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RESEARCH ARTICLE

Insecticide resistance and control failure likelihood of the whitefly *Bemisia tabaci* (MEAM1; B biotype): a Neotropical scenario

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Azadirachtin resistance; bioinsecticide; control failure survey; inadvertent resistance selection; whiteflies.

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Abstract

Insecticide resistance is a standing concern for arthropod pest species, which may result in insecticide control failure. Nonetheless, while insecticide resistance has remained a focus of attention for decades, the incurring risk of insecticide control failure has been neglected. The recognition of both problems is paramount for arthropod pest management and particularly so when invasive species notoriously difficult to control and exhibiting frequent cases of insecticide resistance are considered. Such is the case of the putative whitefly species Middle East-Asia Minor I (MEAM1) (*Bemisia tabaci* B-biotype), for which little information is available in the Neotropics. Thus, the likely occurrence and levels of resistance to seven insecticides were surveyed among Brazilian populations of this species. The likelihood of control failure to the five insecticides registered for this species was also determined. Resistance was detected to all insecticides assessed reaching instances of high (i.e. >100×) to very high levels (>1000×) in all of them. Overall efficacy was particularly low (<60%) and the control failure likelihood was high (>25%) and frequent (70%) for the bioinsecticide azadirachtin, followed by spiromesifen and lambda-cyhalothrin. In contrast, the likelihood of control failure was low for diafenthiuron, and mainly imidacloprid. As cartap and chlorantraniliprole are not used against whiteflies, but are frequently applied on the same host plants, inadvertent selection probably took place leading to high levels of resistance, particularly for the latter. The resistance levels of cartap and chlorantraniliprole correlated with imidacloprid resistance ($r > 0.65$, $P < 0.001$), suggesting that the latter use may have somewhat favoured inadvertent selection for resistance to both compounds not used against the whitefly. A further concern is that chlorantraniliprole use in the reported scenario may allow cross selection to cyantraniliprole, a related diamide with recent registration against whiteflies demanding attention in designing resistance management programmes.

Introduction

Insecticide resistance is arguably the most well-studied consequence of insecticide use for pest control and remains a standing concern for managing pest species for a few decades (Metcalf, 1980; Sparks & Nauen, 2015; Guedes *et al.*, 2016, 2017). The early recognition and initial definition of insecticide resistance recognising it as a population-based and relative phenomenon was later aided by the explicit recognition of its genetic basis (WHO, 1957; Crow, 1960; Sawicki, 1987). More recently though, the concept of insecticide resistance has been

associated with that of control failure as one of its potential causes (Whalon *et al.,* 2008; Guedes, 2017; IRAC, 2017). However, insecticide resistance and control failure are not synonymous and their distinction and practical relationship invites further investigation, which remains a challenge (Guedes, 2017).

Control failure of an insecticide due to insecticide resistance is a significant reduction of efficacy of an insecticidal formulation used at its label rate failing to reach the expected control level (Guedes, 2017). Therefore, the determination of the risk of control failure (or control failure likelihood, CFL) due to insecticide resistance requires specific determination using realistic methods simulating field exposure, commercial insecticide formulations used at their label rate, and a minimum threshold of field efficacy (Gontijo *et al.,* 2013; Guedes, 2017). Previous studies with vegetable arthropod pest species have allowed evolving methods to determine CFL as the frequency of resistant individuals from which the resistance to an insecticide compromises its field efficacy (Silva *et al.,* 2011; Gontijo *et al.,* 2013; Guedes, 2017; Naveen *et al.,* 2017). These recent efforts have not yet reached important invasive species other than the tomato pinworm *Tuta absoluta* (Meyrick), such as the invasive putative species of whitefly target of our study.

The whitefly *Bemisia tabaci* (Gennadius) (Sternorrhyncha: Aleyrodidae) is an invasive species that rose to prominence by the late 1970s and it is currently recognised as a complex of cryptic species with world wide distribution (Brown *et al.,* 1995; De Barro *et al.,* 2011, 2012). This species complex is also recognised as a major concern regarding insecticide resistance figuring among the top five arthropods with most reported cases of insecticide resistance (Sparks & Nauen, 2015). Two putative species of *B. tabaci* are the main focus of attention, the Middle East-Asia Minor I (i.e. MEAM1 or biotype B) and the Mediterranean (MED or biotype Q) species of the complex, which account for both of the major global invasion events recorded for the group (De Barro, 2012). Despite of the wide distribution of both cryptic species displacing native ones (De Barro *et al.,* 2011; McKenzie *et al.,* 2012), MEAM1 currently prevails in the Neotropical region (Barbosa *et al.,* 2014, 2015), while MED seems to be displacing the former from China due to the local levels of host suitability and pattern of insecticide use (Sun *et al.,* 2013; Gao & Reitz, 2017; Zheng *et al.,* 2017).

The putative species MEAM1 and MED are also the main focus of the insecticide resistance studies carried out throughout the world and particularly intensive in the Nearctic and Western Palearctic regions (e.g. Roditakis *et al.,* 2005; Fernández *et al.,* 2009; Castle *et al.,* 2010; Li *et al.,* 2012; Panini *et al.,* 2017). However, insecticide resistance studies on whiteflies are quickly increasing in the Eastern Palearctic and Indo-Malay regions likely due to the enhanced problems detected with the current spreading of the MED species in China and India (Basit *et al.,* 2011, 2013; Yuan *et al.,* 2012; Naveen *et al.,* 2017; Zheng *et al.,* 2017; Wang *et al.,* 2017a, 2017b). In curious contrast, we are aware of only three studies on whitefly resistance to insecticides in Neotropical America, two of them from Central America (Byrne *et al.,* 2003; Satilla-Ortega *et al.,* 2011), and a single study from South America reporting low to moderate levels of resistance to the neonicotinoids imidacloprid and thiamethoxam (Silva *et al.,* 2009). Here we attempted to mitigate this deficiency building on the previous effort and expanding the survey to a broader and more representative number of populations and insecticides, and also estimating the prevailing CFL in Brazil.

The levels of resistance to seven insecticides were surveyed in 11 field-collected populations of whiteflies (*B. tabaci*) from representative agricultural regions from Brazil, and contrasted with a standard susceptible population. The putative species of the sampled individuals were also determined. Five of the insecticides tested, namely azadirachtin, diafenthiuron, imidacloprid, lambda-cyhalothrin and spiromesifen, are registered and commonly used for managing whiteflies in Brazil (MAPA, 2017). In contrast, cartap and chlorantraniliprole are not registered, nor recommended, for whitefly control in the country. However, these two insecticides are frequently used in the same whitefly host plants, particularly on vegetable crops and against other pest species (MAPA, 2017), potentially leading to inadvertent selection for resistance in whiteflies as well.

We expected prevalence of the putative species MEAM1, since the introduction of the species MED is recent in the country (Barbosa *et al.,* 2015), and also expected an increase in incidence and levels of insecticide resistance from the earlier levels detected in the survey by Silva *et al.* (2009). The likelihood of control failure was also expected to be high, particularly for older insecticides such as the pyrethroid lambda-cyhalothrin and imidacloprid, in contrast with compounds of more recent use such as azadirachtin, diafenthiuron and spiromesifen, where the selection for insecticide resistance is consequently more recent. The potential inadvertent selection for resistance to cartap and chlorantraniliprole was expected to lead to lower levels of resistance, if any, and the same was expected for azadirachtin, a botanical (bio)insecticide for which cases of insecticide resistance are rare (Fernández *et al.,* 2009; Siegwart *et al.,* 2015).

Materials and methods

Insects

A total of 12 whitefly populations were used in the study. One of them is a susceptible standard population maintained at the Agronomic Institute of Campinas (IAC, Campinas, SP, Brazil), where it is maintained on collard greens since the early 2000s without insecticide exposure (Silva *et al.,* 2009). The other 11 populations were field-collected between May 2013 and August 2014 at different sites and hosts representative of the main agricultural regions with serious whitefly incidence in Brazil (Fig. 1). The insect populations were established from at least 200 individuals and were maintained for one to three generations within greenhouse in cages of polyvinyl chloride (PVC) covered with organza fabric (134 cm × 134 cm × 134 cm) and containing an assortment of potted *Brassica* plants (broccoli, cabbage and collard greens), which were periodically replaced.

Ten insects from each population were subjected to biotype recognition after DNA extraction following Frohlich *et al.* (1999), as adapted by Ragab (2013). The polymerase chain reactions (PCRs) were performed using the primers CI-J-2195 and L2-N-3014 from Simon *et al.* (1994) to amplify the desired mitochondrial fragment of cytochrome oxidase subunit I (COI) to allow the intended recognition. PCR cycles used the following conditions: an initial 2 min denaturation at 95°C, followed by 34 cycles of 30 s denaturation at 94°C, a 30 s annealing at 52°C, a 2 min elongation at 72°C, and a final extension step of 10 min at 72°C. The obtained amplicons were sequenced using the DNA sequencing services of Macrogen Inc. (South Korea; www.macrogen.com). The biotype recognition was performed based on the COI sequence similarity with the recognised genetic groups of *B. tabaci* using the Basic Local Alignment Search Tool (BLAST) (National Center for Biotechnology Information [NCBI]; https://blast.ncbi.nlm.nih.gov/Blast.cgi), and confirmed by phylogenetic analysis using MEGA ver. 5.0 (Tamura *et al.,* 2011).

Insecticides

Seven insecticides were used in this study, four of which exhibit neuro-muscle activity (the nereistoxin analogue cartap [a nicotinic acetylcholine receptor blocker], the diamide chlorantraniliprole [a ryanodine receptor modulator in muscle fibres], the neonicotinoid imidacloprid [an agonist of nicotinic acetylcholine receptors], and the pyrethroid lambda-cyhalothrin [a sodium channel modulator]). The other three (non-neurotoxic) insecticides were the terpenoid azadirachtin, obtained from extract of the neem tree (*Azadirachta indica* A. Juss) and exhibiting growth-regulation and sterilant activities, the ATPase inhibitor diafenthiuron, and the tetronic acid derivative spiromesifen, which is an inhibitor of acetyl CoA carboxylase. The commercial formulations were as follows: azadirachtin 12 g a.i. L^{-1} [suspendable concentrate; DVA Agro, Campinas, Brazil], cartap 500 g a.i. L^{-1} [soluble powder; Sumitomo Chemical, São Paulo, Brazil], chlorantraniliprole 200 g a.i. L^{-1} [suspendable concentrate; DuPont, São Paulo, Brazil], diafenthiuron 500 g a.i. Kg^{-1} [wettable powder; Syngenta, São Paulo, Brazil], imidacloprid 700 g a.i. L^{-1} [water dispersible granule; Bayer CropScience, São Paulo, Brazil], lambda-cyhalothrin 50 g a.i. L^{-1} [suspendable concentrate; Syngenta, São Paulo, Brazil], and spiromesifen 240 g a.i./L [Syngenta, Bayer CropScience]. A non-ionic surfactant (Tween® 80; Sigma-Aldrich, São Paulo, Brazil) at the concentration of 0.03% v/v was added to the insecticide solutions.

General bioassay procedure

The insecticide bioassay procedure followed Dittrich *et al.* (1985) using leaf disk dipping in insecticide solution. Briefly, plants of jack bean (*Canavalia ensiformis* L.) were pot cultivated and between 3 and 5 weeks after germination they had their leaves removed and cleaned by immersion in distilled water. The leaves were subsequently cut into disks (2.2 cm diameter), which were dipped in insecticide water solution for 10 s. The disks were left to dry and their lower surface was placed upward with the upper surface placed over a 1.5 mL agar–water solution in flat-bottom glass tubes (2.2 cm diameter × 8.3 cm high). Thirty adult insects were released in each glass tube, which were placed in styrofoam supports and covered with voile fabric to prevent the insects from escaping. The insects were maintained in environmental controlled chambers at 25 ± 5°C temperature, 75 ± 5% relative humidity, and 12:12 (L:D) photoperiod. The same general procedure was used for the concentration-mortality bioassays and for the diagnostic bioassays of control failure, which are detailed below.

Concentration-mortality bioassays

The concentration-mortality bioassays were carried out with between seven and 12 concentrations of each insecticide, in addition to a control where only water and surfactant (Tween®80 at 0.03%) were used. The range of concentrations was established after preliminary tests using a broad range of concentrations (10-fold dilutions). Each insecticide bioassay was replicated three times and insect mortality was recorded after 48 h of exposure to the

Sampling site	State	Geographic coordinates	Mean temperature (ºC)	Host plant
Campinas (susceptible)	São Paulo (SP)	22°53′43″S 47°01′47″W	19.3	Collard greens
Araguari	Minas Gerais (MG)	18°29′07″S 48°11′00″W	21.2	Tomato
Brazlândia	Distrito Federal (DF)	15°38′08″S 48°07′47″W	21.5	Tomato/beans
Cristalina	Goiás (GO)	16°46′07″S 47°36′49″W	20.1	Tomato/cotton
Domingos Martins	Espírito Santo (ES)	20°21′36″S 40°39′33″W	21.5	Tomato
Gama	Goiás (GO)	16°01′10″S 48°04′01″W	20.7	Collard greens/Pumpkin
Goiânia	Goiàs (GO)	16°38′34″S 49°15′42″W	23.1	Collard greens/tobacco
Guaíra	São Paulo (SP)	20°19′47″S 48°19′12″W	23.2	Soybean
Jaíba	Minas Gerais (MG)	15°20′46″S 43°40′32″W	24.5	Tomato/Pumpkin
Patos de Minas	Minas Gerais (MG)	18°36′19″S 46°30′54″W	22.8	Tomato
Taquara	Distrito Federal (DF)	15°37′52″S 47°31′10″W	21.7	Tomato/Beans
Viçosa	Minas Gerais (MG)	20°44′55″S 42°52′47″W	20.6	Tomato/Egg plant

Figure 1 Sampling sites of the populations of the whitefly *Bemisia tabaci*.

treated leaf surface. The insects were considered as dead when unable to coordinately walk at least the length of their body.

Diagnostic bioassays of control failure likelihood

Insecticide control failure was recognised by recording insecticide mortality at the label rate for whiteflies (MAPA, 2017), as follows: azadirachtin at 30 μg a.i. mL^{-1}, diafenthiuron at 1360 μg a.i. mL^{-1}, imidacloprid at 630 μg a.i. mL^{-1}, lambda-cyhalothrin at 750 μg a.i. mL^{-1}, and spiromesifen at 1360 μg a.i. mL^{-1}. As cartap and chlorantraniliprole were not registered nor recommended for whitefly control, they were not subjected to diagnosis of control failure. The indicated label rates were used as discriminating concentrations for adult whiteflies following the methods previously described and using four replicates of 30 insects each to assess each insecticide. Insect mortality was recorded after 48 h exposure, as previously indicated. CFL was estimated following Guedes (2017); CFL = 100-[observed mortality (%) × 100] ÷ expected mortality (considered

as 80% following the recommendation of the Brazilian Ministry of Agriculture for minimum efficacy threshold; MAPA, 1995). The observed mortality was used after correction for natural mortality following Abbott (1925). CFL values ≤0 indicate negligible risk of control failure (i.e. CFL = 0).

Statistical analyses

The concentration-mortality results were subjected to probit analyses (PROC PROBIT; SAS software, SAS Institute, Cary, NC, USA). The levels of insecticide resistance (i.e. resistance ratio) were estimated by dividing the LC_{50} of a given field population by the LC_{50} of the susceptible standard population. Significant insecticide resistance was recognised by estimating the 95% confidence intervals for the resistance ratios, which were considered significant if not including the value 1, following Robertson *et al.* (2007).

The estimated resistance ratios were obtained for each of the three replicated bioassays allowing their use in a canonical variate analysis (CVA; PROC CANDISC; SAS Institute), that also incorporates a multivariate analysis of variance to test if there is significant overall difference in resistance among populations across insecticides. The objective of the CVA analysis was to ordinate and group the insect populations based on their respective levels and profiles of insecticide resistance to allow possible recognition of existing patterns of the phenomenon (e.g. Fragoso *et al.,* 2003). Possible correlations between resistance ratios to different insecticides were tested by correlation analyses (PROC CORR; SAS Institute). No data transformation was necessary for these analyses, as they conform to normality and homoscedasticity assumptions (PROC UNIVARIATE; SAS Institute).

The mortality results of the diagnostic bioassays of CFL were subjected to one-sided *Z*-test at 95% confidence level with correction for continuity to test if their departure from the expected 80% mortality was significant. The procedure was performed following Roush & Miller (1986). Bonferroni correction was also used to correct the *P*-values accounting for the multiple testing performed.

Results

Species identification

The diagnostic recognition of the putative species of whiteflies sampled for our studies of insecticide resistance and CFL indicated that all of the populations belong to the MEAM1 species based on the obtained sequences of COI mitochondrial gene fragment.

Relative toxicity and insecticide resistance

Control mortality was always lower than 10% allowing its proper use to correct for natural mortality in the bioassays. The concentration-mortality results were suitably described by the probit model with goodness-of-fit tests providing low χ^2-values (<15.10) and high *P*-values (>0.05), allowing the estimation of the desired toxicological endpoints (i.e. LC_{50}s) and subsequent calculation of resistance ratios and their 95% confidence intervals. Insecticide resistance was detected for all tested compounds regardless if registered, or not, for use against whiteflies. Among the insecticides exhibiting neuro-muscle activity, insecticide resistance reached up to over 170-fold for cartap, over 600-fold for chlorantraniliprole, over 200-fold for imidacloprid, and over 1500-fold for lambda-cyhalothrin (Table 1). The instances of insecticide resistance were frequent for all of these compounds, and particularly so for imidacloprid and lambda-cyhalothrin with 9 and 8 out of 11 populations, respectively exhibiting significant levels of resistance.

Insecticide resistance was also frequent for the non-neurotoxic compounds (Table 2), although the wide fiducial intervals for the susceptible LC_{50} obtained with spiromesifen led to a greater level of uncertainty about the confidence intervals estimated for the resistance ratios of this insecticide compromising the resolution in detecting spiromesifen resistance. As a consequence, a few instances of high levels of resistance to spiromesifen (>100-fold) were not detected as significant. The Taquara population exhibited the highest level of resistance detected in the study, which was over 6000-fold for spiromesifen. Resistance to azadirachtin and diafenthiuron were broadly distributed, but with high levels (between 100 and 1000-fold) prevailing for azadirachtin and low (≤10) to moderate (>10- and <100-fold) levels prevailing for diafenthiuron.

Insecticide resistance patterns and correlations

The ordination and grouping of insect populations based on the pattern of insecticide resistance obtained with CVA provided a significant canonical axis (i.e. whose correlation differ from zero at $P < 0.05$), which was able to explain 58% of the observed variance (Table 3). Unlike the first canonical axis, the second axis was not significant and was only used in the CVA diagram to improve its visual display (Table 3). Azadirachtin and diafenthiuron resistance were the main contributors of the first canonical axis exhibiting higher inter-population variation (Table 3) allowing the recognition of three groups of insect populations (Fig. 2). One group encompassed the field populations of Cristalina and Goiânia, both

Table 1 Relative toxicity of neurotoxic insecticides to adults of Brazilian populations of MEAM1 whiteflies (*Bemisia tabaci* B biotype); the asterisk in the resistance ratio indicate significant difference from the standard susceptible population when the confidence interval does not include the value 1, following Robertson *et al.* (2007)[a]

Insecticide	Population	No.	Slope ± SE	LC_{50} (95% Fiducial Interval) μg i.a/L	χ^2 (degrees of freedom)	*P*	Resistance ratio at LC_{50} [RR_{50} (95% Confidence Interval)]
Cartap	Susceptible	840	0.51 ± 0.06	0.22 (0.82–4.83)	9.03 [6]	0.17	1.00
	Araguari	840	0.63 ± 0.07	2.50 (0.96–50.80)	8.72 [6]	0.19	14.49 (2.41–54.97)*
	Brazlândia	930	2.58 ± 0.29	16.00 (12.47–19.45)	9.77 [7]	0.20	73.53 (22.34–242.12)*
	Cristalina	930	0.56 ± 0.08	2.38 (0.40–8.70)	13.86 [6]	0.05	10.94 (1.78–67.18)*
	Domingos Martins	930	0.47 ± 0.06	0.51 (0.07–2.00)	12.10 [7]	0.10	2.37 (0.36–15.74)
	Gama	1020	0.86 ± 0.13	1.98 (0.88–4.06)	14.59 [8]	0.07	9.10 (2.16–38.16)*
	Goiânia	848	1.55 ± 0.22	15.48 (8.06–24.80)	13.37 [8]	0.10	71.14 (19.51–259.72)*
	Guaíra	870	0.39 ± 0.05	0.23 (0.02–1.27)	12.16 [6]	0.06	1.07 (0.12–9.30)
	Jaíba	930	1.51 ± 0.30	35.34 (18.05–52.90)	13.52 [7]	0.06	162.41 (40.66–648.70)*
	Patos de Minas	1080	0.35 ± 0.03	0.01 (0.00–001)	12.80 [9]	0.17	0.02 (0.00–0.14)
	Taquara	840	1.47 ± 0.30	5.81 (3.09–10.11)	12.32 [6]	0.06	26.70 (7.01–101.89)*
	Viçosa	1080	1.68 ± 0.29	37.21 (24.52–50.51)	15.94 [9]	0.07	171.00 (46.94–623.35)*
Chlorantraniliprole	Susceptible	1110	0.19 ± 0.02	24.81 (4.45–130.55)	6.93 [8]	0.54	1.00
	Araguari	1110	0.23 ± 0.03	301.95 (71.51–1309.53)	5.74 [8]	0.68	12.17 (0.08–1844.08)
	Brazlândia	1110	0.16 ± 0.02	140.80 (21.11–1111.64)	9.22 [8]	0.32	5.68 (0.03–1021.65)
	Cristalina	1068	0.24 ± 0.03	5159.00 (1321.51–25 917.83)	6.60 [8]	0.58	207.94 (1.47–44 661.77)*
	Domingos Martins	1200	0.18 ± 0.02	31.74 (5.27–165.39)	10.05 [9]	0.35	1.28 (0.01–185.92)
	Gama	870	0.35 ± 0.05	124.27 (20.74–727.42)	11.02 [6]	0.09	5.01 (0.08–297.69)
	Goiânia	799	0.99 ± 0.20	3410.60 (1587.07–5454.84)	8.06 [6]	0.23	137.47 (4.35–4353.29)*
	Guaíra	1110	0.20 ± 0.03	125.04 (23.48–583.46)	9.38 [8]	0.31	5.04 (0.04–675.55)
	Jaíba	1020	0.27 ± 0.04	7837.65 (1948.07–45 593.09)	4.60 [7]	0.70	315.91 (2.24–44 661.77)*
	Patos de Minas	1080	0.27 ± 0.03	180.26 (50.63–593.09)	11.26 [8]	0.19	7.27 (0.10–505.41)
	Taquara	1080	5.44 ± 1.10	14 914.33 (11 666.76–17 039.63)	15.10 [8]	0.6	601.14 (21.42–16 898.19)*
	Viçosa	1020	0.26 ± 0.03	99.64 (29.68–303.63)	10.95 [7]	0.14	4.02 (0.06–283.73)
Imidacloprid	Susceptible	840	0.48 ± 0.04	0.14 (0.05–0.33)	8.65 [6]	0.19	1
	Araguari	1050	0.49 ± 0.06	14.54 (5.88–30.02)	7.29 [8]	0.51	102.17 (18.85–553.79)*
	Brazlândia	1140	0.45 ± 0.04	1.01 (0.45–1.97)	7.65 [9]	0.57	7.10 (1.75–28.72)*
	Cristalina	1069	0.96 ± 0.11	14.64 (8.72–23.86)	14.81 [9]	0.10	102.88 (34.58–306.05)*
	Domingos Martins	1110	0.48 ± 0.04	5.55 (2.70–10.31)	8.31 [9]	0.50	39.00 (0.07–151.45)
	Gama	1020	0.62 ± 0.06	2.25 (1.18–3.90)	10.65 [8]	0.22	15.81 (4.67–53.46)*
	Goiânia	770	1.23 ± 0.22	22.95 (11.25–41.39)	12.35 [6]	0.05	161.28 (49.47–525.70)*
	Guaíra	1020	0.39 ± 0.04	0.03 (0.1–0.07)	11.96 [8]	0.15	0.21 (0.05–0.89)
	Jaíba	1170	0.52 ± 0.07	34.01 (12.17–68.99)	9.781 [9]	0.37	239.00 (39.78–1435.23)*
	Patos de Minas	1047	0.42 ± 0.05	1.14 0.34–2.78)	11.54 [8]	0.17	8.01 (1.35–47.90)*
	Taquara	1140	0.35 ± 0.04	2.38 (0.90–5.27)	9.93 [9]	0.36	16.73 (2.79–99.79)*
	Viçosa	1140	0.62 ± 0.06	1.76 (0.87–3.13)	9.53 [9]	0.39	12.37 (3.64–42.07)*
Lambda-cyhalothrin	Susceptible	1110	0.33 ± 0.03	0.38 (0.12–1.00)	14.38 [9]	0.11	1
	Araguari	870	1.11 ± 0.14	67.94 (46.71–98.04)	9.58 [6]	0.14	179.64 (36.49–882.07)*
	Brazlândia	870	0.50 ± 0.05	3.78 (1.70–8.70)	8.39 [6]	0.21	9.99 (0.55–17.70)
	Cristalina	870	0.51 ± 0.05	13.52 (5.50–28.79)	9.67 [6]	0.14	35.75 (5.66–225.35)*
	Domingos Martins	990	0.33 ± 0.04	0.88 (0.09–4.99)	12.09 [7]	0.10	2.34 (0.18–30.43)
	Gama	960	0.86 ± 0.12	24.65 (9.92–48.02)	13.23 [7]	0.07	65.18 (11.19–378.65)*
	Goiânia	904	1.00 ± 0.11	575.38 (321.35–894.87)	12.61 [9]	0.18	1521.36 (90.70–25 448.60)*
	Guaíra	990	0.25 ± 0.03	9.13 (2.02–30.45)	11.96 [7]	0.10	24.14 (1.22–475.90)*
	Jaíba	1110	1.02 ± 0.11	126.46 (87.85–172.19)	8.98 [9]	0.44	334.37 (65.79–1694.82)*
	Patos de Minas	870	0.43 ± 0.06	1.64 (0.25–8.07)	11.10 [6]	0.09	4.34 (0.49–38.33)
	Taquara	960	1.08 ± 0.15	137.81 (89.80–206.40)	11.71 [8]	0.16	364.38 (69.45–1906.39)*
	Viçosa	1080	0.48 ± 0.06	47.36 (19.59–102.21)	8.80 [8]	0.36	125.22 (15.16–1032.00)*

[a]Low χ^2 and high corresponding *P*-values (i.e. $P > 0.05$) indicate suitable fit to the probit model, whose slope (± SE) is indicated, as is the degrees of freedom for the χ^2 goodness of fit estimates; log transformation was used for the probit model fitting.

Table 2 Relative toxicity of non-neurotoxic insecticides to adults of Brazilian populations of MEAM1 whiteflies (*Bemisia tabaci* B biotype); the asterisk in the resistance ratio indicate significant difference from the standard susceptible population when the confidence interval does not include the value 1, following Robertson *et al.* (2007)[a]

Insecticide	Population	No.	Slope ± SE	LC_{50} (95% Fiducial Interval) μg i.a L^{-1}	χ^2 [degrees of freedom]	*P*	Resistance ratio at LC_{50} [RR_{50} (95% Confidence Interval)]
Azadirachtin	Susceptible	930	0.41 ± 0.05	0.51 (0.11–1.91)	13.27 [7]	0.07	1
	Araguari	780	1.80 ± 0.28	37.95 (24.08–54.36)	8.56 [5]	0.13	74.53 (15.55–357.63)*
	Brazlândia	780	1.90 ± 0.52	67.32 (29.40–119.80)	11.05 [5]	0.05	132.21 (25.23–694.08)*
	Cristalina	990	3.07 ± 0.32	158.73 (132.23–183.12)	9.76 [8]	0.28	311.72 (69.61–1398.25)*
	Domingos Martins	960	1.55 ± 0.23	130.41 (69.09–195.04)	12.35 [7]	0.09	256.11 (48.42–1356.66)*
	Gama	750	0.60 ± 0.09	1.33 (0.19–5.71)	9.66 [5]	0.08	2.61 (0.36–19.24)
	Goiânia	888	3.79 ± 0.82	134.56 (107.72–177.62)	10.60 [7]	0.16	264.26 (58.94–1186.62)*
	Guaíra	1050	0.20 ± 0.03	125.03 (23.48–583.45)	9.38 [8]	0.31	245.54 (5.10–11 861.50)*
	Jaíba	930	0.87 ± 0.14	27.96 (5.00–69.72)	13.33 [7]	0.06	54.91 (7.33–412.23)*
	Patos de Minas	960	0.43 ± 0.07	2.05 (0.19–9.05)	13.30 [7]	0.06	4.03 (0.32–51.73)
	Taquara	1110	1.32 ± 0.20	44.60 (30.36–59.71)	11.01 [9]	0.27	87.59 (17.49–439.34)*
	Viçosa	960	1.23 ± 0.16	23.72 (10.09–43.01)	13.67 [7]	0.06	46.58 (9.03–240.81)*
Diafenthiuron	Susceptible	1140	0.39 ± 0.04	1.87 (0.73–4.19)	13.04 [9]	0.16	1
	Araguari	930	0.93 ± 0.10	5.97 (3.58–8.85)	9.70 [7]	0.21	3.19 (0.72–14.07)
	Brazlândia	1050	0.54 ± 0.09	12.37 (2.01–36.71)	14.08 [8]	0.08	6.61 (0.72–60.77)
	Cristalina	840	4.13 ± 0.53	364.07 (310.25–409.80)	9.78 [6]	0.13	194.69 (48.49–779.34)*
	Domingos Martins	1050	0.79 ± 0.12	15.14 (3.41–36.93)	14.34 [8]	0.07	8.10 (1.29–50.57)*
	Gama	870	1.18 ± 0.16	42.79 (30.23–57.26)	8.22 [6]	0.22	22.88 (5.28–98.85)*
	Goiânia	1040	1.48 ± 0.16	175.48 (131.44–220.51)	7.97 [10]	0.63	93.84 (21.98–399.36)*
	Guaíra	930	0.22 ± 0.03	8.97 (1.88–43.67)	2.47 [6]	0.87	4.80 (0.14–166.24)
	Jaíba	990	1.41 ± 0.16	34.34 (26.41–42.84)	5.23 [8]	0.73	18.36 (4.49–74.89)*
	Patos de Minas	960	0.62 ± 0.11	8.54 (1.09–25.48)	12.14 [7]	0.10	4.57 (0.51–41.09)
	Taquara	870	1.97 ± 0.50	54.45 (27.77–81.38)	11.83 [6]	0.07	29.12 (6.45–130.98)*
	Viçosa	1110	1.90 ± 0.27	72.86 (60.32–88.87)	13.75 [9]	0.13	38.96 (9.79–154.56)*
Spiromesifen	Susceptible	1110	0.17 ± 0.02	7.64 (1.08–45.80)	2.14 [8]	0.98	1
	Araguari	1110	0.24 ± 0.03	450.97 (95.44–2064.87)	5.10 [8]	0.75	59.03 (0.13–263.33)
	Brazlândia	1110	0.26 ± 0.04	23.86 (2.04–152.71)	14.35 [8]	0.07	3.12 (0.03–338.46)
	Cristalina	1080	0.38 ± 0.09	913.20 (510.80–1375.00)	9.42 [8]	0.31	119.53 (11.37–1080.54)*
	Domingos Martins	1110	0.12 ± 0.02	39.63 (3.69–517.97)	2.06 [8]	0.98	5.19 (0.05–57.13)
	Gama	930	0.25 ± 0.03	14.63 (4.41–50.95)	2.93 [6]	0.82	1.91 (0.03–118.37)
	Goiânia	1066	0.70 ± 0.09	1181.89 (696.08–1799.31)	13.46 [9]	0.14	154.70 (0.16–1483.93)
	Guaíra	1080	0.15 ± 0.02	2.22 (0.28–14.28)	7.02 [8]	0.53	0.29 (0.01–68.43)
	Jaíba	1110	0.28 ± 0.03	883.98 (288.82–2972.24)	4.42 [8]	0.82	115.70 (0.01–1588.22)
	Patos de Minas	1110	0.20 ± 0.02	15.98 (3.44–79.26)	10.09 [8]	0.26	2.09 (0.03–148.82)
	Taquara	1020	0.45 ± 0.12	46 578.39 (20 106.33–207 412.95)	9.55 [8]	0.30	6096.65 (65.38–567 196.71)*
	Viçosa	1200	0.25 ± 0.03	64.96 (21.31–193.27)	7.42 [9]	0.59	8.50 (0.15–475.42)

[a]Low χ^2 and high corresponding *P*-values (i.e. $P > 0.05$) indicate suitable fit to the probit model, whose slope (± SE) is indicated, as is the degrees of freedom for the χ^2 goodness of fit estimates; log transformation was used for the probit model fitting.

exhibiting resistance to all insecticides and noticeably so for azadirachtin and diafenthiuron. A second group was formed by all of the other populations, while an intermediate group interconnected these two clusters of populations encompassing the populations from Goiânia and all of the others, except Araguari and Patos de Minas, which were both susceptible to diafenthiuron, but only the later was susceptible to azadirachtin (Table 2; Fig. 2). Nonetheless, the patterns of insecticide resistance among whitefly populations differ considerably from site to site, as reflected in the multivariate analysis of variance performed (Wilks' lambda = 0.0182, $F_{77,115} = 1.43$, $P = 0.04$), and the CVA ordination is a summarised simplification of the resistance patterns observed potentially over-emphasising azadirachtin and diafenthiuron resistance. Regardless, the grouping patterns obtained are suggestive of higher insecticide resistance problems in central rather than eastern and southeastern Brazil.

Correlation analyses testing the association between resistance ratios of cartap and chlorantraniliprole, insecticides exhibiting resistance in whiteflies but not used for their control, and the other compounds were performed to indicate potential cross-resistance that may be favouring their occurrence (Table 4). Resistance to cartap and chlorantraniliprole were significantly correlated

Table 3 Main canonical axes and their canonical loadings (canonical correlations) from the canonical variate analysis (CVA) for the spectrum and level of insecticide resistance in 12 populations of the MEAM1 whiteflies (*Bemisia tabaci* B biotype)[a]

	Canonical loadings	
Variables (resistance ratios)	1st axis	2nd axis
Azadirachtin	**0.38**	0.05
Cartap	−0.07	−0.65
Chlorantraniliprole	−0.04	−0.10
Diafenthiuron	**0.82**	−0.03
Imidacloprid	−0.01	0.02
Lambda-cyhalothrin	−0.07	0.27
Spiromesifen	0.01	−0.07
F	1.43	1.00
$d.f._{num;den}$	77; 115	60; 105
P	0.04	0.49
Eigenvalue	4.15	0.96
Squared canonical correlation	0.81	0.49

[a]Only the 1st axis was significant and its main contributors are indicated in bold.

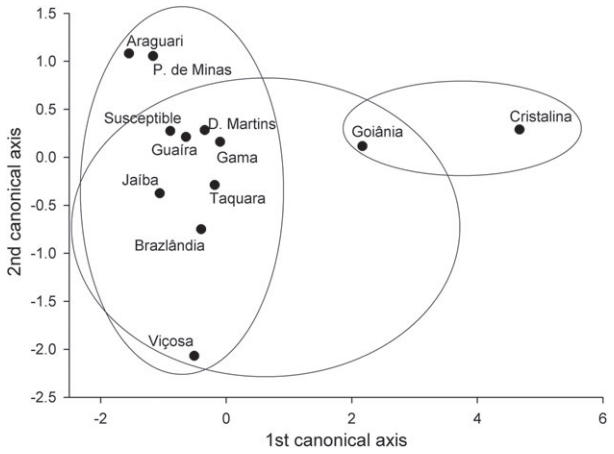

Figure 2 CVA ordination diagram showing the divergence in patterns of insecticide resistance among populations of whitefly of the putative species MEAM1 (see Table 3). The symbols are centroids representing the mean canonical variates of levels of resistance to different insecticides for each population. The large circles indicate clusters of populations that are not significantly different by the approximate *F* test ($P < 0.05$), based on the Mahalanobis distance (D^2) between class means in the CVA ordination. The 2nd CVA axis was not significant and was used here only to improve the display of the populations surveyed.

($r = 0.58$, $P < 0.001$) suggesting that both compounds may be profiting from cross-selection to another insecticide. In addition, resistance to these compounds was significantly correlated with resistance to imidacloprid (cartap: $r = 0.65$, $P < 0.001$; chlorantraniliprole: $r = 0.83$, $P < 0.001$), but chlorantraniliprole resistance was also marginally correlated with spiromesifen resistance ($r = 0.35$, $P = 0.04$).

Table 4 Correlations testing the association between resistance ratios of cartap and chlorantraniliprole, insecticides exhibiting resistance in MEAM1 whiteflies (*Bemisia tabaci* B biotype), but not used for their control, and the other compounds ($n = 12$)[a]

	Cartap		Chlorantraniliprole	
Insecticides	*r*	*P*	*r*	*P*
Azadirachtin	0.05	0.79	0.06	0.74
Cartap	–	–	0.58	< 0.001*
Chlorantraniliprole	0.58	< 0.001*	–	–
Diafenthiuron	0.07	0.67	0.02	0.91
Imidacloprid	0.65	< 0.001*	0.83	< 0.001*
Lambda-cyhalothrin	−0.08	0.66	- 0.04	0.81
Spiromesifen	−0.03	0.86	0.35	0.04*

[a]The asterisk indicates significant correlation at $P < 0.05$.

Control failure likelihood

The diagnosis for control failure indicated significant departure from the expected target mortality of 80%, which is the minimum efficacy threshold for the context under investigation (Table 5). Such instances were rare for diafenthiuron with two expected instances of control failure and absent for imidacloprid (Table 5). In contrast, control failure is expected against most of the populations for azadirachtin, and four populations for both lambda-cyhalothrin, and spiromesifen even using the rather restrictive Bonferroni correction to account for multiple comparisons (Table 5). The mortality range for azadirachtin was below 50% on average with over 35% risk of control failure incurring in about 75% of the populations, which was the most critical situation, followed by spiromesifen and lambda-cyhalothrin (Fig. 3).

Discussion

Two putative whitefly species, MEAM1 and MED, were responsible for the two major global invasion events within the group (Brown *et al.,* 1995; De Barro *et al.,* 2011). They are currently widespread and the former, MEAM1, prevails in Neotropical America although MED was recently introduced and is experiencing rapid spread in the region (Barbosa *et al.,* 2014, 2015). As their respective prevalence seems dependent on local levels of host suitability and pattern of insecticide use with consequences for their management (Sun *et al.,* 2013; Gao & Reitz, 2017; Zheng *et al.,* 2017), the recognition of the prevailing species is important and was carried out in our study. We expected the prevalence of MEAM1 due to the still recent detection of MED in the country, which took place by 2013 after its detection in Argentina and Uruguay (Grille *et al.,* 2011; Barbosa *et al.,* 2015). Indeed, all of the field-collected populations from our study belong to the putative species MEAM1 without exception and no mixture with MED individuals was observed. This seems good

Table 5 Estimated insecticide mortality (%) and control failure likelihood (%) of populations of the MEAM1 whiteflies (*Bemisia tabaci* B biotype) using Brazilian recommended label rates[a]

Populations	Mortality [Control failure likelihood] (%)				
	Azadirachtin	Diafenthiuron	Imidacloprid	Lambda-cyhalothrin	Spiromesifen
Susceptible	84.17 [0.00]	100.00 [0.00]	100.00 [0.00]	100.00 [0.00]	97.50 [0.00]
Araguari	48.33 [39.59]*	95.00 [0.00]	79.17 [1.04]	41.67 [47.91]*	65.83 [17.71]
Brazlândia	34.17 [57.29]*	96.67 [0.00]	98.33 [0.00]	93.33 [0.00]	95.00 [0.00]
Cristalina	34.17 [57.29]*	35.83 [55.21]*	77.50 [3.13]	90.83 [0.00]	30.83 [61.46]*
Domingos Martins	43.33 [45.84]*	100.00 [0.00]	85.00 [0.00]	81.67 [0.00]	62.50 [21.46]
Gama	77.50 [3.13]	96.67 [0.00]	89.17 [0.00]	86.67 [0.00]	95.83 [0.00]
Goiânia	28.33 [64.59]*	36.67 [54.16]*	79.17 [1.04]	43.33 [45.84]*	57.50 [28.13]*
Guaíra	36.67 [54.16]*	95.00 [0.00]	100.00 [0.00]	62.50 [21.88]	95.00 [0.00]
Jaíba	64.17 [19.79]	95.00 [0.00]	65.00 [18.75]	53.33 [33.34]*	44.17 [44.79]*
Patos de Minas	75.00 [6.25]	84.17 [0.00]	95.83 [0.00]	75.83 [5.21]	87.50 [0.00]
Taquara	68.33 [14.59]	89.17 [0.00]	92.50 [0.00]	54.17 [32.29]	38.33 [52.09]*
Viçosa	30.83 [61.46]*	83.33 [0.00]	91.67 [0.00]	38.33 [52.09]*	73.33 [8.34]

[a]Mortalities followed by an asterisk are significantly lower (one-sided Z-test at 95% confidence level with correction for continuity and also Bonferroni correction; $n = 120$) than the minimum efficacy threshold of 80%, as required by the Brazilian legislation (MAPA, 1995).

news as MED is usually regarded as an even more serious concern regarding insecticide resistance than MEAM1, at least in China (Xie *et al.,* 2014; Wang *et al.,* 2017b).

We also expected an increase in incidence and levels of insecticide resistance from the earlier levels detected in Brazil (Silva *et al.,* 2009). This expectation was confirmed as only low levels of resistance (≤10-fold) and only to the neonicotinoids imidacloprid and thiamethoxam were earlier detected by Silva *et al.* (2009). The situation has greatly worsened and although imidacloprid resistance was detected in all but one of the insect populations from our study, reaching high levels of resistance (i.e. >100-fold) in four of them, the situation of the other compounds was worse, except for diafenthiuron. Among the recommended neurotoxic insecticides resistance to the pyrethroid lambda-cyhalothrin was either high (>100-fold), or very high (i.e. >1000-fold) for nearly half of the whitefly populations surveyed here. High levels of lambda-cyhalothrin resistance have also been reported in undetermined biotypes in China (He *et al.,* 2007), where resistance to neonicotinoids is also frequent particularly in populations of MED (or biotype Q) (Yuan *et al.,* 2012; Zheng *et al.,* 2017; Wang *et al.,* 2017b), and moderate resistance was reported in Egypt and Israel again of undetermined putative species (Ahmad *et al.,* 2002; El Kady & Devine, 2003; He *et al.,* 2007).

Regarding the insecticides that do not target either nerve or muscle cells, the results obtained also sparked concerns regarding insecticide resistance among the MEAM1 populations surveyed. The incidence of the phenomenon was high for azadirachtin and diafenthiuron (nine and seven out of 11 populations exhibiting significant resistance, respectively), and less so for spiromesifen. The latter exhibited two out of 11 instances

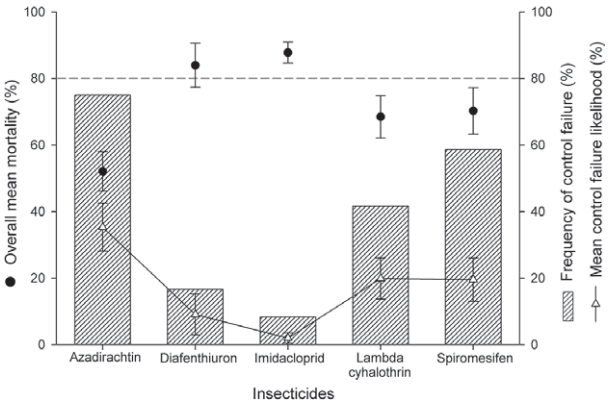

Figure 3 Mean mortality and control failure likelihood (%; ± SEM), and frequency of control failure for each registered insecticide across 12 populations of whitefly of the putative species MEAM1. The dashed horizontal line indicates the minimum field-efficacy threshold for insecticide registration, as required by the Brazilian legislation (MAPA, 1995).

of significant resistance with low resolution for three additional cases potentially exhibiting moderate to high levels of resistance, but with the highest levels of resistance detected in our survey (>6000-fold). Spiromesifen resistance among whiteflies has been detected in Florida (USA) and Spain, but the resistance levels were low to moderate (Fernández *et al.,* 2009; Mann *et al.,* 2012). Instances of diafenthiuron resistance among whiteflies are rare and only low levels were reported in Pakistan (Basit *et al.,* 2013), unlike what we observed in our survey with Brazilian populations of MEAM1 although at no lower levels than the other insecticides.

Azadirachtin resistance among Brazilian populations of MEAM1 was frequent and the levels were high, which

came as a surprise. This is so because this bioinsecticide exhibits more complex and still poorly known modes of action that allegedly delays the evolution of resistance (Mordue (Luntz) & Nisbet, 2000; Mordue (Luntz) *et al.*, 2010; Sparks & Nauen, 2015; Siegwart *et al.*, 2015). Nonetheless, aphid resistance to azadirachtin has been demonstrated (Feng & Isman, 1995), and low to moderate levels of azadirachtin resistance (<31-fold) were detected among Spanish populations of MED whiteflies (Fernández *et al.*, 2009). The rather prevalent resistance to azadirachtin among MEAM1 Brazilian whiteflies adds to the concern with the phenomenon and adds risk to organic cultivation in the country, which tends to rely on this insecticide for whitefly management particularly in vegetable crops (MAPA, 2017).

The scenario observed for cartap and chlorantraniliprole resistance also calls for attention. Neither insecticide holds registration against whiteflies in Brazil (MAPA, 2017). Nonetheless, they are used in this species' host plants for the control of other arthropod pest species allowing the inadvertent selection for resistance among whitefly populations. In addition, the significant correlations observed between resistance to both compounds, and their resistance with that of imidacloprid, suggest that cross-selection may also be taking placing allowing cross resistance among these insecticides, although the occurrence of multiple resistance cannot be ruled out. This is another issue deserving of attention for resistance management as it limits the alternative insecticides available for tactics such as insecticide rotation. Low chlorantraniliprole resistance among whiteflies was detected in nymphs of populations of MEAM1 and MED from the United States and China (Li *et al.*, 2012; Caballero *et al.*, 2013; Xie *et al.*, 2014), but we are unaware of reports of cartap resistance in whiteflies reinforcing our surprise with the rather frequent and high levels of resistance to both compounds detected among the surveyed whitefly populations from Brazil in the current study.

As insecticide resistance may result in insecticide control failure, the latter was also assessed in our study where it was also expected to be high, particularly for older insecticides (i.e. lambda-cyhalothrin and imidacloprid), in contrast with compounds of more recent use and to which selection for insecticide resistance is consequently more recent. The patterns of insecticide resistance observed lay credence to the concern with potential insecticide field control failures. Curiously though, the risk of control failure is consistently higher and more widespread for azadirachtin and spiromesifen, rather than to imidacloprid and diafenthiuron, also compromising suitable overall field efficacy. These results likely reflect the decrease in imidacloprid use against whiteflies and the still modest use of diafenthiuron compared with the other insecticides in Brazil, particularly azadirachtin and spiromesifen. Again, the situation in central Brazil is particularly serious with higher risk of insecticide control failure in this region where most of its whitefly populations sampled exhibited high risks of control failure.

In summary, the putative whitefly species MEAM1 still prevails in the main vegetable producing areas in Brazil and insecticide resistance greatly increased in the country to a far broader range of insecticides. Resistance to azadirachtin, spiromesifen, and lambda-cyhalothrin are even more serious and widespread leading to greater CFL. Central Brazil exhibits an even more critical situation with higher levels of resistance and higher likelihood of control failure. Another issue of concern is the detected resistance to cartap and chlorantraniliprole, which are not even registered and used targeting whiteflies. Therefore, the resistance to these insecticides is due to inadvertent selection upon their use against other arthropod pest species on the same host plants, and potentially assisted by cross-selection to imidacloprid, which deserves further attention. Resistance to chlorantraniliprole reverts in an additional concern, which is the potential cross-resistance that it may lead to the related anthranilic diamide cyantraniliprole that was recently registered for whitefly control, whose use may be compromised. Again, this possibility also requires attention in designing resistance management programmes for this pest species.

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References

Abbott W.S. (1925) A method of computing the effectiveness of an insecticide. *Journal of Economic Entomology*, **18**, 265–266.

Ahmad M., Arif M.I., Ahmad Z., Denholm I. (2002) Cotton whitefly (*Bemisia tabaci*) resistance to organophosphate and pyrethroid insecticides in Pakistan. *Pest Management Science*, **58**, 203–208.

Barbosa L.F., Marubayashi J.M., De Marchi B.R., Yuki V.A., Pavan M.A., Moriones E., Navas-Castillo J., Krause-Sakate R. (2014) Indigenous American species of the *Bemisia tabaci* complex are still widespread in the Americas. *Pest Management Science*, **70**, 1440–1445.

Barbosa L.F., Yuki A., Massaharu J., De Marchi R., Perini L., Pavan M.A., De Barros R., Ghanim M., Moriones E., Navas-Castillo J., Krause-Sakate R. (2015) First report of *Bemisia tabaci* Mediterranean (Q biotype) species in Brazil. *Pest Management Science*, **71**, 501–504.

Basit M., Saeed S., Saleem M.A., Denholm I., Shah M. (2013) Detection of resistance, cross-resistance, and stability of resistance to new chemistry insecticides in *Bemisia tabaci* (Homoptera: Aleyrodidae). *Journal of Economic Entomology*, **106**, 1414–1422.

Basit M., Sayyed A.H., Saleem M.A., Saeed S. (2011) Cross-resistance, inheritance and stability of resistance to acetamiprid in cotton whitefly, *Bemisia tabaci* Genn. (Hemiptera: Aleyrodidae). *Crop Protection*, **30**, 705–712.

Brown J.K., Frohlich D.R., Rosell R.C. (1995) The sweet-potato or silverleaf whitflies: biotypes of *Bemisia tabaci* or species complex? *Annual Review of Entomology*, **40**, 511–534.

Byrne F., Castle S., Prabhaker N., Toscano N. (2003) Biochemical study of resistance to imidacloprid in B biotype *Bemisia tabaci* from Guatemala. *Pest Management Science*, **59**, 347–352.

Caballero R., Cyman S., Schuster D. (2013) Monitoring insecticide resistance in biotype B of *Bemisia tabaci* (Hemiptera: Aleyrodidae) in Florida. *Florida Entomologist*, **96**, 1243–1256.

Castle S., Palumbo J., Prabhaker N., Horowitz A., Denholm I. (2010) Ecological determinants of *Bemisia tabaci* resistance to insecticides. In *Bionomics and Management of a Global Pest*, pp. 423–465. Eds P. Stnsly and S. Naranjo. Amsterdam, The Netherlands: Springer.

Crow J.F. (1960) Genetics of insecticide resistance: general considerations. *Miscellaneous Publications of the Entomological Society of America*, **2**, 69–74.

De Barro P.J. (2012) The *Bemisia tabaci* species complex : questions to guide future research. *Journal of Integrative Agriculture*, **11**, 187–196.

De Barro P.J., Liu S., Boykin L.M., Dinsdale A.B. (2011) *Bemisia tabaci*: a statement of species status. *Annual Review of Entomology*, **56**, 1–19.

Dittrich V., Hassan S., Ernst G. (1985) Sudanese cotton and the whitefly: a case study on the emergence of a new primary pest. *Crop Protection*, **4**, 161–176.

El Kady H., Devine G. (2003) Insecticide resistance in Egyptian populations of the cotton whitefly, *Bemisia tabaci* (Hemiptera: Aleyrodidae). *Pest Management Science*, **59**, 865–871.

Feng R., Isman M. (1995) Selection for resistance to azadirachtin in the green peach aphid, *Myzus persicae*. *Experientia*, **51**, 831–833.

Fernández E., Grávalos C., Haro P.J., Cifuentes D., Bielza P. (2009) Insecticide resistance status of *Bemisia tabaci* Q-biotype in south-eastern Spain and Pablo Bielza. *Pest Management Science*, **65**, 885–891.

Fragoso D.B., Guedes R.N.C., Rezende S.T. (2003) Glutathione *S*-transferase detoxification as a potential pyrethroid resistance mechanism in the maize weevil, *Sitophilus zeamais*. *Entomologia Experimentalis et Applicata*, **109**, 21–29.

Frohlich D., Torres-Jerez I., Bedford I., Markham P., Brown J. (1999) A phylogeographical analysis of the *Bemisia tabaci* species complex based on mitochondrial DNA markers. *Molecular Ecology*, **8**, 1683–1691.

Gao Y., Reitz S.R. (2017) Emerging themes in our understanding of species displacements. *Annual Review of Entomology*, **62**, 165–183.

Gontijo P.C., Picanço M.C., Pereira E.J.G., Martins J.C., Chediak M., Guedes R.N.C. (2013) Spatial and temporal variation in the control failure likelihood of the tomato leaf miner, *Tuta absoluta*. *Annals of Applied Biology*, **162**, 50–59.

Grille G., Gauthier N., Buenahora J., Basso C., Bonato O. (2011) First report of the Q biotype of *Bemisia tabaci* in Argentina and Uruguay. *Phytoparasitica*, **39**, 253–238.

Guedes R.N.C. (2017) Insecticide resistance, control failure likelihood, and the first law of geography. *Pest Management Science*, **73**, 479–484.

Guedes R.N.C., Smagghe G., Stark J.D., Desneux N. (2016) Pesticide-induced stress in arthropod pests of optimized integrated pest management programs. *Annual Review of Entomology*, **61**, 43–62.

Guedes R.N.C., Walse S.S., Throne J.E. (2017) Sublethal exposure, insecticide resistance, and community stress. *Current Opinion in Insect Science*, **21**, 47–53.

He Y., Weng Q., Huang J., Liang Z., Lin G., We D. (2007) Insecticide resistance of *Bemisia tabaci* field populations. *Chinese Journal of Applied Ecology*, **18**, 1578–1582.

IRAC, Insecticide Resistance Action Committee. (2017) Resistance: Definition, Background, Development. URL http://www.irac-online.org/about/resistance [accessed on 1 April 2017].

Li X., Degain B., Harpold V., Marcon P., Nichols R., Fournier A., Naranjo S., Palumbo J., Ellsworth P. (2012) Baseline susceptibilities of B- and Q-biotype *Bemisia tabaci* to anthranilic diamides in Arizona. *Pest Management Science*, **68**, 83–91.

Mann R., Schuster D., Cordero R., Toapanta M. (2012) Baseline toxicity of spiromesifen to biotype B of *Bemisia tabaci* in Florida. *Florida Entomologist*, **95**, 95–98.

MAPA, Ministério da Agriccultura, Pecuária e Abastecimento. (1995) Normas e Exigências para Execução de Testes de Produtos Químicos para Fins de Registro no MAPA. Brasília, Brazil: Ministério da Agricultura e Reforma Agrária.

MAPA, Ministério da Agriccultura, Pecuária e Abastecimento. (2017) *AGROFIT: Sistema de Agrotóxicos Fitossanitários*. Brasília, Brazil: MAPA/CGAF/DFIA/DAS. URL http://agrofit.agricultura.gov.br/agrofit_cons/principal_agrofit_cons [accessed on 1 April 2017].

McKenzie C.L., Bethke J.A., Byrne F.J., Chamberlin J.R., Dennehy T.J., Dickey A.M., Gilrein D., Hall P.M., Ludwig S., Oetting R.D., Osborne L.S., Schmale L., Shatters R.G. Jr (2012) Distribution of *Bemisia tabaci* (Hemiptera: Aleyrodidae) biotypes in North America after the Q invasion. *Journal of Economic Entomology*, **105**, 753–766.

Metcalf R.L. (1980) Changing role of insecticidddes in crop protection. *Annual Review of Entomology*, **25**, 219–256.

Mordue (Luntz) A.J., Morgan E.D., Nisbet A.J. (2010) Azadirachtin, a natural product in insect control. In *Insect Control*, pp. 185–204. Eds L.I. Gilbert and S.S. Gill. London, UK: Academic Press.

Mordue (Luntz) A.J., Nisbet A.J. (2000) Azadirachtin from the neem tree *Azadirachta indica*: its action against insects. *Anais da Sociedade Entomológica do Brazil*, **29**, 615–632.

Naveen N.C., Rahul C., Kumar D., Reb K.B., Rajagopal R. (2017) Insecticide resistance status in the whitefly, *Bemisia tabaci* genetic groups Asia-I , Asia-II-1 and Asia-II-7 on the Indian subcontinent. *Scientific Reports*, **7**, 40634.

Panini M., Tozzi F., Zimmer C.T., Bass C., Field L., Borzatta V., Moores G. (2017) Biochemical evaluation of interactions between synergistic molecules and phase I enzymes involved in insecticide resistance in B- and Q-type *Bemisia tabaci* (Hemiptera: Aleyrodidae). *Pest Management Science*, **73**, 1873–1882. https://doi.org/10.1002/ps.4553.

Ragab A. (2013) Genetic variability of the whitefly *Bemisia tabaci* and its secondary endosymbionts in the Arabian Peninsula. M.Sc. Thesis. Thuwal, Saudi Arabia: King Abdullah University of Science and Technology.

Robertson J.L., Russell R.M., Preisler H.K., Savin N.E. (2007) *Bioassays with Arthropods*. 2nd edn. Boca Raton: CRC Press.

Roditakis E., Roditakis N.E., Tsagkarakou A. (2005) Insecticide resistance in *Bemisia tabaci* (Homoptera: Aleyrodidae) populations from Crete. *Pest Management Science*, **582**, 577–582.

Roush R.T., Miller G.L. (1986) Considerations for design of insecticide resistance monitoring programs. *Journal of Economic Entomology*, **79**, 293–298.

Santillán-Ortega C., Rodríguez-Maciel J., Lopex-Collados J., Díaz-Gomez O., Lagunes-Tejeda A., Carrillo-Martínez J., Bernal J., Robles-Bermúdez A., Aguilar-Medel S., Silva-Aguayo G. (2011) Susceptibility of females and males of *Bemisia tabaci* (Gennadius) B-biotype and *Trialeurodes vaporariorum* (Westwood) to thiamethoxam. *Southwestern Entomologist*, **36**, 167–176.

Sawicki R.M. (1987) Definition, dettection and documentation of insecticide resistance. In *Combating Resistance to Xenobiotics: Biological and Chemical Approaches*, pp. 105–107. Eds M.G. Ford, D.W. Holloman, B.P.S. Khambay and R.M. Sawicki. Chichester, UK: Ellis Horwood.

Siegwart M., Graillot B., Lopez C.B., Besse S., Bardin M., Nicot P.C., Lopez-Ferber M. (2015) Resistance to bio-insecticides or how to enhance their sustainability: a review. *Frontiers in Plant Science*, **6**, 1–19.

Silva G.A., Picanço M.C., Bacci L., Crespo A.L.B., Rosado J.F., Guedes R.N.C. (2011) Control failure likelihood and spatial dependence of insecticide resistance in the tomato pinworm, *Tuta absoluta*. *Pest Management Science*, **67**, 913–920.

Silva L.D., Omoto C., Bleicher E., Dourado P.M. (2009) Monitoring the susceptibility to insecticides in *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae). *Neotropical Entomology*, **38**, 116–125.

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

Sparks T.C., Nauen R. (2015) IRAC: mode of action classification and insecticide resistance management. *Pesticide Biochemistry and Physiology*, **121**, 122–128.

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

Tamura K., Peterson D., Peterson N., Stecher G., Nei M., Kumar S. (2011) MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Molecular Biology and Evolution*, **28**, 2731–2739.

Wang R., Fang Y., Mu C., Qu C., Li F., Wang Z., Luo C. (2017a) Baseline susceptibility and cross-resistance of cycloxaprid, a novel cis-nitromethylene neonicotinoid insecticide, in *Bemisia tabaci* MED from China. *Crop Protection*. https://doi.org/10.1016/j.cropro.2017.02.012.

Wang S., Zhang Y., Yang X., Xie W., Wu Q. (2017b) Resistance monitoring for eight insecticides on the sweet potato whitefly (Hemiptera: Aleyrodidae) in China. *Journal of Economic Entomology*, **110**, 660–666.

Whalon M.E., Mota-Sanchez D., Hollingworth R.M. (2008) Analysis of global pesticide resistance in arthropods. In *Global Pesticide Resistance in Arthropods*, pp. 5–31. Eds M.E. Whalon, D. Mota-Sanchez and R.M. Hollingworth. Wallingford, UK: CABI.

WHO, World Health Organization. (1957) *Expert Committee on Malaria, seventh report*. WHO Technical Report Series No. 125. Geneva, Switzerland: WHO.

Xie W., Liu Y., Wang S., Wu Q., Pan H., Yang X., Guo L., Zhang Y. (2014) Sensitivity of *Bemisia tabaci* (Hemiptera: Aleyrodidae) to several new insecticides in China: effects of insecticide type and whitefly species, strain, and stage. *Journal of Insect Science*, **14**, 261.

Yuan L., Wang S., Zhou J., Du Y., Zhang Y., Wang J. (2012) Status of insecticide resistance and associated mutations in Q-biotype of white fly, *Bemisia tabaci*, from eastern China. *Crop Protection*, **31**, 67–71.

Zheng H., Xie W., Wang S., Wu Q., Zhou X., Zhang Y. (2017) Dynamic monitoring (B versus Q) and further resistance status of Q-type *Bemisia tabaci* in China. *Crop Protection*, **94**, 115–122.