

# Chemical class rotations for control of *Bemisia tabaci* (Hemiptera: Aleyrodidae) on poinsettia and their effect on cryptic species population composition

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## Abstract

**BACKGROUND:** *Bemisia tabaci*, a polyphagous insect with over 900 host plants, is an effective vector of more than 100 plant viruses. Being highly fecund, *B. tabaci* has the potential to develop insecticide resistance rapidly, as demonstrated by reports of use failures with MEAM1 and MED cryptic species (commonly known as biotypes B and Q respectively). Insecticide resistance management is a key component of pest management practices. The research herein studied season-long rotational management programs on poinsettia and their impact on the ratio of MEAM1:MED cryptic species in the surviving treated populations.

**RESULTS:** In all four experiments, only three of the treatments completely eliminated the adult or immature whiteflies, but all significantly reduced the populations. Out of 18 active ingredients tested, dinotefuran (applied as a soil drench) was the most efficacious against both MEAM1 and MED cryptic species compared with the other chemical or biorational insecticides evaluated. Reduced susceptibility of MED was reported against a variety of treatment regimes.

**CONCLUSION:** Rotations can be used to manage MEAM1 and MED cryptic species and maintain a very low population level or completely eliminate *Bemisia* on poinsettia. It is imperative to continue to emphasize the importance of rotating among different modes of action in pest management programs in order to retain effective chemistries for as long as possible in the market place. Published 2014. This article is a U.S. Government work and is in the public domain in the USA.

**Keywords:** MEAM1; MED; biotype B; biotype Q; insecticides; biopesticides

## 1 INTRODUCTION

*Bemisia tabaci* (Gennadius) feeds upon over 900 host plants<sup>1,2</sup> and vectors over 111 plant virus species<sup>3</sup> and is considered to be a major invasive species worldwide.<sup>4</sup> The taxonomic status of *B. tabaci* remains debated between 36 previously identified biotypes and the newly proposed 24 discrete species.<sup>5–8</sup> Losses in agricultural production have increased owing to *B. tabaci* as new, more virulent and less pesticide-sensitive cryptic species have spread to all continents except Antarctica.<sup>9</sup> Very few countries have escaped its cosmopolitan distribution and subsequent establishment of at least one of the *B. tabaci* cryptic species, with the exception of a small portion of the European Union (Finland, Ireland, the United Kingdom and certain regions of Portugal).<sup>10</sup> The two most invasive members of the cryptic species complex posing the greatest threat to growers are Middle East–Asia Minor 1 (MEAM1) and Mediterranean (MED) (commonly known as biotypes B and Q respectively).<sup>7</sup>

After the introduction of MEAM1 into the United States around 1985, unprecedented losses began occurring on poinsettia in the late 1980s in Florida, followed by high infestations in field-grown tomato crops.<sup>11–13</sup> MEAM1 rapidly spread across the southern

United States to Texas, Arizona and California, where extreme field outbreaks occurred during the early 1990s on melons, cotton and vegetable crops.<sup>14–17</sup>

Indistinguishable morphologically from MEAM1, MED is extremely problematic to agricultural production because populations are highly prone to develop resistance to insect growth regulators (IGRs)<sup>18</sup> and neonicotinoid insecticides.<sup>19</sup> Both classes of insecticides are widely used for controlling whiteflies in many cropping systems, including cotton,<sup>20</sup> vegetables<sup>21</sup> and

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ornamentals.<sup>22,23</sup> Since its discovery in Spain in 1997<sup>24</sup> and in Israel in 1999,<sup>25</sup> MED has caused severe crop damage in the Mediterranean Basin in both protected and open agriculture<sup>26,27</sup> and has exhibited an invasive ability arguably matched only by MEAM1. MED has recently been reported in Argentina and Uruguay,<sup>28</sup> China,<sup>29</sup> Costa Rica,<sup>30</sup> France,<sup>31</sup> Guatemala,<sup>32</sup> Japan,<sup>33</sup> Korea,<sup>34</sup> Mexico,<sup>35</sup> New Zealand,<sup>36</sup> Taiwan<sup>37</sup> and Tunisia,<sup>38</sup> as well as in the United States.<sup>39–42</sup> In the United States, nuclear microsatellite markers exhibiting a high degree of concordance with mitochondrial markers uncovered a major east–west phylogeographic break within MED populations invading that country.<sup>42</sup>

Determined to be essentially unaffected by pyriproxyfen in egg bioassays, MED whiteflies collected in the United States also had noticeably reduced susceptibility to acetamiprid, buprofezin, mixtures of fenpropathrin and acephate (normally synergistic against MEAM1), imidacloprid and thiamethoxam.<sup>43</sup> With multiple reports of resistance across different modes of action, MED has a reputation for being particularly capable of developing resistance under intensive insecticide use.<sup>18,19,26,43–45</sup> Following the initial detection of MED in Arizona in 2004,<sup>39</sup> an APHIS-coordinated multistate, multiagency, multi-industry, multicommodity and multi-institutional Q-biotype Task Force initiative was formed and coordinated a whole-country survey. From 2005 to this day, 26 states have reported the presence of MED.<sup>41</sup> Thus, rotational programs as part of best management practices targeted at propagated ornamentals<sup>22</sup> and plants for planting intended for export<sup>23</sup> needed to be verified for managing MED and mixed MEAM1 and MED whitefly populations. It was theorized that mixed populations of MEAM1 and MED under insecticide pressure would shift to mostly, if not entirely, MED by the end of a poinsettia crop cycle. The objectives of these experiments were (1) to determine the efficacy of rotating insecticides with different modes of action against the two cryptic species (MEAM1 and MED) infesting poinsettia, (2) to determine the chemical class rotation effect on the cryptic species population composition on poinsettia and (3) to determine the cryptic species population composition over time on poinsettia in the absence of insecticide pressure.

## 2 MATERIALS AND METHODS

### 2.1 Insect culture

Adult male and female *B. tabaci* MEAM1 (mtCO1 GenBank accession number HQ877585) were obtained from laboratory cultures maintained by the College of Agricultural Science, the University of Georgia, Griffin, Georgia. All stages of whitefly have been maintained on poinsettias (*Euphorbia pulcherrima* cv. Freedom Red) since 2005 by serial transfer and housed in a large poly-greenhouse with ambient temperature, light and humidity. *B. tabaci* MED (mtCO1 GenBank accession number HQ877514) were originally obtained from an ornamental grower in Georgia in 2009<sup>41</sup> and reared in a separate poly-greenhouse at the Griffin campus of the University of Georgia, following MEAM1 colony maintenance procedures. The MED culture is the haplotype Q1, as reported by McKenzie *et al.*,<sup>41</sup> and has recently been determined to be Eastern MED.<sup>42</sup>

### 2.2 Whitefly DNA extraction

DNA was extracted from individual whiteflies by placing a single whitefly in a 1.5 mL Eppendorf tube, adding 50  $\mu$ L of DNA lysis buffer (50 mM KCl, 50 mM Tris–HCl, pH 8.4, 0.45% Tween 20, 0.45% NP40) and grinding with a pestle. The pestle was rinsed with an

additional 50  $\mu$ L of DNA lysis buffer and collected in the same tube. Tubes were placed in a metal boiling rack and boiled at 95 °C for 5 min, then placed directly in ice for 5 min. Tubes were then centrifuged at 8000  $\times g$  for 30 s, and the supernatant (crude DNA lysate) was transferred to another tube and stored at –80 °C for future polymerase chain reaction (PCR) analysis.

### 2.3 PCR analysis

The PCR amplifications for the mtCOI gene were performed using the Btab-Uni primer set and the B (MEAM1) and Q (MED) biotype specific primer sets described by Shatters *et al.*<sup>46</sup> The 30  $\mu$ L PCR reactions were run using a MJ Research PTC-200 Peltier thermal cycler (MJ Research, Inc., Waltham, MA) under the conditions described by Shatters *et al.*<sup>46</sup>

### 2.4 Gel electrophoresis

B (MEAM1) and Q (MED) biotype specific primer sets amplify a specific fragment size of 478 and 303 bp respectively,<sup>46</sup> and product size was used to determine species difference on a weekly basis during each greenhouse trial. Crude DNA preps were used for visualization of bands. Gel electrophoresis of samples was performed using either the Cambrex Flashgel system (Cambrex, East Rutherford, NJ) for comparison of 12 or fewer samples or the 2% Invitrogen Egel 48 (Invitrogen, Carlsbad, CA) for analysis of 48 samples.

### 2.5 Mitochondrial cytochrome oxidase I (COI) amplification sequence analysis

MEAM1 and MED whitefly laboratory cultures used for the infestation source of all greenhouse trials were sequenced prior to the initiation of each of the four greenhouse trials. Mitochondrial COI sequence analysis was performed first by PCR amplification of an approximately 748 bp mtCOI DNA fragment and then sequencing the PCR amplified DNA. Prior to sequencing, the amplified products were cleaned using Montage<sup>®</sup> PCR clean-up filters (Millipore, Billerica, MA). A quantity of 50 ng of total whitefly genomic DNA was used in BigDye<sup>®</sup> sequencing reactions. All sequencing was performed bidirectionally with the amplification primers and BigDye<sup>®</sup> Terminator v.3.1 cycle sequencing kits (Applied Biosystems, Foster City, CA). Sequence reactions were analyzed on an Applied Biosystems 3730XL DNA sequence analyzer and were then compared and edited using Sequencher software (Gene Codes, Ann Arbor, MI).<sup>47</sup> Cryptic species determination was based on direct sequence comparisons using the web-based NCBI BLAST sequence comparison application (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>).

### 2.6 Pesticide materials

Pesticide active ingredient, trade name and formulations, Insecticide Resistance Action Committee (IRAC) classification, manufacturer, application rates and application methods used in the poinsettia greenhouse trials are listed in Table 1.

### 2.7 Experimental procedure

Poinsettia plants were obtained from Davis Floral Greenhouses, Dewy Rose, GA, cv 'Classical Red' (trials 1 and 2), Oglevee Greenhouses, McDonough, GA, cv. 'Eckespoint Red Velvet' (trial 3), and Ecke Greenhouses, Encinitas, CA, cv. 'Prestige Red' (trial 4) and potted into SunGro 300 potting medium (SunGro Horticulture, Agawam, MA) in 15 cm pots. The cages were fabricated with white

**Table 1.** Formulated pesticides evaluated in poinsettia greenhouse trials against cryptic *Bemisia tabaci* species MEAM1 and MED, commonly known as biotypes B and Q respectively

Pesticide active ingredient	Trade name	IRAC <sup>a</sup>	Manufacturer	Rate (100 gal <sup>-1</sup> )	Application method <sup>b</sup>	GH trial
Acetamiprid	TriStar 0.76EC	4A	Cleary Chemical	15 fl oz	F	3
Acetamiprid	TriStar 30SG	4A	Cleary Chemical	5 oz	F	1, 2, 4
Dinotefuran	Safari 20SG	4A	Valent	12 oz	D	1, 2, 3
Dinotefuran	Safari 20SG	4A	Valent	8 oz	F	2, 3, 4
Imidacloprid	Marathon II 2 F	4A	OHP	50 mL 1000 pots <sup>-1</sup>	D	1, 2, 3
Thiamethoxam	Flagship 25WP	4A	Syngenta	8 oz	F	1, 2
Abamectin	Avid 0.15EC	6	Syngenta	8 fl oz	F	1, 2, 3, 4
Pyriproxyfen	Distance 0.86 IGR	7D	Valent	8 fl oz	F	1, 2, 3
Fonicamid	Aria 50WP	9C	FMC	4 oz	F	1, 2, 4
Pyridaben	Sanmite	21	BASF	6 oz	F	1, 2, 3, 4
Spiromesifen	Judo 4 F	23	OHP	4 fl oz	F	1, 2, 3
Spirotetramat	Kontos 2 F	23	OHP	1.7 fl oz	F	1, 2, 3
Azadirachtin	Azatin XL 4.5	UN	OHP	10 fl oz	F	1, 2
Horticultural oil	Sunspray Ultrafine	NA	R.E. Carroll Inc.	128 fl oz	F	1, 2
Neem oil	Triact 70%	NA	OHP	128 fl oz	F	2, 3, 4
Petroleum oil	Purespray Green	NA	Petro-Canada Lubricants Inc.	128 fl oz	F	4
Insecticidal soap	M-Pede	NA	Dow	128 fl oz	F	4
<i>Paecilomyces fumosoroseus</i>	PFR-97 20%WDG	NA	Natural Industries	28 oz	F	4
<i>Metarhizium anisopliae</i>	Met 52EC	NA	Novozymes	60 fl oz	F	4
<i>Beauveria bassiana</i> Strain GHA	BotaniGard 22WP	M	Laverlam	16 oz	F	4

<sup>a</sup> Insecticide Resistance Action Committee (IRAC) classification.

<sup>b</sup> D = drench; F = foliar; foliar treatments were applied as a full-coverage foliar spray using a compressed air hand-held sprayer at 35–40 psi and using an 8003 nozzle. Mycoinsecticides were mixed 30 min prior to application, with regular agitation to make sure the solution was thoroughly dissolved. Drenches were applied by pouring 100 mL of the solution evenly over the surface of each pot.

polyester/nylon netting, mesh size 48 × 48, with 20 cages 61 × 61 × 61 cm tall and 20 cages 61 × 91 × 61 cm tall. The plants were pinched, and for experiment 3 a plant growth regulator (Cycocel) was applied 3 weeks later to promote a more compact plant. After four plants at the 4–6-primary-leaf stage were moved into cages, 25 adult MED and 25 MEAM1 whiteflies were introduced into each cage twice at approximately a 2 week interval to establish populations. Two weeks later, precounts and cryptic species determination were made prior to the first application of any rotational program. The leaf turn method was employed for adult and nymph population estimates so leaves were not removed from the plant. A population estimate was made by counting the number of immature (post-first-instar) and adult whiteflies on the underside of either two leaves (trials 1 and 2) on each of four plants or two leaves (trials 3 and 4) on each of two plants selected at random. Population counts were made at 7 day intervals after the initial application and continued for approximately 8 weeks after the first application. The length of the trial depended on the condition of the plants in the water control. In addition, approximately ten whitefly adults were collected from each cage after each population evaluation, but before application, if scheduled, for cryptic species ratio determination. In some situations, populations were reduced, so there were not ten adults available for collection. Treatments were arranged in an RCB design and replicated 5 times for each experiment.

An application schedule rotating products among IRAC mode of action groups was established, but repeat application depended on whitefly population levels. If an application was successful the second application was delayed, and if not it could be brought forward. See Table 2 for the actual application schedule.

## 2.8 Statistical analysis

A split-block experimental design was used, with time as the main block and treatments as the subplot. The number of adult and immature whiteflies was subjected to analysis of variance (ANOVA). Data were subjected to square root transformation prior to conducting the ANOVA and mean separation procedure. The data presented are the untransformed means. As main effect of treatment × time was significant, separate ANOVAs were conducted each week. Treatment effects that were significant had means separated by the Ryan–Einot–Gabriel–Welsh multiple range test (REGWQ) at  $\alpha = 0.05$ . As the number of whitefly adults and immatures in the efficacy trial was not uniform, Henderson–Tilton's formula was used to calculate corrected mortality.<sup>48</sup> All statistical tests were conducted using PROC GLM procedures (SAS Institute, Cary, NC).<sup>49</sup>

## 3 RESULTS AND DISCUSSION

### 3.1 Cryptic species composition over time without insecticide selection pressure

The target ratio of MEAM1 to MED was 50:50 at the beginning of each experiment. Twenty five adult whiteflies from MEAM1 and MED whitefly cultures were placed into each treatment twice at 2 week intervals; however, both the sex and age of the whitefly were not known, and consequently target ratios could never be exact. Experiments started approximately 1 month after the first whitefly infestation, which equates to about one whitefly generation<sup>50,51</sup> before the first sample for cryptic species composition was taken (week zero for insecticide applications). MEAM1:MED ratios analyzed using all treatment replications ( $n = 40$ ) from each

**Table 2.** Pesticide application schedule<sup>a</sup>

Rotational program	GH trial	Week 0	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7
1	1, 2	Imidacloprid D			Pyriproxifen		Abamectin	Abamectin	
2	3	Imidacloprid D			Spirotetramet		Pyridaben	Pyridaben	
3	3	Dinotefuran D							
4	1, 2	Thiamethoxam		Spiromesifen			Abamectin	Abamectin	
5	3	Acetamiprid					Abamectin	Abamectin	
6	1, 2	Pyridaben	Pyridaben		Spirotetramet		Acetamiprid		
7	4	Pyridaben	Pyridaben			Oil + Pfr	Oil + Pfr	Oil + Pfr	
8	3	Spiromesifen		Spiromesifen					
9	3	Spirotetramet		Spirotetramet				Acetamiprid	
10	1, 2	Pyriproxifen			Dinotefuran D		Flonicamid	Flonicamid	
11	3	Pyriproxifen			Dinotefuran		Dinotefuran		
12	1	Flonicamid	Flonicamid	Thiamethoxam		Spirotetramet			
13	2	Flonicamid	Flonicamid	Dinotefuran			Spirotetramet		
14	4	Flonicamid	Flonicamid				Dinotefuran		Dinotefuran
15	1, 2	Oil	Oil	Imidacloprid D			Spiromesifen		
16	4	Oil	Oil			Oil	Oil	Oil	
17	1	Azadirachtin	Azadirachtin		Acetamiprid		Abamectin	Abamectin	
18	2	Neem oil	Neem oil		Acetamiprid		Abamectin	Abamectin	
19	3	Neem oil	Neem oil		Acetamiprid		Acetamiprid		
20	4	<i>Paecilomyces fumosoroseus</i>	<i>Paecilomyces fumosoroseus</i>			Acetamiprid		Acetamiprid	
21	4	<i>Metarhizium anisopliae</i>	<i>Metarhizium anisopliae</i>			Insecticidal soap	Insecticidal soap	Insecticidal soap	
22	4	<i>Beauveria bassiana</i> Strain GHA	<i>Beauveria bassiana</i> Strain GHA			Neem oil	Neem oil	Neem oil	
23	4	Abamectin	Abamectin			<i>Metarhizium anisopliae</i>	<i>Metarhizium anisopliae</i>	<i>Metarhizium anisopliae</i>	
24	1, 2, 3, 4	Water check							

<sup>a</sup> Drench applications are specifically listed (D); all other treatments were applied as foliar sprays. Pfr = *Paecilomyces fumosoroseus*.

greenhouse trial and compared between trials for percentage MEAM1 and MED for week zero were significantly different from each other (data not shown) and are accurately represented by the untreated water check for each trial. Initial MEAM1:MED ratios for the untreated water check in week zero for trials 1 to 4 were 54:46, 22:78, 65:35 and 78:22 respectively (Tables 3 to 6). The mean number of adult *B. tabaci* cryptic species MEAM1 and MED in the control treatments of the four greenhouse trials are presented in Fig. 1.

The untreated water control in greenhouse trial 1 remained fairly close to a 50:50 ratio throughout the first experiment, which indicated that there was an even population of MEAM1:MED and neither displaced the other on poinsettia (Table 3; Fig. 1). This is contrary to the theory that, if the two cryptic species were put together and no attempt to control them was made, then MEAM1 would outcompete MED whitefly. MEAM1 is an exceptional competitor and has the capability to displace other whitefly cryptic species of the *B. tabaci* complex,<sup>16,51,52</sup> including MED.<sup>53,54</sup> Developmental times on poinsettia also favor MEAM1 (~21 days)<sup>51</sup> over MED (~33 days)<sup>50</sup> by 12 days or half the generation time for MEAM1 on this host plant. Poinsettia greenhouse trials are limited to the crop production cycle, and consequently there are only 12 weeks from start to finish (planted cuttings to shipment), given that cuttings take 3–4 weeks to root before experiments can begin. At the 50:50 ratio, MEAM1 may have displaced MED in following generations had this experiment continued past 63 days (9 weeks). On cotton and cabbage, MEAM1 displaced MED in five generations, and seven generations when reared on tomato when the

initial populations of the two species were equal and no insecticide was applied.<sup>55</sup> However, in the same study, MED displaced MEAM1 on pepper in two generations, indicating that host plants play an important role in whitefly competition, depending on the relative levels of plant suitability for these two cryptic species.<sup>55</sup>

The greenhouse trial 2 week zero ratio favored MED by 3:1 (MEAM1:MED 22:78); however, MEAM1 percentages continued to increase to 56, 69 and 74% for weeks 1, 2 and 3 respectively (Table 4) before declining to near the initial ratio (27:73) at trial termination. This trial was terminated early owing to plant death caused by heavy whitefly infestations in the water check. Greenhouse trial 3 week zero ratios favored MEAM1 by 2:1 (MEAM1:MED 65:35), and MEAM1 clearly dominated MED for the duration of the 8 week trial, almost completely displacing MED at trial termination (MEAM1:MED 97:3) (Table 5). Greenhouse trial 4 week zero was the inverse of trial 2, with week zero ratio favoring MEAM1 by 3:1 instead of MED (MEAM1:MED 78:22). MED percentage reached a high of 12% in week 5 (Table 6) and was 8% or lower for all other weekly evaluations. Further studies on poinsettia are needed to decipher how population composition is affected by initial infestation ratios of these two cryptic species.

### 3.2 Pesticide performance

In earlier experiments, the efficacy of different insecticides against MED whitefly<sup>56</sup> had been determined and compounds were categorized as tier 1, including the most efficacious compounds,

**Table 3.** Mean number of adult and immature *Bemisia tabaci* on the underside of two leaves per poinsettia plant and the percentage ratio of MEAM1 (B) and MED (Q) whiteflies in greenhouse trial 1. Within a cell, the first number signifies the mean number of adults and the second number shows the mean number of immatures. Henderson–Tilton's corrected percentage mortality is presented in parentheses after each mean<sup>a</sup>

Treatment <sup>b</sup> (week of application)	Week 0	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Week 9
	Sep 8	Sep 16	Sep 23	Sep 30	Oct 7	Oct 14	Oct 21	Oct 28	Nov 4	Nov 11
1 Imidacloprid drench (week 0)	3.3 B 33.1 a	2.1 B (75) 24.1 b (50)	5.7 BC (46) 49.9 b (54)	4.7 BC (63) 12.1 de (87)						
Pyriproxyfen (week 3)					4.1 C (76) 9.3 b (74)	4.0 C (72) 10.2 bc (81)				
Abamectin (weeks 5 and 6)							0.9 CD (94) 18.8 c (89)	0.7 C (96) 12.4 c (95)	1.8 CD (97) 14.7 cd (97)	4.3 C (83) 14.2 b (85)
MEAM1(B):MED(Q)	46:54	64:36	50:50	37:63	41:59	26:74	0:100	0:100	2:98	**
4 Thiamethoxam (week 0)	6.4 AB 31.1 a	3.1 B (81) 55.2 a (0)	12.8 AB (37) 40.4 b (60)							
Spiromesifen (week 2)				7.5 B (70) 26.9 cd (68)	5.9 C (82) 14.5 ab (57)	4.2 C (85) 6.1 c (88)				
Abamectin (weeks 5 and 6)							1.1 CD (96) 11.5 cd (93)	1.3 C (97) 5.7 cd (98)	0.5 D (100) 3.5 d (99)	0.5 D (99) 9.5 b (89)
MEAM1(B):MED(Q)	60:40	65:35	60:40	54:46	34:66	19:81	22:78	8:92	0:100	**
6 Pyridaben (weeks 0 and 1)	4.6 AB 35.1 a	4.9 B (58) 45.9 a (11)	16.1 A (0) 44.1 b (62)	16.6 A (7) 53.9 b (44)						
Spirotetramat (week 3)					14.8 B (37) 25.9 a (32)	10.7 B (47) 22.3 b (61)				
Acetamiprid 30SG (week 5)							4.2 B (81) 44.1 b (76)	5.9 B (79) 33.7 b (88)	19.6 B (78) 28.1 bc (95)	25.4 B (30) 29.3 b (70)
MEAM1(B):MED(Q)	60:40	78:22	56:44	55:45	49:51	40:60	23:77	28:72	23:77	**
10 Pyriproxyfen (week 0)	4.5 AB 23.3 a	1.8 B (84) 49.4 a (0)	5.5 BC (62) 44.6 b (42)	6.6 B (62) 25.0 cd (61)						
Dinotefuran drench (week 3)					2.6 C (89) 21.3 ab (16)	1.5 D (92) 4.4 c (88)				
Fonicamid (weeks 5 and 6)							0.4 D (98) 6.5 d (95)	0.5 C (98) 0.3 d (100)	0.7 D (99) 1.9 d (99)	1.2 CD (97) 1.1 c (98)
MEAM1(B):MED(Q)	60:40	38:62	4:96	2:98	2:98	2:98	0:100	0:100	0:100	**
12 Fonicamid (weeks 0 and 1)	4.0 AB 33.8 a	4.3 B (58) 41.6 ab (16)	8.3 BC (35) 38.3 b (66)							
Thiamethoxam (week 2)				6.8 B (56) 28.7 cd (69)	6.4 C (69) 15.1 ab (59)					
Spirotetramat (week 4)						5.2 C (70) 6.5 c (88)	3.2 BC (83) 27.9 bc (84)	5.3 B (78) 36.9 b (86)	5.3 C (93) 40.4 b (92)	17.9 B (43) 78.4 a (18)
MEAM1(B):MED(Q)	45:55	88:12	69:31	68:32	42:58	38:62	13:87	12:88	23:77	**
15 Oil (week 0)	4.1 AB 27.8 a	0.9 B (91) 35.0 ab (14)	2.1 C (84) 34.1 b (63)							
Imidacloprid drench (week 2)				1.6 C (90) 12.3 e (84)	3.8 C (82) 10.8 b (64)	2.0 CD (89) 3.7 c (92)				
Spiromesifen (week 5)							0.7 D (96) 12.8 cd (92)	0.6 C (98) 6.9 cd (97)	1.9 CD (98) 14.4 cd (97)	3.2 CD (90) 15.9 b (80)
MEAM1(B):MED(Q)	64:36	86:14	16:84	8:92	5:95	5:95	0:100	6:94	0:100	**
17 Azadirachtin (weeks 0 and 1)	6.5 A 24.4 a	2.8 B (83) 49.5 a (0)	5.5 BC (73) 47.3 b (41)	7.6 B (70) 32.5 c (51)						
Acetamiprid 30SG (week 3)					5.8 C (82) 16.9 ab (36)	6.1 C (79) 5.7 c (86)				
Abamectin (weeks 5 and 6)							0.8 D (97) 15.9 cd (87)	2.2 C (94) 11.1 c (94)	1.9 D (98) 9.9 d (97)	4.1 CD (92) 11.5 b (83)
MEAM1(B):MED(Q)	51:49	77:23	46:54	50:50	42:58	19:81	15:85	4:96	0:100	**
24 Water check	5.8 AB 31.8 a	14.7 A 46.6 a	18.5 A 104.5 a	22.6 A 86.9 a	29.5 A 34.5 a	25.5 A 51.3 a	28.1 A 164.6 a	34.9 A 254.0 a	110.6 A 496.5 a	45.8 A 89.9 a
MEAM1(B):MED(Q)	54:46	71:29	53:47	70:30	71:29	46:54	45:55	57:43	55:45	**

<sup>a</sup> Mean values followed by different letters in a column are significantly different (Ryan–Einot–Gabriel–Welsch multiple range test,  $P < 0.05$ ). In each column, different letters (A, B) indicate significant differences between adult means across eight treatments; different letters (a, b) indicate significant differences between immature means across eight treatments. \*\* Whitefly samples were not available for biotyping.

<sup>b</sup> According to the numbering in Table 2.



**Table 4.** Mean number of adult and immature *Bemisia tabaci* on the underside of two leaves per poinsettia plant and the percentage ratio of MEAM1 (B) and MED (Q) whiteflies in greenhouse trial 2. Within a cell, the first number signifies the mean number of adults and the second number shows the mean number of immatures. Henderson–Tilton's percentage corrected mortality is presented in parentheses after each mean<sup>a</sup>

Treatment <sup>b</sup> (week of application)		Week 0 Nov 18	Week 1 Nov 24	Week 2 Dec 2	Week 3 Dec 8	Week 4 Dec 16	Week 5 Dec 22	Week 6 Dec 30
1	Imidacloprid drench (week 0)	2.4 B 29.3 ab	2.5 AB (0) 33.7 a (37)	3.0 ABC (30) 80.0 ab (33)	9.5 A (5) 92.0 a (31)			
	Pyriproxyfen (week 3)					11.8 A (0) 82.3 ab (37)	10.4 B (64) 61.6 ab (53)	
	Abamectin (weeks 5 and 6)							7.3 BC (80) 26.4 b (78)
	MEAM1(B):MED(Q)	34:66	47:53	77:23	59:41	42:58	22:78	0:100
4	Thiamethoxam (week 0)	4.4 AB 27.8 ab	2.0 B (39) 31.6 a (37)	7.9 A (0) 98.3 a (14)				
	Spiromesifen (week 2)				7.5 A (59) 68.3 a (46)	11.6 A (42) 52.5 abcd (58)	17.4 B (67) 109.6 a (12)	
	Abamectin (weeks 5 and 6)							4.0 BCD (94) 11.8 b (90)
	MEAM1(B):MED(Q)	39:61	69:31	89:11	65:35	24:76	12:88	4:96
6	Pyridaben (weeks 0 and 1)	5.5 A 31.4 ab	1.6 B (61) 28.9 a (49)	5.4 AB (45) 92.6 ab (28)	9.8 A (57) 46.8 ab (67)			
	Spirotetramat (week 3)					13.3 A (47) 44.9 bcd (68)	11.6 B (82) 53.4 ab (62)	
	Acetamiprid 30SG (week 5)							12.3 B (86) 20.6 b (84)
	MEAM1(B):MED(Q)	28:72	45:55	73:27	43:57	22:78	19:81	0:100
10	Pyriproxyfen (week 0)	3.2 AB 40.1 a	4.9 A (0) 36.6 a (50)	2.7 ABC (53) 119.1 a (28)	8.0 A (40) 86.2 a (53)			
	Dinotefuran drench (week 3)					14.4 A (1) 77.3 abc (57)	22.3 B (42) 62.8 ab (65)	
	Fonicamid (weeks 5 and 6)							8.8 BC (82) 18.1 b (89)
	MEAM1(B):MED(Q)	32:68	67:33	59:41	34:66	8:92	0:100	0:100
13	Fonicamid (weeks 0 and 1)	4.1 AB 26.7 ab	1.3 B (57) 30.0 a (38)	1.9 BC (74) 102.7 a (6)				
	Dinotefuran (week 2)				1.8 B (90) 71.7 a (41)	8.4 A (55) 73.0 abc (39)	17.2 B (65) 72.0 ab (40)	
	Spirotetramat (week 5)							14.9 B (76) 30.4 b (73)
	MEAM1(B):MED(Q)	24:76	25:75	78:22	87:13	28:72	14:86	10:90
15	Oil (weeks 0 and 1)	2.8 AB 25.4 ab	1.8 B (13) 24.6 a (47)	1.5 C (70) 36.9 b (65)				
	Imidacloprid Drench (week 2)				1.3 B (89) 24.9 b (78)	7.1 A (44) 26.4 d (77)	8.4 B (75) 29.4 b (74)	
	Spiromesifen (week 5)							4.1 CD (91) 19.6 b (81)
	MEAM1(B):MED(Q)	13:87	23:77	63:37	42:58	21:79	14:86	0:100
18	Neem oil (weeks 0 and 1)	4.9 A 29.8 ab	2.2 B (39) 32.2 a (41)	3.1 ABC (64) 81.1 ab (34)	5.5 AB (73) 52.4 ab (61)			
	Acetamiprid 30SG (week 3)					8.1 A (64) 37.1 cd (72)	6.0 B (90) 54.6 ab (59)	
	Abamectin (weeks 5 and 6)							0.8 D (99) 7.0 b (94)
	MEAM1(B):MED(Q)	29:71	80:20	86:14	81:19	15:85	4:96	0:100
24	Water check	2.7 AB 20.8 b	2.0 B 37.8 a	4.8 ABC 85.4 ab	11.3 A 94.7 a	12.3 A 93.3 a	32.5 A 93.7 a	41.7 A 86.7 a
	MEAM1(B):MED(Q)	22:78	56:44	69:31	74:26	43:57	23:77	27:73

<sup>a</sup> Mean values followed by different letters in a column are significantly different (Ryan–Einot–Gabriel–Welsch multiple range test,  $P < 0.05$ ). In each column, different letters (A, B) indicate significant differences between adult means across eight treatments; different letters (a, b) indicate significant differences between immature means across eight treatments. \*\* Whitefly samples were not available for biotyping.

<sup>b</sup> According to the numbering in Table 2.

**Table 5.** Mean number of adult and immature *Bemisia tabaci* on the underside of two leaves per poinsettia plant and the percentage ratio of MEAM1 (B) and MED (Q) whiteflies in greenhouse trial 3. Within a cell, the first number signifies the mean number of adults and the second number shows the mean number of immatures. Henderson–Tilton's percentage corrected mortality is presented in parentheses after each mean<sup>a</sup>

Treatment <sup>b</sup> (week of application)		Week 0 Aug 25	Week 1 Sep 1	Week 2 Sep 8	Week 3 Sep 15	Week 4 Sep 22	Week 5 Sep 29	Week 6 Oct 6	Week 7 Oct 13	Week 8 Oct 20
2	Imidacloprid (week 0)	4.6 A 14.9 a	1.1 ABC (61) 14.9 ab (43)	5.3 A (0) 9.7 bc (75)	11.1 AB (22) 4.7 b (90)					
	Spirotetramat (week 3)					4.0 B (76) 3.9 b (95)	2.0 B (91) 3.9 bc (95)			
	Pyridaben (weeks 5 and 6)							0.9 B (98) 2.5 b (98)	0.6 B (98) 2.3 c (97)	1.2 B (97) 3.0 b (97)
	MEAM1(B):MED(Q)	59:41	59:41	40:60	38:62	33:67	24:76	8:92	0:100	8:92
3	Dinotefuran drench (week 0)	4.8 A 16.4 a	0.3 C (90) 10.9 b (62)	0.5 B (90) 3.7 cd (91)	0.0 D (100) 2.7 b (95)	0.0 C (100) 0.2 b (100)	0.0 B (100) 0.1 c (100)	0.0 B (100) 0.4 b (100)	0.0 B (100) 0.2 c (100)	0.0 B (100) 0.5 b (100)
	MEAM1(B):MED(Q)	64:36	63:37	12:88	33:67	**	**	**	**	**
	Acetamiprid 30SG (week 0)	5.7 A 18.9 a	0.6 BC (83) 19.1 ab (42)	1.8 AB (79) 9.7 bc (81)	4.0 BC (77) 3.6 b (94)	3.6 B (82) 3.2 b (97)	3.0 B (89) 12.8 b (87)			
5	Abamectin (weeks 5 and 6)							1.0 B (98) 3.8 b (97)	2.4 B (95) 13.7 b (85)	3.9 B (92) 8.6 b (93)
	MEAM1(B):MED(Q)	64:36	56:44	42:58	14:86	23:77	13:87	9:91	13:87	8:92
	Spiromesifen (weeks 0 and 2)	6.6 A 12.1 a	1.9 AB (53) 10.1 b (52)	0.6 B (91) 2.1 d (93)	0.0 D (100) 1.5 b (96)	0.1 C (100) 0.1 b (100)	0.2 B (99) 1.0 bc (98)	0.0 B (100) 0.5 b (99)	0.1 B (100) 0.0 c (100)	0.1 B (100) 0.8 b (99)
8	MEAM1(B):MED(Q)	53:47	80:20	75:25	33:67	0:100	**	**	100:0	100:0
	Spirotetramat (weeks 0 and 2)	10.3 A 14.1 a	2.8 AB (56) 11.9 ab (52)	1.5 AB (86) 8.4 bcd (77)	0.9 CD (97) 4.2 b (91)	1.5 BC (96) 1.0 b (99)	1.1 B (98) 1.8 bc (98)			
	Acetamiprid 0.76EC (weeks 5)							1.5 B (98) 0.7 b (99)	0.7 B (99) 1.6 c (98)	0.0 B (100) 0.1 b (100)
9	MEAM1(B):MED(Q)	79:21	92:8	70:30	66:34	70:30	87:13	72:28	0:100	50:50
	Pyriproxyfen (week 0)	7.1 A 20.4 a	2.9 AB (34) 24.9 a (31)	11.9 A (0) 16.3 b (70)	17.1 A (22) 6.8 b (89)					
	Dinotefuran (weeks 3 and 5)					1.3 BC (95) 2.0 b (98)	1.2 B (96) 4.7 bc (96)	0.4 B (99) 3.1 b (98)	1.6 B (97) 1.9 c (98)	1.8 B (97) 7.5 b (94)
11	MEAM1(B):MED(Q)	73:27	72:28	29:71	8:92	2:98	5:95	0:100	33:67	0:100
	Neem oil (weeks 0 and 1)	4.1 A 18.1 a	1.1 ABC (56) 12.0 ab (62)	2.1 AB (50) 7.3 bcd (85)	8.0 AB (37) 4.3 b (92)					
	Acetamiprid 0.76EC (weeks 3 and 5)					1.3 BC (91) 1.6 b (98)	0.8 B (96) 0.9 bc (99)	0.1 B (100) 0.6 b (100)	0.3 B (99) 1.1 c (99)	0.7 B (98) 3.0 b (97)
19	MEAM1(B):MED(Q)	73:27	73:27	53:47	51:49	43:57	30:70	13:87	0:100	0:100
	Water check	5.2 A 11.9 a	3.2 A 20.9 ab	5.3 AB 31.4 a	16.1 A 37.6 a	18.5 A 64.6 a	24.2 A 62.5 a	50.0 A 81.5 a	43.9 A 58.5 a	45.1 A 76.0 a
	MEAM1(B):MED(Q)	65:35	86:14	75:25	80:20	79:21	66:34	91:9	71:29	97:3

<sup>a</sup> Mean values followed by different letters in a column are significantly different (Ryan–Einot–Gabriel–Welsch multiple range test,  $P < 0.05$ ). In each column, different letters (A, B) indicate significant differences between adult means across eight treatments; different letters (a, b) indicate significant differences between immature means across eight treatments. \*\* Whitefly samples were not available for biotyping.

<sup>b</sup> According to the numbering in Table 2.

and tier 2, the compounds that reduce whitefly populations but not quite as successfully. The first (Table 3) and second (Table 4) experiments started with tier 2 compounds (imidacloprid, thiamethoxam, pyridaben, pyriproxyfen, flonicamid, oil and azadirachtin or hydrophobic extract of neem oil), followed in the rotation by tier 1 compounds (spirotetramat, dinotefuran, acetamiprid and spiromesifen). This allowed the possibility of having at least a few whiteflies left in the second half of the experiment to evaluate the impact of the insecticide regimes on the ratio of the two cryptic species (Fig. 2). Tier 1 compounds were used in either

the second or third application in the rotation, but not both. In experiment 3 (Table 5), this order was reversed and started with the tier 1 compounds, with the exception of the standard (imidacloprid), pyriproxyfen and neem oil rotations, which were followed in the rotation by a tier 1 compound. Consequently, treatments did not have many adult whiteflies left to sample and determine the ratio of the two cryptic species. In the fourth experiment (Table 6), different environmentally friendly biological or mycoinsecticides were applied. These compounds are usually slow acting and target sensitive, and often just suppress the population. In this final trial,

**Table 6.** Mean number of adult and immature *Bemisia tabaci* on the underside of two leaves per poinsettia plant and the percentage ratio of MEAM1 (B) and MED (Q) whiteflies in greenhouse trial 4. Within a cell, the first number signifies the mean number of adults and the second number shows the mean number of immatures. Henderson–Tilton’s corrected percentage mortality is presented in parentheses after each mean<sup>a</sup>

Treatment <sup>b</sup> (week of application)	Week 0	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Week 9
	Dec 8	Dec 15	Dec 22	Dec 29	Jan 4	Jan 12	Jan 19	Jan 26	Feb 2	Feb 9
7 Pyridaben (weeks 0 and 1)	7.7 A 21.5 ab	2.5 A (55) 23.8 a (44)	0.6 C (88) 14.8 b (75)	0.3 B (91) 69.2 a (21)	1.4 B (86) 32.0 ab (68)					
Oil + <i>Paecilomyces fumosoroseus</i> (weeks 4, 5 and 6)						4.2 B (71) 47.1 b (68)	1.5 D (93) 21.0 bc (80)	0.1 D (100) 8.0 d (94)	1.4 D (96) 8.7 d (94)	0.1 C (100) 7.4 c (93)
MEAM1(B):MED(Q)	82:18	76:24	100:0	89:11	100:0	96:4	85:15	100:0	67:33	**
14 Fonicamid (weeks 0 and 1)	13.5 A 16.9 ab	5.0 A (49) 29.4 a (12)	0.6 C (93) 33.0 ab (30)	1.9 AB (67) 78.3 a (0)	7.8 AB (56) 61.9 a (21)	23.0 A (8) 152.6 ab (0)				
Dinotefuran (weeks 5 and 7)							6.8 BCD (83) 37.2 abc (55)	6.5C (86) 38.4 bc (66)	3.8 CD (94) 26.8 bc (75)	4.3 C (93) 20.0 bc (74)
MEAM1(B):MED(Q)	73:27	82:18	81:19	100:0	97:3	97:3	94:6	88:12	100:0	**
16 Oil (weeks 0, 1, 4, 5 and 6)	17.9 A 35.0 a	3.4 A (74) 53.5 a (22)	0.5 C (96) 22.4 ab (77)	0.5 B (93) 51.7 a (64)	0.9 B (96) 31.2 ab (81)	3.7 B (89) 61.8 ab (74)	3.2 CD (94) 24.9 bc (86)	3.7 CD (94) 12.1 d (95)	3.8 CD (95) 14.8 cd (93)	3.9 C (95) 11.3 bc (93)
MEAM1(B):MED(Q)	90:10	84:16	100:0	100:0	100:0	71:29	60:40	87:13	81:19	**
20 <i>Paecilomyces fumosoroseus</i> (weeks 0 and 1)	14.1 A 16.1 ab	3.9 A (62) 33.8 a (0)	2.5 ABC (73) 23.8 ab (47)	2.3 AB (62) 100.6 a (0)	9.1 AB (51) 50.2 ab (33)					
Acetamiprid 30SG (weeks 4 and 6)						8.3 AB (68) 133.4 ab (0)	13.6 BC (68) 54.7 ab (31)	8.8 BC (82) 43.8 bc (60)	18.3 BC (71) 30.1 bc (71)	8.4 BC (87) 28.4 b (62)
MEAM1(B):MED(Q)	68:32	87:13	80:20	98:2	97:3	84:16	82:18	63:37	58:42	**
21 <i>Metarhizium anisopliae</i> (weeks 0 and 1)	11.1 A 22.3 ab	4.7 A (42) 42.0 a (4)	6.8 AB (8) 20.5 ab (67)	6.6 A (0) 41.9 a (54)	6.4 AB (56) 21.8 b (79)					
Insecticidal soap (weeks 4, 5 and 6)						2.7 B (87) 43.9 b (71)	6.1 CD (82) 20.3 c (81)	2.2 CD (94) 18.1 cd (88)	2.3 CD (95) 24.4 cd (83)	1.2 C (98) 11.9 bc (88)
MEAM1(B):MED(Q)	77:23	87:13	93:7	97:3	100:0	98:2	83:17	50:50	81:19	**
22 <i>Beauveria bassiana</i> Strain GHA (weeks 0 and 1)	10.5 A 21.1 ab	9.0 A (0) 29.2 a (30)	3.3 ABC (53) 33.8 ab (42)	4.3 AB (4) 48.6 a (43)	11.4 A (18) 62.1 a (37)					
Neem oil (weeks 4, 5 and 6)						26.0 A (0) 163.0 a (0)	18.4 AB (41) 60.9 a (41)	20.3 AB (44) 51.6 ab (64)	28.5 AB (39) 51.2 b (62)	22.1 B (56) 26.2 b (73)
MEAM1(B):MED(Q)	90:10	94:6	84:16	100:0	93:7	98:2	83:17	89:11	70:30	**
23 Abamectin (weeks 0 and 1)	15.0 A 10.4 b	3.9 A (64) 34.8 a (0)	2.5 BC (75) 27.8 ab (4)	2.1 AB (67) 66.8 a (0)	8.7 AB (56) 60.1 a (0)					
<i>Metarhizium anisopliae</i> (weeks 4, 5 and 6)						8.8 AB (68) 91.5 ab (0)	8.4 BCD (81) 42.4 abc (17)	5.3 CD (90) 29.1 bcd (58)	11.0 CD (83) 18.9 cd (71)	8.3 BC (88) 24.9 bc (48)
MEAM1(B):MED(Q)	83:17	95:5	100:0	100:0	98:2	69:31	87:13	92:8	71:29	**
24 Water check	11.3 AB 15.0 a	8.2 A 29.5 a	7.5 A 41.6 a	4.8 A 60.9 a	14.9 A 69.9 a	20.9 A 103.0 a	33.8 A 73.6 a	39.3 A 101.1 a	50.2 A 95.3 a	53.7 A 69.5 a
MEAM1(B):MED(Q)	78:22	92:8	93:7	97:3	99:1	92:8	93:7	88:12	92:8	**

<sup>a</sup> Mean values followed by different letters in a column are significantly different (Ryan–Einot–Gabriel–Welsch multiple range test,  $P < 0.05$ ). In each column, different letters (A, B) indicate significant differences between adult means across eight treatments; different letters (a, b) indicate significant differences between immature means across eight treatments. \*\* Whitefly samples were not available for biotyping.

<sup>b</sup> According to the numbering in Table 2.

the biological insecticides were the least effective against *Bemisia*; however, much of this reduced activity was a result of the population of whiteflies being too great when this experiment was initiated for mycoinsecticides to be effective. These compounds are most successful as early curative insecticides, applied when population levels are low.

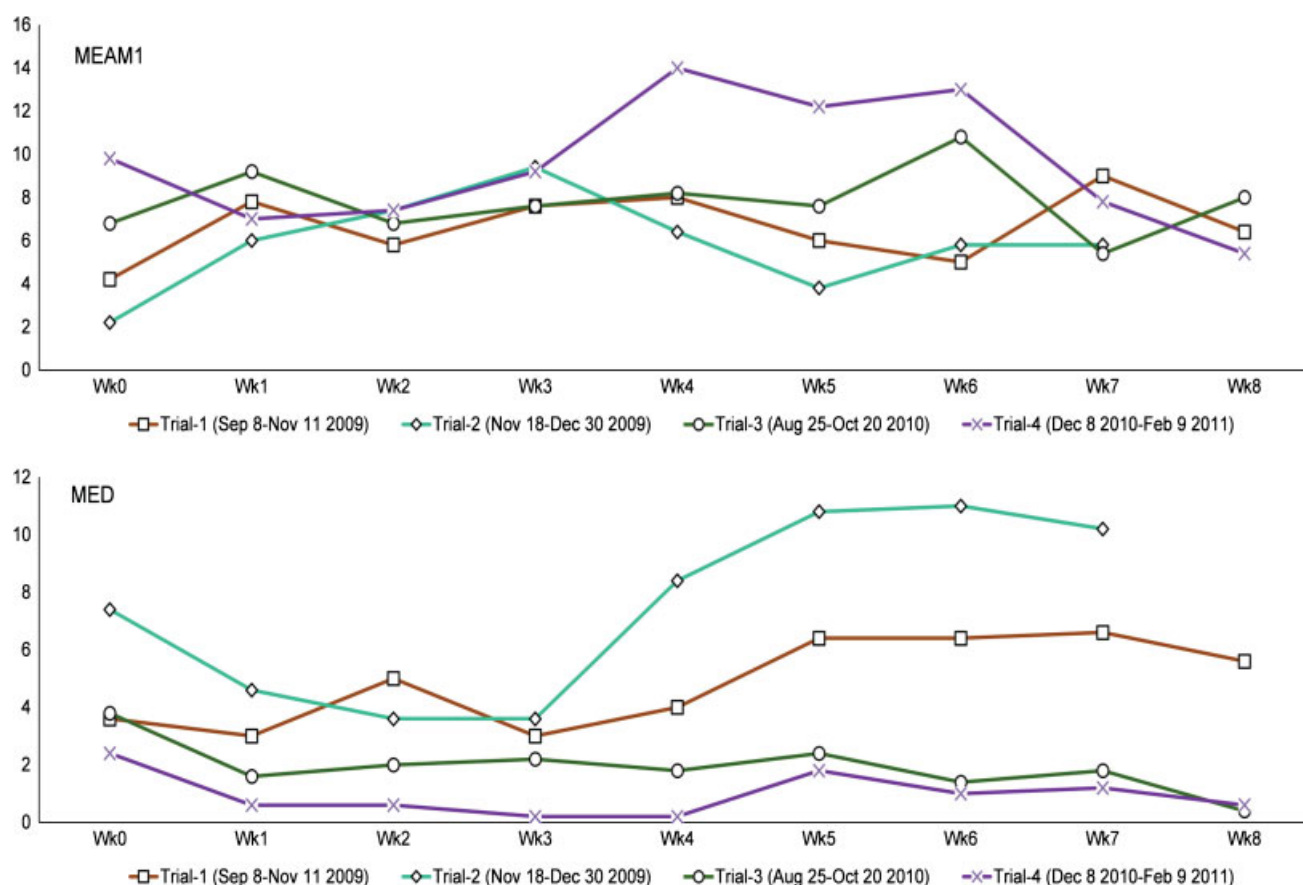
In the following results, each insecticide that was included in the trials will be evaluated in terms of how it performed in the situation in which it was applied. Did it make a difference whether it was a first-in or a follow-up compound in the rotation? What was a compound’s activity against each cryptic species, and how did that change the population composition? Most were

efficacious, and many had different activity against the different cryptic species.

### 3.2.1 Imidacloprid (*Marathon*)

The first commercially available insecticide from the neonicotinoid class was imidacloprid, and not surprisingly MED resistance was detected in Spain shortly after its debut in the mid-1990s<sup>57</sup> and has since been reported in other parts of the world.<sup>26,39,43–45</sup> Imidacloprid was the standard used in the first three experiments because it had been the answer to the resistance problem encountered with the older insecticides when MEAM1 was introduced into





**Figure 1.** Mean number of adult *Bemisia tabaci* cryptic species MEAM1 (B) and MED (Q) in the untreated water checks in four poinsettia greenhouse trials.

the United States. When imidacloprid came on the market in the early to mid-1990s, growers switched to this insecticide, and excellent control of MEAM1 was achieved.<sup>58</sup> At the present time, almost two decades later, imidacloprid still has some activity against MEAM1, but many populations express reduced susceptibility.<sup>59</sup> In North America, reduced susceptibility and resistance have been reported in Florida,<sup>60</sup> Arizona and California.<sup>59,61</sup> In the first three experiments (Tables 3 to 5), it was observed that imidacloprid did not have very good activity on immatures (average control across trials 61%) or adults (average control across trials 34%). The activity that it did have was against MEAM1, because the ratio switched to a MED-dominated population by the second insecticide application (Fig. 2; Tables 3 to 5). This compound is only useful in managing susceptible MEAM1 whitefly populations.

### 3.2.2 Thiamethoxam (Flagship)

Thiamethoxam is a neonicotinoid precursor converted to clothianidin in insects and plants,<sup>62</sup> and resistance in whitefly has been reported.<sup>19,39,45</sup> This product was used as the first step in the rotation in experiments 1 and 2 (Tables 3 and 4), and in neither situation were satisfactory results obtained. In experiment 1, a significant reduction in immatures compared with the water check was obtained, but there was no significant reduction in adults (Table 3). In experiment 2 there was no reduction in either adults or immatures (Table 4), and in fact there was a higher population of adults and immatures than in the water check. In both experiments, thiamethoxam was followed by an application of spiromesifen (Judo) in 2 weeks, so it could be that thiamethoxam still had

activity against the population as a residual along with spiromesifen. There was a reduction in immatures following the spiromesifen application (Tables 3 and 4), but not enough for growers to be satisfied with this rotation. In addition, there was no reduction in MEAM1 (Fig. 2), indicating that MEAM1 is resistant, or at least has reduced susceptibility, to thiamethoxam. When spiromesifen was applied, a change from a MEAM1-dominated population to a MED-dominated population was observed.

### 3.2.3 Pyridaben (Sanmite)

Pyridaben is both an acaricide and an insecticide, with some activity against *Bemisia* that belongs to the mitochondrial electron transport inhibitor pyridazine group.<sup>63</sup> It has not been a primary insecticide used against whiteflies, but has been a part of many rotations. In earlier experiments, mixed results have been obtained with this compound.<sup>56</sup> However, it has shown promise against the MED. Pyridaben was included as a tier 2 insecticide and applied as a first insecticide in three experiments (Tables 3, 4 and 6). Two applications were made at a 7 day interval. In each situation, there was an increase in the population of immatures until the third or fourth week, and then a significant drop in numbers. In one experiment there was a drop in adults in the second week (Table 6), but in the others the population stayed the same (Tables 3 and 4). Pyridaben did not prove to be a reliable compound for the rotation, but it did suppress the population and could be included in a rotation once the population was knocked down and a grower wanted to maintain control.

There did not appear to be any selectivity for cryptic species with pyridaben (Fig. 2; Tables 3, 4 and 6). There was some change in the

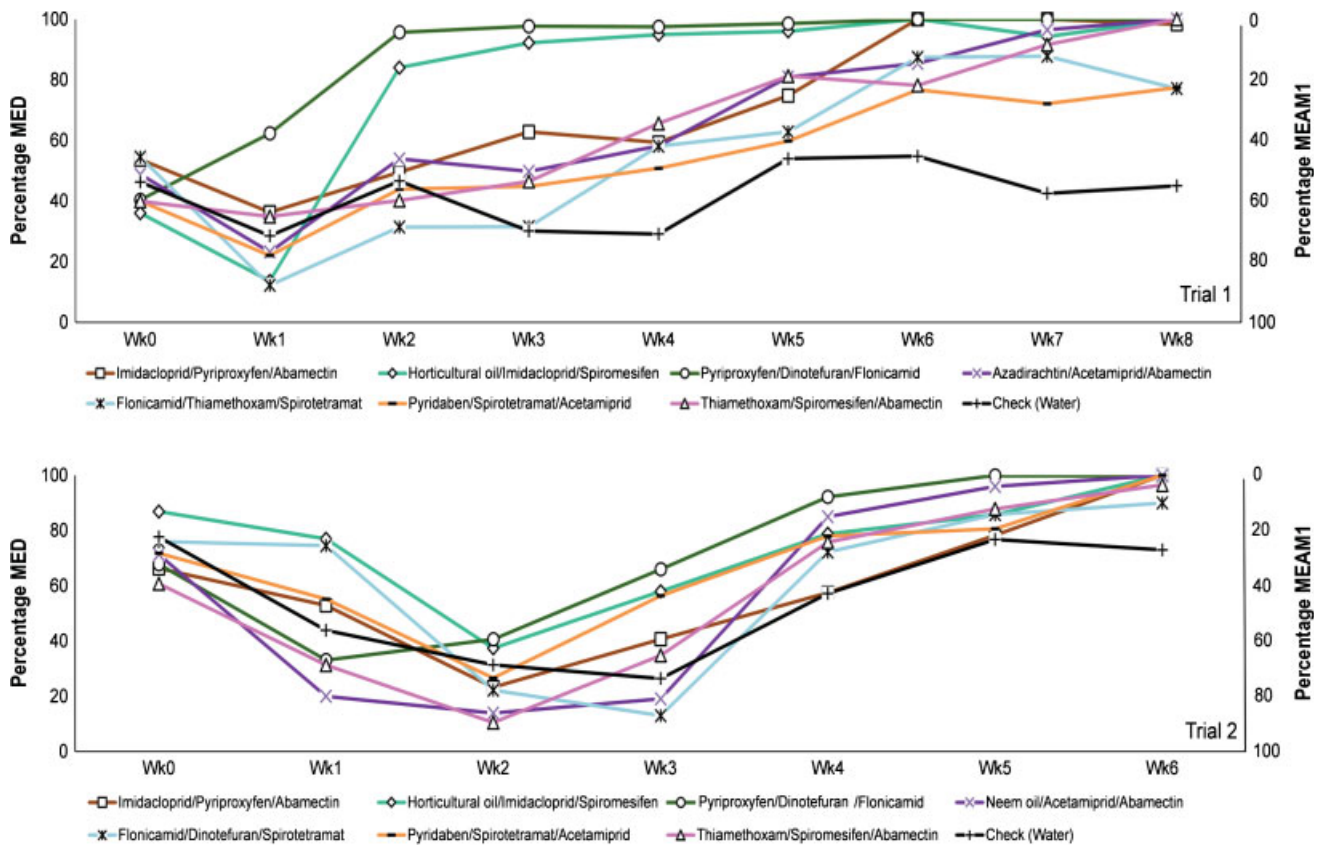


Figure 2. Percentage of adult *Bemisia tabaci* cryptic species MEAM1 (B) and MED (Q) in greenhouse trials 1 and 2.

ratio of MEAM1 to MED, but it was not consistent, nor did it appear to favor either cryptic species.

3.2.4 Pyriproxyfen (Distance)

Pyriproxyfen is a powerful insect growth regulator (IGR) that mimics a juvenile growth hormone that suppresses embryogenesis and adult emergence.<sup>64</sup> This IGR was used as a measure of MED resistance. Pyriproxyfen is still very effective against MEAM1 in the United States, and it is a compound that is often used in rotations by commercial greenhouses. However, with the introduction of the MED whitefly it is no longer as promising, as this cryptic species is supposedly resistant to pyriproxyfen.<sup>18,26,39</sup> In the first two experiments, imidacloprid was followed by pyriproxyfen (Tables 3 and 4); this was a very common practice among greenhouse growers. It was not a successful management practice with the present population. Satisfactory reduction was not achieved, and the ratio of cryptic species continued to move towards 100% MED (Fig. 2). Pyriproxyfen was not effective against MED, but it appeared to eliminate MEAM1. Pyriproxyfen was used as a first application treatment in experiment 3, and population reduction was slow (Table 5). As an IGR, these results were not unexpected. The population was reduced before rotating to another compound, but it took 3 weeks. There was also a change in cryptic species ratio from over 70% MEAM1 to over 70% MED (Table 5), demonstrating the selectivity of this chemistry.

3.2.5 Flonicamid (Aria)

Flonicamid is another insecticide where mixed results in efficacy against *Bemisia* have been observed.<sup>56</sup> This compound belongs

to the pyridine carboxamide group and is systemic, with selective activity against hemipterans, including whiteflies and aphids, by rapidly inhibiting feeding.<sup>65</sup> In these experiments, a significant reduction in adult but not in immature whiteflies was observed (Tables 3, 4 and 6). The present results indicate that there was only efficacy for adults. This compound could still be used in a rotation, on the understanding that it is primarily an adulticide. It is interesting that it did appear to be more efficacious against MED; there were more MEAM1 adults in the samples following the flonicamid applications (Fig. 2; Tables 3, 4 and 6).

3.2.6 Abamectin (Avid)

Abamectin, a broad-spectrum pesticide with translaminar ability, is a mixture of avermectins B<sub>1a</sub> and B<sub>1b</sub>, a group of macrocyclic lactones isolated from fermentation of the soil actinomycete microorganism *Streptomyces avermitilis* Burg.<sup>66</sup> Abamectin appeared to be more active against MED than MEAM1 in earlier trials.<sup>56</sup> In these experiments, abamectin was favored as a final compound in the rotation because it is a very safe insecticide that does not cause any phytotoxicity when applied to poinsettias in full bract color. Therefore, it is an excellent choice for the final application prior to shipment. In experiment 1 it was applied twice, but only once in experiment 2 because it was close to the end of the trial. In experiment 3 it was applied twice following an acetamiprid (TriStar) treatment. In each situation, reduction in immatures and adults was observed (Tables 3 to 5), but there was an indication of recovery of the population when applied 3 weeks before sampling. Long residual is not expected with abamectin, and this could be an indication of reduction in residual activity.<sup>66</sup> In experiment 4, abamectin was applied as the first insecticide in the rotation, and the number of adults

was reduced but the immature population increased (Table 6). Obviously, if adults were reduced, a reduction in the immature population later in the next generation would be noticed. However, the initial application of abamectin was followed by an entomopathogen Met52, and the population of immatures increased in the first week and then declined in the following weeks. Slow activity would be expected from an entomopathogen, so this could be the case in this experiment. The population never did drop to an acceptable level in experiment 4 (Table 6).

In all experiments with abamectin as the final insecticide in the rotation there was a change to a ratio of 100% or near 100% MED (Fig. 2; Tables 3 to 6). It was extremely difficult to determine whether this was the result of earlier applications or of abamectin. In most situations the change to MED was already present before the abamectin application. In experiment 4, where abamectin was the first in the rotation, the ratio started favoring MEAM1 (Table 6) and continued to be dominated by MEAM1 until the end of the trial. This would agree with earlier findings that abamectin was more effective against MED.

### 3.2.7 Dinotefuran (*Safari*)

Dinotefuran is a neonicotinoid used by growers to manage populations of whiteflies exhibiting resistance to imidacloprid,<sup>67</sup> is especially effective against MED populations<sup>56</sup> and is considered here as a tier 1 compound. It is also very efficacious against MEAM1 on poinsettia.<sup>67</sup> Earlier trials indicate that dinotefuran is most efficacious as a drench against both cryptic species.<sup>56,67</sup> In the first two experiments it was applied as a drench following the pyriproxyfen (*Distance*) treatment and as a foliar spray following flonicamid (*Aria*). When dinotefuran was applied as a drench, excellent control was obtained within 4 weeks in experiment 1 (Table 3), but the control was not as good in experiment 2 (Table 4). In experiment 2 the application was followed up by flonicamid (*Aria*), and there was good knockdown the following week, but it is hard to tell whether it was the dinotefuran or the flonicamid that resulted in the reduction. Most probably it was both. The single foliar spray was only applied in experiment 2 (Table 4), and satisfactory reduction was not achieved. Usually, two applications of dinotefuran are needed to be effective as a foliar spray. The foliar spray was followed by spirotetramat (*Kontos*), with good reduction the next week. In both of these experiments the population of *Bemisia* changed to 100% MED (Fig. 2), indicating that MEAM1 is the most susceptible cryptic species to these rotations.

In experiment 3, dinotefuran drench was used as a first application in the rotation; excellent results were obtained, and no follow-up application was required (Table 5). Here again, the ratio of MEAM1 to MED changed from 64% MEAM1 to 88% MED and a final 67% before all adults were killed. A foliar spray of dinotefuran was used to follow pyriproxyfen, and this time two applications 14 days apart were used. In this case, very good control was achieved following significant reduction with pyriproxyfen (primarily MEAM1), and again the population changed from predominantly MEAM1 to MED (Table 5). Dinotefuran is an excellent insecticide for both cryptic species, but most efficacious as a drench.<sup>56,67</sup>

### 3.2.8 Acetamiprid (*TriStar*)

Acetamiprid is a tier 1 neonicotinoid that has been efficacious against both cryptic species.<sup>19,39,43,60,61</sup> It was used as a follow-up to azadirachtin (*Azatin*) and then neem oil (*Triact*) in the first three experiments, as the third treatment following spirotetramat (*Kontos*) in experiments 1 and 2 and as the second treatment after

spirotetramat in the third experiment (Table 2). In all of these treatments, good reduction in whitefly populations was achieved (Tables 3 to 5), but with a rotation it is often hard to key in on what was the most important component of the rotation. It definitely was effective when used behind spirotetramat, and two applications were applied 14 days apart. In all other trials it was only applied once before rotating to another treatment. It has to be one of the best foliar treatments used in the experiments. In experiment 4 it was applied twice following an extreme build-up of whiteflies, and acetamiprid still reduced the population tremendously (Table 6). However, it was not at an adequate level at the end of the trial. Once a population has reached a very high level, it is nearly impossible completely to eliminate this pest.

In all situations the rotation containing acetamiprid eliminated MEAM1, and the few whiteflies left were 100% or nearly all MED (Fig. 2; Tables 3 to 6). Thus, this neonicotinoid is another insecticide where MED is more tolerant to available insecticides than MEAM1. Most of the MED were also eliminated, so the numbers represent only a few remaining adults, or the application was late in the rotation and there was insufficient time to obtain the final mortality of adults.

### 3.2.9 Spiromesifen (*Judo*)

Spiromesifen and spirotetramat (*Kontos*) belong to the new IRAC 23 Ketoenol group which includes both derivatives of tetroneic acids (spiromesifen) and tetramic acids (spirotetramat). Both were used as tier 1 compounds in these experiments. Spiromesifen is reported to act effectively on the egg and early nymphal stages of *B. tabaci* (both MEAM1 and MED), but adults and late nymphal instars are reported to be only moderately controlled.<sup>68,69</sup> Spiromesifen was applied as the second or third compound in the rotation in experiments 1 and 2, and as the initial compound in experiment 3. In experiment 1, efficacy was not great against immature whiteflies – there was reduction but not elimination of nymphs (Table 3). Although spiromesifen is not considered to be an adulticide, there was significant reduction in adults in both treatments (Tables 3 and 4). In experiment 2, the application was late in the experiment, and reduction in adults and immatures was observed 1 week after application (Table 4), but the treatment could not be followed long enough to obtain a good result from the application owing to experiment termination. In experiment 3, spiromesifen was the first compound applied in the rotation and the only one applied because excellent control of adults was achieved in 2 weeks and of immatures by week 4 (Table 5).

In all experiments where spiromesifen was applied third, the ratio of the biotypes was at or near 100% MED (Fig. 2; Tables 3 to 5). However, as it was the third compound applied, it is unclear whether it was spiromesifen or a previous insecticide that changed the cryptic species ratio. Having said that, in experiment 3, spiromesifen was the first in and the only insecticide in the treatment, and the ratio was changed from near 50:50 to 100% MED by the fourth week (Table 5). There were no adults in weeks 5 and 6, but on weeks 7 and 8 the ratio changed to 100% MEAM1. Again, it is unclear whether this was a result of only one or two specimens collected on these weeks, as the average number of adults sampled was 0.1 per leaf (Table 5).

### 3.2.10 Spirotetramat (*Kontos*)

Spirotetramat is the newest insecticide on the market and can be applied as a soil drench, but in these trials it was applied as a foliar spray. This compound is principally effective on immature stages



but also significantly reduces fecundity and fertility of *B. tabaci*.<sup>70</sup> Spirotetramat is a true systemic, possessing full ambimobility or two-way systemicity (phloem and xylem transport) that can penetrate through the leaf cuticle and translocate up to growing shoots and down to roots, thereby protecting all areas of the plant.<sup>70</sup> Spirotetramat was applied as the second compound in the rotation in experiments 1 and 2 and as the third application in experiment 3 (Table 2). Each time it was followed by acetamiprid (TriStar), when applied second, only 2 weeks after application. In one situation there was no reduction (Table 3), and in the other a 50% reduction (Table 4) was observed. In both situations it was significantly less than the check. However, 2 weeks is a short period of time to obtain a good evaluation. When it was applied third there was no reduction for the 5 weeks following the application, and there was a significant increase on the last week. Once again, the treatment was much less than the water check. In experiment 3, spirotetramat was applied as the initial treatment and repeated 2 weeks later, resulting in a significant reduction in both immatures and adults (Table 5), and good control was achieved. The treatment was followed by acetamiprid and the result was excellent control.

Spirotetramat is a new insecticide with no cross-resistance to any other insecticide<sup>70</sup> reported. It is doubtful whether resistance has had time to develop in either cryptic species, and there was no indication that one cryptic species was suppressed by the spirotetramat treatment (Fig. 2). In the trial where it was the first treatment there was no change in cryptic species composition, but when it was followed by acetamiprid there was a noticeable change to a MED-dominated population (Table 5).

### 3.2.11 Oil (plant or petroleum)

Oils represent a completely different mode of action than the other insecticides used in these experiments. Oils physically kill by suffocating pests, regardless of whether they are plant or petroleum derived. Therefore, it is very important that complete coverage is achieved because the oil must actually get on the insect for it to be effective. Because of this physical activity, selectivity towards one cryptic species over another was not expected, and no clear favoritism could be detected. Oil is much more effective early in a rotation, when the population is low, plants are small and the plant canopy is open, so better coverage can be achieved. Oil was included in three experiments, either plant-derived or petroleum oils, and in almost every situation suppression of the population was observed (Tables 3 to 6), but without any dramatic reduction. In the first experiment (Table 3), oil significantly reduced immature populations by the second week after application and provided significant reduction for adult populations by the first week. In the second trial (Table 4), immature control averaged 56% and adult control was 42%. In experiment 4 (Table 6), oil was applied alone in five repeat applications within 7 weeks with no other insecticide (Table 6). Average seasonal adult control was 92%, and average immature control was 76%; however, there was phytotoxicity following this oil-alone treatment. Oil was also combined with a mycoinsecticide (Pfr-97), and control of both whitefly stages was slightly better, although not significantly better than with oil by itself; however, phytotoxicity was not as severe. Based on the present observations, oil will either have to be repeated early in the rotation or rotated with another insecticide to achieve good control and avoid phytotoxicity to poinsettias.

### 3.2.12 Insecticidal soap (M-Pede)

Insecticidal soaps are contact poisons derived from potassium salts that are totally degradable, environmentally friendly and

considered to be a staple in organic pest control. Insecticidal soap was used in the fourth experiment following the application of an entomopathogen (*Metarhizium anisopliae*; Met-52) and was applied for three consecutive weeks (Table 6). The average mean percentage mortality over 5 weeks for immatures was 82% and for adults 91%. Cryptic species composition did not appear to be affected by this treatment, and the ratio reverted to 50:50 MEAM1:MED for one of the evaluations during that 5 week period.

### 3.2.13 Azadirachtin or neem oil (Azatin or Triact)

Azadirachtin is a steroid-like tetranortriterpenoid derived from neem trees, whereas Triact is a clarified hydrophobic extract of neem oil, and both are considered to be effective against *B. tabaci* by preventing molting and reducing growth, development and oviposition in adults.<sup>71</sup> Azadirachtin was used in the first trial and neem oil was used in the remaining three greenhouse trials (Table 2). When either compound was used first in the rotation and sprayed twice (Tables 3 to 5), performance was moderate with an average mean percentage mortality ranging from 48 to 75 for immature whitefly and 31 to 80 for adult whitefly. Performance was better when neem oil was used in the rotation after an entomopathogen and sprayed 3 times, with an average mean percentage mortality for immatures of 82 and for adults of 91 (Table 6). Increased performance could be due to the extra application.

Cryptic species composition remained close to 50:50 (Table 3), favored MEAM1 (Table 4) or moved towards equilibrium (Tables 5 and 6), indicating that these compounds may not select for one cryptic species over another on poinsettia, as no clear favorite could be discerned.

### 3.2.14 Mycoinsecticides: *Paecilomyces fumosoroseus* (Pfr-97), *Metarhizium anisopliae* (Met-52) and *Beauveria bassiana* Strain GHA (Botanigard)

Neonicotinoids are at great risk of being removed from the market place in the United States owing to concerns over toxicity to pollinators, which has sparked a renewed interest in mycoinsecticide use in pest management programs, especially in the EU where the use of this class of insecticides has already been banned.<sup>10,72</sup> In greenhouse trial 4, three entomopathogenic fungi were evaluated against a population of whitefly that favored MEAM1 over MED by 3:1. Cryptic species composition pushed towards MEAM1 in all treatments, but this was probably due to the initial ratio favoring MEAM1 at the beginning of this trial and not due to the individual treatment. Each was sprayed twice back to back as the first treatment of their respective treatment regimes and followed for 4 weeks before the next treatment in the rotation was applied. *Paecilomyces fumosoroseus* (Pfr-97) performed best for adult control (62% average mean mortality) and *Metarhizium anisopliae* (Met-52) performed best for immature control (51% average mean mortality), but all three performed poorly when applied alone (Table 6). *Beauveria bassiana* Strain GHA (Botanigard) provided an average mean mortality of 19% for adult whitefly and 38% for immatures. *M. anisopliae* was also used as the second application following abamectin, and performance was better for adult control (82% average mean mortality) but remained the same for immature control. The efficacy of *P. fumosoroseus* increased when oil was combined for three consecutive sprays, resulting in an average mean mortality of immature and adult whitefly of 86 and 92% respectively.

## 4 CONCLUSION

Best management practices<sup>22,23</sup> for controlling whitefly cryptic species MEAM1 and MED infesting poinsettia were evaluated in the greenhouse. The objectives of these experiments were to determine the efficacy of different insecticide treatment regimes rotating different insecticide modes of action and how that selective efficacy for either MEAM1 or MED whitefly affected the population composition and effective management over time. Dinotefuran, spiromesifen and acetamiprid were the only three treatments (Table 5) that completely eliminated the adult or immature whiteflies during at least one evaluation in their rotation, but all treatments significantly reduced the whitefly populations. In the first two experiments, all rotations favored MED, with five of the seven rotation regimes producing ~100% MED by the last evaluation. The treatment regimes that did not completely favor MED included one of the two newer insecticides (spiromesifen or spirotetramat) in the rotation. The absence of cross-resistance between these two compounds and other major commonly used insecticides from different chemical classes, such as neonicotinoids and pyriproxyfen, suggests the possible use of these compounds in insecticide resistance management programs.<sup>68–70</sup>

In trial 3, rotations with compounds that were the most efficacious were used first; thus, rotations were not always necessary because *Bemisia* was eliminated early. There was a shift to the MED whitefly that was similar to that seen in trials 1 and 2, and with most of the insecticides this is the most resistant species. Most of the insecticides were efficacious against MEAM1 whitefly; however, there were indications of reduced susceptibility with a few of the compounds. The biorational pesticides, in the last experiment, were not as efficacious. Most of the insecticides have some reduced susceptibility against MED whitefly, and rotations are absolutely necessary to prolong the inevitable development of resistance. Rotations can be used to manage both cryptic species and maintain a very low population level or completely eliminate *Bemisia* on poinsettia. In these trials, it is obvious that resistance problems with the MED whitefly already exist. The newer insecticides, such as spirotetramat, to which the MED whitefly had not been exposed, and the biorational compounds were equally effective against both cryptic species. Dinotefuran applied as a drench was the most efficacious insecticide against MED whitefly in these experiments.

Rotating to different modes of action reduces the rate of resistance development, and the importance of using a rotation of different modes of action in pest management programs must continue to be emphasized.

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