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Development of a bioassay system for monitoring susceptibility in *Thrips tabaci*

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Abstract: A system is described for collecting adult and larva of *Thrips tabaci* from onion foliage into insecticide-treated vials to evaluate susceptibility to insecticides. The thrips insecticide bioassay system (TIBS) allows one to treat vials and store them for 3 weeks before thrips are collected. Depending on the population density in the field, collection of the insects for the test required from 3–6 h for one person. Assays are read after 24 h. This system was used in 1997 and 1998 in commercial onion fields in Honduras and Nicaragua, and TIBS was sensitive enough to detect differences to the insecticides tested, to thrips life stages and to different generations within an onion-growing season. Data collected suggest that there were not serious problems with thrips insecticide resistance, with the possible exception of cypermethrin in Nicaragua which had a resistance ratio (RR) value of 26.9 for adult thrips. The largest RR values were observed at the end of the growing season, and this may be caused by the season-long selection by insecticide sprays. The mortality of adults and larvae followed the same general pattern, but the ratio between larvae and adults differed for each chemical group.

Keywords: Insecta; resistance; onion thrips; *Thrips tabaci*

1 INTRODUCTION

The onion thrips, *Thrips tabaci* Lindeman, is a pest of onions and related allium species throughout the world,[1] and is a major pest of onions in Honduras and throughout Central America.[2] In Honduras *T tabaci* populations increase dramatically during the drier and hotter months of the year when most onions are planted. Populations of *T tabaci* can reach >100 per plant during the dry season and form visible clouds that land on field workers.[3] Reports indicate that thrips can reduce onion yield and bulb size by more than 55% when they are not controlled.[4–6] The major control strategy for onion thrips is the frequent use of insecticides, and growers may apply treatments weekly, resulting in 9–12 applications per crop.[2] There is concern that such intensive treatments may result in the development of resistance.

In the case of *T tabaci*, changes in field performance have been reported in Texas,[7] Colorado,[8] Michigan[9,10] and New York.[11] There have been no studies documenting resistance in onion thrips in Honduras or elsewhere in Central America, even though some authors have suggested such resistance.[3,6] Such field studies should be combined with a bioassay which can accurately measure changes in phenotypic response to an insecticide.

A bioassay procedure for evaluating insecticide resistance requires large numbers of healthy insects and a reliable procedure that yields repeatable results. A common approach for conducting insecticide resistance assays is to culture the insect populations under laboratory conditions until sufficient numbers are available for testing, but this presents several problems. Thrips are difficult to contain in cages because of their small size (<2 mm) and can contaminate (and be contaminated by) other colonies within the greenhouse and laboratory. Additionally, since colonies are reared on plant material, one must pay special attention to making sure that the plant material is not contaminated already, and this is especially difficult with thrips. Finally, because resistance may change over time, there should be concern about collecting a field sample of insects, rearing them for generations to obtain sufficient numbers of individuals and then conducting a bioassay on these later generations.

A common bioassay procedure for plant feeding insects is a leaf-dip assay, and this has been used in at least one study for *T tabaci* on onions.[11] However, collecting and rearing sufficient numbers of thrips and then conducting a bioassay cannot provide the grower with the knowledge needed to make an informed decision on using an insecticide. To

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overcome these concerns, we sought to develop a field procedure for assaying tolerance to some insecticides commonly used aginast *T tabaci* on onions.[2] We named this system TIBS (thrips insecticide bioassay system) and herein describe its components and how it was evaluated against populations of *T tabaci* collected in Honduras and Nicaragua.

2 MATERIALS AND METHODS

2.2 Bioassays

2.2.1 The container

We used a standard 0.5-ml microcentrifuge tube with a flexible plastic cap (Fig 1; Laboratory Product Sales, Rochester, NY). On the inside of the cap was a small well into which three drops of a 10% sugar-water solution containing red food colorant (USDA red dye # 4) were deposited. The solution was sealed into the well with a small piece of stretched parafilm through which the thrips could feed on the solution. The 10% sugar solution was used as the food source for thrips because it greatly prolonged thrips survival.[2] Red food colorant was added to the food to facilitate determining whether the parafilm membrane became broken and the solution contaminated the vial when the assay was read. The flexible cap only served as a container for the solution and to seal the vial. The vial tube, but not the cap, was treated with insecticide, as described below. After the vials were treated with the insecticide and allowed to dry for 8 h, an opening was made at the small end of the vial using a heated sewing pin. It was through this opening that thrips were collected in the field.

2.2.2 The power source

This bioassay procedure required a vacuum power source to aspirate the thrips from the onion plants through the small pin hole and into the insecticide-coated microcentrifuge tubes. We used a blower/mulching vacuum model SV22 Barracuda from Weed Eater® (WCI Outdoor Products Inc, Shreveport, LA) with a small gasoline two-cycle engine. The blower could be placed on a wooden tripod in the center of the field to be sampled. The air intake of the blower was sealed with a piece of a rubber inner tube to generate suction. Into this rubber tube, one end of one or more plastic hoses (0.625 cm diam × 5 m long) were inserted (one hose could be used for each sampler in the field, and multiple hoses did not significantly decrease the suction). At the other end of the hose a small (6 cm^2) piece of screen, through which thrips could not pass, was placed between the insecticide-treated plastic microcentrifuge tube (flexible cap moved aside) and the hose. This screen prevented thrips from being sucked from the microcentrifuge tube into the hose. The suction was adjusted so that thrips were dislodged from the plant, entered the vial, but were not harmed by the screen.

Figure 1. Standard 0.5-ml microcentrifuge vial with plastic cap used for TIBS. Thrips are collected through a hole at the narrow end of the vial. The well of the cap is filled with a sugar-water solution and sealed with parafilm.

2.2.3 Insecticides

We selected insecticides commonly used for control of thrips in Honduras and Nicaragua and tested them against adult and larval stages. The following pyrethroids were used: two formulations of cypermethrin (Ammo®, FMC Corp, Agricultural Products Group, Philadelphia PA; Cymbush 25® Syngenta, UK) and one formulation of permethrin (Ambush® 10EC; Syngenta, UK). The following organophosphates were tested: malathion (Malation®; Agroquimicos Versa, SA de C.V, Torreon, Coahuila, Mexico) and oxydemeton-methyl (Metasystox-R®, Bayer de México SA México DF, Mexico). The carbamate methomyl (Lannate 90 WP®; DuPont Agricultural Products, Wilmington, DE) was also tested. All the products tested were acquired as 'over-the-counter' products in Honduras.

2.2.4 Treating the containers

Previous studies[2] indicated no differences between water and acetone as a solvent, so water was used in these tests. Bond® (Loveland Industries, Loveland, CO) was used as a sticker agent and a 4-h impregnation time was sufficient for coating the vial.

For each insecticide and collection site, nine insecticide concentrations and a control were prepared. Five vials (replicates) were tested for each insecticide concentration (50 vials total). To coat the microcentrifuge tubes, the insecticide solution was poured into each microcentrifuge tube and allowed to remain for 4 h. The insecticide solution was then drained and an opening was made at the narrow end of the microcentrifuge tube using a hot needle. The vials were allowed to dry at room temperature, assisted with a room fan for at least 8 h. Coated microcentrifuge tubes were stored for up to 3 weeks in a refrigerator (4 °C) inside a plastic bag (for each insecticide concentration) since previous studies had shown no differences in mortality for recently-prepared and 3-week-old vials.[2]

2.2.5 Thrips collection

Thrips collections were made after 0900 h to avoid collecting water drops that were often present on leaves from the night dew. Both thrips life stages

(adult and larva) were collected into the same vials. The small opening at the tip of the tube was then sealed with a parafilm membrane until the assay was read. After collection the vials were stored in a cooler until they were returned to the laboratory, where they were placed at room temperature in a dark and ventilated chamber until the assay was read.

2.2.6 Collection sites

In 1997 and 1998 samples were collected from fields in two different locations in Honduras and one in Nicaragua, and in August 1996 one sample was collected from greenhouses at Cornell University's Agricultural Experiment Station in Geneva, New York. The New York population was used in the development of TIBS. In Honduras the first location was Finca Guanacaste (Comayagua), a farm at the Fundación Hondureña de Investigación Agrícola (FHIA) vegetable experimental station. For the last 5 years onions have been planted on this farm from August to May for cultivar evaluation. The second population was from Zamorano (Francisco Morazan). In this locality there has been uninterrupted vegetable production (including onions) for teaching, research and commercial purposes for the last 45 years. Insecticide resistance has already been documented in some crops (eg diamondback moth in cabbage.[12] The area sampled in Nicaragua was Finca Barbacoa (Sebaco), where 100–250 ha of onions are planted annually from June to May. Thrips samples for this study were taken in Honduras and Nicaragua during May 1997 to March 1998.

2.2.7 Analysis

Thrips mortality within the vials was assessed at 24 h after the collection with the help of a dissecting stereoscope. For each vial the number of dead and live adults and larvae was counted. Data were analyzed using the Probit procedure to calculate the mean lethal concentration to kill 50% of the population (LC_{50}) and its respective confidence interval (CI) in mg litre^{-1}.[13] Data from trials where the Probit analysis did not calculate the CI or when the resulting χ^2 statistic was not significant ($P < 0.05$) were discarded. The resistance ratio (RR), used to compare LC_{50} values between populations, was calculated for each insecticide and thrips life stage by using the lowest LC_{50} of the group as the comparison base line. Additionally, insecticide susceptibility for each insect stage for each insecticide was determined by calculating the RR between larva and adult mortality (LC_{50} larva/LC_{50} adult) for each insecticide collection. Two RR values were considered different if their respective CI values did not overlap. To determine any changes of insecticide resistance within the growing season, data were sorted by locality and trial date within the season (the onion-growing season in Honduras starts in August and ends in May). For seasonal changes, the RR value of each insecticide per location was calculated using the base of the LC_{50} at the beginning of the season.

3 RESULTS AND DISCUSSION

3.1 Susceptibility of *Thrips tabaci* adults to insecticides

Control mortality using TIBS was <2%, thus providing good data for the Probit procedure. Data analysis demonstrated that TIBS was sensitive enough to detect differences in adult thrips insecticide susceptibility for all insecticides tested (Table 1). Organophosphate insecticides had the least variability in RR values between the tested populations, but still there were some significant differences between populations. The highest RR values for malathion and oxydemeton-methyl were 2.1 and 5.6, respectively. For the carbamate insecticide, methomyl, the highest RR value was 12.6. This compound is used regularly in onion crops to control *Spodoptera exigua* (Hübner) larvae, but it is not used directly to control thrips.

There were significant differences in the population responses to pyrethroids. Permethrin is not easily found in the Honduras pesticide market and the RR value detected for it was 1.9. For cypermethrin, the most common insecticide used to control thrips in Central America, the maximum RR value detected was 26.9. Whether a RR value of 26.9 would result in a control failure in the field needs to be clarified, but growers claimed to have difficulty controlling thrips in this field when pyrethroids were used (F Mansell, pers comm, 1998). Reviewing thrips scouting records of this farm, the control efficacy (population after the application/population before the application) for cypermethrin applications was <53% compared with control efficacy >73% when methomyl was used.[2] Thus, the insecticide application method was not likely responsible for the lack of thrips control on this farm.

3.2 Susceptibility of *Thrips tabaci* larvae to insecticides, and differences between adult and larval stages

As with the adults, TIBS was sensitive enough to detect differences in insecticide susceptibility in larval populations (Table 2). Susceptibility of larvae followed the same general trend as in adults (Table 1), but there were some differences in the ratio of LC_{50} values between adults and larvae. The organophosphate insecticides controlled adults and larvae equally well with differences ≤2.4 (Table 2). Pyrethroids were more effective in suppressing adults with an average ratio of larval LC_{50}/adult LC_{50} of 2.9 and 3.9 for cypermethrin and permethrin, respectively. Of the seven cases compared for cypermethrin (Table 2), in four cases adult and larval mortalities were different in their LC_{50} values and for permethrin the two compared cases were different. Methomyl was more effective for larval control, with an average ratio of larval LC_{50}/adult LC_{50} of 0.7.

Table 1. Susceptibility of *Thrips tabaci* adult populations to various insecticides

Population	Collection date	*n*	Slope (SE)	LC_{50} (mg litre^{-1})	95% CL	RR[a]
Cypermethrin						
Comayagua	1 May 97	119	7.00 (1.55)	36.8	30.4–41.9	4.5 b
Comayagua	1 Oct 97	134	2.80 (0.45)	9	7.0–11.6	1.1 a
Comayagua 1	1 Nov 97	223	2.70 (0.34)	35.4	26.0–46.8	4.3 bc
Comayagua 2	1 Nov 97	166	3.23 (0.45)	40.1	28.8–65.7	4.9 b–d
Comayagua	7 Jan 98	260	2.86 (0.30)	28.3	20.8–41.7	3.5 b
Comayagua	8 Jan 98	194	4.43 (0.48)	46.8	39.6–55.3	5.7 b–d
NY	1 Aug 96	467	2.03 (0.19)	8.2	6.0–10.7	1 a
Nicaragua	30 Nov 97	194	4.65 (1.07)	75.7	54.3–91.6	9.2 cd
Nicaragua	13 Feb 98	329	1.22 (0.17)	220	168.2–302.1	26.9 e
Zamorano	1 Sep 97	396	4.93 (0.62)	10.8	8.7–13.0	1.3 a
Zamorano	14 Jan 98	156	5.00 (0.68)	57.3	43.4–73.4	7 cd
Malathion						
Comayagua	5 Mar 98	27	7.99 (3.35)	282.8	203.8–368.0	1.5 ab
Comayagua	22 Jan 98	173	7.93 (1.15)	281.3	255.0–310.1	1.5 b
Nicaragua	13 Feb 98	286	1.81 (0.26)	207.2	72.0–335.5	1.1 ab
Zamorano	1 Sep 97	591	2.60 (0.20)	188.2	151.5–232.6	1 a
Zamorano	14 Jan 98	301	4.21 (0.51)	391.9	284.5–505.1	2.1 b
Zamorano	19 Feb 98	96	9.52 (1.69)	287	260.5–314.2	1.5 b
Methomyl						
Comayagua	7 Jan 98	336	4.54 (0.42)	8.4	7.4–9.5	5.5 b
Comayagua	8 Jan 98	235	2.34 (0.27)	17.5	11.9–24.8	11.5 c
Nicaragua	30 Nov 97	301	1.38 (0.25)	1.5	0.1–3.4	1 a
Zamorano	1 Sep 97	359	2.46 (0.23)	7.9	6.5–9.4	5.1 b
Zamorano	14 Jan 98	256	2.31 (0.25)	19.3	15.2–24.6	12.6 c
Oxydemeton-methyl						
Comayagua	1 May 97	250	3.61 (0.38)	230	174.9–302.6	5.6 b
Zamorano	1 Sep 97	350	2.34 (0.30)	40.9	32.0–49.3	1 a
Permethrin						
NY	1 Aug 96	502	2.11 (0.21)	244.4	181.5–337.4	1 a
Zamorano	1 Sep 97	218	2.86 (0.40)	463.5	346.4–572.1	1.9 b

[a] Values followed by different letters signify significant differences ($P = 0.05$) between populations within a given insecticide.

Table 2. Susceptibility of larval populations of *Thrips tabaci* to various insecticides and a comparison of adult and larval susceptibility

Population	Collection date	*n*	Slope (SE)	LC_{50} (mg litre^{-1})	95% CL	RR[a]	Larva/adult LC_{50} (mg litre^{-1})[a]
Cypermethrin							
Comayagua	1 May 97	338	2.81 (0.35)	225.6	181.6–273.5	19.6 f	6.1*
Comayagua	1 Oct 97	462	3.34 (0.32)	26.5	21.7–31.7	2.3 cd	2.9*
Comayagua	7 Jan 98	509	3.60 (0.33)	143	100.8–232.9	12.4 f	5.0*
Lejamani	1 Aug 97	364	0.68 (0.10)	11.5	2.9–19.2	1 a–c	
NY	1 Aug 96	334	1.72 (0.28)	26.1	7.5–67.2	2.3 a–e	3.2
Nicaragua	30 Nov 97	319	2.82 (0.31)	46.4	35.0–57.4	4 b–e	0.6
Nicaragua	13 Feb 98	445	1.41 (0.18)	173	122.4–241.7	15 f	0.8
Zamorano	1 Sep 97	367	2.83 (0.30)	20.3	14.0–26.7	1.8 a–d	1.9*
Zamorano	19 Feb 98	490	1.90 (0.19)	27.9	19.2–36.8	2.4 a–e	
Malathion							
Comayagua	1 May 97	300	2.07 (0.23)	581.9	463.2–727.0	3 d	
Org Comayagua	22 Jan 98	638	4.99 (0.36)	268.9	247.3–290.8	1.4 bc	1
Nicaragua	13 Feb 98	248	5.97 (1.54)	504.5	309.6–602.2	2.6 b–d	2.4
Zamorano	1 Sep 97	302	2.07 (0.20)	196	154.1–246.2	1 a	1

Table 2. Continued

Population	Collection date	*n*	Slope (SE)	LC_{50} (mg litre^{-1})	95% CL	RR[a]	Larva/adult LC_{50} (mg litre^{-1})[a]
Zamorano	14 Jan 98	311	4.94 (0.60)	360	264.2–480.6	1.8 b–d	0.9
Zamorano	19 Feb 98	410	7.98 (0.70)	258.6	166.3–366.6	1.3 a–c	0.9
Methomyl							
Comayagua	1 May 97	241	2.04 (0.26)	2.4	1.5–3.4	1.7 ab	
Org. Comayagua	7 Jan 98	480	4.64 (0.38)	6.3	4.7–8.4	4.6 c	0.7
Comayagua	8 Jan 98	559	2.42 (0.19)	7.3	6.0–9.1	5.4 c	0.4*
Org. Comayagua	22 Jan 98	371	2.29 (0.42)	1.4	0.3–2.0	1 a	
Nicaragua	30 Nov 97	286	2.27 (0.49)	2.1	0.9–3.2	1.6 ab	1.4
Zamorano	1 Sep 97	624	3.97 (0.32)	3.1	2.3–3.9	2.3 b	0.4*
Zamorano	19 Feb 98	386	2.75 (0.48)	15.1	9.0–21.2	11.1 c	
Oxydemeton-methyl							
Lejamani	1 Aug 97	517	2.27 (0.19)	150.1	124.6–178.6	3.9 b	
Zamorano	1 Sep 97	358	2.9 (0.42)	38.6	24.4–49.5	1 a	0.9
Permethrin							
NY	1 Aug 96	553	1.9 (0.20)	1028.7	721.1–1420.1	1 a	4.2*
Zamorano	1 Sep 97	332	2.33 (0.31)	1701.7	1419.8–2139.8	1.6 a	3.7*

[a] Values followed by different letters or by *signify significant differences ($P = 0.05$) between populations within a given insecticide or between LC_{50} ratios of larva/adult.

With four adult to larval comparison cases, two were significantly different.

3.3 Seasonal changes in susceptibility

Our results show a tendency for decreased susceptibility as the growing season progresses. In Central America, the onion-growing season begins in August–September with the planting of seedbeds, and continues until April–May when the last onions are harvested. For the period of May–July, most of the onion land is planted with maize or beans as a rotation practice and because onions do not grow well during these months due to heavy rains and long day-length. To assess changes over the season we also sorted the data by month of the growing season (August–May) for each locality, and then the RR was calculated for each insecticide and locality using the LC_{50} of the first trial of the season in each locality as the base.

The highest RR values for most insecticides tested in each locality occurred at the end of the onion-growing season, and for six of seven comparisons for adult thrips and five of seven for larval thrips, the RR values increased significantly over the growing season (Tables 1 and 2). In some cases the decline in insecticide susceptibility was significantly higher in the thrips life stage where the insecticide tolerance was higher. For example, in Comayagua with cypermethrin, which provided better control of adults than larvae, the adult RR values at the end of the season increased 4.1-fold while the larval RR value increased 19.6-fold. The same tendency was observed for cypermethrin in Nicaragua, but not at Zamorano.

It seems that thrips insecticide resistance increases over the growing season as a consequence of the selection pressure of the insecticides used to control this pest. In Comayagua, where thrips were controlled mainly by pyrethroid applications, and methomyl was used in the same fields for control of *S exigua*, thrips insecticide susceptibility significantly decreased over the season by 19.6- and 2-fold for cypermethrin and methomyl, respectively. At the end of the season, however, resistant thrips may not be able to survive in abundance because there is not sufficient green vegetation in the surrounding areas, April and May being the driest months of the year. From June to September onions are not planted and heavy rains may maintain thrips populations at low levels in native vegetation where they are not treated. Each year at the beginning of the onion season (September–October), non-insecticide-selected thrips may migrate from natural vegetation or other crops to colonize the onion fields, but only in December–January when the rains stop do populations increase and farmers start to use insecticides to control thrips.[2] Thus, intense selection for resistance may last for only 3–4 months of the year (January–April) and this may prevent higher levels of resistance than we observed. Further study is necessary to clarify the seasonal development of resistance and the relationship between dose–mortality assessments using TIBS and field performance, but having a rapid method such as TIBS will help growers in developing an insecticide resistance management strategy.

4 CONCLUSION

The TIBS system proved efficient for detecting differences in insecticide susceptibility between populations. The time required to collect the thrips for each insecticide (50 vials in total) was variable depending on

the thrips population in the field. With populations ≤ 2 thrips per plant, it took around 6 h for one person to complete the process. With thrips populations of 5–10 thrips per plant, the process required 3 h. When four or more people were collecting thrips at the same time (each one using a different suction hose) in fields with large thrips infestation, the collection for each insecticide was completed within 1 h. Before using this procedure, however, it is important that the storage life of treated vials with any insecticide be evaluated and that care be taken to ensure there is little control mortality.

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