EFFECT OF MICROMITE® ON THE EGG PARASITOIDS CERATOGRAMMA ETIENNEI (HYMENOPTERA: TRICHOGRAMMATIDAE) AND QUADRASTICHUS HAITIENSIS (HYMENOPTERA: EULOPHIDAE)

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Source: Florida Entomologist, 87(2) : 222-224

Published By: Florida Entomological Society

URL: https://doi.org/10.1653/0015-4040(2004)087[0222:EOMOTE]2.0.CO;2

EFFECT OF MICROMITE® ON THE EGG PARASITOIDS *CERATOGRAMMA ETIENNEI* (HYMENOPTERA: TRICHOGRAMMATIDAE) AND *QUADRASTICHUS HAITIENSIS* (HYMENOPTERA: EULOPHIDAE)

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The Diaprepes root weevil, *Diaprepes abbreviatus* (L.), was first detected near Apopka, Florida in 1964 (Woodruff 1964). Since then, it has spread throughout the citrus growing areas of the state causing growers millions of dollars in losses each year. In south Florida, *D. abbreviatus* is also a problem in root crops and ornamental plants (Peña & Amalin 2000). One of the components of the pest management program for this weevil is the use of Micromite® (diflubenzuron), a chitinase inhibitor that sterilizes the egg by interrupting the formation and deposition of chitin in developing embryos. Early studies indicated that diflubenzuron significantly reduces the reproductive potential of *D. abbreviatus* when applied to citrus foliage (Schroeder et al. 1976; Lovestrand & Beavers 1980; Schroeder et al. 1980). Later, Schroeder (1996) reported that residues of diflubenzuron significantly affected the reproductive potential of *D. abbreviatus* for more than one month after application to citrus foliage.

Micromite has been described as a foundation product for reducing citrus root weevil populations, in part due to its compatibility with root weevil natural enemies (Anonymous 1996). However, there are no reports on the effect of micromite on egg parasitoids that have been imported and established for biological control of *D. abbreviatus* (Hall et al. 2001; Peña et al. 2000; Peña et al. 2003). A study was initiated to evaluate the impact of Micromite on *Ceratogramma etiennei* Delvare and *Quadrastichus haitiensis* (Gahan), two egg parasitoids of Diaprepes root weevil.

Green buttonwood (*Conocarpus erectus* L.) seedlings were grown from cuttings in 3.7-liter pots. Adult *D. abbreviatus* were collected from an insecticide-free ornamental orchard in Homestead, Florida. A Guadeloupe strain of *C. etiennei* was obtained from J. Etienne, Institut National de la Recherche Agronomique (INRA). The culture was maintained at the Tropical Research and Education Center (TREC) insectary, Homestead, Florida. Insect cultures were maintained at 26.5 ± 1.0°C, 12:12 L:D and approximately 78% RH, on eggs of *D. abbreviatus* laid on strips of wax paper using the methodology of Etiennei et. al (1990). *Quadrastichus haitiensis* originally from Puerto Rico was obtained from Ru Nguyen, Division of Plant Industry, Gainesville, Florida, and maintained as above.

The effect of Micromite ingestion by the adult Diaprepes root weevil plus absorption of residues from leaves into eggs was evaluated in experiment 1. Three green buttonwood seedlings planted separately on 3.7-liter pots were sprayed with Micromite to run-off using the simulated field rate (0.485 g/1 liter of water). Another three seedlings were sprayed with water as control checks. All the seedlings were enclosed separately in screen cages (240 cm × 120 cm × 120 cm). One hundred adults of *D. abbreviatus* were introduced inside each cage for oviposition. After 3 days, 20 egg masses on leaves still attached to branches were collected from each seedling, arranged as bouquets in flasks of water and placed in Plexiglass cages (30 cm × 30 cm × 30 cm) separately. Six cages, 3 treated and 3 untreated, were prepared for each parasitoid species. One hundred 2- to 3-d-old *C. etiennei* and *Q. haitiensis* adults were introduced into each Plexiglass cage. Bouquets were removed after 3 days and portions of the leaves with individual egg masses laid between two leaf surfaces (covered with two leaf layers intact) were placed in culture tubes (12 mm × 75 mm). After 7 d, egg masses were exposed by removing one leaf surface and parasitized eggs counted. Parasitized eggs were recognized by the characteristic golden egg chorion for *C. etiennei* and silver transparent egg chorion for *Q. haitiensis* (Peña et al. 2000).

Effects of ingestion of micromite by adult *D. abbreviatus* alone were evaluated in a separate experiment. Ten adult *D. abbreviatus* females were exposed to Micromite -treated seedlings and to untreated foliage for 3 days as described above. The adults were allowed to oviposit on to double strips of wax paper inside Plexiglass cages (30 cm × 30 cm × 30 cm). After 2 d, paper strips containing 20 egg masses were collected from each cage and each egg mass was placed separately inside culture tubes (12 mm × 75 mm). One 2-d-old mated female wasp was introduced into each tube (20 tubes per treatment for each parasitoid species). After 7 d, eggs were examined as described above.

In a third test, 10 untreated female weevils were allowed to oviposit on wax paper for 2 days. Wax papers containing approximately 20 egg

TABLE 1. EFFECT OF MICROMITE EXPOSURE BY ADULT FEEDING ON TREATED FOLIAGE (INGESTION), OVIPOSITION ON TREATED SURFACE (FOLIAGE), OR BY DIPPING EGG MASSES IN PESTICIDE (DIPPED) ON *CERATOGRAMMA ETIENNEI* PARASITISM ON *D. ABBREVIATUS* EGG MASSES.

	Source of Micromite exposure*			Percent Parasitized	
Experiment	Female treatment	Substrate	Egg mass	Control	Treated
1	Ingestion	Treated (foliage)	None	78.6 a**	0.0 b
2	Ingestion	None (wax paper)	None	95.0 a	0.0 b
3	None	None (wax paper)	Dipped	40.0 a	30.0 a

*Treatments were applied prior to parasitoid oviposition and all egg masses were sandwiched two between layers of substrate throughout the experiments.

**Means in rows followed by the same letter are not significantly different according to DMRT.

masses were dipped for 30 sec in 0.485 g/1 liter Micromite suspension and 20 egg masses were dipped in water as control. The strips of wax paper were left in place leaving the eggs covered on both sides. Wax papers were air dried and individual egg masses were placed singly in culture tubes, exposed to parasitoids and evaluated as above.

The effect of direct sprays on *C. etiennie* pupae was evaluated in a grove with Hamlin orange (*Citrus sinensis* [L.] Osbeck) trees in Hendry County Florida. Treatments were (1) Micromite 80 WG@ 5 oz ai per acre (6.25 oz/acre) plus 1% F433-66 horticulture oil, (2) F433-66 horticulture oil only @ 5%, and (3) untreated control. Plots consisted of single rows, each with 20-23 trees separated by 4 guard rows. Wax paper strips containing *D. abbreviatus* eggs exposed to *C. etiennei* 12, 14, and 16 days earlier and thus containing parasitoid pupae, were stapled on leaves. There were 22 parasitized egg masses of each age group within each of the 3 treatments. Trees were sprayed on 27 September 2000 from both sides using a Durand Wayland 3P 100-32 airblast speed sprayer equipped with 3 nozzles #3 T-Jet stainless steel nozzles, operating at 400 psi and calibrated to 91 GPA. Wax papers were collected the following day and allowed to incubate in the laboratory as above. Emerged wasps were counted.

Mean percent parasitization was computed and compared by Analysis of Variance (ANOVA), followed by Duncan's Multiple Range Test (DMRT) at $P = 0.05$.

No successful development of *C. etiennei* embryos was observed in egg masses from female *D. abbreviatus* fed on Micromite-treated seedlings, whether oviposition occurred on leaves or wax paper (Table 1). Although, there was no significant difference ($df = 39, 1, F = 3.23, P = 0.08$) between Micromite-treated egg masses and the untreated control in emergence of *C. etiennei*, a trend toward less emergence from treated egg masses was observed. In contrast, no reduction of *Q. haitiensis* emergence was observed in response to host feeding on treated leaves ($df = 39, 1, F = 0.02, P = 0.87$), nor in response treatment of egg masses ($df = 39, 1, F = 2.44, P = 0.12$) compared to untreated controls.

In the field experiment, both Micromite and oil sprays reduced emergence of *C. etiennei*, although the influence of parasitoid age depended on the treatment (Table 2). Oil had its greatest impact on the older parasitoids (treatment 14 and 16 d after parasitization) with no significant effect on eggs parasitized 12 d prior to treatment. This might be due to a greater effect of suffocation on older parasitoids presumably respiring at a higher rate. In contrast, Micromite had its greatest impact on the younger parasitoids (initiated 12 and 14 d prior to treatment). No significant effect was observed on emergence from eggs parasitized 16 d before treatment, presumably because parasitoids had already synthesized sufficient chitin to complete development. This result confirmed the sensitivity of *C. etiennei* to Micromite, particularly of younger parasitoids.

These results suggest that Micromite interferes with development of *C. etiennei* but not *Q. haitiensis* in *D. abbreviatus* eggs. Furthermore, the effect on *C. etiennei* is age dependent, with greatest impact on young (developing) parasitoids and young pupae as compared to old pupae. This incompatibility may be one of the reasons for the continuing recovery of *Q. haitiensis* in south Florida in contrast to *C. etiennei* (Peña et al., un-

TABLE 2. PERCENT EMERGENCE OF *CERATOGRAMMA ETIENNEI* AT DIFFERENT AGE AT TIME OF TREATMENT (LENGTH OF INTERVAL FROM PARASITIZATION TO EXPOSURE IN THE FIELD).

Treatment	% Emergence by parasitoid age (d)		
	12	14	16
Micromite	0.62 b	1.48 b	7.27 ab
Oil	2.86 ab	5.09 b	4.42 b
Untreated	8.19 a	13.01 a	14.10 a

Means in columns followed by the same letters are not significantly different ($P \leq 0.05$).

published data). Although Micromite is known to be relatively safe to beneficial insects, *C. etiennei* proved to be highly sensitive. Thus, extensive use of this pesticide to control *D. abbreviatus* could be at least partially responsible for difficulties experienced in establishing *C. etiennei* (Hall et al. 2001). Perhaps more successful biological control could be achieved in the absence of this pesticide.

We thank Drs. Nancy Epsky and Paul Kendra for their comments and review of this manuscript. This research was partially supported by a T-STAR grant to J. E. Peña. Experiment Station Journal Series R-09925.

Summary

This study reports the impact of Micromite on parasitization of *D. abbreviatus* egg masses by the parasitoids *Ceratogramma etiennei* and *Quadrastichus hatiensis*. *Diaprepes abbreviatus* egg masses treated with Micromite resulted in lower egg parasitization by *C. etiennei*. Micromite did not appear to affect egg parasitization by *Q. hatiensis*. A field test confirmed the sensitivity of *C. etiennei* to Micromite as well as to horticulture oil.

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