Demographic Trends in Mixed *Bemisia tabaci* (Hemiptera: Aleyrodidae) Cryptic Species Populations in Commercial Poinsettia Under Biological Control- and Insecticide-Based Management

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ABSTRACT *Bemisia tabaci* (Gennadius) is an economically important pest of agricultural and ornamental plants worldwide and is now widely recognized as a cryptic species complex. In North America, *B. tabaci* is a particularly important pest of greenhouse poinsettia. In poinsettia production, two cryptic species from the *B. tabaci* complex, *Mediterranean* and *Middle East Minor 1*, often infest crops simultaneously. Differences in pesticide susceptibility between these two cryptic species have the potential to influence growers' management decisions, including the use of biological control or insecticides, and the choice of insecticide active ingredient. However, the demographic behavior of mixed-species infestations in commercial greenhouses has yet to be investigated. We conducted a survey of *B. tabaci* populations in commercial greenhouses in Ontario, Canada, and provide evidence that under biological control-based management, *Middle East Minor 1* can displace *Mediterranean*, whereas under insecticide-based management *Mediterranean* populations will persist. Furthermore, we comment on implications of this behavior on the management of *B. tabaci*, and comment on methods used to identify *B. tabaci* cryptic species.

KEY WORDS biotype, asymmetric mating interaction, DNA barcode

The whitefly *Bemisia tabaci* (Gennadius; Hemiptera: Aleyrodidae) is a small phloem-feeding insect that predominantly feeds on herbaceous plants, although its host range includes upwards of 600 species (Oliveira et al. 2001). *B. tabaci* has a global distribution (Dinsdale et al. 2010), and throughout much of its range, it is a significant pest of ornamentals, vegetables, legumes, and cotton (Oliveira et al. 2001). In many crops, plant damage is caused by direct feeding, transmission of plant viruses (Jones 2003), or growth of sooty-mold fungus as a result of the secretion of honeydew. However, for ornamentals such as poinsettia (*Euphorbia pulcherrima*), which are marketed for their esthetic value, the presence of any life stage (larva, prepupa, pupa, adult, or exuvia) on the plants may result in entire consignments being rejected by buyers/wholesalers, causing significant financial loss for the grower. These market conditions present an important management challenge for poinsettia producers, as *B. tabaci* densities must be reduced to levels typically lower than nonornamental commodities.

It is now widely recognized that *B. tabaci* is a cryptic species complex consisting of at least 24 morphologically indistinguishable yet behaviorally and physiologically distinct species (Boykin et al. 2007, Dinsdale et al. 2010, De Barro et al. 2011, Liu et al. 2012, McKenzie et al. 2012, Tay et al. 2012). To account for this variation, a convention of assigning a unique "biotype" designation to distinct *B. tabaci* populations was adopted, of which 36 biotypes are reported in the literature (De Barro et al. 2011). However, for various reasons, including the taxonomic appropriateness of the term "biotype," the biotype naming convention for *B. tabaci* has fallen out of favor (Tay et al. 2012) and has been replaced by a classification system that represents the presumed phylogeographic origins of the proposed cryptic species (Dinsdale et al. 2010). This classification system is based on a partial cytochrome c oxidase subunit I (COI) sequence derived from the 3′ region of the gene. For the purposes of this manuscript, we follow this phylogeographic naming convention. Of the 24 putative cryptic species, *Mediterranean* (formerly *"biotype Q,"* or sweet potato whitefly) and *Middle East-Asia Minor 1* (MEAM1: formerly *"biotype B,"* or *Bemisia argentifolii* Bellows & Perring, silver leaf whitefly) are the most commonly encountered in the pest management literature (Liu et al. 2007, McKenzie et al. 2012) and often infest ornamental crops in North America, including poinsettia. Both of these species have experienced significant range expansions as a result of international trade (Cheek and Macdonald 1994, Dalton 2006). A third cryptic species, *New World* (formerly, *"biotype A, C, D,*

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F, Jatropha, N, R, Sida"), is present in North America, but it rarely infests crop plants and is generally not considered economically significant (Dinsdale et al. 2010, McKenzie et al. 2012). Of practical importance is the fact that *Mediterranean* is more resistant to, and likely has a greater capacity to develop resistance to, various insecticides than other *B. tabaci* cryptic species, in particular MEAM1 (Dennehy et al. 2010, Li et al. 2012). Also, both the *Mediterranean* and the MEAM1 cryptic species frequently occur on the same crop (McKenzie et al. 2012). Together this can present a significant pest management challenge for growers owing to the uncertainty surrounding the identity and hence resistance status of their *B. tabaci* infestations.

In Ontario, Canada (where this study was conducted), poinsettia crops are produced from imported vegetative cuttings. *B. tabaci* is not known to overwinter outdoors in Ontario, and thus infestations in poinsettia result from the import of infested vegetative cuttings. In Ontario, typical *B. tabaci* management programs are based on either insecticides or biological control. Insecticides registered for control of whitefly in poinsettia in Canada include neonicotinoids (i.e., imidacloprid and acetamiprid), insect growth regulators (IGRs; i.e., pyriproxifen and kinoprene), organophosphorates (i.e., dichlorvos, malathion, chlorpyrifos, acephate, and naled), and pyrethroids (i.e., permethrin), as well as endosulfan, spiromesifen, pyridaben, pymetrozine, and insecticidal soap. Insecticide-based management programs are initiated in August (at the earliest) and conclude in October–November before shipping of the finished crop. Of these classes of insecticides, it has been demonstrated that *Mediterranean* is more tolerant to IGRs, neonicotinoids, and pyrethroids than MEAM1 (Horowitz et al. 2005, Dennehy et al. 2010). Alternatively, many growers use one or more commercially available biological control agents: *Eretmocerus eremicus* Rose & Zolnerowich, *Eretmocerus mundus* Mercet, *Encarsia formosa* Gahan, *Amblyseius* (= *Typhlodromips*) *swirskii* (Athias-Henriot), or *Delphastus catalinae* (Horn). Under both biological control- and insecticide-based management schemes, it may be necessary to apply a "clean-up" application of a foliar insecticide toward the end of the season to ensure the marketability of the crop.

In mixed laboratory populations, it has been shown that MEAM1 will displace *Mediterranean* via asymmetric mating interference (Pascual and Callejas 2004, Crowder et al. 2010). Similarly, MEAM1 has displaced other *B. tabaci* cryptic species on a landscape scale in both China and Australia (Liu et al. 2007). However, in the presence of insecticide selection pressures, *Mediterranean* will persist in mixed populations (Horowitz et al. 2005, Crowder et al. 2010). Therefore, if season-long application of insecticides promotes the proliferation or maintenance of *Mediterranean* individuals in mixed *Bemisia* populations, it could limit the utility of late-season "clean-up" insecticide applications, jeopardizing the marketability of the crop. At this point, little is known about the population dynamics of mixed populations under biological control compared with insecticide-based management regimens in commercial greenhouses. Therefore, understanding how compositions of mixed populations of *Mediterranean* and MEAM1 change under realistic greenhouse production conditions could help improve *B. tabaci* management. We report results from a *B. tabaci* survey from commercial poinsettia greenhouses in Ontario, Canada, using either biological control- or insecticide-based *B. tabaci* management programs, and discuss potential management implications of the observed trends. Furthermore, we generate full COI sequences for both *Mediterranean* and MEAM1 collected in Ontario, to bridge the gap between the 5′ COI marker used for the current classification of *B. tabaci* cryptic species and the marker used for DNA barcoding.

Materials and Methods

***B. tabaci* Survey.** *B. tabaci* were hand-collected from six commercial poinsettia greenhouses throughout the Niagara Peninsula in Ontario, Canada, during the 2012 production season. Three of the greenhouses used biological control as their primary strategy for *Bemisia* management, and although programs varied between greenhouses, all three relied heavily on *E. eremicus* and *E. formosa.* The remaining three greenhouses exclusively used insecticides for *B. tabaci* management. These programs varied by greenhouse but included neonicotinoids, IGRs (primarily pyriproxifen), and pyridaben. Insecticide applications were initiated between 7 and 22 August. Each greenhouse was regularly monitored during the poinsettia production cycle from 5 July to 21 November, by inspecting ≈10% of the crop during each visit. The data collected from these greenhouses comprised the seasonal data set. For the seasonal data set, the proportion of *Mediterranean* individuals in each greenhouse collection event was arcsine square-root transformed to normalize the data and subjected to analysis of variance in R version 2.14.1 (R Development Core Team 2011). Variance was partitioned by greenhouse management method (i.e., biological control- or insecticide-based), week of sampling, and the greenhouse sampled. On 12 December, a number of one-time-only surveys were conducted in an additional seven greenhouses from the Niagara Peninsula, two of which exclusively used insecticide-based *Bemisia* management, and five used biological control-based *Bemisia* management. These data comprised the end-of-season survey.

During surveys, plants were individually inspected and whiteflies were aspirated directly into 95% ethanol. The majority of individuals collected were adults; however, on occasion, nymphs/pupae were collected if they were the only life stage present on a given plant. If multiple individuals were collected from a single plant, they were combined in a single collection vial. Whitefly samples were stored at −20°C before DNA extraction. DNA was extracted from whole specimens with XytXtract Insect (ANDE; Xytogen; Perth, Australia) DNA extraction kits using manufacturer-recommended protocols (Castalanelli et al. 2010). When

Table 1. Primers and PCR cycling conditions used in this study

Primer mixture	Sequence 5′–3′	Cycling conditions	Source
Biotype			
BioB-L	CTAGGGTTTATTGTTTGAGGTCATCATATATTC	94°C for 2 min 35 cycles of, 94°C for 30s, 64°C for 1 min, 72°C for 1 min, 72°C for 10 min	Shatters et al. (2009)
BioB-R	AATATCGACGAGGCATTCCCCCT		
New World-L	TACTGTTGRAATAGATGTTGACACTCGGG		
New World-R	GGAAAAAATGTAAGRTTTACTCCCWCAAATATT		
BioQ-L	CTTGGTAACTCTTCTGTAGATGTGTGTT		
BioQ-R	CCTTCCCGCAGAAGAAATTTTGTTC		
COI(42–699*)			
WF-F	ATTCAACCAATCAYAARGATATYGG	94°C for 1 min 5 cycles of, 94°C for 40s, 45°C for 40 s, 72°C for 1 min 35 cycles of, 94°C for 40 s, 51°C for 40 s, 72°C for 1 min, 72°C for 10 min	This Study
WF-R	TAAACTTCTGGATGHCCAAARAAYCA		
COI(581–1003*)			
BtabINT-F	GATTTCTCTYCCTGTTCTTGCA	94°C for 2 min 35 cycles of, 94°C for 30s, 57.3°C for 1 min, 72°C for 1 min, 72°C for 10 min	This Study
BtabINT-R	TCCTGTAAATCAAAGGCCAAGRG		
COI(801–1537*)			
Btab-Uni-L	GAGGCTGRAAAATTARAAGTATTTGG	94°C 2 min 35 cycles of, 94°C for 30s, 46°C for 1 min, 72°C for 1 min, 72°C for 10 min	Shatters et al. (2009)
Btab-Uni-R	CTTAAATTTACTGCACTTTCTGCCAYATTAG		

*Binding sites on GenBank Accession AY521259 *Bemisia tabaci* (*New World* biotype) complete mitochondrial genome.

possible, whitefly specimens were retained and stored individually in 95% ethanol. DNA extracts were stored at −20°C for a maximum of 3 d before analysis.

Cryptic Species Identification. The cryptic species' identity of *B. tabaci* individuals was determined using a method developed by Shatters et al. (2009). DNA extracts from unidentified *B. tabaci* specimens were subjected to a diagnostic multiplex polymerase chain reaction (PCR) amplification containing three sets of primers designed to amplify fragments of different lengths from each of the "biotypes" or cryptic species: 303 bp from *Mediterranean*, 405 bp from *New World*, and 478 bp from MEAM1. Fragments were then visualized on a 2% agarose gel prestained with SYBR Safe DNA gel stain (Life Technologies).

COI Sequence Bridging. Near-complete 1,466-bp COI sequences were generated for six *Mediterranean* and six MEAM1 individuals, randomly selected from all collections. This sequence encompasses both the 5′ COI marker used for *B. tabaci* cryptic species classification and the DNA barcoding region. Pairwise distances were computed between each haplotype and a consensus sequence for each *B. tabaci* cryptic species (Dinsdale et al. 2010) in MEGA5 (Tamura et al. 2011) to verify cryptic species designation. All PCR reactions included 6.25 μl of 10% trehalose, 2.00 μl of water, 1.25 μl of 10× PCR Buffer (Life Technologies), 0.625 μl $MgCl_2$ (50 mM), 0.25 μl of dNTP (10 mM), 0.0625 μl of Platinum *Taq* (Life Technologies), 0.1 μl total volume of each forward and reverse primers, and 2 μl of DNA template (Ivanova et al. 2009). A complete list of primers and PCR conditions can be found in Table 1.

Results

In total, 632 *B. tabaci* were collected from 319 poinsettia plants derived from 43 separate greenhouse collection events (Table 2) that included both seasonal and end-of-year sampling. The vast majority, 89% (n = 564) of individuals were identified as MEAM1; the remainder were identified as *Mediterranean*, no *New World* individuals were found. Of the 171 plants from which multiple whiteflies were collected, only one harbored individuals of both MEAM1 and Mediterranean; this detection was made in a greenhouse with

Table 2. Summary of *Bemisia tabaci* cryptic species' surveys conducted in Ontario, Canada, in commercial poinsettia greenhouses

Data set/greenhouse management	No. greenhouses	No. *B. tabaci*	No. MEAM1	No. *Mediterranean*
Seasonal data set	6	512	453 (88%)	59 (12%)
Biological control	3	366	353 (96%)	13 (4%)
Insecticide	3	146	100 (68%)	46 (32%)
End-of-season survey	7	119	111 (93%)	8 (7%)
Biological control	5	96	95 (99%)	1 (1%)
Insecticide	2	23	16 (70%)	7 (30%)
Total	11	632	564 (89%)	67 (11%)
Biological control	8	462	448 (97%)	14 (3%)
Insecticide	5	170	116 (68%)	54 (32%)

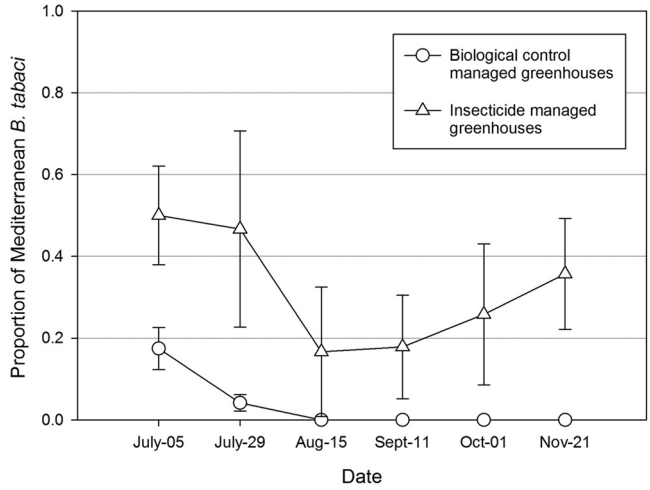

Fig. 1. Proportion ± SE of *Mediterranean B. tabaci* detected in six commercial poinsettia greenhouses surveyed between 5 July and 22 November 2012. Three greenhouse used biological control-based management, and three used insecticide-based management.

biological control-based *Bemisia* management as part of the end-of-season survey on 12 December. Individual greenhouse collections were predominantly MEAM1 (60% of greenhouse visits), followed by mixed MEAM1–*Mediterranean* (28%), and *Mediterranean* (12%). The majority (81%) of all *Mediterranean* individuals found in this survey were in greenhouses using insecticide-based *Bemisia* management programs.

Seasonal Data Set. The seasonal data set consisted of 512 individuals collected during 36 greenhouse visits. The majority (88%) of individuals were identified as MEAM1. The proportion of *Mediterranean* individuals in biological control-managed greenhouses decreased from 17% on 5 July to 0% on 21 November. In the insecticide-managed greenhouses, the proportion of *Mediterranean* individuals was 50% on 5 July and 36% on 21 November (Fig. 1). *Mediterranean* individuals were detected in two of the three biological control- and all of the insecticide-managed greenhouses on the first sampling date on 5 July. After 29 July, no *Mediterranean* individuals were detected in any of the bx-biological control-managed greenhouses. In the insecticide-managed greenhouses, *Mediterranean* individuals were found in two of the greenhouses for the duration of the survey, but were not detected in the third after 5 July. Greenhouse management method had a significant effect on the proportion of *Mediterranean* individuals in sampled populations ($F = 18.10$, df = 1,32, $P < 0.05$), whereas sampling week and the greenhouse did not.

End-of-Season Survey. In total, 119 whiteflies were collected from the end-of-season survey on 12 December. The majority (93%, $n = 111$) of individuals were MEAM1, with the remainder being *Mediterranean.* Collections from five of the biological control-managed greenhouses were predominantly composed of MEAM1, with the exception of a single *Mediterranean* individual. Collections from one of the insecticide-managed greenhouses was entirely composed of *Mediterranean* ($n = 8$) individuals, whereas the other was entirely composed of MEAM1 ($n = 14$).

COI Sequencing. Two COI haplotypes were identified, one corresponding to MEAM1 and the other to *Mediterranean.* Identifications inferred from the fragment length assay (Shatters et al. 2009) and the consensus sequence approach (Dinsdale et al. 2010) were in complete agreement. That is, individuals identified as MEAM1 and *Mediterranean* via the fragment length assay were identical (i.e., 0.00% divergent) to their respective consensus sequence (Supp Table 1[online only]). Sequence, trace-file, and specimen images for these specimens can be found in BOLD project BTB, BOLD Process-ID: BTB004-13 to BTB0015-13. GenBank accession numbers for these specimens are KJ591609 to KJ591620. All sequences derived from *Mediterranean* individuals were assigned to Barcode Index Number (BIN) BOLD:AAG4846 and MEAM1 to BOLD:AAT8875 by the Barcode of Life Data Systems (Ratnasingham and Hebert 2007, 2013).

Discussion

Our data suggest that MEAM1 is capable of displacing *Mediterranean* populations in a greenhouse environment, similar to observations in both laboratory populations and in the field (Liu et al. 2007, Crowder et al. 2010, Dinsdale et al. 2012, Luan et al. 2013). This trend is evident in the biological control-managed greenhouses, as indicated by our inability to detect any *Mediterranean* individuals after 29 July in the seasonal data set. This is supported by observations made at the end-of-season survey, where only a single *Mediterranean* individual was found. Our data also suggest that MEAM1 displaced *Mediterranean* populations in the insecticide-managed greenhouses until the start of insecticide programs in August. Before the initiation of insecticide-based management programs, the pro-

portion of *Mediterranean* individuals decreased; however, after insecticide-based management programs began, the proportion of *Mediterranean* individuals increased (Fig. 1). This increase likely occurred owing to insecticide applications selecting for *Mediterranean* individuals in those greenhouses, demonstrating the potential for pest resurgence due to insecticide resistance. Overall our data support the predictions made by Crowder et al. (2010) regarding the competitive exclusion of *B. tabaci* cryptic species in the presence and absence of insecticides. Together, these observations emphasize the importance of periodically determining the composition of *Bemisia* populations to inform management decisions, particularly insecticide active ingredient selection in the context of a resistance management program in mixed-species infestations.

Our results suggest that mixed-species infestations in commercial greenhouses can revert to MEAM1 under biological control-based management. Therefore, biological control may be the preferred management technique if reduction of *Mediterranean* individuals is an important pest management objective, which may be the case in some ornamental production systems (e.g., poinsettia). The decline in *Mediterranean* individuals in biological control-based management greenhouses was likely a result of asymmetric mating interference between MEAM1 and *Mediterranean* individuals (Crowder et al. 2010). This would occur more rapidly in the biological control greenhouses owing to the greater proportion of MEAM1 individuals compared with the insecticide-managed greenhouses (Fig. 1). Alternatively, it is possible that the biological control agents used by growers may preferentially parasitize or prey on the *Mediterranean* individuals. However, to our knowledge, no comparable data exist on the host preferences or functional response of parasitoids against *B. tabaci* cryptic species. The only example found in the literature dealt with *Encarsia sophia* (Girault & Dodd), a parasitoid with a cosmopolitan distribution, which showed a preference for MEAM1 (Wang et al. 2011). Given the economic importance of *B. tabaci*, we encourage more studies to determine if any host preferences exist for the commercially available *B. tabaci* biological control agents.

To date, the majority of DNA sequence data generated for *B. tabaci* have been for the 3′ region of the COI gene (Frohlich et al. 1999, Boykin et al. 2007, Shatters et al. 2009, Dinsdale et al. 2010, Ahmed et al. 2012). Information gleaned from these data was used to describe the genetic variation within the *B. tabaci* cryptic species complex (Frohlich et al. 1999, Shatters et al. 2009, Dinsdale et al. 2010, De Barro et al. 2011), and has been used by the current authors and others (Ahmed et al. 2012) to identify *B. tabaci* cryptic species. However, a 650-bp fragment of the 5′ region of this gene (also known as the DNA barcode) has been widely adopted for use as a genetic marker for the identification of animals, including pest insects, in both an applied and a research context (Hebert et al. 2003, Armstrong 2010, Bonants et al. 2010, Frewin et al. 2013). At the time of writing this article, DNA barcodes have been generated for 138,537 insect species (refer to BOLD: www.boldsystems.org, Ratnasingham and Hebert 2007). Given the widespread adoption of DNA barcoding as a tool for species identification, we would encourage researchers generating COI sequence data for *B. tabaci* to consider including both the 5′ and 3′ regions, and to follow DNA barcoding metadata standards (Hanner 2009). This will ensure that data collected with one marker can be unambiguously linked to data collected from the other and that the data will be useful to the largest community possible.

Our study suggests that MEAM1 is capable of displacing *Mediterranean* in greenhouses in a single growing season, which encompasses approximately five to six generations of *B. tabaci.* This knowledge may help growers to more efficiently manage mixed *B. tabaci* populations. For example, the ability of MEAM1 *B. tabaci* to naturally displace *Mediterranean* under biological control-based management may increase the efficacy of "clean-up" insecticide applications when necessary, which can be important for ornamental crops. These data also support the use of biological control-based *B. tabaci* management, as it can effectively reduce *B. tabaci* populations below economic levels while allowing MEAM1 to displace *Mediterranean.* Conversely, repeated use of insecticides may promote the persistence of the *Mediterranean* cryptic species.

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