Extraordinary Resistance to Insecticides Reveals Exotic Q Biotype of *Bemisia tabaci* in the New World

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ABSTRACT A strain of the whitefly *Bemisia tabaci* (Gennadius) possessing unusually high levels of resistance to a wide range of insecticides was discovered in 2004 in the course of routine resistance monitoring in Arizona. The multiply resistant insects, collected from poinsettia (*Euphorbia pulcherrima* Willd. ex Klotzsch) plants purchased at a retail store in Tucson, were subjected to biotype analysis in three laboratories. Polyacrylamide gel electrophoresis of naphthyl esterases and sequencing of the mitochondrial cytochrome oxidase I gene (780 bp) confirmed the first detection of the Q biotype of *B. tabaci* in the New World. This U.S. Q biotype strain, referred to as Poinsettia'04, was highly resistant to two selective insect growth regulators, pyriproxyfen and buprofezin, and to mixtures of fenpropathrin and acephate. It was also unusually low in susceptibility to the neonicotinoid insecticides imidacloprid, acetamiprid, and thiamethoxam, relative to B biotype whiteflies. In 100 collections of whiteflies made in Arizona cotton (*Gossypium* spp.), vegetable, and melon (*Cucumis melo* L.) fields from 2001 to 2005, no Q biotypes were detected. Regions of the United States that were severely impacted by the introduction of the B biotype of *B. tabaci* in the 1980s would be well advised to promote measures that limit movement of the Q biotype from controlled environments into field systems and to formulate alternatives for managing this multiply-resistant biotype, in the event that it becomes more widely distributed.

KEY WORDS *Bemisia tabaci*, insect growth regulators, insecticide resistance, neonicotinoids, Q biotype

The whitefly *Bemisia tabaci* (Gennadius) is a severe pest of cotton (*Gossypium* spp.), vegetables, melons (*Cucumis melo* L.), tomatoes (*Lycopersicon* spp.), and other crops in the southern United States. It has long been thought to comprise morphologically indistinguishable biotypes that do not interbreed for the most part and that vary with respect to host preference, virus–vector efficiency, dispersal behavior, and insecticide resistance (Brown et al. 1995, Byrne et al. 1995b, Brown 2001, Perring 2001, Viscarret et al. 2003, but see Pascual 2006). Recent interbiotype mating and phylogenic studies show that most of these biotypes represent genetically distinct cryptic species (Dinsdale et al. 2010, Elbaz et al. 2010, Xu et al. 2010, De Barro et al. 2011; but for connection of the current study to the literature, we retain the use of the term biotype). Invasions by exotic biotypes can lead to explosive reproduction and increases in population size, especially in the U.S. desert southwest, where overlapping cropping seasons made possible by the warm, dry climate much of the year, allow opportunities for this pest and virus vector to feed and reproduce on multiple host crops.

A case in point is the unprecedented outbreak of whitefly in the late 1980s and early 1990s, when a new biotype of *B. tabaci*, the B biotype, was introduced by human-mediated movement of contaminated greenhouse-grown ornamentals (Costa and Brown 1991, Brown et al. 1995) in the United States and displaced the native A biotype among low desert growing regions of Mexico, California, and Arizona (Costa et al. 1993). The B biotype, which originated in the Middle East, Arabian Peninsula, or northern Africa (Kirk et al. 2000), is extremely polyphagous with a wide range of host plants (Brown et al. 1995). Unlike the native A biotype, the B biotype was resistant to broad-spectrum insecticides, including organophosphates, carbam-

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ates, and pyrethroids (Costa et al. 1993, Denholm et al. 1998). Seemingly overnight, producers were faced with unprecedented infestations of an insect that previously was easy to control. Initial attempts to control the B biotype whitefly with broad-spectrum insecticides such as pyrethroids synergized with organophosphates resulted in severe problems with whitefly resurgences in cotton (*Gossypium* spp.), severe development of resistance (Dennehy et al. 1996a,b; Dennehy and Williams 1997; Castle et al. 2001), natural enemy destruction (Naranjo 2001; Naranjo et al. 2003, 2004), secondary pest outbreaks (Ellsworth and Martinez-Carrillo 2001, Palumbo et al. 2001) and loss of profitability due to crop damage and expenses related to high numbers of insecticide applications (Ellsworth and Jones 2001, Naranjo and Ellsworth 2009). The difficult-to-control B biotype was so problematic that a national 5-yr plan of research and action was undertaken by the U.S. Department of Agriculture (DeQuattro et al. 2007).

In Arizona, whitefly control changed dramatically for the better, first in melon and vegetables in 1993, and then in cotton in 1996, with the introduction of new management recommendations (Dennehy et al. 1996a,b; Palumbo et al. 2003; Naranjo and Ellsworth, 2009). Intensive investments were made into research and education that improved monitoring, treatment thresholds, conservation of beneficial insects, and use of insecticides (Dennehy et al. 1996a,b; Ellsworth et al. 1996; Palumbo et al. 2001, 2003; Ellsworth and Martinez-Carillo 2001; Naranjo and Ellsworth 2009). Insecticides that were highly toxic to key natural enemies of whiteflies were supplanted by limited early season use of insect growth regulators (IGRs) in cotton and of neonicotinoids in melon and vegetables. Two highly effective selective IGRs, pyriproxyfen (a juvenile hormone analog) and buprofezin (a chitin synthesis inhibitor), and several reduced-risk neonicotinoids, including imidacloprid, thiamethoxam, dinotefuran, and acetamiprid, replaced much of the previously widespread use of synergized pyrethroid insecticides (Dennehy et al. 1996a), pyrethroids mixed with organophosphate or carbamate insecticides. Since 1996, insecticide treatments in Arizona cotton have declined to averages of less than two or three treatments per year (Agnew and Baker 2001; Ellsworth and Martinez-Carrillo 2001; Shanley and Baker 2002, 2003). This represents a dramatic change from 1995, when producers were making 6 to 12 insecticide treatments per acre of cotton (Naranjo and Ellsworth 2009).

When reports began to emerge on another *B. tabaci* biotype, the Q biotype (Guirao et al. 1997), associated with severe IGR or neonicotinoid resistance problems in southern Europe (Nauen et al. 2002, Rauch and Nauen 2003, Nauen and Denholm 2005, Prabhaker et al. 2005) and Israel (Horowitz et al. 2003a,b, 2005), we recognized it as a potential threat to our hard-won success in whitefly management. Thus, in 2001, we began doing biotype assessments of whiteflies collected for routine resistance monitoring throughout Arizona. Here, we describe the first detection of the Q biotype in the New World. We demonstrate that this new biotype was unlike any whiteflies we had tested from Arizona, with respect to its greatly elevated levels of resistance to a broad range of insecticides. On this basis, we conclude that the Q biotype may have the potential to disrupt the same production systems that were severely impacted by previous movement into the United States of the B biotype of *B. tabaci*. We also provide evidence the Q biotype was detected before potential establishment in field systems in Arizona.

Materials and Methods

Collection of Whiteflies. Collections of *B. tabaci* were made in 2001 ($n = 27$), 2003 ($n = 28$), 2004 ($n = 30$), and 2005 ($n = 37$), principally from the irrigated agricultural areas of Arizona (Fig. 1; also see Supp Table S1a–d [online only]). One or two of these samples each year were from field locations in California (Supp Table S1a–c [online only]). Although the same geographical regions were sampled each year, whiteflies did not occur in densities permitting collection at each site each year. When infestations permitted, collections were made from melons, vegetables, cotton, and nursery and ornamental crops. Collections from ornamentals focused on poinsettias in retail nurseries and stores, and they were made during November and December. Our objective was to obtain enough whiteflies from each site to permit insecticide bioassays and molecular biotyping. The former required ≈5,000 adult whiteflies, whereas the latter required as few as five or 10 adults. Low whitefly densities, often resulting from treatment of fields with insecticides, predation, parasitism, or a combination prevented testing of some collections with some insecticides. Adult whiteflies were collected in modified plastic vials by vacuuming plant foliage with a Makita cordless vacuum (model 4071D; Makita Corp., Anjo, Aichi, Japan). Samples were transported to the laboratory in Tucson and were released into cages containing several cotton plants of variety DPL-50, at the five to seven true-leaf stage.

Bioassays of Susceptibility to Insecticides. Insecticide bioassays were typically conducted on adults within 12–36 h of making field collections. Most samples from ornamental plants and some samples from other crops were obtained as nymphs from which adults were reared in the laboratory. In such cases, infested leaves or potted plants were transported to the laboratory and placed in cages containing potted cotton plants, to permit adults to emerge. Bioassays were conducted after adequate numbers of adults had emerged, which was typically between 1 and 4 wk after collection. Bioassays of susceptibility to six insecticides were conducted on each collection of whiteflies by using a published method for each insecticide evaluated (Table 1). Bioassay methods for the IGRs pyriproxyfen and buprofezin were described by Li et al. (2003) and involved dipping of small cotton plants containing whitefly eggs or first-instar nymphs, respectively. The residual leaf disk bioassay used for

Fig. 1. Locations at which whiteflies were sampled in 2004. Field-collected whiteflies were placed in cages in the laboratory and tested for susceptibility to key insecticides. Adults of the field-collected generation were also preserved in 95% ethanol for molecular biotype determinations.

fenpropathrin + acephate mixtures was described by Dennehy and Williams (1997) and included 1,000 μg/ml acephate as a synergist in all concentrations of fenpropathrin tested. This concentration of acephate was not toxic to adult whiteflies in our bioassay. The three neonicotinoid insecticides, imidacloprid, thiamethoxam, and acetamiprid, were tested using leaf disk bioassays (Li et al. 2000). However, to conform with prevailing routes of exposure in the field, imidacloprid was tested using disks cut from leaves that had been treated using a 24-h hydroponic uptake procedure, whereas thiamethoxam and acetamiprid were tested as residual leaf dips (Table 1). The following formulated insecticides were used:, Admire 2F (imidacloprid, Bayer Crop Sciences, Research Triangle Park, NC), Centric 40 WG (thiamethoxam, Syngenta Crop Protection, Greensboro, NC), Courier 40 SC (buprofezin, Nichino America, Inc., Wilmington, DE), Danitol 2.4 EC (fenpropathrin, Valent USA Corp., Walnut Creek, CA), Intruder 70 WP (acetamiprid, DuPont Agricultural Products, Wilmington, DE), Knack 0.86 EC (pyriproxyfen, Valent USA Corp.), and Orthene 97S (acephate, Valent USA Corp.).

Biotype Identification. Depending on the abundance of whiteflies in samples, 10–100 adults from each collection were placed in 95% ethanol and held at −20°C until biotype evaluations could be conducted. In 2001, the first biotype analyses were conducted using individuals obtained directly from control groups of insecticide bioassays as the samples were tested in the laboratory. Thereafter, we preserved adults directly from field-collected individuals or as soon as adults emerged in the laboratory from nymphs

Table 1. Summary of bioassay methods used for the insecticides tested against *B. tabaci* in 2004

Insecticides	Pyriproxyfen	Imidacloprid	Fenpropathrin	Buprofezin	Thiamethoxam	Acetamiprid
Formulation	Knack 0.86 EC	Admire 2F	Danitol 2.4 EC, Orthene 97S	Courier 40 SC	Centric 40 WG	Intruder 70 WP
Concn μg/ml	Control, 0.01, 0.1, 1.0	Control, 1, 10, 100, 1,000	Control, 10, 100 (+1,000 acepahte)	Control, 8, 100, 1,000	Control, 1, 10, 100, 1,000	Control, 1, 10, 100, 1,000
Replication	6 plant reps, >20 eggs per leaf	10 vial reps, 25 adults per vial	6 vial reps, 25 adults per vial	6 plant reps, >20 nymphs per plant	6 vial reps, 25 adults per vial	6 vial reps, 25 adults per vial
Method	Seedling in vial, dipped after oviposition	Seedling, 24 h hydropnc uptake, infest leaf disc	Leaf disc, dipped before infestation	Infested seedling in vial, dipped	Leaf disc, dipped before infestation	Leaf disc, dipped before infestation
Stage treated	Egg	Adult	Adult	N1 (crawler) stage	Adult	Adult
Treatment method	Leaf dip, 20 s	24 h hydroponic uptake	Leaf dip, 10 s	Leaf dip 20 s	Leaf dip 10 s	Leaf dip 10 s
Duration	7-d exposure	48-h exposure	48-h exposure	9-d exposure	48-h exposure	48-h exposure
Notes	24-h ovip period, followed by 20-s leaf dip, read 7 d after dipping	Small seedling (2–4 true leaf-stage), cut stem above root line; put into imdaclopnd solution for 24 h	Small seedling (2–4 true-leaf stage), cut leaf discs and dip for 10s into solution	24-h oviposition period, followed by 8 d to develop to N1, 20-s leaf dip, read 9 d after dipping	Small seedling (2–4 true-leaf stage), cut leaf discs and dip for 10 s into solution	Small seedling (2–4 true-leaf stage), cut leaf discs and dip for 10 s into solution

collected in the field. The mitochondria cytochrome oxidase (*mtCOI*) gene was used as a molecular marker to identify haplotype-specific DNA differences. The mtCOI fragment (850 bp) was polymerase chain reaction (PCR)-amplified using the primers C1-J-2195 MTD-10 (5′-TTG ATT TTT TGG TCA TCC AGA AGT-3′) and L2-N-3014 MTD-12 (5′-TCC AAT GCA CTA ATC TGC CAT ATT A-3′) (Simon et al., 1994, Fröhlich et al. 1999, Ma et al. 2009). PCR products were separated on 1% agarose gels, and bands were visualized by ethidium bromide staining. PCR products were cleaned, quantified, and the DNA sequence was determined (bidirectionally) at the Biotechnology Core Facility of The University of Arizona, Tucson, AZ. The sequences were aligned with reference *mtCOI* sequences for A, B, Q, and other biotypes or haplotypes available in the reference collection by using MegAlign (Lasergene, DNASTAR, Madison, WI) or DNAMAN version 4.0. The biotype/haplotype identity was determined based on comparative sequence analysis in relation to a suite of reference *mtCOI* sequences available in the Brown laboratory (J.K.B., some data unpublished).

DNA Extraction and PCR Amplification and Sequencing of mtCOI Gene: 2001. Total nucleic acids were extracted from individual adult female whiteflies by placing them on a section of parafilm and grinding in 5 μl of ice-cold lysis buffer with a plastic pestle. Lysis buffer was made fresh and consisted of 1 mM Tris-HCl, pH 8.0, containing 0.5 mM EDTA, 0.5% Nonidet P-40, and 1 mg/ml proteinase K. Extracts were then incubated at 65°C for 15 min and 95°C for 10 min before a brief centrifugation (10,000 $\times$ *g*) to pellet debris. The aqueous supernatant was used as the DNA templates for PCR amplification of *mtCOI* gene. PCR, DNA sequencing, and sequence analysis were carried out as described above.

DNA Extraction and PCR Amplification and Sequencing of mtCOI Gene: 2003–2005. Total nucleic acids were extracted from individual adult male or female whiteflies by placing them on a section of parafilm and grinding in 15 μl of DNAzol and 5 μl of polyacryl carrier with a plastic pestle. Each homogenate was then transferred to a 1.5-ml microcentrifuge tube containing 0.48 ml of DNAzol and 2.5 μl of proteinase K. Samples were held at room temperature for 30 min before precipitation of DNA by the addition of 0.25 ml of 100% ethanol. After centrifugation at 13,000 rpm for 10 min, the supernatant was removed, and the resulting DNA pellet was washed with 75% ethanol and centrifuged for 5 min at 6,500 rpm. Excess ethanol was removed from the tube before repeating the ethanol wash. The DNA pellet was air-dried before being resuspended in 40 μl of prewarmed low TRIS-EDTA (TE) buffer and stored at −20°C. PCR, DNA sequencing, and sequence analysis were carried out as described above except that PCR products were recovered from 1% agarose gels using the QIAquick gel extraction kit (QIAGEN, Valencia, CA), cleaned using QIAQuick spin columns (QIAGEN), and sequenced in one direction (5′ end).

Polyacrylamide Gel Electrophoresis (PAGE) Analysis of Naphthyl Esterases. Extraordinary survival of the Poinsettia'04 strain in insecticide bioassays prompted us to expedite biotype analysis of this culture in advance of our routine molecular biotyping of the 2004 collections. The first biotype analyses of the Poinsettia'04 strain were conducted using conventional PAGE of naphthyl esterases (Byrne et al. 1995a, 2000) at the University of California, Riverside, CA. This method permitted rapid visual identification of the biotype of the Poinsettia'04 strain. Analyses were done on individual adult whiteflies using 1-naphthyl butyrate as substrate (Byrne et al., 1995a, 2000). Ten adult female whiteflies were collected from the Poinsettia'04 culture on 1 March 2005; frozen; and sent to the University of California, Riverside, for analysis. Individual insects were homogenized initially in microtiter plates in 5 μl of 1.6% Triton X-100 containing 10% sucrose. A further 15 μ1 was then added, the homogenates were mixed, and an aliquot (15 μl, equivalent to 0.75 insect) was loaded directly from the microtiter plate onto a PAGE gel. Specially designed combs were used to cast PAGE wells with 4.5-mm spacing in the gel, which enabled direct loading of samples from the microtiter plate using a multichannel pipette (5–50 μ1 of Finnpipette). A 20% concentration of Triton X-100 was used in the stacking gel; a 0.05% concentration was used in the resolving gel. Gels were run at 250 V for 2 h at 4°C; stained with 0.50 mM 1-naphthyl butyrate and 0.2% Fast Blue RR in phosphate buffer, pH 6.0, for 30 min in darkness at room temperature; fixed in 7% acetic acid; and photographed. Banding patterns of the Poinsettia'04 strain were contrasted with other 2004 field samples and internal controls of frozen reference samples of B and Q biotypes of *B. tabaci.*

Data Analyses. For each insecticide evaluated, the mean and SD of corrected mortality of the Poinsettia'04 strain was compared with that of all other collections of *B. tabaci* using box plots (JMP IN 5.0, SAS Institute, Cary, NC) with corresponding means and 95% confidence intervals (CI) for the grand means. Box plots allowed all means of corrected mortality to be visualized graphically for all strains and concentrations tested. The ends of the box represent the 25th and 75th quantiles. The line across the middle of the box identifies the median sample value. Each box has vertical lines or whiskers showing the inner quartiles. Whiskers extend to the outermost data point that falls within the outer quartiles. Means diamonds illustrate the grand mean and 95% CI for grand mean mortality of all strains tested, except for the Poinsettia'04 strain. The line across each diamond represents the grand mean mortality. The vertical span of each diamond represents the 95% CI for grand mean mortality. For each strain, chemical, and concentration evaluated, one-tailed Wilcoxon signed-rank tests were used to estimate the probability that mean mortality of the Poinsettia'04 strain was significantly different from the mean mortality of all other strains tested (JMP IN 5.0, SAS Institute).

Results

Detection of the Poinsettia'04 Strain. Of the total of 28–34 populations of *B. tabaci* bioassayed for susceptibility to the two IGRs (pyriproxyfen and buprofezin), three neonicotinoids (imidacloprid, acetamiprid, and thiamethoxam), and one synergized pyrethroid (fenpropathrin + acephate) in 2004, only the poinsettia collection Tucson Retail Greenhouse #3 (GPS ID 04-134; Supp Table S1c [online only]), named Poinsettia'04 hereafter, was dramatically different from all the other strains. Relative to the average susceptibility of all the other strains, Poinsettia'04 possessed significantly reduced mortality to all discriminating doses of the six insecticides tested (Wilcoxon signed ranks tests, $P < 0.0001$; Table 2). The resistance levels of Poinsettia'04 to the six insecticides are reported below.

Pyriproxyfen. Except for Poinsettia'04, in total 34 populations or strains, including 18 from cotton, 11 from melon, and five from ornamentals were bioassayed in 2004 by the leaf dip bioassay dipping leaves with eggs in 0.0 (water control), 0.01, 0.1, or 1 μg/ml pypriproxyfen solution for 20 s (Table 1). Poinsettia'04 was strikingly different from the 34 populations in terms of control mortality and resistance levels. Poinsettia'04 eggs had significantly higher control mortality (26.4%) than the 34 populations (grand mean, 7.43%; data not shown), suggestive of a fitness cost at the egg stage for Poinsettia'04. Its corrected egg mortalities at the three discriminating concentrations were <10% (0.0, 2.38, and 7.78%, respectively; Fig. 2; Table 2), whereas the corrected egg mortalities of the 34 populations at the three concentrations ranged from 1.11 to 60.8%, from 33.0 to 99.3%, and from 55.7 to 100.0%, respectively (Fig. 2). Subsequent bioassays conducted with 10 and 100 μg/ml pyriproxyfen only yielded corrected egg mortality of <20% (data not shown), suggesting that the LC_{50} of pypriproxyfen against Poinsettia'04 was >100 μg/ml. Relative to the baseline LC_{50} (0.0020–0.0067 μg/ml; Li et al. 2003) of pypriproxyfen against the Arizona field populations of *B. tabaci* collected in 1996, Poinsettia'04 had a resistance of >14,925.4-fold (Poinsettia'04 LC_{50}/the baseline LC_{50}) to pypriproxyfen.

Buprofezin. Twenty-nine of the 34 populations and Poinsettia'04 were bioassayed by dipping leaves with first instar nymphs (crawlers) for 20 s (Table 1) in 0.0 (control), 8.0, 100.0, or 1,000.0 μg/ml buprofezin solution. The control mortality of Poinsettia'04 (20.4%) was slightly higher than those of the 29 populations (13.83%, grand mean; data not shown). Its corrected crawler mortality at the three concentrations were 4.14, 17.1, and 34.5%, respectively, suggesting that the LC_{50} of buprofezin against Poinsettia'04 was >1,000 μg/ml. By contrast, the corrected crawler mortalities of the 29 populations at the three concentrations ranged from 43.8 to 71.8%, from 71.4 to 94.7%, and from 98.0 to 100%, respectively (Fig. 3; Table 2). Compared with the baseline LC_{50} of buprofezin against the Arizona field populations collected in 1996 ($\approx$1 μg/ml; Dennehy et al. 2005), Poinsettia'04 had a resistance of

Table 2. Susceptibility to insecticides of the Poinsettia'04 strain (Q biotype) versus all other collections of *B. tabaci* evaluated from collections made in 2004

	Concn (μg/ml)	Poinsettia'04		All 2004 strains except Poinsettia'04		Wilcoxon signed ranks		
		Mean corrected mortality (%)	SD of corrected mortality (%)	Mean corrected mortality (%)	SD of corrected mortality (%)	df	Test statistic	*P* value (prob > *t*)
Pyriproxyfen	0.01	0.000	0.000	19.7	16.1	33	297	<0.0001
	0.1	2.38	5.40	78.0	20.4	33	297	<0.0001
	1	7.78	15.1	93.3	9.54	33	297	<0.0001
Buprofezin	8	4.14	5.61	59.8	7.62	28	217	<0.0001
	100	17.1	23.6	83.4	6.42	28	217	<0.0001
	1,000	34.5	27.1	99.8	0.329	28	217	<0.0001
Fenpropathrin + acephate	10	4.84	6.49	79.6	16.4	29	63	<0.0001
	100	7.61	8.53	94.8	5.54	29	232	<0.0001
Imidacloprid	1	1.24	3.03	83.2	17.1	29	232	<0.0001
	10	18.8	15.7	97.4	3.83	29	232	<0.0001
	100	64.6	18.2	99.6	1.03	29	232	<0.0001
	1,000	79.3	14.5	99.3	1.69	29	232	<0.0001
Thiamethoxam	1	3.53	3.42	22.0	17.9	27	196	<0.0001
	10	3.47	11.5	62.8	23.5	27	203	<0.0001
	100	11.5	4.86	91.4	9.79	27	203	<0.0001
	1,000	78.3	15.0	99.2	1.04	27	203	<0.0001
Acetamiprid	1	3.64	5.04	17.3	13.3	27	187	<0.0001
	10	1.93	4.37	70.0	23.2	27	203	<0.0001
	100	25.8	8.34	95.1	5.17	27	203	<0.0001
	1,000	62.0	24.6	99.0	1.27	27	203	<0.0001

Wilcoxon signed ranks (one-tailed) tests were used to estimate statistical significance of differences between means. All differences were highly significant.

>1,000 fold to buprofezin (Poinsettia'04 LC_{50} [>1,000 μg/ml]/the baseline LC_{50} [≈1 μg/ml]).

Fenpropathrin + Acephate. Thirty of the 34 populations and Poinsettia'04 were bioassayed by exposing adults to leaf discs treated with 0 (control), 10, or 100 μg/ml fenpropathrin + acephate mixture for 48 h (Table 1). Unlike the egg (pyriproxyfen) and crawler (buprofezin) bioassays, no differences in control mortality were observed between Poinsettia'04 (3.32%) and the 30 populations (4.99%, grand mean; data not shown), suggestive of no fitness cost at adult stage. The corrected adult mortalities of Poinsettia'04 at the two diagnostic concentrations were <10% (4.84 and 7.61%, respectively), suggesting that the LC_{50} of the fenpropathrin + acephate mixture against Poinsettia'04 was >100 μg/ml. By contrast, the corrected mortalities of the 30 populations ranged from 20.8 to 97.8% and from 88.7 to 100%, respectively (Fig. 4; Table 2).

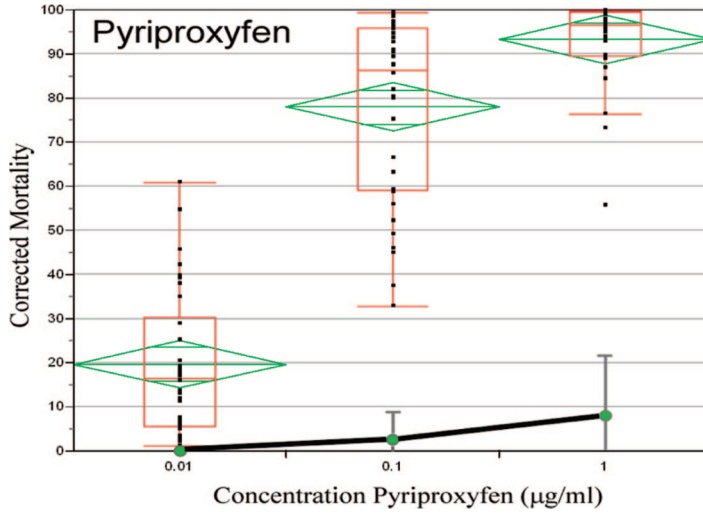

Fig. 2. Reduced pyriproxyfen susceptibility in Poinsettia'04. Susceptibility of the Poinsettia'04 strain (lower line, ±SD), was significantly less than that of the 34 field populations evaluated in 2004. All collections except Poinsettia'04 were the B biotype. Box plots show the range, quartiles, and 95% CI for grand mean mortality observed in pyriproxyfen bioassays of 18 collections from cotton, 11 collections from melons, and five collections from ornamentals. (Online figure in color.)

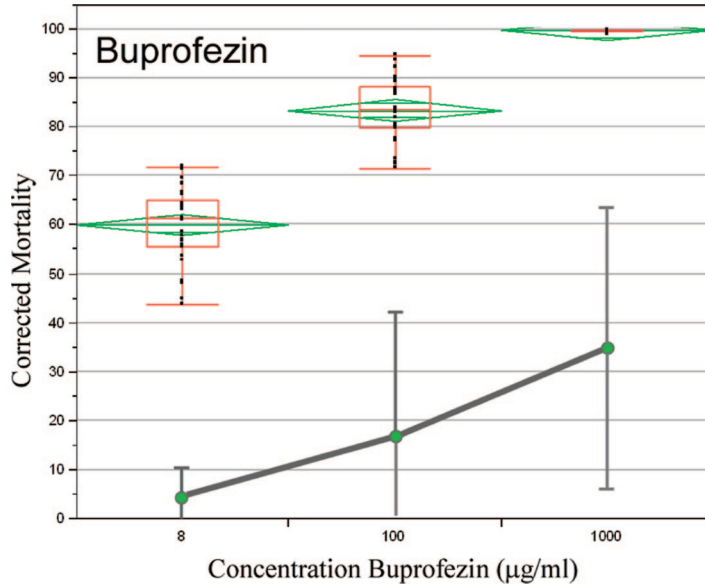

Fig. 3. Reduced buprofeszin susceptibility in Poinsettia'04. The Poinsettia'04 strain (lower line, ±SD) was significantly less susceptible to buprofezin than were 29 whitefly populations evaluated in 2004. All collections except Poinsettia'04 were the B biotype. Box plots show the range, quartiles, and 95% CI for grand mean mortality observed in buprofezin bioassays of 15 collections from cotton, 11 collections from melons, and three collections from ornamentals. (Online figure in color.)

Because the LC_{50} of this mixture against Arizona-collected susceptible B Biotype whiteflies is ≈1 μg/ml (Dennehy and Williams 1997), we estimate that Poinsettia'04 was at least 100-fold resistant to this mixture (Poinsettia'04 LC_{50} [>100 μg/ml]/the baseline LC_{50} [1 μg/ml]).

Neonicotinoid Insecticides. Adults of 30 (imidacloprid) or 28 (acetamiprid and thiamethoxam) of the 34

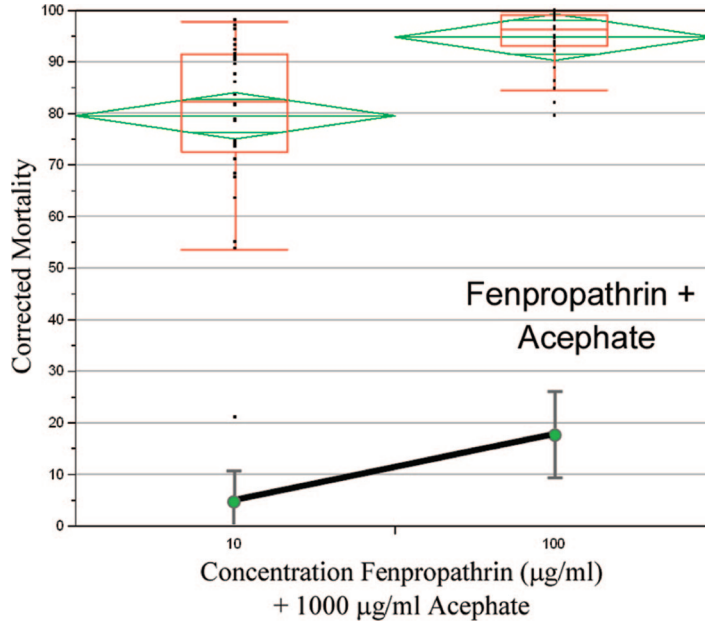

Fig. 4. Reduced susceptibility to fenpropathrin and acephate in Poinsettia'04. The Poinsettia'04 strain (lower line, ±SD) was less susceptible to mixtures of fenpropathrin and acephate than were 30 whitefly populations evaluated in 2004. All collections except Poinsettia'04 were the B Biotype. Box plots show the range, quartiles, and 95% CI for grand mean mortality observed in bioassays of 15 collections from cotton, 11 collections from melons, and four collections from ornamentals. (Online figure in color.)

populations and Poinsettia'04 were bioassayed with 0 (water control), 1, 10, 100, and 1,000 μg/ml of imidacloprid, acetamiprid, or thiamethoxam. No differences in control mortality of adults were observed between Poinsettia'04 and all the other populations (data not shown). But the corrected mortalities of Poinsettia'04 at all the four concentrations (1, 10, 100, and 1,000 μg/ml) of the three neonicotinoids were dramatically lower than those of any other 2004 field and ornamental population tested and their grand means (Fig. 5; Table 2). Imidacloprid at 1 μg/ml killed ≈83% adults of most other populations, whereas 1,000 μg/ml imidacloprid only killed 79% adults of Poinsettia'04. This suggests that Poinsettia'04 had ≈1,000-fold (Poinsettia'04 LC_{79} [1,000 μg/ml]/other population LC_{83} [1 μg/ml]) resistance to imidacloprid. By the same reasoning, Poinsettia'04 also had roughly 100-fold resistance to acetamiprid and thiamethoxam because 10 μg/ml of either killed 65–70% adults of most of other populations, whereas 1,000 μg/ml of the two neonicotinoids killed 60–80% adults of Poinsettia'04.

Biotype Identification of the Poinsettia'04 Isolate. Based on the significantly higher control mortality of the Poinsettia'04 egg and crawler stages, and its high level of resistance to multiple insecticides, compared with those of all the other 2004 populations, we hypothesized that Poinseetia'04 could be a Q biotype population, whereas all other isolates or populations were probably the B biotype. To test this hypothesis, we determined the biotype identity of all isolates/populations, including Poinsettia'04 by analysis of the *mtCOI* sequence (Fröhlich et al., 1999), electrphoretic analysis of naphthyl esterase banding patterns (Byrne et al. 1995a, 2000; Poinsettia'04 only), or both. An analysis of the esterase banding patterns identified seven of 10 adults taken from the Poinsettia'04 strain on 1 March 2005 as Q biotype of *B. tabaci* and three of 10 adults as the B biotype *B. tabaci* (Fig. 6).

mtCOI sequence analysis of 20 adults that had been preserved in 95% ethanol shortly after the Poinsettia'04 strain was isolated in the laboratory in December 2004, conducted independently in two laboratories (UA, 10 adults per laboratory), revealed that the unique esterase banding profile obtained for the Poinsettia'04 sample subjected to esterase analysis corresponded to the Q biotype based on haplotype analysis. Sequence analysis by each laboratory revealed that the *mtCOI* sequences of all tested individuals from Poinsettia'04 were more closely related to the standard Q biotype *mtCOI* sequence (Spain Q, GenBank accession DQ302946) than to the standard B-biotype *mtCOI* gene sequence (Arizona B, GenBank accession AY057123; see phylogenetic tree in Fig. 7; only four representative individuals are shown in the tree), indicating that Poinsettia'04 was a Q biotype population. Sequence analysis also confirmed that all other 2004 populations were the B biotype (Supp Table 1c [online only]). These results confirmed the first identification of the Q biotype *B. tabaci* in the United States.

Distribution of the Q Biotype in Arizona. In 2001, we began routine biotyping of *B. tabaci* populations by preserving samples that we obtained for resistance monitoring. No Q biotypes were detected in field collections made in 2001 (Supp Table S1a [online only]), 2003 (Supp Table S1b [online only]), 2004 (Supp Table S1c [online only]), or 2005 (Supp Table S1d [online only]). Thus, we seem to have detected the Q biotype before it has impacted the principal field and row crop hosts of *B. tabaci* in Arizona. However, of the 13 poinsettia collections obtained in 2005, six were the Q biotype (Supp Table S1d [online only]).

Discussion

A whitefly collection, named Poinsettia'04, possessing unusually high levels of resistance to a wide range of insecticides was discovered in 2004, in the course of conducting annual resistance monitoring in Arizona. The multiply resistant strain was obtained from poinsettia plants purchased at a retail store in Tucson, AZ. It was subjected to biotype identification in three laboratories. PAGE electrophoresis of naphthyl esterases (Byrne et al. 1995a, 2000) and sequencing of the *mtCOI* gene (780 bp) (Fröhlich et al. 1999, Brown 2001) confirmed the first finding of the Q biotype of *B. tabaci* in the New World. *mtCOI* sequence analysis of a sample of 20 adults initially preserved in December 2004 found no B biotype individuals, whereas esterase profile analysis of a sample of 10 adults taken from the Poinsettia'04 strain 3 mo later revealed the presence of three B biotype individuals. Apparently the B biotypes detected by esterase banding pattern analysis were either contaminants introduced on the cotton plants we placed in rearing cages or they were at sufficiently low levels in the initial Poinsettia'04 strain that our sample size of 20 did not detect them.

It seems that the Q biotype was detected at a relatively early stage after its entry into the United States. This conclusion is based on the absence of the Q biotype in 100 separate field collections of *B. tabaci* from irrigated host crops in Arizona. That Q biotypes were found only on retail or wholesale poinsettias provides evidence that this exotic biotype was transported within the United States on ornamental plants. Subsequent investigations by regulatory agencies in 2005 found Q biotypes at the wholesale nursery that produced the plants from which the Poinsettia'04 Q biotypes were collected in Tucson in 2004, as well as at the location from which the wholesaler obtained poinsettia propagation material. The conclusion from these findings was that Q biotypes were disseminated on poinsettia propagation material, a deduction that has since that time been supported by surveys conducted by cooperating states. However, we were unable to track down the original source of this strain due to the diversity of locations from which poinsettia materials were imported at the poinsettia propagation facility. By 2005, the Q biotype had been detected in >20 U.S. states (Osborne 2005) and in Guatemala (Bethke et al. 2009).

The Poinsettia'04 strain was highly resistant to two IGRs, pyriproxyfen and buprofezin. These highly selective insecticides have provided the foundation for

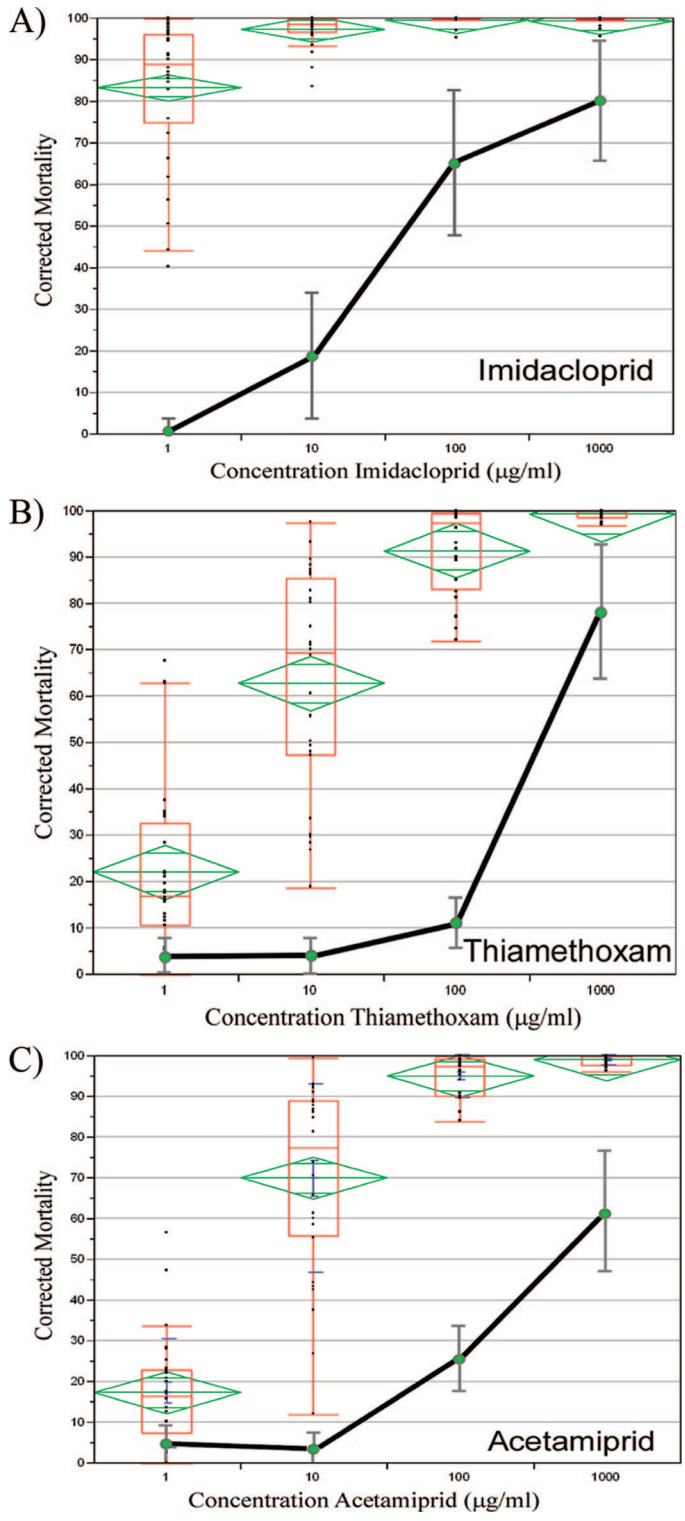

Fig. 5. Reduced neonicotinoid susceptibility in Poinsettia'04. The Poinsettia'04 strain (lower lines, ±SD) was significantly less susceptible to three neonicotinoid insecticides than were the 30 field populations evaluated in 2004. All collections except Poinsettia'04 were the B Biotype. Box plots show the range, quartiles, and 95% CI for grand mean mortality observed in bioassays of 15 collections from cotton, 11 collections from melons, and four collections from ornamentals. (A) Imidacloprid. (B) Thiamethoxam. (C) Acetamiprid. (Online figure in color.)

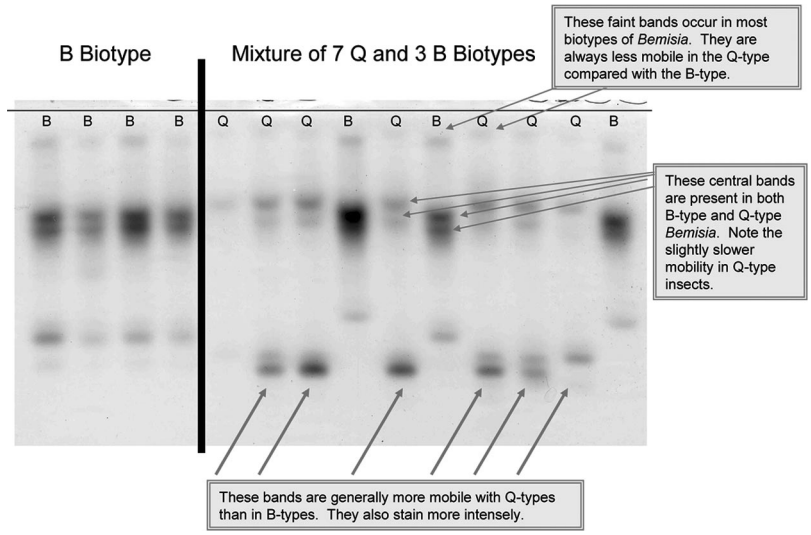

Fig. 6. PAGE electrophoresis of naphthyl esterases of the first Q biotype of *B. tabaci* identified in the Americas. The B biotypes to the left of the vertical line were collected in 2004 from Arizona fields. The mixture of seven Q biotypes and three B biotypes to the right of the vertical line were sampled from Poinsettia'04 strain on 1 March 2005, 2 mo after it was placed in culture in the laboratory. The distinct differences in esterase banding patterns of B and Q types are noted.

a highly successful resistance management program in Arizona cotton for more than a decade (Dennehy et al. 1996a, Ellsworth and Martinez-Carrillo 2001). Poinsettia'04 was virtually unaffected by pyriproxyfen in egg bioassays, had strikingly reduced susceptibility to buprofezin in nymphal bioassays, and possessed unusually low susceptibility to the important neonicotinoid insecticides acetamiprid, imidacloprid, and thiamethoxam. Although many B biotype populations were highly resistant to synergized pyrethroids and could not be controlled by foliar sprays of synergized pyrethroid mixtures (Dennehy and Williams 1997), Poinsettia'04 had the lowest mortality in bioassays of fenpropathrin and acephate that we have recorded in over a decade of monitoring resistance to pyrethroid insecticides in whiteflies (Dennehy et al. 2005). Furthermore, because Sivasupramaniam et al. (1997) demonstrated that susceptibility to fenpropathrin + acephate mixtures reflected susceptibility to all synergized pyrethroid mixtures being used against whiteflies in Arizona, Poinsettia'04 also may have high levels of resistance to other synergized pyrethroids and organophosphates.

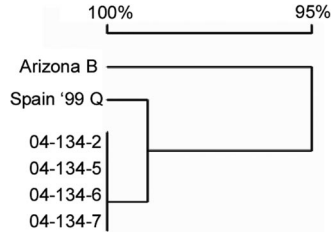

Fig. 7. Phylogenetic tree of *mtCOI* from Poinsettia'04 whiteflies collected from infested poinsettias at a retail outlet in Tucson, AZ, in December 2004 were biotyped by using PCR and DNA sequencing of the *mtCOI* gene. Representative sequences of four individuals (04-134-2, 04-134-5, 04-134-6, 04-134-7; other individuals not included) from Poinsettia'04 (GPS ID 04-134) had >99% correspondence of *mtCOI* nucleic acid sequences with the reference *B. tabaci* sequence of the Q biotype, Spain 99, and only 95% correspondence with the Arizona B reference sample.

The high levels of resistance measured in Poinsettia'04 to IGRs, neonicotinoids, and synergized pyrethroids are not comparable with the other Q biotype strains found in Europe or Israel, the two possible sources of Poinsettia'04. This suggests that the unusually high levels of multiple resistance of Poinsettia'04 have probably resulted from the aggressive use of insecticides in whitefly management programs both before and after its entry into the United States within the ornamental industry (Byrne et al. 2010). Poinsettias are vulnerable to attack by whiteflies during each stage of the production process, and in facilities where phytosanitary measures are inadequate to prevent outbreaks, chemical control measures are relied upon almost exclusively. The Q biotype has shown an extraordinary ability to develop resistance to a wide range of insecticides. Several Q biotype strains from Spain, Italy, and Germany, possible sources of Poinsettia'04, have expressed >100-fold resistance to neonicotinoids (acetamiprid, imidacloprid, and thiamethoxam) through increased cytochrome P450 monooxygenase-mediated detoxification (Nauen et al. 2002; Rauch and Nauen 2003; Nauen and Denholm 2005; Prabhaker et al. 2005; Karunker et al. 2008, 2009). In Israel, another possible source of Poinsettia'04, the Q biotype has been associated with severe resistance to pyriproxyfen via a single locus (Horowitz et al. 2003a,b). Laboratory selection of Israeli populations containing a mixture of B and Q biotypes with either

pyriproxyfen or neonicotinoids increased the percentage of the Q biotype individuals (Horowitz et al. 2005), suggesting that the Q biotype individuals possessed resistance to both pyriproxyfen and neonicotinoids. Nonetheless, Poinsettia'04 is the first reported Q biotype strain that has high levels of resistance to broad-spectrum synergized pyrethroids, IGRs (pyriproxyfen and buprofezin), and neonicotinoids. The mechanisms conferring this multiple resistance in Poinsettia'04 have yet to be elucidated.

Severe economic losses to agriculture, resulting from the introduction of the B biotype of *B. tabaci*, have been chronicled by pest managers in many areas of the United States (Henneberry and Nichols 2002). And the Q biotype of *B. tabaci* is the dominant biotype in Europe and coexists with the B in Israel and also recently in China (Horowitz et al. 2003a; Simón et al. 2003; Chu et al. 2007, 2010). As of 2009, the Q biotype is still not a pest outside of greenhouse environments in the United States (McKenzie et al. 2009; X.L. et al., unpublished data), but the potential threat posed by the multiply resistant Q biotype is indisputable. Although it is not possible to predict the future spread of the Q biotype within the United States, or the severity of associated control and virus problems, there is a compelling need to formulate contingency plans for its management. Information regarding the geographic distribution, insecticide resistance, pest status, relative fitness, and competitiveness compared with current field populations, and virus–vector relationships of this invasive biotype will be essential for formulating such plans. In addition, regulatory and educational efforts to limit the further spread of the Q biotype within the United States and to thwart further importation on plant materials produced offshore will be critical for management of this new problem.

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