Biological Control **17,** 243–249 (2000)
doi:10.1006/bcon.1999.0795, available online at http://www.idealibrary.com on IDEAL®

Disruptive Sublethal Effects of Insecticides on Biological Control: Altered Foraging Ability and Life Span of a Parasitoid after Feeding on Extrafloral Nectar of Cotton Treated with Systemic Insecticides

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Received April 20, 1999; accepted October 25, 1999

Predictions in integrated pest management on the compatibility of an insecticide with biological control often are based on incomplete screening tests. While measuring levels of mortality from direct insecticide exposure is a very common screening method, possible sublethal effects as a result of either direct or indirect insecticide exposure remain relatively unknown. The impact of sublethal effects on the success of biological control can be as deleterious as mortality. Here, we report the reduced host foraging ability and longevity of the parasitoid *Microplitis croceipes* Cresson (Hymenoptera: Braconidae) after feeding on extrafloral nectar from cotton (*Gossypium hirsutum* L., Malvaceae) plants that were treated with systemic insecticides. The insecticides used in this study are regularly applied in cotton-growing areas in the United States. For all tested insecticides, longevity of *M. croceipes* females that fed on nectar from cotton was affected for at least 10 days after plants were treated with insecticides. Moreover, the parasitoid's host foraging ability was severely affected for periods ranging from 2 days (imidacloprid) to 18 days (aldicarb) after insecticide application. The consequences of these sublethal effects on the success of biological control are discussed.

Key Words: **biological control; systemic insecticides; pesticides; beneficial insects; natural enemies; *Microplitis croceipes;* parasitoid; mortality; sublethal effects; wind tunnel; flight response; longevity; IPM.**

INTRODUCTION

In integrated pest management it is important to determine which insecticides are compatible with key biological control agents and to identify the possible disruptive effects on beneficial insects. Unfortunately, predictions about the compatibility of an insecticide with biological control often are based on incomplete screening tests in which three factors are generally overlooked. (1) Beneficial insects in screening tests are usually directly exposed to the tested insecticide, thereby ignoring indirect routes of insecticide exposure that also may exist, e.g., feeding on contaminated prey (Granett and Weseloh, 1975; Wiles and Jepson, 1993; De Cock *et al.*, 1996). (2) Many tests are performed on only one developmental stage of a beneficial insect (mostly adults), while other stages may also be affected, e.g., effects of insecticide-contaminated hosts on endoparasitic larvae (Teague *et al.*, 1985; Obrycki *et al.*, 1986; Furlong and Wright, 1993; Gerling and Sinai, 1994; Jones *et al.*, 1998). (3) Mortality frequently is the only effect that insecticides are screened for, while more conspicuous sublethal effects in beneficial insects, such as altered behavior, reduced reproduction, and reduced longevity, are largely overlooked (Elzen, 1990). Like mortality, sublethal effects can severely reduce the performance of the biological control agent (Jacobs *et al.*, 1984; Elzen *et al.*, 1989; Roger *et al.*, 1995).

In contrast with nonsystemic insecticides, many systemic insecticides and their metabolites are claimed to be fairly safe for beneficial insects because direct exposure to these chemicals occurs only when insects feed on plant tissue. However, systemic insecticides can potentially contaminate floral and extrafloral nectar when systemically distributed throughout the plant (Lord *et al.*, 1968) and cause high mortality to nectar-feeding parasitoids for as long as 6 weeks after insecticide application (Cate *et al.*, 1972). Also, high mortality and severely reduced fertility were reported in honey bees (*Apis mellifera* L.) after feeding on sugar solutions containing insecticide in even lower concentrations than that found in nectar of treated onion plants (Waller and Barker, 1979). Therefore, there is a serious risk that many nontarget insects that feed on nectar and/or pollen of treated plants can be indirectly exposed to systemic insecticides.

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1049-9644/00 $35.00

Nectar is a vital food source for adult parasitoids because it contains both amino acids and sugars (Baker and Baker, 1973; Hanny and Elmore, 1974). Many adult parasitoid species feed almost exclusively on nectar (Jervis *et al.,* 1993), and without nectar parasitoids show a dramatic reduction in their ability to parasitize insect pests (Stapel *et al.,* 1997; Lewis *et al.,* 1998). Nectar can be found in floral nectaries and, in some plant species like cotton, in extrafloral nectaries, glands located outside of the flower. Extrafloral nectaries in cotton are located under the leaves in the largest midribs, on the squares between the bracts, and at the bases of bracts. These nectaries are easily accessible and the odor of this nectar is readily detected by parasitoids (Stapel *et al.,* 1997).

In this study we investigated the detrimental effects on host foraging ability and longevity of the parasitoid *Microplitis croceipes* Cresson that occur after parasitoids feed on extrafloral nectar from cotton plants treated with systemic insecticides.

MATERIALS AND METHODS

Cotton plants. The cotton plants (*Gossypium hirsutum,* variety DPL90; Delta and Pineland Co., Scott, MS) were grown in a greenhouse (25–35°C, 30–70% RH, 16:8 (L:D) h) in 1-L pots containing a mix of potting soil, peat moss (Promix; Premier Horticulture, Inc., Red Hill, PA), and fertilizer (Osmocote; Grace Sierra Co., Milpitas, CA). The cotton plants used in the experiments were 1.5 months old, with 8–10 leaves.

Parasitoids. M. croceipes (Hymenoptera: Braconidae) was reared on *Helicoverpa zea* Boddie (Lepidoptera: Noctuidae) larvae as described by Lewis and Burton (1970) and held in a climate-controlled room (25°C, 70% RH, 14:10 (L:D) h). All females used in the experiments were mated, 2 days old, and starved. The parasitoids were starved by holding newly emerged adults in cages with only water for 2 days.

Hosts. Eggs of *H. zea* were obtained from our rearing facility at the Insect Biology and Population Management Research Laboratory, USDA-ARS, Tifton, GA. The larvae were reared on an artificial pinto bean diet (Burton, 1969) in a rearing room (25°C, 70% RH, 14:10 (L:D) h). Third-instar larvae were used to damage cotton leaves for the wind tunnel experiments.

Insecticide application. Three systemic insecticides were tested: acephate (Orthene 75S; Valent, Inc., Walnut Creek, CA), imidacloprid (Provado; Bayer Corp., Kansas City, MO), and aldicarb (Temik 15G grit; Rhone-Poulenc AG Corp., Research Triangle Park, NC). Acephate and imidacloprid solutions were applied to the plants with a regulator-controlled aerial spray applicator, using CO_2 as a propellant at a constant pressure of 70 kPa. Per plant, a 5-ml solution of acephate (0.375 mg/L) or imidacloprid (1 ml/L) was sprayed. Aldicarb was mixed in the upper soil layer at a rate of 1 mg/plant. Control plants were sprayed with 5 ml of water/plant. The insecticide application methods and rates used on the cotton plants were recommended by the manufacturers.

Nectar collection. Before the cotton plants were treated with insecticides, all extrafloral nectaries were checked and nectar was removed with 10-µl pipets. After insecticide treatment extrafloral nectar was collected from all leaves every other day and stored in a freezer for a limited time (maximum 2 weeks) until parasitoids were available to conduct the experiments.

Experiments. Parasitoid flight response in a wind tunnel was used as a measure of host foraging ability. Two-day-starved *M. croceipes* females were individually allowed to feed on a 2-µl nectar droplet until they were satiated. The wasps were then individually placed in glass vials with honey/water solution using cotton to close the vials. The following day, the wasps were individually exposed to hosts and frass, allowing them to sting one host before testing their flight response in a wind tunnel. To stimulate a flight response in the wind tunnel, a cotton leaf that was damaged overnight by two third-instar *H. zea* was placed upwind and wasps were individually released 1 m downwind. A response was recorded when a flight was initiated within 5 min and the wasp finished the flight by landing on the leaf. Wasps were given three chances to complete a flight. After the wind tunnel experiments, the wasps were individually held in vials with honey/water solution to determine their longevity.

Statistical analyses. Flight response in the wind tunnel of parasitoids that fed on nectar from treated cotton plants was compared with the response of those that fed on nectar from control plants and analyzed using Fisher's Exact Test. Longevity of the parasitoid was compared in a similar manner and analyzed with *F* tests for variance followed by applicable *t* tests.

RESULTS

Flight response to host-associated odors. Only 23.5% of the *M. croceipes* females that were fed cotton extrafloral nectar collected 2 days after imidacloprid treatment responded to host-associated odors in the wind tunnel, while flight response of the control wasps was 79.2% (Fig. 1a). This reduction in response was significant ($P < 0.05$) and lasted for as long as 4 days after treatment. Nectar collected from acephate-treated cotton also caused a lower flight response of *M. croceipes* in the wind tunnel (Fig. 2a). No females were able to respond when fed nectar collected 2 days after treatment, and response was 23.1 and 60.4% when fed nectar collected 4 and 6 days, respectively, after treatment ($P < 0.05$). A flight response similar to that of control wasps was observed only in wasps that were fed

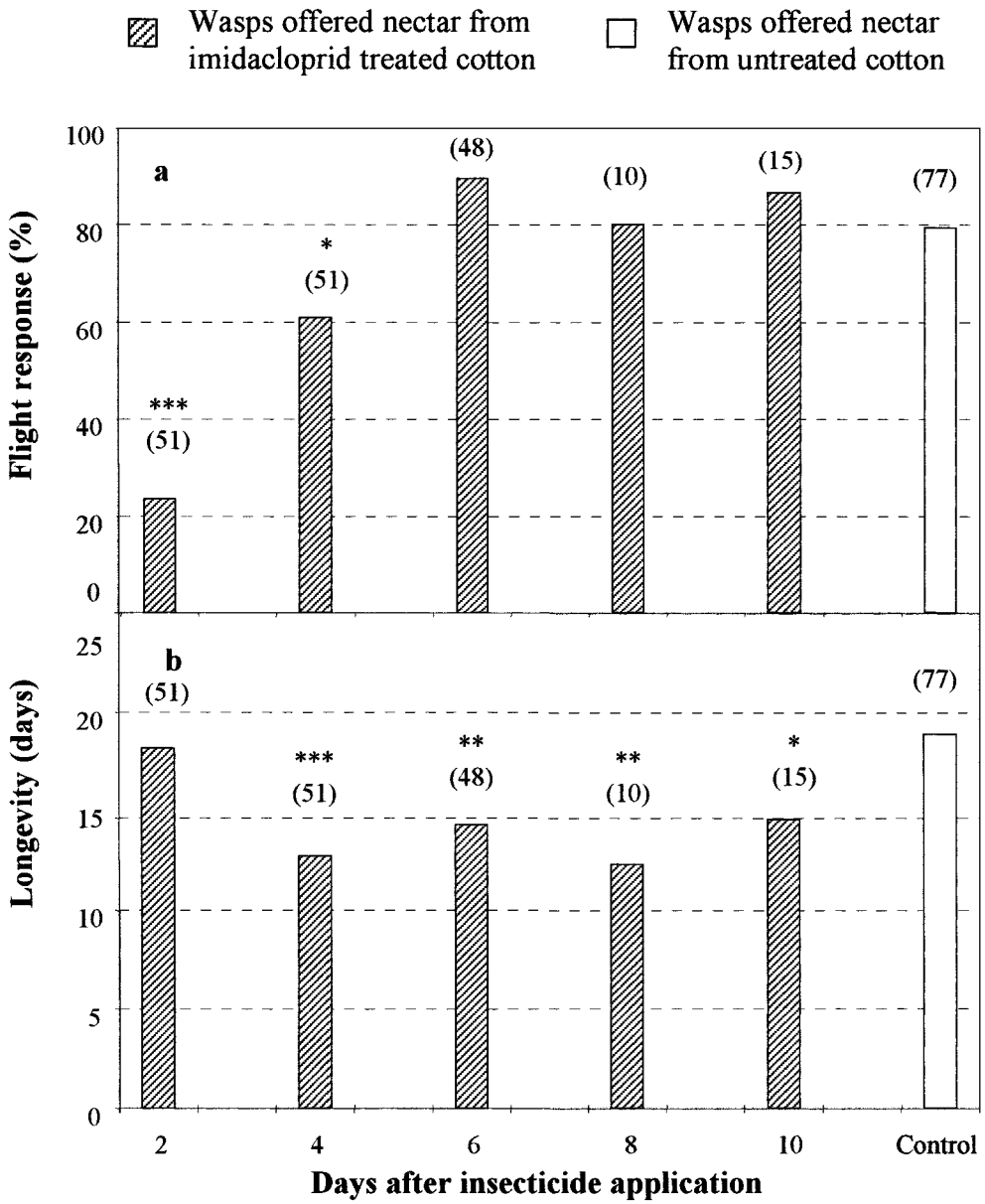

FIG. 1. Flight response to caterpillar-damaged cotton leaves in a wind tunnel (a) and longevity (b) of *Microplitis croceipes* females offered nectar from imidacloprid-treated cotton. Nectar was collected 2–10 days after insecticide application and fed to wasps. Asterisks indicate significant difference from control at levels $P = 0.05$ (*), $P = 0.01$ (**), or $P = 0.001$ (***). Numbers between brackets represent the sample sizes.

nectar that was collected 8 days or more after treatment. The most dramatic reduction in flight response was observed when wasps were fed nectar from aldicarb-treated cotton plants (Fig. 3a). The flight response of these wasps was similar to that of control wasps when fed nectar that was collected 2 days after application, but from day 4 to day 18 a sharp decline occurred. This delayed and then prolonged decline in flight response was probably caused by the application method. This was the only insecticide that was applied to the soil. On day 20, the flight response was similar to that of control wasps.

Longevity. *M. croceipes* females that fed on nectar collected between 4 and 10 days after imidacloprid treatment of the cotton plants showed a reduced longevity compared to that of control wasps ($P < 0.05$, Fig. 1b). However, nectar collected 2 days after imidacloprid treatment did not affect longevity ($P = 0.71$). Unfortunately, due to limited availability of extrafloral nectar, we were not able to determine when parasitoid longevity returned to the control level. Lower longevity also was observed in wasps that fed on nectar collected between 2 and 10 days after acephate application ($P < 0.05$, Fig. 2b) and in wasps that fed on nectar

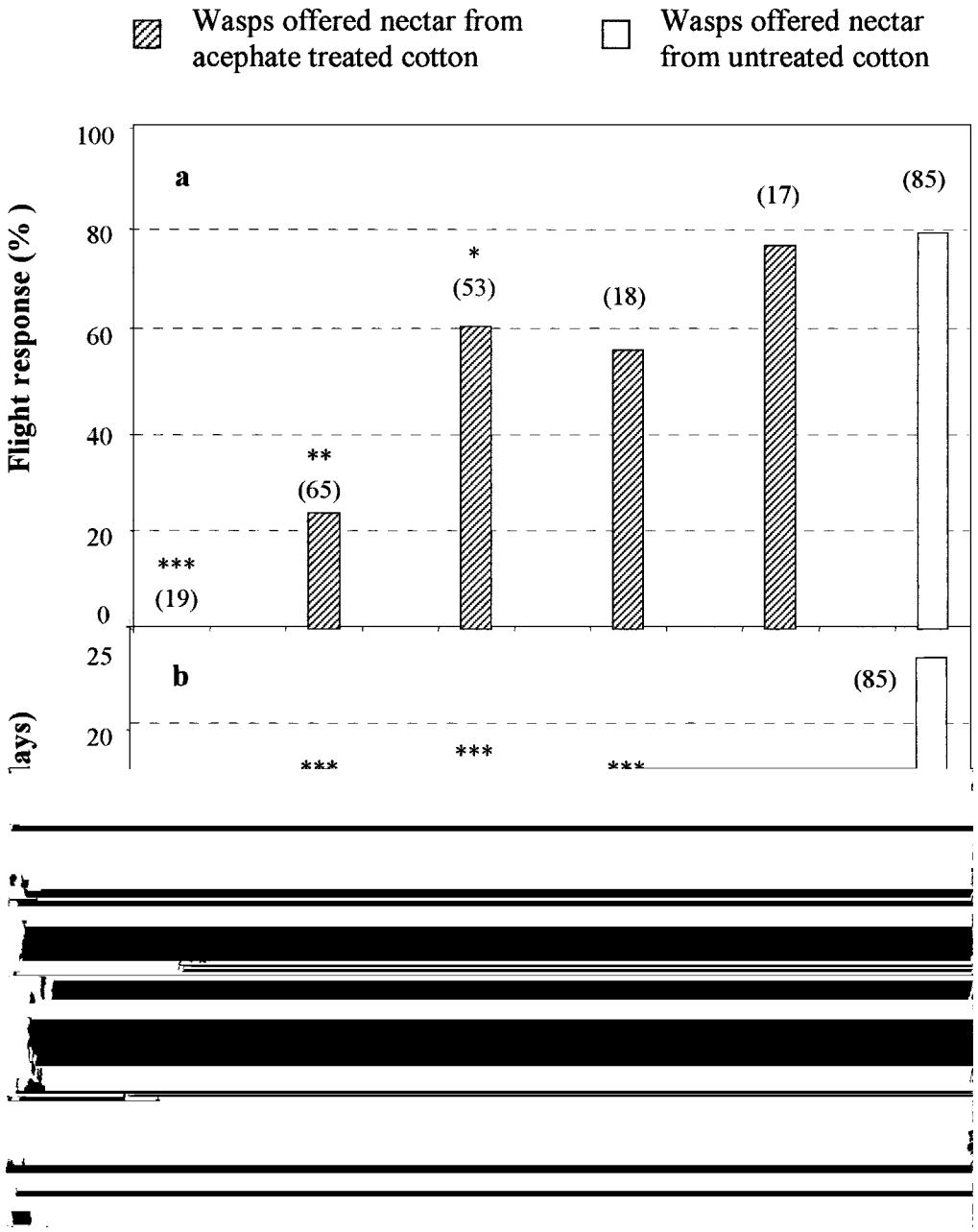

FIG. 2. Flight response to caterpillar-damaged cotton leaves in a wind tunnel (a) and longevity (b) of *Microplitis croceipes* females offered nectar from acephate-treated cotton. Nectar was collected 2–10 days after insecticide application and fed to wasps. Asterisks indicate significant difference from control at levels $P = 0.05$ (*), $P = 0.01$ (**), or $P = 0.001$ (***). Numbers between brackets represent the sample sizes.

collected between 2 and 20 days after aldicarb application ($P < 0.05$, Fig. 3b).

DISCUSSION

We have demonstrated here that, through feeding on extrafloral nectar of insecticide-treated cotton, both host foraging ability and longevity of *M. croceipes* were seriously affected for an extended period of time. In our wind tunnel experiments the three tested insecticides differentially affected the parasitoid's flight response. Nectar from imidacloprid-treated cotton reduced the flight response of this parasitoid for 4 days, acephate for as long as 6 days, and nectar from aldicarb-treated cotton had the strongest effect, lasting as long as 18 days after application. In this study, host foraging ability was measured 1 day after nectar feeding. Preliminary data indicate that parasitoids intoxicated with

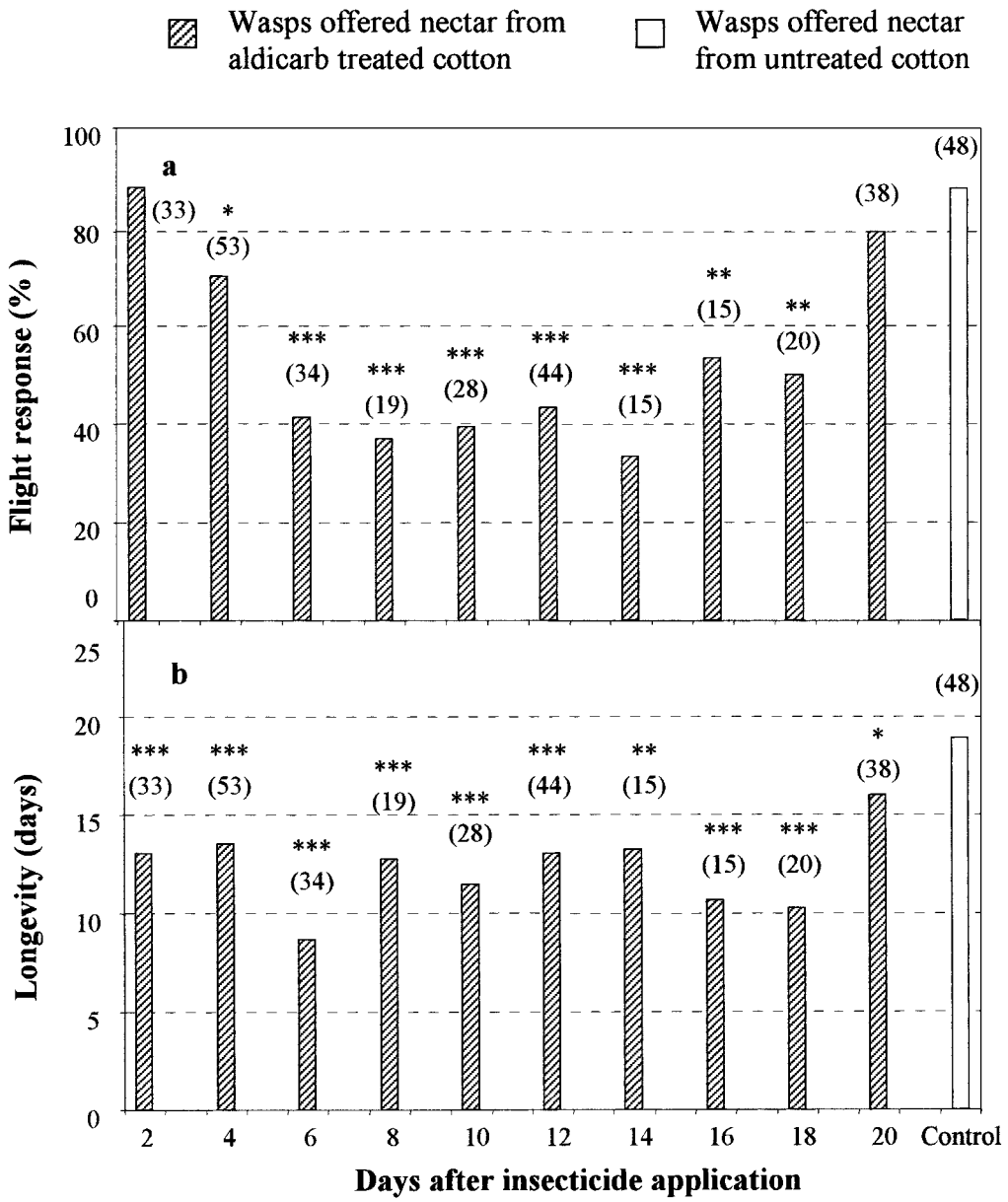

FIG. 3. Flight response to caterpillar-damaged cotton leaves in a wind tunnel (a) and longevity (b) of *Microplitis croceipes* females offered nectar from aldicarb-treated cotton. Nectar was collected 2–20 days after insecticide application and fed to wasps. Asterisks indicate significant difference from control at levels $P = 0.05$ (*), $P = 0.01$ (**), or $P = 0.001$ (***). Numbers between brackets represent the sample sizes.

imidacloprid regained their foraging ability 2 days after nectar feeding, whereas no recovery was observed in acephate- and aldicarb-intoxicated wasps (J. O. Stapel, unpublished). Parasitoid longevity was affected for at least 10 days with imidacloprid and acephate and at least 18 days with aldicarb. The insecticide application method may have influenced the duration of sublethal effects. Aldicarb was the only insecticide that was applied to the soil. Similar application of the two other insecticides might also result in longer periods of sublethal effects.

The results obtained in the present study show that some insecticides can indirectly affect the action of nectar-feeding parasitic Hymenoptera. Due to the high detectability and accessibility of cotton extrafloral nectaries (Stapel *et al.*, 1997) these glands may become serious death traps through contamination by these insecticides. Therefore, we predict that certain systemic insecticides may depress the impact of parasitoids attacking lepidopteran pests in cotton. Apparently, the adverse impact of insecticides on beneficial insects can be far more subtle than just mortality. The

more inconspicuous sublethal effects are recognized only when further investigation of an insect's biology is conducted. In other laboratory studies, for example, high mortality was not observed in *Trichogramma* spp. exposed to the insecticide esfenvalerate, but egg parasitization in treated fields was significantly reduced due to the parasitoid's ability to avoid pyrethroid-treated plants (Campbell *et al.*, 1991).

Reports dealing with sublethal insecticide effects on beneficial insects are relatively scarce. Croft (1990) reported that more than 75% of the studies from 1950 to 1986 measured mortality of beneficial insects, while median lethal dose, concentration, or time were measured in another 20% of the studies; only 5% of the studies reported sublethal effects. The sublethal effects reported mostly are reduced fecundity and longevity (Grosch, 1970, 1975; O'Brien *et al.*, 1985; Hsieh and Allen, 1986; Rosenheim and Hoy, 1988). Studies, such as the present one, that also monitor altered foraging behavior of beneficial insects as a result of insecticide exposure, are rarely performed, even though this is a key behavior for beneficial insect effectiveness. Elzen *et al.* (1989) showed a decrease in flight activity toward cotton plants by *M. croceipes* females sprayed directly with a fenvalerate/chlorodimeform mixture. Flight activity in this insect is usually considered to be an indicator of foraging efficiency. The same authors further observed that unsprayed parasitoid females were less attracted to cotton treated either with a fenvalerate/chlorodimeform mixture or with methomyl. The latter finding suggests that odors from some insecticide-treated plants may invoke avoidance behavior in beneficial insects that can potentially modify their effectiveness as biological control agents. Similar findings on insecticide-induced avoidance behavior were reported by Gu De Jiu and Waage (1990) and Umoru *et al.* (1996) in the aphid parasitoid *Diaeretiella rapae* M'Intosh and by Wiles and Jepson (1994) in *Coccinella septempunctata* L. In these studies, the behavioral effects were likely caused by both contact experience with the insecticide and insecticide odor. The question then arises whether avoidance behavior in the field will prevent *M. croceipes* from being exposed to the three insecticides that we examined in our study. In additional dual choice tests, we did not observe reduced responses to insecticide-treated cotton, nor did we notice avoidance behavior to insecticide-contaminated extrafloral nectar in *M. croceipes* females (A. M. Cortesero, unpublished). This suggests that in the field these parasitoids probably are not able to detect cotton or nectar that is contaminated with the insecticides that we tested. However, further field testing is needed to support our findings.

Sublethal effects of insecticides may ultimately cause beneficial insects to become less effective as biological control agents in the field due to their lower performance in parasitizing and preying on hosts. Therefore, in addition to mortality, an assessment of the impact of an insecticide on beneficial insects should include sublethal effects, together with information on the residual activity of insecticides, as we and others have shown that certain insecticides can cause sublethal effects on beneficial insects for many days after their application (Tipping and Burbutis, 1983). At the moment, many pesticides are regarded as highly selective while not all side effects are known. A new framework for insecticide research as described by Jepson (1989) is necessary to understand the overall impact of insecticides on multitrophic systems. Predicting the overall effects of insecticide use, including mortality and sublethal effects in beneficial insects, confirmed by field data, will enable us to develop and use truly selective insecticides that cause minimal disruption to naturally occurring biological control agents.

ACKNOWLEDGMENTS

Our thanks go to Drs. Walker A. Jones (USDA), Catherine Mannion (Tennessee State University), Charlie E. Rogers (deceased, USDA), and David Rogers (Bayer, Inc.) for reviewing earlier drafts of the manuscript.

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