,641 individuals analyzed from 517 collections that include data from our previous work, only 16 individuals contained genetic or zymogram evidence of possible hybridization of the Q and B biotypes, and there was no evidence that rare hybrid B-Q marker co-occurrences persisted in any populations.

KEY WORDS *Bemisia argentifolii*, microsatellite marker, mitochondrial cytochrome oxidase I subunit, New World biotype

Bemisia *tabaci* (Gennadius) (Hemiptera: Aleyrodidae) is considered one of the top 100 global invasive species (Global Invasive Species Database, http://www.issg.org/database) with its ability to attack >900 host plants (Oliveira et al. 2001, Simmons et al. 2008) and vector >111 plant virus species (Jones 2003). *B. tabaci* is actually a species complex (De Barro et al. 2011) with at least 36 previously identified biotypes

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(Perring 2001; Simon et al. 2003a,b; Qiu et al. 2006; Zang et al. 2006); however, recent phylogenetic studies suggest genetic delineation into 24 discrete species may be possible (Dinsdale et al. 2010). Because the species delineation has not been officially recognized, we continue to use biotype designations in this report.

Agricultural production losses due to *B. tabaci* infestations have escalated over the past 25 yr as new and more virulent biotypes have spread to all continents except Antarctica (De Barro et al. 2005). Few countries with the exception of only a handful of European Union countries (Finland, Ireland, Sweden and the United Kingdom, and certain regions of Portugal) (Cuthbertson et al. 2011), have escaped its cosmopolitan distribution and subsequent establishment of at least one member of the *B. tabaci* species complex. The two most invasive biotypes that pose the greatest threat to growers are the B and Q biotypes (Perring 2001).

The inherent ability of *B. tabaci* to disperse locally and migrate (Byrne et al. 1996) is exacerbated by its extensive movement through global commerce of primarily ornamental plant products (Chu et al. 2006, Dalton 2006, Drayton et al. 2009, Cuthbertson et al. 2011). After the introduction of biotype B into the United States ≈1985, unprecedented losses began occurring in the late 1980s in Florida on poinsettia followed by high infestations in field-grown tomato (*Solanum lycopersicum* L.) crops (Hamon and Salguero 1987, Schuster et al. 1989, Hoelmer et al. 1991). Biotype B rapidly spread across the southern United States to Texas, Arizona, and California where extreme field outbreaks occurred during the early 1990s on melons, cotton, and vegetable crops (Perring et al. 1991, 1992, 1993; Gonzalez et al. 1992). In addition to an expanded host range and a more aggressive colonizer of crops, other traits identified at the morphological (Bellows et al. 1994, Costa et al. 1995, Rosell et al. 1997), biochemical (Costa and Brown 1991, Perring et al. 1992, Brown et al. 2000), and molecular (Gawel and Bartlett 1993, De Barro et al. 2005, Boykin et al. 2007) levels were considered sufficiently different from the indigenous populations to warrant a new species designation (i.e., *Bemisia argentifolii* Bellows & Perring) (Perring et al. 1993, Bellows et al. 1994). However, for purposes of discussion, we are continuing with the biotype B designation because there has yet to be an official species delineation of the entire complex.

Indistinguishable in appearance from *B. argentifolii* (*B. tabaci* biotype B), biotype Q is extremely problematic to agricultural production because populations are highly prone to develop resistance to insect growth regulators (Horowitz et al. 2003b) and neonicotinoid insecticides (Horowitz et al. 2004). Both classes of insecticides are widely used for controlling whiteflies in many cropping systems including cotton (*Gossypium hirsutum* L.) (Ellsworth and Martinez-Carrillo 2001), vegetables (Palumbo et al. 2001), and ornamentals (http://mrec.ifas.ufl.edu/lso/documents/Export%20Mgmt%20Plan-7-07.pdf). Since its discovery in Spain in 1997 (Guirao et al. 1997) and Israel in 2003 (Horowitz et al. 2003a), biotype Q has caused severe crop damage in the Mediterranean Basin in both protected and open agriculture (Horowitz et al. 2003b, Simon et al. 2003b, Vassiliou et al. 2008) and proven an invasive ability arguably matched only by biotype B. Biotype Q has recently been reported in Argentina and Uruguay (Grille et al. 2011), China (Chu et al. 2006), Costa Rica (Guevara-Coto et al. 2011), France (Dalmon et al. 2008), Guatemala (Bethke et al. 2009), Japan (Ueda and Brown 2006), Korea (Lee et al. 2005), Mexico (Martinez-Carrillo and Brown 2007), New Zealand (Scott et al. 2007), Taiwan (Hsieh et al. 2008), Tunisia (Gorsane et al. 2011), and the United States (Dennehy et al. 2005, McKenzie et al. 2009). Biotype Q was first detected in the United States in December 2004 on poinsettias (*Euphorbia pulcherrima* Willd. ex Klotzschfrom) from a retail outlet in Arizona during routine resistance monitoring surveys (Dennehy et al. 2005). Determined to be essentially unaffected by pyriproxyfen in egg bioassays, these whiteflies also had noticeably reduced susceptibility to acetamiprid, buprofezin, mixtures of fenpropathrin and acephate (normally synergistic against biotype B), imidacloprid, and thiamethoxam (Dennehy et al. 2010). Other examples of resistance in biotype Q have helped foster a reputation that biotype Q is especially capable of developing resistance under intensive insecticide use conditions (Nauen et al. 2002, Horowitz et al. 2005, Nauen and Denholm 2005). Concurrent with the appearance of biotype Q in the United States were reports from ornamental plant growers of increasing problems in controlling whitefly infestations that were almost certainly related to the spread of the Q biotype. As a result, there was great concern over the possibility of the development of a B-Q hybrid whitefly possessing the high insecticide resistance of the Q biotype with the more aggressive invasive characteristics of the B biotype and possible spread to cotton and field-grown vegetables. Growers and researchers in the southern United States were very concerned about experiencing déjà vu with respect to the control failures that occurred when the B biotype was introduced in the mid-1980s. This added urgency and a need to understand how the Q biotype spread throughout the United States and its interaction with the B biotype. As part of an Animal and Plant Health Inspection Service (APHIS)-coordinated multistate, -agency, -industry, -commodity, and -institutional Q-biotype Task Force initiative and coordinated whole-country survey, this biotype was reported in 26 states in the United States during the past 6 yr. After the initial detection of biotype Q in Arizona in 2004 (Dennehy et al. 2005), it was detected in 19 states in 2005, two states each in 2006 and 2007, and single detections in 2008 and 2010, with no new detections in 2009 and 2011 reported to the Q-biotype Task Force http://mrec.ifas.ufl.edu/LSO/BEMISIA/positive_states.htm). As part of that survey, the distribution of *B. tabaci* biotypes was determined across North America from January 2005 to December 2011, including Bermuda and Hawaii (islands of Hawaii and Oahu), Canada, and Mexico, with the primary objective to monitor the introduction of the Q biotype and distribution of all *Bemisia* biotypes, including B, Q, and the New World (includes biotype A and closely related

populations not yet categorized). Here, we present the results of this extensive survey of *B. tabaci* biotypes in North America to investigate and document the "Q" invasion around the continent.

Materials and Methods

Whitefly Collection. The majority of the samples were provided through cooperation with local, state, and country agricultural agencies, industry representatives, crop consultants, University and Agricultural Research Service entomologists, and vegetable and ornamental growers from across North America. Due to the perception of growers concerning regulatory constraints and the involvement of APHIS and state agencies, it became very apparent early on that a mechanism for submitting whitefly samples anonymously must be implemented for growers to participate. Grower participation was crucial to the success of determining *B. tabaci* biotype distribution rapidly so that local management programs tailored by biotype could be developed and implemented (http://mrec.ifas.ufl.edu/lso/documents/Export%20Mgmt%20Plan-7-07.pdf). To protect growers from regulatory action if biotype Q was detected at their nursery or field, samples were only reported at the state level and the specific location within the state was kept confidential. In Florida, a different scenario existed because for each positive biotype Q find from a grower, corresponding staff with the Florida Department of Agriculture and Consumer Services Division of Plant Industry or an entomologist (state or Agricultural Research Service) also provided a collection from the same county so that locations could be reported at the county level for that state. Florida detections reported here are continuations from McKenzie et al. (2009), but the specific location is only be reported here at the state level.

Whitefly sampling guidelines were provided to ensure sampling bias did not occur, but the actual sample technique depended on the individual sampler and bias could have resulted if guidelines were not followed. Adults or immature stages collected were immediately placed in 95% ethanol for molecular analysis. *B. tabaci* are haplodiploid with 2N females and 1N males (Byrne and Devonshire 1996); therefore, female whiteflies were identified and selected for further analysis to allow microsatellite genotyping of homozygous and heterozygous individuals within populations. At least 12 adult female whiteflies were processed from each sample if available. If 12 adult females were not available, adult males or whitefly nymphs collected from leaves of host plants were used for mitochondrial cytochrome oxidase I small subunit (mtCOI) sequence analysis and genotyped but removed before population genetic analysis of microsatellites. If 12 whiteflies (females, males, nymphs) were not available, we analyzed the number of individuals that were provided. In subsequent analysis of data, where collections were grouped by biotype(s) present in each collection, we used only populations that had five or more females. We understand that we may miss biotypes present at something <20% of the population, but for the purposes of this general survey we deemed this an acceptable cutoff given the cost and time associated with deeper sampling.

Host Plants and Locations Surveyed. The whitefly surveys included multiple locations across North America representing 25 of the United States, Bermuda, Canada, and Mexico and 37 different host plants (Table 1). Host plants were split between ornamentals and culinary herb (67%) and vegetable and field crop (33%) commodities (Table 1). The same crops were surveyed across multiple locations, when possible, and many locations were sampled multiple times. Florida (62 collections), Georgia (56 collections), Arizona (34 collections), New York (16 collections), Canada (15), California and Texas (12 collections each), and Mexico (11 collections) represented the most extensively sampled locations. Poinsettia was the most extensively sampled host, with 130 collections from 23 greenhouse or nursery locations (state or country) representing 46% of all the samples collected (Table 1). Tomato was the second most sampled host with 32 collections (11%) followed by cotton with 23 collections (8%). Cotton was sampled from the field 100% of the time, and tomato was split between 18 field collections (78%) and five commercial greenhouse collections (22%).

Methods Used to Determine Biotype. Molecular techniques used to distinguish whitefly biotypes included either esterase zymogram assays (Byrne and Devonshire 1991), or genetic analysis of mtCOI DNA sequence (Frohlich et al. 1999, Shatters et al. 2009), and microsatellite fragment analysis (De Barro et al. 2003). Q biotype *Bemisia* can be distinguished from B biotype insects based on the esterase electrophoretic banding patterns (Byrne and Devonshire 1991, Dennehy et al. 2010), and this method was used to routinely confirm biotype status results using mtCOI sequence and microsatellite data analysis as well as report detections at the state level. Genotyping at two microsatellite markers identified by De Barro et al. (2003), BEM6-$(CA)_8$imp, and BEM23-$(GAA)_{31}$imp, was found to be diagnostic for B and Q biotypes by McKenzie et al. (2009) and used to determine biotype status in conjunction with analysis of mtCOI DNA sequence. Zymogram data were included for a subset of individuals from some populations to verify concordance with genetic data because esterase zymograms were used as the original basis on which to separate biotypes. Although both identification methods were not performed on the same individual, in populations where both genetic identification and esterase zymogram identification were performed, the two methods were in 100% agreement (data not shown).

Electrophoresis of Esterase Zymograms. Individual whitefly adults were homogenized in a final volume of 20 μl of 10% sucrose prepared in a 0.1% aqueous solution of Triton X-100. To facilitate the processing of large numbers of insects, individual adults were placed in the wells of a microtiter plate and then homogenized using a multiple homogenizer (Burkard Scien-

Table 1. *B. tabaci* host plants and states or countries surveyed in North America agricultural ecosystems, 2005–2010

Host plant[a]		State or country (no. collection sites)
Common name	Scientific name	
Ornamentals and culinary herbs		
Anthurium	*Anthurium andraeanum* Schott	Florida (1)
Basil	*Ocimum basilicum* L.	Mexico (7)
Begonia	*Begonia* L. spp.	Oregon (1)
Caryopteris	*Caryopteris divaricata* Maxim.	South Carolina (1)
Datura	*Datura wrightii* Regel	Nevada (1)
Echinacea	*Echinacea purpurea* (L.) Moench	Michigan (1), Pennsylvania (1), Virginia (1)
Fuchsia	*Fuchsia triphylla* L.	Oregon (1)
Gerbera daisy	*Gerbera jamesonii* Bolus ex Hook. f.	California (7), Canada (1), Georgia (1), Hawaii (1), Michigan (1), Pennsylvania (1), Texas (1)
Heather	*Calluna vulgaris* (L.) Hull	Canada (1)
Hibiscus	*Hibiscus moscheutos* L.	Florida (1), Georgia (3), Hawaii (1), New York (1), South Carolina (1)
Hypoestes	*Hypoestes phyllostachya* Baker	Texas (1)
Lantana	*Lantana camara* L.	Bermuda (1), Georgia (1), Texas (2)
Mum	*Chrysanthemum xmorifolium* Ramat.	Georgia (2)
Penstemon	*Penstemon* spp.	Michigan (1)
Pentas	*Pentas lanceolata* (Forssk.) Deflers	Pennsylvania (1)
Poinsettia	*Euphorbia pulcherrima* Willd. ex Klotzsch	Alabama (1), Arizona (10), Bermuda (1), California (2), Canada (13), Connecticut (3), Florida (24), Georgia (22), Illinois (5), Indiana (2), Maine (1), Maryland (1), Massachusetts (3), Michigan (4), Mexico (1), New Hampshire (1), New Jersey (3), New York (15), Oregon (6), Pennsylvania (1), Texas (4), Vermont (6), Washington (1)
Poinsettia (annual)	*Euphorbia heterophylla* L.	Bermuda (1)
Sage	*Salvia* L. spp.	Florida (1), Georgia (1)
Thyme	*Thymus vulgaris* L.	Mexico (1)
Verbena	*Verbena* L. spp.	Pennsylvania (1)
Veronica	*Veronica* L. spp.	Michigan (2)
'Ornamentals'	No specific host given	Alabama (1), California (1), Georgia (1), Kentucky (2)
Vegetable and row crops		
Bean (snap)	*Phaseolus vulgaris* L.	Georgia (1)
Bell pepper	*Capsicum annuum* L.	Florida (1), Georgia (3)
Brassicaceae	*Brassica* sp. L.	California (1)
Cabbage	*Brassica oleracea* L. var. *capitata* L.	Arizona (1)
Cantaloupe	*Cucumis melo* L. var. *cantalupensis* Naudin	Arizona (2), Florida (1)
Cauliflower	*Brassica oleracea* L. var. *botrytis* L.	Arizona (2)
Collard Greens	*Brassica oleracea* L. var. *acephala* DC	Georgia (3), South Carolina (1)
Cotton	*Gossypium hirsutum* L.	Arizona (19); California (1); Georgia (3)
Cucumber	*Cucumis sativus* L.	Florida (1), Georgia (2)
Cucurbit	Cucurbitaceae	Florida (1)
Eggplant	*Solanum melongena* L.	Florida (3), Georgia (5)
Kale	*Brassica oleracea* L.	Texas (1)
Peanut	*Arachis hypogaea* L.	Georgia (1)
Potato	*Solanum tuberosum* L.	Florida (1)
Squash (crook-neck)	*Cucurbita moschata* (Duchesne) Duchesne ex Poir.	Florida (1), Georgia (2)
Squash (zucchini)	*Cucurbita pepo* L.	Florida (2)
Tomato	*Lycopersicon esculentum* Mill.	Florida (22), Georgia (5), Mexico (2), Texas (3)
Watermelon	*Citrullus lanatus* (Thunb.) Matsum. & Nakai	Florida (2)

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

tific, Uxbridge, Middlesex, United Kingdom). Fifteen microliters from each homogenate was then transferred to the wells of a 7.5% polyacrylamide gel (Byrne and Devonshire 1991). Gels were electrophoresed at 200 V for 90 min at 4°C. After electrophoresis, esterases were localized on gels by staining for 30 min with 0.5 mM 1-naphthyl butyrate prepared in 0.2% Fast Blue RR salt at pH 6.0. Esterase bands were fixed by immersing the stained gel in 7% acetic acid. B biotype *B. tabaci* were included on each gel for reference purposes (Dennehy et al. 2010).

Whitefly DNA Extraction. 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 (De Barro and Driver 1997), 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 and boiled at 95°C for 5 min and then placed directly in ice for 5 min. Tubes were then centrifuged at 8000 $\times$ *g* for 30 s, and the supernatant (crude DNA lysate) was transferred to another tube and stored at −80°C for future processing. Aliquots from the same individual whitefly DNA extract were used for both mtCOI and microsatellite marker analysis so that both methods could be directly compared.

mtCOI Sequence Analysis. Polymerase chain reaction (PCR) amplifications for the mtCOI gene were performed using either the universal COI primers C1-J-2195 and L2-N-3014 (Simon et al. 1994) as originally used by Frohlich et al. (1999) for *B. tabaci* or amplified with the Btab-Uni primer set described by Shatters et al. (2009). mtCOI sequence analysis was performed first by PCR amplification of an ≈700–800-bp mtCOI DNA fragment (depending on the primer set) and then sequencing the PCR amplified DNA. The 30-μl PCR reactions were run using a PTC-200 Peltier thermal cycler (MJ Research, Watertown, MA) under the conditions described by Shatters et al. (2009). Conditions were the same for both primer sets with the exception of the annealing temperatures. Before sequencing, 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 a 3730XL DNA sequence analyzer (Applied Biosystems) 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 when necessary by inference from neighbor joining methods of phylogenetic analysis of the mtCOI sequences by using CLUSTALW alignments of each sequence type (Boykin et al. 2007).

Microsatellite Marker Analysis. PCR amplifications for microsatellite primers developed by De Barro et al. (2003), BEM6-$(CA)_8$imp and BEM23-$(GAA)_{31}$imp, were conducted in 13-μl reactions composed of 6.5 μl of Immomix (catalog no. BIO-25019, Bioline, Taunton, MA), 5.0 μl of sterile water, 0.25 μl of forward primer (10 pmol), 0.25 μl of reverse primer (10 pmol), and 1 μl of DNA template. The forward primer was labeled with the fluorescein derivative, FAM (Eurofins MWG Operon, Huntsville, AL), for microsatellite scoring, and the 13-μl PCR reactions were run with the thermal regime of 94°C for 7 min followed by 35 cycles of 1 min at 94°C (denaturation), 1 min at 47°C (annealing), 1 min at 73°C (extension), and a final step of 72°C for 1 h. One microliter of the FAM-labeled PCR product was added to a mix of 13.75 μl of formamide (code size K295–100 ml, Ameresco, Solon, OH) and 0.25 μl of GENESCAN 500 ROX Size Standard (part no. 401734, Applied Biosystems) and loaded onto a 3730XL DNA analyzer. Whiteflies were genotyped, and their amplicons were sized and characterized using GENEMAPPER 4.0 (Applied Biosystems). All whitefly collections from McKenzie et al. 2009 were included in the microsatellite analysis.

Cloning and Microsatellite Sequencing Analysis. Due to concern about possible hybridization between biotypes B and Q, several individuals showing evidence of shared microsatellite alleles were regenotyped. If the putative shared allele was confirmed, microsatellite products were direct sequenced on a 3730XL DNA analyzer by using a BigDye Terminator v3.1 Cycle Sequencing kit in the case of homozygote samples. Heterozygote samples were cloned using an Invitrogen TOPO TA Cloning (Invitrogen, Carlsbad, CA), and a minimum of 16 colonies were sequenced. Sequences were manually aligned in Sequencher 4.7 (Gene Codes) and compared with sequenced individuals homozygous for a given allele.

Statistical Analysis. Three mitochondrial haplotypes of biotype Q were documented by McKenzie et al. (2009). We tested whether whiteflies possessing these haplotypes were genetically differentiated from one another based on the two nuclear markers. Males and individuals of unknown sex were removed to be certain only diploid individuals were analyzed. The resulting microsatellite data set contained 899 samples, Q1 ($n = 570$), Q2 ($n = 227$), and Q3 ($n = 102$), with missing data for 87 individuals at the Bem6 locus and 67 individuals at the Bem23 locus. The data were analyzed using the analysis of molecular variance module in GenAlEx 6.41 (Peakall and Smouse 2006) with significance of differentiation (Fst) estimates among biotype Q haplotype groups tested by permutation. Migration/gene flow (Nm) estimates among haplotype groups also were calculated when Fst significantly differed from zero.

Results

Whitefly Collections. In total, 2,971 individual whiteflies from 280 different collections were analyzed. Of those individuals, 1,799 (61%) from 198 collections were biotype B, 1,101 (37%) individuals from 127 collections were biotype Q, and 56 (2%) individuals from five collections were the New World biotype.

New World Detections. The New World biotype was detected on field-grown tomatoes in Mexico (two) and in commercial greenhouses in Texas (three) and represented 100% of these collections. To our knowledge, the latter identification represents the first report of the New World biotype in the United States since its rapid displacement in the late 1980s after the introduction of biotype B (Perring 1996). The U.S. New World biotype was designated the A biotype when it was shown to have a distinct esterase zymogram pattern (Costa and Brown 1991); however, subsequent comparisons of mtCOI sequence data from southern North America and Central America have shown a somewhat diverse group of variants closely related to that for the A biotype (Bethke et al. 2009). Because it is unknown whether these represent variants of the A biotype, or represent closely related yet distinct biotypes, we have chosen to collectively call them New World. The diversity in New World mtCOI sequences is shown in Fig. 1, where the nucleotide sequence for the PCR-amplified fragment is aligned for the Mexican (GenBank accessions JF754916, JF754917) and Texan (GenBank accessions HQ877602, JF754907, JF754908) collections described previously, as well as collections from Colombia (Shat-

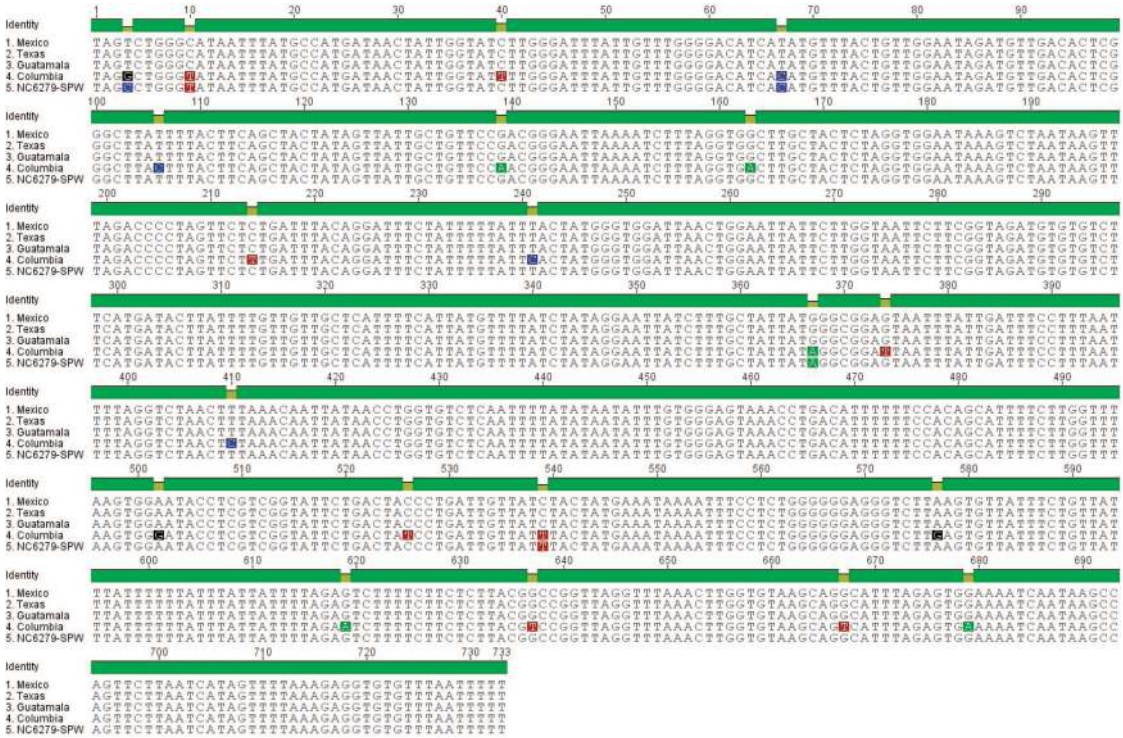

Fig. 1. Nucleotide ClustalW alignment of the 733-bp mtCOI PCR fragment from four *B. tabaci* New World biotype collections from Texas (GenBank accession HQ877602), Mexico (GenBank accession EU427729), Guatemala (GenBank accession EU881709), and Colombia (GenBank accession EU427728) and one sequence representing the native U.S. *B. argentifolii* (A biotype) taken from the published mitochondrial genome sequence (NC6279-SPW). The green bar represents locations where all are identical. Polymorphisms that differ from the majority base for the five sequences are indicated by color. The only polymorphism leading to an amino acid substitution was the G-to-T transversion in the Colombia sequence at position 374.

ters et al. 2009, GenBank accession EU427728), Guatemala (Bethke et al. 2009, GenBank accession EU881709), and the sequence taken from the complete "sweet potato whitefly" mitochondrial sequence present in GenBank (Reference Sequence: NC_006279.1). These results show that the Mexican, Texan, and Guatemalan sequences are identical but the originally described "sweet potato whitefly" also known as biotype A (Costa and Brown 1991), and Colombian sequences contain five and 15 single-nucleotide polymorphisms (SNPs), respectively. All SNPs were synonymous substitutions (did not change the amino acid coding sequence) with the exception of one SNP in the Colombian sequence that cause an amino acid change from valine to leucine. This New World diversity and the discovery of what seems to be new incursions into Texas from Mexico underscores the need for a systematic analysis of the New World biotype complex that will further improve our understanding of *B. tabaci* global movement and its associated threat to agriculture.

Biotype B and Q Detections. Seventy-one percent of all collections contained at least one biotype B individual and 53% of all collections contained only biotype B whiteflies. Forty-five percent of all collections were found to contain at least one biotype Q individual sampled from ornamentals, herbs, and tomato transplants (single collection in Georgia) located in protected commercial horticultural greenhouses, with no detections in open agriculture (field crops) (data not shown). When biotype Q was detected, 18% of the collections contained a mixture of both biotypes B and Q and 28% of all collections contained only biotype Q whiteflies. Biotype Q was detected in all states surveyed except Nevada and Virginia, but these states only had a single collection each. In addition, Louisiana, North Carolina, and Ohio were reported to the Q-biotype Task Force by Dr. J. Brown (University of Arizona) by using mtCOI markers as having the Q biotype (http://mrec.ifas.ufl.edu/LSO/BEMISIA/positive_states.htm) for a total of 26 states with the Q biotype. Ten of the 15 collections (67%) from Canada and a single collection from Bermuda contained biotype Q, representing the first report of biotype Q for both countries. Biotype Q also was detected in Mexico on poinsettia confirming an earlier report from Martinez-Carrillo and Brown (2007).

Biotype B and Q mtCOI Diversity. Almost all biotype B whitefly individuals analyzed from North America (>3,300 individual whiteflies) had the identical mtCOI sequence in the region amplified and

Table 2. SNPs detected in North American *B. tabaci* B biotypes

Host plant	Sample location	GenBank accession	Base no. on the amplified mtCOI fragment[a]							
			136	268	294	496	524	676	704	801
Tomato	USHRL	FJ188651	T	G	C	A	T	G	T	C
Potato	Florida	HQ877510	*C*	G	C	A	T	G	T	C
Cotton	Arizona	HQ877499	T	*A*	C	A	T	G	T	C
Poinsettia	Arizona	HQ877500	T	*A*	C	A	T	G	T	C
Melons	Arizona	HQ877501	T	*A*	C	A	T	G	T	C
Cotton	Arizona	HQ877502	T	*A*	C	A	T	G	T	C
Poinsettia	Arizona	HQ877533	T	*A*	C	A	T	G	T	C
Tomato	Florida	HQ877512	T	G	*T*[b]	A	T	G	T	C
Pepper	Florida	JN547217	T	G	C	*T*	T	G	T	C
Poinsettia	Georgia	HQ877516	T	G	C	A	*C*[b]	G	T	C
Poinsettia	Canada	HQ877505	T	G	C	A	T	*A*	T	C
Poinsettia	Florida	HQ877506	T	G	C	A	T	G	*C*[b]	C
Poinsettia	Canada	HQ877504	T	G	C	A	T	G	T	*T*[b]

[a] All North American biotype B sequences analyzed (>3,300 individual whiteflies including McKenzie et al. 2009) were identical to the USHRL whitefly laboratory colony, GenBank accession FJ188651, that was used as a reference for comparison.
[b] Bases represent SNPs that are nonsynonymous.

sequenced (Table 2). However, eight SNPs occurred at different locations within the mtCOI sequences of seven individual whiteflies from seven different collections and from three individual whiteflies within one collection. Four of the SNPs were nonsynonymous. Arizona had five collections, where eight whiteflies in total shared a unique synonymous SNP that did not occur in any of the other B biotypes (two whiteflies from each of three collections and a single whitefly from each of two Arizona collections). Poinsettia was the host for five of the collections that represented four of the different SNPs detected, indicating that host plant selection was unlikely to have contributed to the differences.

Three distinct mitochondrial variants of *B. tabaci* biotype Q (designated as Q1, Q2, and Q3) whiteflies identified by McKenzie et al. (2009) were abundantly distributed throughout North America. All SNPs that distinguished these haplotypes were from synonymous substitutions. *B. tabaci* biotype Q haplotype groups were significantly differentiated based on nuclear microsatellite genotypes. The estimated differentiation between haplotype groups Q2 and Q3 was 0.054 ($P = 0.0001$), with an estimate of 4.4 migrants per generation between them. The estimated differentiation between haplotype groups Q1 and Q3 was 0.28 ($P = 0.0001$), with a migration estimate of 0.66. The estimated differentiation between haplotype groups Q1 and Q2 was 0.40 ($P = 0.0001$), with an estimated 0.37 migrants per generation between them. It should be noted that pair-wise nuclear differentiation among mtCO1 haplotypes is only based on two nuclear loci in this report, and more loci should be added to confirm these results. The stronger nuclear differentiation found between Q1 and Q2/Q3 is not surprising because haplotype Q1 probably originated from an eastern Mediterranean locale, e.g., Israel, whereas Q2/Q3 probably originated from western Mediterranean locale(s), e.g., Spain or Morocco (Tsagkarakou et al. 2007). Interestingly, at least four genetic groups of biotype Q in North America are resolved using a seven locus nuclear data set (C.L.M. unpublished data), suggesting the presence of additional genetic substructure beyond that described in this report. The estimated number of migrants per generation between Q2 and Q3 is more than four, suggesting higher connectivity between them. In contrast, the differentiation between Q1 and the other two populations is much higher and the estimated number of migrants per generation is less than one. The three haplotypes rarely co-occurred (see host plant distribution), which could reduce gene flow among them. Haplotype Q1 was only documented to co-occur with Q2 and Q3 in one sample from California. The infrequency of co-occurrence, coupled with divergence at both nuclear and mitochondrial DNA is consistent with the inference of at least three independent invasions of biotype Q.

Biotype B and Q Nuclear Diversity. A previous report (McKenzie et al. 2009) showed that genotypes at two nuclear microsatellite markers proved relatively diagnostic for the B and Q biotypes. Increased sampling has yielded a larger number of rare alleles in the current report (Table 3) and additional rare instances of putative allele sharing between biotypes. Despite this, microsatellite genotyping at these loci

Table 3. *B. tabaci* biotype mtCOI haplotypes correlated to diagnostic microsatellite primers BEM6 and BEM23

mtCOI haplotype	Size in bases of the amplified fragment (frequency %)	
	BEM6 $(CA)_8$ imp	BEM23 $(GAA)_{31}$ imp
New World	195(7), 202(51), 209(16), 216(24), 223(1)	
Biotype B	216(96), 223(4), 210, 230(<1)	224(100), 217, 219, 221, 222, 227, 230, 233(<1)
Biotype Q	203(7), 210(91), 217(1), 196, 197(<1)	407(49), 410(50), 224, 229,387,401, 403(<1)
Q1	203(1), 210(97), 217(1), 196(<1)	387(1), 407(74), 410(25), 224, 401, 403 (<1)
Q2	197(1), 203(22), 210(76), 196, 217(<1)	407(3), 410(97), 229(<1)
Q3	203(7), 210(93)	407(16), 410(84)

yields the common biotype associated alleles >98% of the time (Table 3).

Biotype B and Q Hybridization. Because, based solely on allele size, there seemed to be a rare occurrence of shared Bem6 microsatellite alleles between B and Q biotypes, these loci of interest were sequenced for more conclusive evidence of field hybridization between B and Q biotypes.

Bem6 Allele 217 in Biotype Q Samples. Two biotype Q individuals that genotyped as a 210/217 heterozygote at the Bem6 locus were cloned and sequenced. The sequence of the putatively shared 216–217-bp fragment was identical to that of a biotype Q individual homozygous for the 217 allele (data not shown). The biotype B 216 allele and the biotype Q 217 allele differ by two heptanucleotide insertions at one tandem repeat and two heptanucleotide deletions at a second tandem repeat. Thus there is no evidence for a shared 217 allele between biotypes B and Q at the Bem6 locus.

Bem6 Allele 210 in Biotype B Samples. One biotype B individual that genotyped as a 210/217 heterozygote at the Bem6 locus was cloned and sequenced and a second individual that genotyped as a 210 homozygote was sequenced directly. Each individual had a different 210 allele sequence, but both differed by single heptanucleotide deletions from the B 216 allele. In contrast, the B 210 alleles differed from the Q 210 allele by a minimum of four heptanucleotide insertions/deletions occurring at two to three different tandem repeats (data not shown). Thus, there is no evidence for a shared 210 allele between biotypes B and Q at the Bem6 locus.

Bem23 Allele 224 in Biotype Q Samples. One biotype Q individual that genotyped as a 224 407 heterozygote at the Bem23 locus was cloned and sequenced. The sequence of the putatively shared 224-bp fragment was identical to that found in several biotype B individuals homozygous for the 224 allele (data not shown). This provides evidence for a shared 224 allele between biotypes B and Q in a single case at the Bem23 locus.

Of 4,151 whiteflies genotyped at the microsatellite loci, only one showed evidence of mixed ancestry between biotypes B and Q after sequencing the apparent shared allele. The sequencing effort also demonstrated that several apparently shared alleles at the Bem6 locus were homoplasious, having evolved independently via different insertions and deletions at multiple heptanucleotide tandem repeats, but producing DNA fragments of identical size. Biotypes B and Q belong to genetically distinct but morphologically indistinguishable species in the *Bemisia tabaci* species complex (De Barro et al. 2011) and are reproductively incompatible (Elbaz et al. 2010, Sun et al. 2011). Hybrids are rarely produced in the laboratory and are sterile (Sun et al. 2011). Therefore, maladaptive hybridization of B and Q in North America is predicted to be rare and selected against (Servedio and Noor 2003) as supported by our data. Of the 4,641 individuals analyzed from 517 collections (includes data from McKenzie et al. 2009), as well as samples analyzed by esterase zymogram (data not shown), only 16 individuals contained genetic (one individual)/zymogram (15 individuals) signatures that suggested possible hybridization of the Q and B biotypes and there was no evidence that rare hybrid B-Q marker cooccurrences persisted in any populations.

Biotype B and Q Geographical and Host Plant Distribution. Our geographical distribution data support at least three introductions of biotype Q into North America (Fig. 2; Supp Table 1 [online only]). Alabama, Hawaii, Michigan, South Carolina, and Texas had only Q1 detections. In this study, Florida had 11 collections that were 100% Q1, combining with earlier data (McKenzie et al. 2009) to give a total of 35 collections of this haplotype. Despite earlier detections of haplotypes Q2 and Q3 (McKenzie et al. 2009), these haplotypes were not detected in Florida during this sampling period. Florida Q1 detections reported here occurred in late 2010 and were the first biotype Q detections since 2006. In Pennsylvania, four collections were 100% Q1 and one collection was a mixture of Q2 and Q3. In Georgia, detections shifted from 100% Q2 (five collections) to 100% Q1 (10 collections) beginning in summer 2007. There were four Q3 collections from Georgia that were sampled from the same grower over time, suggesting that this grower had a resident population. In New York, one collection was 100% Q1 and three collections were 100% Q2. Arizona, Connecticut, Kentucky, Indiana, Illinois, New Hampshire, and New Jersey had only Q2 detections. Oregon had two collections that were 100% Q2, and one collection that was a mixture of Q2 and Q3. Canada had nine collections (n = 63 whiteflies) that were 100% Q3, one collection that was 100% Q2, and one collection whose haplotype composition was duplicated in Mexico that was 9% Q1 and 91% Q3 (n = 12 whiteflies). Bermuda had a single collection that was 100% Q3. Of 231 collections that were analyzed with mtCOI, only a single collection from California contained all three haplotypes (Q1:Q2:Q3 = 17:2:3; n = 22 whiteflies). These data combined with the nuclear data strongly support the inference of multiple, independent biotype Q invasions into North America from at least three different sources of infestation.

The host plant distribution of biotypes (B, Q, or B/Q mixture) on collections of poinsettias were almost equally divided between B alone (30%), B/Q mixture (32%), and Q alone (38%) (Fig. 3) indicating there was ample possibility for the two biotypes to hybridize. Collections included those analyzed by either mtCOI or esterase zymogram. Poinsettia (111 collections) and nonpoinsettia collections (42) were counted as B only or Q only if they had five or more individuals sampled, and B/Q mixed collections were counted regardless of the number of individuals sampled in the collection as long as there was at least one individual of each biotype. The percentage of collections on nonpoinsettia ornamentals that contained either B, Q, or a mixture of biotypes was similar to poinsettia collections in that there were substantial numbers of B only (24%), B/Q mixture (33%), and Q only (43%) collections (Fig. 3). However, there was

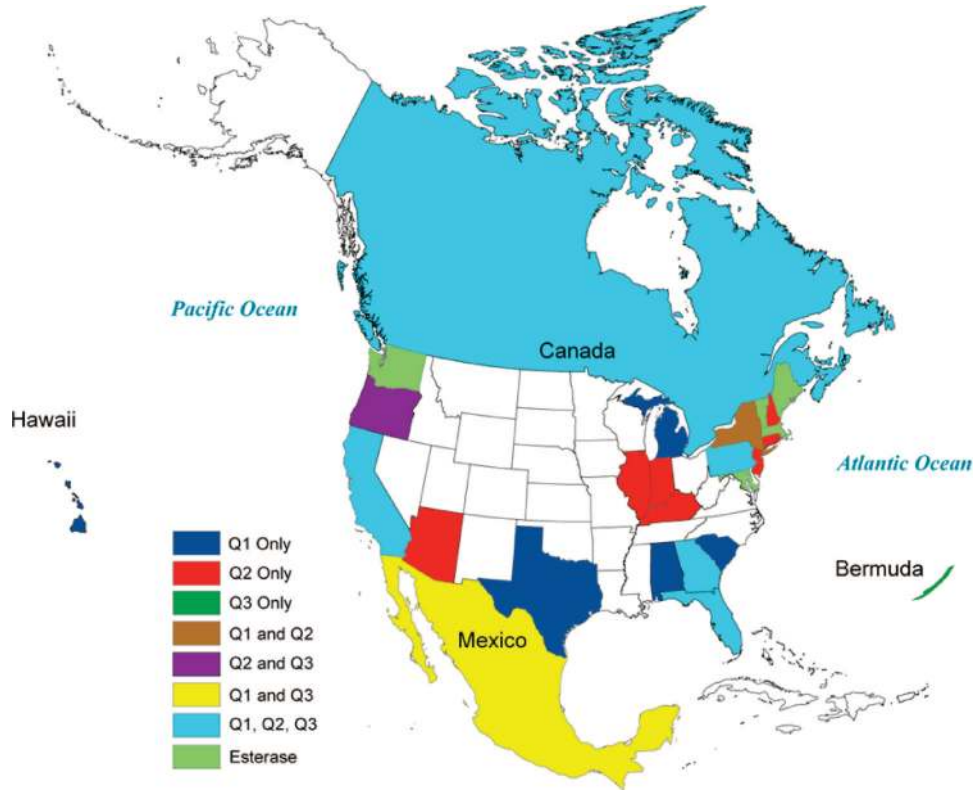

Fig. 2. *B. tabaci* biotype Q distribution in North America.

clearly a higher percentage of Q alone on all other ornamentals compared with poinsettias.

The host plant distribution data for Q biotype haplotypes within collections from poinsettias (40) and nonpoinsettia hosts (20) only included those collections analyzed by mtCOI (Fig. 4). All collections with a Q haplotype were counted irrespective of the presence of biotype B in the collection, and collections containing only a single haplotype were only counted if five or more individuals were sequenced. On poinsettias, Q1 only and Q2 only haplotypes were equally represented, with 33% of the distribution of Q biotype haplotypes within collections of poinsettia (Fig. 4). Haplotype Q3 only comprised 20% of the collections. Together, pure haplotype collections (Q1, Q2, or Q3 only) comprised 86% of all poinsettia collections, supporting at least three separate independent introductions into North America. Mixed haplotype collections (three each) of Q1/Q3 and Q2/Q3 represented the remaining 14% (7% each) of the distribution of haplotypes within collections of poinsettia. Even though Q1 and Q2 were the most abundant haplotypes, they were never found together in a single poinsettia collection, strongly suggesting multiple separate independent introductions into North America. In nonpoinsettia collections, Q1 only was the predominant haplotype (70%) followed by Q2 only (20%) (Fig. 4). Interestingly, Q1 was found with Q2 in two collections from gerbera (*Gerbera* spp.) in different locations in California in summer 2005, which was never duplicated again in sampling of any host plant. In one of the two California gerbera collections, all three haplotypes were detected which was also never duplicated again.

Discussion

Earlier surveys of *B. tabaci* populations collected primarily from vegetables in Florida (McKenzie et al. 2004) by using random amplification of polymorphic DNA-PCR techniques indicated the presence of only the B biotype of *B. tabaci.* More recent surveys in Florida (McKenzie et al. 2009) expanded the host range to include 17 herb and ornamental plant hosts, in addition to 13 preferred whitefly field-grown vegetable hosts and detected biotype Q on five different ornamental plant hosts and one herb in greenhouse and nursery locations. In Arizona, extensive sampling with >100 collections was conducted (Dennehy et al. 2010), with an emphasis on cotton and field-grown vegetables in addition to retail nurseries; biotype Q was only detected in retail nurseries. Combined, these data indicated that the biotype Q has not moved into field crops in the United States since its first detection in 2004 until 2011, despite somewhat common coexistence on nursery plants. There was great concern among growers and researchers alike that biotype Q would move from protected ornamental greenhouse

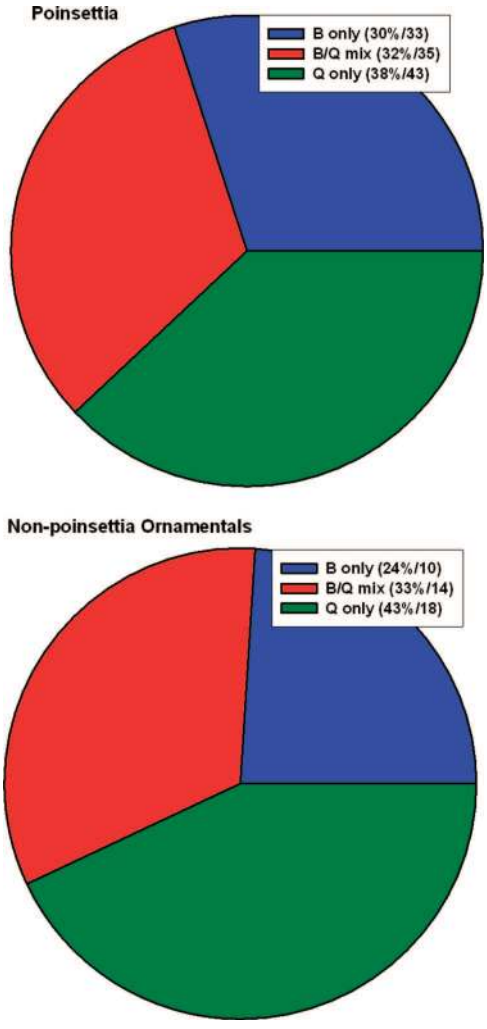

Fig. 3. Percentage of whitefly collections on poinsettia and nonpoinsettia ornamentals that contained either B, Q, or mix of biotypes. Collections were counted as B only or Q only if they had five or more individuals sampled. B/Q mix collections were counted regardless of the number of individuals sampled. Values in parentheses are the percentage and number of collections that make up the category.

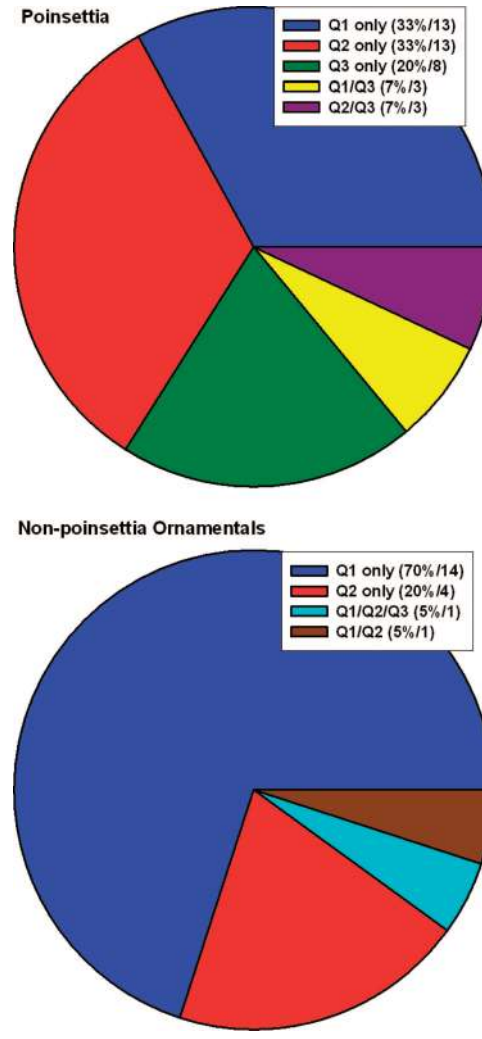

Fig. 4. Host plant distribution of Q biotype haplotypes within collections on poinsettia and nonpoinsettia ornamentals. All collections with Q haplotype were counted irrespective of the presence of B biotype. Collections containing only a single Q haplotype were only counted if five or more individuals were sequenced. There was no limit to the number of individuals sequenced for collections containing more than one haplotype. Values in parentheses are the percentage and number of collections that make up the category.

production to open agriculture (Dalton 2006, Hu et al. 2011) as it has done in other Mediterranean and Asian countries including Spain (Pascual and Callejas 2004), Israel (Horowitz et al. 2005), and China (Pan et al. 2011). Tomato transplants for field production can be grown in the same greenhouses, with ornamental plants providing ample opportunities for biotype Q to infest tomato transplants destined for the field. In fact, biotype Q was detected on tomato transplants growing in a commercial greenhouse in Georgia (Supp Table 1 [online only]). However, there were no detections of biotype Q in field-grown crops in the United States (McKenzie et al. 2009, Dennehy et al. 2010). In Taiwan (Hsieh et al. 2011) and Japan (Kijima et al. 2011), biotype Q has been detected on multiple occasions in commercial greenhouses after its initial introduction into those countries; however, there have been no detections in open fields. In caged studies, adult whiteflies reared on poinsettia as nymphs would lay eggs on six field crops (alfalfa, cabbage, cotton, cowpea, melon, and tomato), but resulting F1 adults had shorter life spans and extremely low fecundities (Hu et al. 2011). So, although whiteflies migrating from infested greenhouses to the field or infesting vegetable transplants before being planted in the field is possible, whiteflies reared on poinsettia seem to be incapable of rapidly adapting to the representative field crops. Poor adaptation to field crops may explain in

part why the Q biotype that is being dispersed on poinsettia and other ornamental hosts throughout North America has not established in the U.S. field system (Hu et al. 2011). The distribution and frequency of whitefly biotypes across regions also could be affected by insecticide use (Horowitz et al. 2005, Khasdan et al. 2005). In experiments with B and Q from Israel, but not the United States, Q predominated to the exclusion of B when insecticides were used. Regardless of whether insecticides were applied or not, the percentage of B individuals increased from 50 to 100% in four (untreated) or five (treated) generations, respectively, for B whitefly from the United States (Crowder et al. 2010). These results may help explain why Q coexists with B in Israel (Horowitz et al. 2005, Khasdan et al. 2005) but has been excluded from the field in the United States (McKenzie et al. 2009, Dennehy et al. 2010).

This study showed growers how rapidly resistant populations can be spread internationally, within a very short time frame (one growing season), occurring in a single crop, in one isolated portion of a nursery. As a result, there were many growers that were diligent in implementation of modified control strategies and this too may have aided in the prevention of the Q biotype moving to the field. A "Management Program for Whiteflies on Propagated Ornamentals with an Emphasis on the Q-Biotype" was developed in 2006 (Bethke et al. 2006) and continues to be distributed to >10,000 ornamental plant growers and propagators (http://www.mrec.ifas.ufl.edu/LSO/bemisia/bemisia.htm).

In-depth analysis of insecticide resistance profiles indicates substantial variation among different Q-biotype populations (Nauen et al. 2002; Dennehy et al. 2005, 2010; Horowitz et al. 2005; Nauen and Denholm 2005); therefore, the ability to identify the Q haplotype is of practical importance to growers for selecting among control strategies. The haplotype Q2 insecticide resistance profile has been evaluated in depth (Dennehy et al. 2010), and haplotype Q1 is currently being characterized (McKenzie et al., unpublished data). This also may be useful in detecting population variation or changes, including new introductions if source haplotypes are adequately characterized. Future work coordinating mtCOI genotyping with insecticide resistance profiles will be conducted to determine whether these genotyping methods can be used as a predictor of insecticide resistance profiles. Furthermore, the use of these molecular tools will allow investigators to track the likely origin(s) of whitefly biotypes allowing for the implementation of management efforts targeting the insects before and after they arrive in the United States.

In summary, the appearance of biotype Q in the United States in 2004 was associated with reports from ornamental growers of increasing problems in controlling whitefly infestations. As part of an APHIS-coordinated multistate, -industry, -commodity, -agency, and -institutional Q biotype Task Force initiative, a coordinated whole-country survey was conducted across North America from January 2005 to December 2011 and included Bermuda and Hawaii (islands of Hawaii and Oahu), Canada, and Mexico. Our findings show the following: 1) the New World biotype was detected in Texas and represents the first report of this biotype in the United States since its rapid displacement in the late 1980s by biotype B; 2) biotype Q was detected in 23 U.S. states and Mexico, with first reports of biotype Q in Canada and Bermuda; 3) biotype Q 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 in open-field agriculture; 4) genetic markers identified three distinct haplotypes of biotype Q whiteflies supporting the inference of independent invasions from at least three different sources; 5) although we provide data of rarely occurring B-Q hybrids, these showed no evidence of persistence; and 6) unlike other countries where the Q biotype has invaded field crops, in the United States rapid detection and implementation of improved control strategies targeting the Q biotype has prevented the establishment of this pest beyond greenhouse production.

Nucleotide Sequence Accession Numbers. The GenBank accession numbers for the mtCOI fragment amplified from biotype B, Q, and New World whiteflies from 20 of the United States, Bermuda, Canada, and Mexico and various host plants across North America are HQ198596–HQ198617, HQ198623–HQ198640, HQ198642–HQ198645, HQ198647–HQ198650, HQ198656–HQ198677, HQ198679–HQ198687, HQ198689, HQ198690, HQ198692–HQ198703, HQ198707–HQ198730, HQ198732–HQ198743, HQ198745–HQ198750, HQ198752, HQ198755, HQ198758, HQ198765, HQ198767, HQ198772–HQ198806, HQ877499–HQ877510, HQ877512–HQ877520, HQ877522–HQ877546, HQ877548–HQ877568, HQ877570, HQ877572, HQ877573, HQ877575, HQ877578, HQ877588-HQ877590, HQ877592 though HQ877602, JF754907–JF754912, JF754914–JF754925, and JN547217. Biotype Q specific accession numbers are also listed in Supp. Table 1 (online only).

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