CONTRASTS OF THREE INSECTICIDE RESISTANCE MONITORING METHODS FOR WHITEFLY

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Abstract

Three resistance monitoring methods were tested to evaluate their relative reliability, discriminating ability, convenience, and practicality for monitoring insecticide resistance in Arizona whiteflies. Adult whiteflies were collected from the field and tested in the laboratory with three methods: leaf disk, sticky trap, and vial. Each method was evaluated using a mixture of Danitol® + Orthene® and two single chemicals, Thiodan® and Danitol®, against two populations divergent in susceptibility. The Yuma population was relatively susceptible and the Gila River Basin population highly resistant. Correlations of field efficacy and leaf disk bioassays were conducted with the Yuma population and a comparatively resistant Maricopa population. At each location egg, immature, and adult whitefly densities were monitored before and after Danitol® + Orthene® treatments and resistance estimates were also monitored in the populations using leaf disk bioassays.

Our results illustrated that the leaf disk method had the greatest discriminating ability between susceptible and resistant populations. The results also indicated that the vial method was the most practical, and that the sticky trap method was good at discriminating between populations which have large differences in susceptibility. The field efficacy trials indicated results from leaf disk assays reflected what had occurred in the field.

Introduction

Since 1990, whitefly, Bemisia tabaci (Gennadius), (a.k.a. Bemisia argentifolii Bellows and Perring) has been a very serious threat to production of cotton, vegetables, and melons in Arizona (Byrne et al. 1990). The increased severity of whitefly in the Southwest has been attributed to the establishment and subsequent predominance of a distinctly new form of the pest (Brown et al. 1995). This new biotype (or species) has proven to be much more refractory to insecticides than populations were previously. Statewide monitoring of whitefly resistance has been conducted by our laboratory since 1994 in cooperation with the USDA Western Cotton Research Laboratory. These statewide surveys of resistance have confirmed the existence of serious resistance problems in some areas of Arizona (Dennehy et al. 1995).

Monitoring insecticide resistance in whitefly with conventional bioassay is costly and difficult but is currently the only effective way to detect and manage resistance in this pest. Worldwide, three very different bioassays have been used for this purpose: the leaf disk method, the sticky trap method, and the vial method. These methods, however, have not been strenuously contrasted or statistically validated regarding their precision and accuracy in reflecting efficacy of pesticides against whitefly in cotton fields. Each of these methods offers clear advantages and disadvantages. Here we present results of using all of these methods to estimate susceptibility of two Arizona whitefly populations to three different insecticide treatments. Based on these contrasts we draw conclusions regarding the relative reliability, discriminating ability, convenience, and practicality of these commonly used methods. We then use the most reliable of the three methods to correlate bioassay results with field efficacy trials at two locations in Arizona that are divergent in susceptibility to Danitol® + Orthene®.

Materials and Methods

Collection of Whitefly

Statewide monitoring of resistance identified two field populations, Yuma and Gila River Basin, with widely differing susceptibilities to the mixture of Danitol® + Orthene®. Adult whiteflies were vacuum- collected directly from field foliage at each site using plastic vials with fitted screen bottoms and a Makita® cordless vacuum (4071D). The samples were transported in ice chests directly to the Extension Arthropod Resistance Management Laboratory (EARML), in Tucson, where they were released into rearing cages containing young cotton (Pima S-7) plants. The whitefly were maintained in these cages until they were placed in bioassays (<7 days).

Leaf Disk Method

The leaf disk method (Rowland et al. 1991) used leaf punches taken from cotton plants 18 to 26 days old. The leaf disks were dipped for 10 s in formulated insecticide diluted in water. After drying, the disks were placed individually on a base of agar (1.6%) in 20ml glass scintillation vial. Within 2h of dipping, 20-30 adult whitefly were aspirated into each vial. Assays were then held in an incubator at 27¡C for 48 h, after which they were scored using a binocular microscope. Scintillation vials were tapped on the counter 10 times after which whiteflies not exhibiting repetitive movement of more than one appendage were scored as dead. At least five different concentrations were evaluated of each formulated insecticide. These treatments were within the following ranges: 0-10000 µg/ml Danitol® (2.4 EC); 0-32µg/ml Thiodan® (3 EC); the mixture of and 1000µg/ml Orthene® (90S) + 0-32µg/ml Danitol® (2.4 EC).

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Sticky Trap Method

The Sticky Trap method (Prabhaker et al. 1992) uses 3" x 5" plastic yellow cards coated on one side with a very thin layer of Tanglefoot®. Three ml of formulated insecticide diluted in water was sprayed over each card using a Potter Precision Spray Tower. The cards were allowed to dry and were then passively infested with approximately 50 whitefly each. Passive infestation comprised using a glass jar, with a square approximately 3.5 cm on side cut in the jar lid and a fiber optic light. A sticky card with Tanglefoot®-side facing inside the jar was positioned on the lid of the horizontal jar. The light source, placed so that the sticky card's infesting area was illuminated, attracted whitefly to the exposed sticky card. Infested cards were then placed on foam racks in 13 gallon coolers containing at least a 3 cm depth of water. After 24 hours mortality was assessed with the aid of a binocular microscope. To do this the sticky card was tapped on the countertop 10 times after which individuals not exhibiting repetitive movement of more then one appendage, when probed with a #5 camel hair brush, were scored as dead. The concentrations of formulated insecticide evaluated were in the ranges of: 0-10000 µg/ml Danitol® (2.4 EC); 0-3200 µg/ml Thiodan® (3 EC); and 1000 µg/ml Orthene® (90S) + 0-3200 µg/ml Danitol® (2.4 EC).

Vial Method

The Vial Assay (Cahill and Hackett, 1992) involved placement of 0.25 ml of technical grade insecticide dissolved in acetone into 20 ml glass scintillation vials. Vials were rolled for 10 min. using a conventional hotdog roller to evenly coat the inner surfaces as the acetone evaporated. The vials were then placed in a ventilation hood for 2 h to fully dry. Twenty whitefly were aspirated into each vial and vials were then placed in an incubator at 27°C. Mortality was assessed after 6 h, using a binocular microscope. This involved tapping vials on the countertop ten times. Individuals unable to right themselves were scored as dead. Concentrations of formulated insecticide evaluated were in the ranges of: 0-1000 µg/ml Danitol® (2.4 EC); 0-100 µg/ml Thiodan® (3 EC); and for the mixture 1000 µg/ml Orthene® (90S) + 0-100 µg/ml Danitol® (2.4 EC).

Field Evaluations of the Leaf Disk Method

Field trials were conducted at Yuma and Maricopa, Arizona. At each site, 5 insecticide threshold treatments x 5 replication were arranged in a Latin square design. Each block was 50 x 12 rows and row spacing was 1 foot. Buffers of 10 feet width separated replicates at Maricopa. Only two of the treatments were used for this evaluation: the unsprayed (control) group and a treatment that triggered sprays at 10 adult whitefly per leaf. When adult whitefly reached 10 per leaf, the corresponding blocks were sprayed using a commercial mixture 0.2 lb. of Danitol® + 0.5 lb. Orthene® per gallon and a volume of 20 gallons of water per acre. Whiteflies for leaf disk bioassays were removed from each plot before spraying and every two days after treatment. This continued for 14 days at the Yuma site and 6 days at the Maricopa site. Adult, egg and immature whitefly densities were estimated for each of the five replicates of the control and treated plots. Adult whitefly densities were sampled using the leaf-turn method (Ellsworth et al. 1994). Eggs and immature densities were sampled by collecting 15 leaves per plot from the fifth main stream node of cotton plants located throughout each plot. The collected leaves were taken to the EARML facility. On each leaf a 1 cm diameter circle was drawn near the leaf base, between the main vein and the second lateral vein. Immature and egg stages within these arenas were counted using a binocular microscope. Collection of leaves were made every two days until plots required re-treatment. This was 14 days at Yuma and 6 days at Maricopa.

Results and Discussion

Contrasts of Methods

Tables 1-3 contrast the results of the leaf disk, sticky trap, and vial methods estimates of the susceptibility of two populations to mixtures of Danitol® + Orthene®, Danitol® alone and Thiodan® alone.

The leaf disk method generally produced the lowest LC50 values, the highest slopes of the response lines and the least overlap in responses of the Yuma and Gila River Basin populations. The sticky trap method produced LC50 values 6- to 118-fold higher than the leaf disk method, except for Danitol® susceptibility of the Gila River Basin population, in which case the sticky trap method was moderately more toxic than the leaf disk method (Table 2). The sticky trap method was less able to discriminate between the two populations than was the leaf disk method. This is illustrated in tables 2 and 3, where the 95% FL of LC50 of the two populations overlap for the sticky trap but not for the leaf disk method. The sticky trap method discriminated well between Yuma and Gila River Basin populations susceptibility to Danitol® + Orthene®, however the slopes produced were not as high as those produced with the leaf disk method (Table 1).

Table 1: Response of Yuma and Gila River, Arizona, whitefly to leaf disk (LD), sticky trap (ST), and vial (V)bioassays of Danitol® (varying rates) mixed with afixed rate of 1000µg/ml Orthene®.

	Yuma			Gila River Basin		
Method	LD	ST	V	LD	ST	V
N	3235	2217	2240	1623	1448	889
Slope	2.03	1.05	1.45	2.30	1.22	0.583
X^2/df	4.26	3.44	3.80	3.95	1.45	2.19
G (.95)	0.054	0.029	0.60	0.44	0.038	0.77
LC_{50}	0.157	2.14	0.210	14.3	309	1690
95% FL	.11-.19	1.4-3.0	.15-.27	5.7-19	220-410	--
RR*(LC_{50})	91.1	144	8000	91.1	144	8000

*LC_{50} Gila River Basin/LC_{50} Yuma

Table 2: Response of Yuma and Gila River, Arizona, whitefly to leaf disk (LD), sticky trap (ST), and vial (V)bioassays of Danitol® (varying rates).

	Yuma			Gila River Basin		
Method	LD	ST	V	LD	ST	V
N	1054	2572	1562	1057	1399	1506
Slope	1.64	1.23	0.581	1.34	0.651	0.120
X^2/df	3.30	1.60	4.22	1.72	1.97	5.45
G (.95)	0.054	0.029	0.16	0.052	0.11	9.9
LC_{50}	111	658	339	4400	1720	—
95% FL	81.0-147	479-858	123-1270	3284-6270	758-3520	—
RR*(LC_{50})	39.6	2.60	—	39.6	2.60	—

*LC_{50} Gila River Basin/LC_{50} Yuma

Table 3: Response of Yuma and Gila River, Arizona, whitefly to leaf disk (LD), sticky trap (ST), and vial (V)bioassays of Thiodan® (varying rates).

	Yuma			Gila River Basin		
Method	LD	ST	V	LD	ST	V
N	1204	1922	1880	829	2016	2393
Slope	3.33	1.49	3.21	2.63	1.82	2.54
X^2/df	1.88	1.80	2.96	1.84	2.94	3.82
G (.95)	0.045	0.045	0.057	0.074	0.073	0.043
LC_{50}	9.06	635	10.8	4.49	531	6.90
95% FL	7.62-10.4	493-783	9.18-12.5	3.42-5.49	362-697	5.45-8.40
RR*(LC_{50})	0.49	0.83	0.63	0.49	0.83	0.63

*LC_{50} Gila River Basin/LC_{50} Yuma

The vial method did not discriminate as well between populations as did the other methods when using Danitol® + Orthene® or Orthene® alone. However, the vial method did produce higher slope values than the sticky trap method and had no overlap of the 95% FL of LC50 for Thiodan® alone (Table 3).

Each bioassay method has distinct advantages and disadvantages. The leaf disk assay had the greatest ability to discriminate differences in susceptibility of populations, produced the steepest slopes, and did not require the use of Tanglefoot®. However, it requires healthy uninfested cotton plants of correct age. It is expensive, not portable, and is relatively difficult and time consuming.

The sticky trap assay, on the other hand, is fairly inexpensive, and portable. It discriminated moderately well between susceptibility of the Yuma and Gila River Basin populations. But this method requires the use of Tanglefoot®, is moderately time consuming (for construction of assays and their spraying) and requires considerable space and quantity of pesticide.

The vial assay is the easiest and cheapest method. Additional advantages include the short duration of the test, portability and the advantage of not having to use Tanglefoot®. However, with the pesticides we evaluated, this method was the least accurate and precise. This appeared to be related to the highly resistant Arizona whitefly we evaluated. The vial bioassay estimated susceptibility of the Yuma population nicely but the high resistance exhibited by the Gila River Basin population yielded very low slopes, except with Thiodan® (Table 3). Stickiness of vials treated with high concentrations of pyrethroids and pryrethroid mixtures further limited the utility of this method in Arizona.

Field Evaluation of the Leaf Disk Method

Differences in field performance of Danitol® + Orthene® were reflected nicely in leaf disk bioassay results (Figures 1-8). That is, when the leaf disk assay showed the greatest susceptibility, field performance was the best, and vice versa. This indicates that the leaf disk method provides acceptable precision in reflecting field performance of Danitol® + Orthene®.

Figure 1 and figure 5 demonstrate the striking differences between leaf disk resistance estimates at Yuma and at Maricopa after the three treatments of Danitol® + Orthene®. At the latter site the population's susceptibility was reduced greater than hundred-fold. Susceptibility of the Yuma population was changed little after three treatments of Danitol® + Orthene®. The densities of eggs, immatures, and adult whiteflies at both sites reflected the susceptibility collected from bioassays (figures 2-4 for the Maricopa site and figures 6-8 for the Yuma site). At Yuma, the spray application suppressed all stages counted for greater than two weeks while the control remained constant or grew in number. The Maricopa populations, on the other hand, appeared to be almost unaffected the treatment of Danitol® + Orthene®. This result shows vividly that the resistance to synergized pyrethroids in Arizona whiteflies renders them nearly immune to these insecticides.

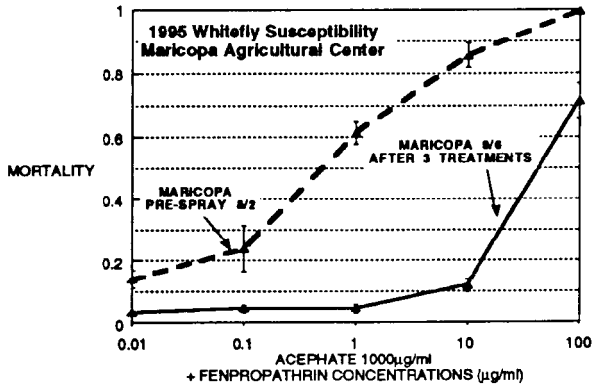

Figure 1

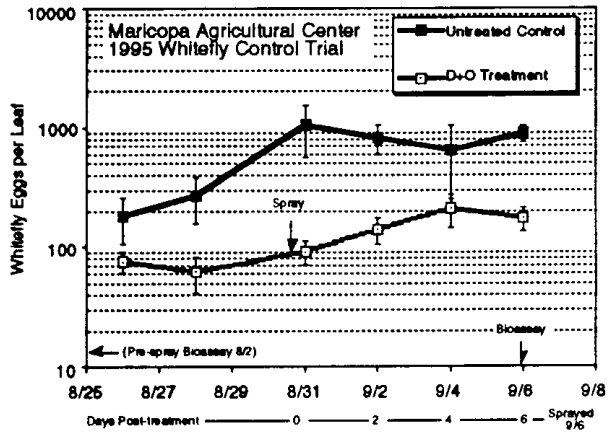

Figure 2

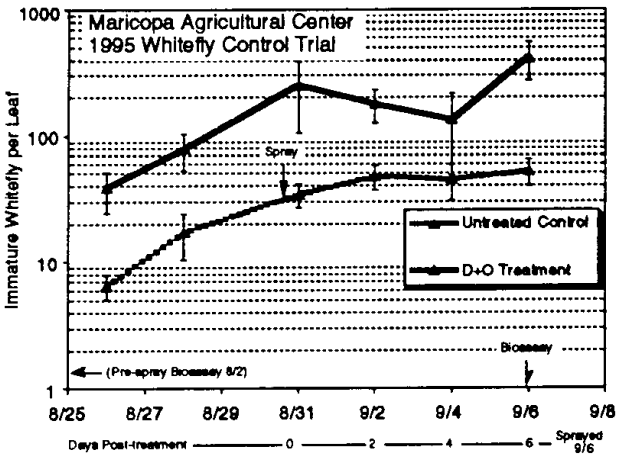

Figure 3

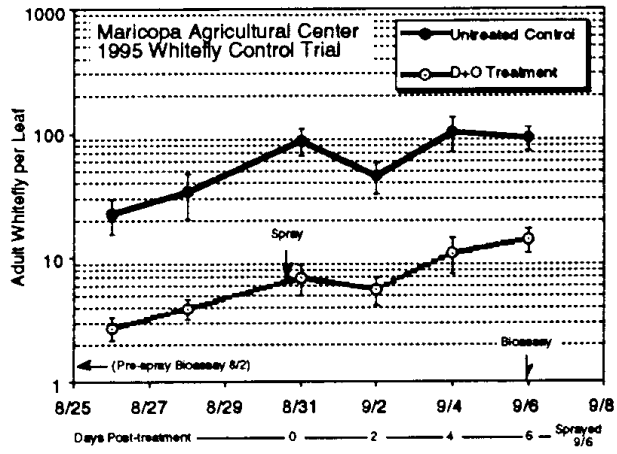

Figure 4

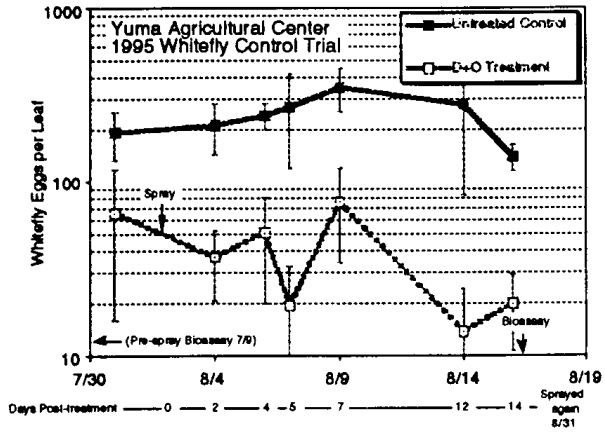

Figure 6

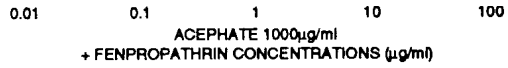

Figure 5

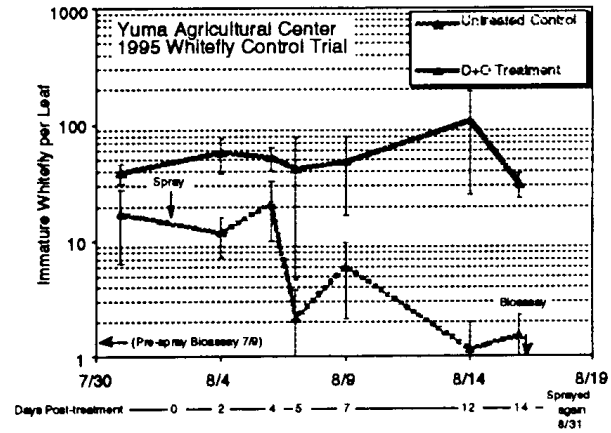

Figure 7

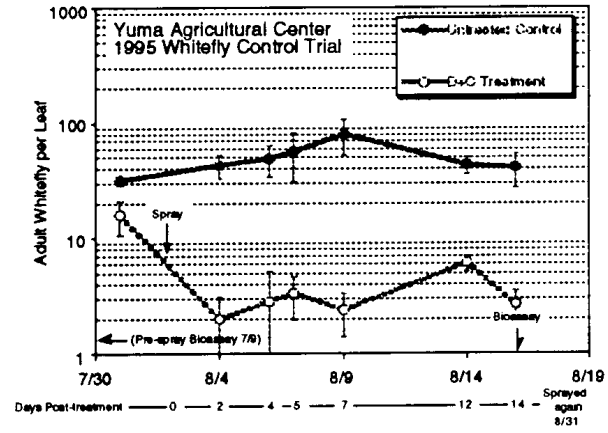

Figure 8

Conclusion

The three principal methods used for whitefly monitoring worldwide produced significantly different estimates of susceptibility and resistance intensity for the three insecticide treatments evaluated. The leaf disk method generally produced the lowest LC50 values, the highest slopes of the response lines and the least overlap in responses of the Yuma and Gila River Basin populations. The sticky trap method produced LC50 values 6 to 118-fold higher than the leaf disk method, except for Danitol® bioassays of the Gila River Basin population, in which case the sticky trap method was moderately more toxic than the leaf disk method. The sticky trap method discriminated well between populations differing in susceptibility to Danitol® + Orthene®, but was less effective than the leaf disk method at discriminating susceptibility to Danitol® or Thiodan® alone. The vial method was the least effective at discriminating between the Yuma and Gila River Basin populations. Additionally, it posed technical problems stemming from stickiness of Danitol®, especially at the high concentrations required to kill the Gila River Basin population. The sticky trap bioassay represented a practical compromise between difficulty and discriminating ability. It also offered the advantage of portability.

The field trials demonstrated excellent concordance between leaf disk bioassay results and field performance of Danitol® + Orthene®. This study suggests that the leaf disk method is the most discriminating and accurate of the three methods. However, it is clearly the most difficult.

To determine which whitefly monitoring method is the best for any given situation many factors must be taken into account. From our findings it appears that the most important distinction is whether monitoring is being done either: 1) to detect individuals that survive the LC95 of susceptible populations or 2) to estimate the intensity of resistance in field populations. In the former case, the vial bioassay may be the simplest and most efficient method. In the latter case, when precision in estimating LC50 values is essential, the leaf disk bioassay is clearly the best choice, based on our results. However, the sticky trap bioassay offers many advantages over the vial and leaf disk methods and may prove to be the optimal compromise between practicality and discriminating ability for many whitefly resistance monitoring programs.

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References

Brown, J.K., D.R. Frohlich, and R.C. Rosell. 1995. The sweetpotato or silverleaf whiteflies: biotypes of Bemisia tabaci : or a species complex? Annu. Rev. Entomol. 40: 511-534.

Byrne, D.N., T.S. Bellows and M.P. Parrella. 1990. Whiteflies in agricultural systems pp. 227-261 in D. Gerling (ed.). Whiteflies: their bionomics, pest status and management. Incept, Andover, UK.

Cahill, M.R. and B. Hackett. 1992. Insecticidal activity and expression of pyrethroid resistance in adult Bemisia tabaci:: using a glass vial bioassay. Brighton Crop Protection Conference Pests and Diseases. 3C-15:251-256.

Dennehy, T.J., A. Simmons, J. Russell, and D. Akey. 1995. Establishment of a whitefly resistance documentation and management program in Arizona. Arizona Cotton Report. University of Arizona. 1995:287-297.

Ellsworth, P., J. Diehl, T. Dennehy, and S. Naranjo. 1994. Sampling sweetpotato whiteflies in cotton. Cooperative Extension- IPM Series Number 2, Bulletin.

Prabhaker, N., N.C. Toscano, T.M. Perring, G. Nuessley, K. Kido, and R.R. Youngmen. 1992. Resistance monitoring of the sweetpotato whitefly (Homoptera: Aleyrodidae) in the Imperial Valley of California. Journal of Economic Entomology. 85 (4): 1063-1068.

Rowland, M., B. Hackett and M. Stribley. 1991. Evaluation of insecticides in field-control simulators and standard laboratory bioassays against resistant and susceptible Bemisia tabaci (Homoptera:Aleyrodidae) from Sudan. Bull. of Entomol. Res. 81:189-199.