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# EFFECT OF MALATHION ON BENEFICIAL INSECTS

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## ABSTRACT

Susceptibility of four natural enemies, *Geocoris punctipes* (Say), *Cotesia marginiventris* (Cresson), *Bracon mellitor* Say, and *Cardiochiles nigriceps* Vierick, to several insecticides was determined. In topical toxicity tests, malathion ultra-low-volume (ULV 95 %) was applied undiluted at 1.36 and at 1.02 kg (AI)/ha with cottonseed oil in a total volume of 1.17 liter/ha. Fipronil and cyfluthrin were applied at 0.043 and 0.037 kg (AI)/ha, respectively, plus cottonseed oil in a total volume of 1.17 liter/ha. Emulsifiable concentrate (EC) formulations of malathion and fipronil at 1.12 and 0.043 kg (AI)/ha, respectively, also were applied with water in a total volume of 46.8 liter/ha. All of the insecticides were highly toxic to all four insect species when applied topically. In 1996 insecticide residue tests, malathion at 1.02 kg (AI)/ha, fipronil at 0.056 kg (AI)/ha, and cyfluthrin at 0.037 kg (AI)/ha plus cottonseed oil were applied in a total volume of 1.17 liter/ha. Toxicity of residues of these insecticides to *C. nigriceps* and *G. punctipes* was determined at 0, 24, and 48 h after treatment (HAT). Exposure to malathion residues at 0 HAT resulted in lowest survival for the two insects. Toxicity of malathion residues decreased sharply at 48 HAT for both insect species. Cyfluthrin was less toxic than malathion at 0 HAT for both insects. Fipronil was less toxic to *C. nigriceps* than to *G. punctipes* at 24 HAT. In 1997 insecticide residue tests, undiluted malathion ULV at 1.36 kg (AI)/ha and malathion ULV at 0.85 kg (AI/ha), fipronil at 0.028 kg (AI/ha), and cyfluthrin at 0.022 kg (AI)/ha plus Orchex® 796 were applied in a total volume of 1.17 liter/ha. Residues of undiluted malathion ULV were highly toxic to the insects through 48 HAT except for *C. nigriceps*, while residues of malathion ULV at the lower rate were highly toxic to only *B. mellitor* and *C. marginiventris* at 48 HAT. Residues of fipronil were less toxic to *C. nigriceps* than to the other natural enemies 0 to 48 HAT. Residues of cyfluthrin were less toxic to all insects than the other two insecticide treatments for each time regime.

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[2] Mention of a proprietary product does not constitute a guarantee or warranty of the product by the USDA and does not imply its approval to the exclusion of other products.

## INTRODUCTION

Currently malathion is being applied ultra-low-volume (ULV) for the control of the boll weevil, *Anthonomus grandis grandis* Boheman, in the Boll Weevil Eradication Program. The uniquely designed ULV ground sprayer developed by the Agricultural Research Service researchers at Stoneville, MS permits the application of volumes as low as 500 ml/ha, which is impossible with conventional hydraulic ground sprayers (Hanks and McWhorter 1991). The ULV formulation of malathion gives better boll weevil control and has greater longevity than both azinphosmethyl and malathion emulsifiable concentrate formulations sprayed with a conventional system (Mulrooney et al. 1997). Earlier field work demonstrated that azinphosmethyl, methyl parathion, and malathion were effective in controlling damaging levels of the boll weevil (Cleveland et al. 1966, McGarr and Wolfenbarger 1968, Lloyd et al. 1972). The efficacy of fipronil and cyfluthrin has been studied to determine the possibility if using them as alternative insecticides for control of the boll weevil (Hopkins and Taft 1967, Burris et al. 1994, Mulrooney et al. 1997, Page et al. 1997, Sparks et al. 1997a).

Insecticides highly toxic to beneficial arthropods can disrupt their populations causing outbreaks of secondary pests. Falcon et al. (1971) and Eveleens et al. (1973) demonstrated that heavy outbreaks of beet armyworms could be generated by insecticide treatments used to suppress the plant bug, *Lygus hesperus* Knight in cotton in California. Ehler and Endicott (1984) determined that secondary outbreaks of three species of scales were attributed to destruction of natural enemies when malathion was used in the eradication program against the Mediterranean fruit fly in northern California. Since resurgence of key pests and outbreaks of secondary pests can occur with treatments that destroy natural enemies, the use of insecticides which are more toxic to the pest than to natural enemies could be a useful tool in an integrated pest management program. Therefore, our research goal was to determine the tolerance of four natural enemies, *Geocoris punctipes* (Say), a general predator of many lepidopteran species, *Cotesia marginiventris* (Cresson), a parasitoid of the beet armyworm, *Bracon mellitor* Say, a native parasitoid of the boll weevil, and *Cardiochiles nigriceps* Vierick, a host-specific parasitoid of the tobacco budworm, *Heliothis virescens* (F.), to some of the current and potential insecticides which can be applied ULV for boll weevil control.

## MATERIALS AND METHODS

*Insects.* *C. marginiventris* females, *B. mellitor* females, and *C. nigriceps* females used in this test were young adults (1-2 days old) reared by USDA, ARS at Mississippi State, MS. The *C. marginiventris* colony was obtained from cocoons from a colony in Tifton, GA. The *C. nigriceps* colony originated from *H. virescens* larvae collected from cotton in 1996. The *B. mellitor* colony originated from boll weevil larvae collected from cotton in 1996. Young adults of *Geocoris punctipes* were collected from an untreated cotton field at Stoneville, MS.

*Topical Toxicity Study.* The test included the following 6 treatments and rates: 1) ultra-low-volume technical malathion (Fyfanon [1.36 kg (AI)/ha], Cheminova, Lemnig, Denmark), 2) malathion ULV (95%) [1.02 kg (AI)/ha], 3) fipronil (Regent [0.043 kg (AI)/ha], Rhone-Poulenc Ag Company, Research Triangle, NC), 4) cyfluthrin (Baythroid [0.037 kg (AI)/ha], Bayer Corp., Atlanta, GA), 5) malathion EC (Malathion 5 EC [1.12 kg (AI)/ha], Micro Flo, Lakeland, FL), and 6) fipronil EC [0.043 kg (AI)/ha]. Treatments 1-4 were applied at ultra-low-volume (1.17 liter/ha) with once refined cottonseed oil as diluent for treatments 2 through 4. EC formulations of malathion and fipronil were applied with water at high volume (46.8 liter/ha). Two controls, water and cottonseed oil, were included in the test.

A laboratory spray chamber (Allen Machine Works, Midland, MI) was used to topically treat adult wasps. The spray chamber used to apply high volume treatments was equipped with a conventional spraying system that was calibrated to deliver 46.8 liter/ha, using a single TX-8 nozzle (Spraying Systems, Wheaton, IL), while maintaining 275 kPa pressure. The laboratory chamber used to apply ultra-low-volume treatments was equipped with an air-assisted ultra-low-volume spraying system (Mulrooney et al. 1997) that was calibrated to apply 16 ml/min (1.17 liter/ha). The liquid was atomized with compressed air (69 kPa) at the nozzle (Bete T-Mizer, Bete Fog Nozzle, Greenfield, MA). Height and speed of the nozzle above the spray surface were 35.6 cm and 6.4 km/h, respectively, for each spraying system.

Predator and parasitoid adult females were aspirated into new plastic petri dishes (100 x 15 mm). A chamber designed by Mulrooney and Adams (1998) was used for immobilizing insects in 6 petri dishes with $CO_2$. A treatment replicate consisted of 30 insects (5 insects per petri dish for 6 dishes). Each treatment was replicated 4 times for a total 120 insects per treatment for each species. Before the test, a hole (55 mm in diameter) was cut in the top of the petri dish and covered with organdy mesh to increase movement of the $CO_2$ into the dish. After the insects were anesthetized lightly, they were immediately placed uncovered in the spray chamber for treatment. After spraying, the insects were transferred to clean petri dishes. Sprayed insects were provided food, honey water for parasitoids and *H. virescens* eggs for the predator, and placed in an environmental chamber maintained at 25 ± 2°C, 50 ± 5% RH, and a photoperiod of 14:10 (L:D) h. After 72 h, these insects were checked for survival. Moribund insects, individuals that moved only when their legs were touched with soft forceps and did not feed on provided diet, were recorded as dead.

*Experimental Design Residual Insecticide Tests.* A John Deere 600 high-clearance sprayer was equipped with an air-assisted spray system (Mulrooney et al. 1997). The liquid was atomized with compressed air (34-55 kPa) at the nozzle (Bete T-Mizer). Transgenic Bt cotton (cultivar NuCOTN $33^B$) containing the Bollgard™ gene (Monsanto Co. St. Louis, MO) was treated when flowers and young fruit were present on plants. A randomized complete block design was used. Plots were 4 (1.02 m) rows x 60.98 m (0.0024 ha). Each treatment was replicated 4 times in 1996 and 6 times in 1997. Weather data were obtained from the National Weather Service at Stoneville, MS.

*1996 Residual Insecticide Test.* This test was conducted on July 16, 1996 and included the following treatments: 1) malathion ULV at 1.02 kg (AI)/ha, 2) fipronil at 0.056 kg (AI)/ha, 3) cyfluthrin at 0.037 kg (AI)/ha, and 4) untreated control. Total volume of the ultra-low-volume applications was 1.17 liter/ha with once-refined cottonseed oil as diluent for treatments 1-3. The minimum-maximum temperatures on the day of application were 21.1 and 32.8°C, respectively. Average minimum-maximum temperatures during the 3-d test were 20.6 and 33.7°C, respectively. There was no precipitation during the test.

*1997 Residual Insecticide Test.* This test was conducted on July 21, 1997 and included the following treatments: 1) malathion ULV at 1.36 kg (AI)/ha, 2) malathion ULV at 0.85 kg (AI)/ha 3) fipronil at 0.028 kg (AI)/ha, 4) cyfluthrin at 0.022 kg (AI)/ha, and 5) untreated control. Total volume of the ultra-low-volume applications was 1.17 liter/ha with Orchex® 796 as diluent with treatments 2-4. The minimum-maximum temperatures on the day of application were 23.4 and 34.5°C, respectively. Average minimum-maximum temperatures during the 3-d test were 23.2 and 36.0°C, respectively. Precipitation did not occur during the test. Mulrooney et al. (1997) reported residues of malathion ULV at 1.36

kg (AI)/ha on upper cotton leaves to be 11.71, 12.89, 6.27 μg/cm$^2$ at 0, 24 and 48 h after treatment, respectively. Residue analysis was determined for malathion ULV at 0.85 kg (AI)/ha for 0 h after treatment following the methods of Mulrooney et al. (1997). A residue analysis for fipronil and cyfluthrin was not developed and therefore was not done.

*Bioassays.* Bioassays for each insect species for the 1996 and 1997 residual insecticide tests were conducted in the laboratory. Cotton leaves were collected from the terminal of ten plants in each replicate at 0, 24, and 48 hours after plants were sprayed. Only upper, fully-exposed leaves were used to assure that the leaves were covered well with residue. After leaves were collected and placed individually in petri dishes, one insect was placed in a petri dish containing one treated leaf. Insects were held at at 25 ± 2°C, 50 ± 5% RH, and a photoperiod of 14:10 (L:D) h. Survival was determined 72 h after exposure to treated leaves.

*Statistical analysis.* Survival data were corrected for control mortality using Abbott's formula (Abbott 1925). Percentage survival data were converted by angular transformation and analyzed using PROC GLM (SAS Institute 1990). Means were separated by a least significant difference test (LSD) (SAS Institute1990) where appropriate.

## RESULTS

*Topical Toxicity Study.* Topical application of each insecticide resulted in low survival for all beneficial insects (Table 1).

TABLE 1. Effect of selected topically-applied insecticides on survival of *Geocoris punctipes*, *Bracon mellitor*, *Cotesia marginiventris*, and *Cardiochiles nigriceps*.

| | | Percentage Survival 72 h after treatment (Mean ± SEM)[1] | | | |
|---|---|---|---|---|---|
| Treatments | Rate[2] | *G. punctipes* | *B. mellitor* | *C. marginiventris* | *C. nigriceps* |
| Cottonseed oil | | 100 ± 0 a | 100 ± 0 a | 93.3 ± 4.2 a | 96.7 ± 3 a |
| Malathion | 1.36 | 0 ± 0 b | 0 ± 0 b | 0 ± 0 b | 0 ± 0 b |
| Malathion+CO[3] | 1.02 | 0 ± 0 b | 0 ± 0 b | 3 ± 3 b | 0 ± 0 b |
| Malathion EC | 1.12 | 0 ± 0 b | 0 ± 0 b | 0 ± 0 b | 0 ± 0 b |
| Fipronil+CO | 0.043 | 0 ± 0 b | 3 ± 3 b | 0 ± 0 b | 0 ± 0 b |
| Fipronil EC | 0.043 | 3 ± 3 b | 0 ± 0 b | 0 ± 0 b | 0 ± 0 b |
| Cyfluthrin+CO | 0.037 | 0 ± 0 b | 0 ± 0 b | 0 ± 0 b | 0 ± 0 b |

[1] Mortality is corrected for control mortality using Abbott's formula (1925). Values within a column followed by the same lower case letter are not significantly different (LSD; $P > 0.05$) between insecticides for a single species.

[2] kg(AI)/ha

[3] cottonseed oil

*1996 Residual Insecticide Test.* Exposure to malathion ULV residues resulted in low survival for both insects at 0 and 24 HAT (Table 2). Toxicity of malathion residues decreased sharply at 48 HAT for both insect species. Toxicity of fipronil residues was lower for the two natural enemies at 24 HAT compared to 0 HAT. Also, this insecticide was less toxic to *C. nigriceps* than to big-eyed bugs at 24 HAT. Cyfluthrin was less toxic than malathion at 0 HAT for both insect species. Overall, malathion was the most toxic insecticide to the natural enemies.

TABLE 2. Effect of residues of ultra-low volume cyfluthrin, fipronil, and malathion 0, 24, and 48 hours after treating (HAT) cotton leaves on survival of *Geocoris punctipes* and *Cardiochiles nigriceps*.

| | | Percentage Survival 72 h after exposure (Mean ± SEM)[1] | | |
|---|---|---|---|---|
| Species | HAT | Cyfluthrin[2] (0.037 kg/ha) | Fipronil[2] (0.056 kg/ha) | Malathion[2] (1.02 kg/ha) |
| *G. punctipes* | 0 | 35.0 ± 2.2 a, 1 | 14.0 ± 5.2 a, 1, 2 | 5.6 ± 2.1 a, 2 |
| *C. nigriceps* | 0 | 32.5 ± 4.9 a, 1 | 21.7 ± 9.8 a, 1, 2 | 0 ± 0 a, 2 |
| *G. punctipes* | 24 | 30.5 ± 4.2 a, 2 | 54.1 ± 2.2 b, 1 | 15.0 ± 3.3 a, 2 |
| *C. nigriceps* | 24 | 27.5 ± 5.7 a, 2 | 90.0 ± 4.4 a, 1 | 12.5 ± 3.7 a, 2 |
| *G. punctipes* | 48 | 90.0 ± 1.7 a, 1 | 92.5 ± 1.3 a, 1 | 97.5 ± 1.7 a, 1 |
| *C. nigriceps* | 48 | 90.0 ± 2.2 a, 1 | 100.0 ± 0 a, 1 | 100.0 ± 0 a, 1 |

[1] Mortality is corrected for control mortality using Abbott's formula (1925). Values within a column followed by the same lower case letter are not significantly different (LSD; $P >$ 0.05) between species for a single treatment for a single treatment period (0, 24, 48 HAT). Values within a row followed by the same number are not significantly different (LSD; $P >$ 0.05) between insecticides for a single species.

[2] With cottonseed oil

*1997 Residual Insecticide Test.* Exposure to residues of undiluted malathion ULV at 1.36 kg (AI)/ha 0, 24, and 48 h after being applied to cotton leaves resulted in low survival for all beneficial insects except *C. nigriceps* at 48 h after treatment (Table 3). Mean (± SE)

TABLE 3. Effect of residues of ultra-low volume cyfluthrin, fipronil, and malathion 0, 24, and 48 hours after treating (HAT) cotton leaves on survival of *Geocoris punctipes* (GP), *Bracon mellitor* (BM), *Cotesia marginiventris* (CM), and *Cardiochiles nigriceps* (CN).

| | | Percentage Survival 72 h after exposure (Mean ± SEM)[1] | | | |
|---|---|---|---|---|---|
| Species | HAT | Cyfluthrin[2] (0.022 kg/ha) | Fipronil[2] (0.028 kg/ha) | Malathion[2] (0.85 kg/ha) | Malathion (1.36 kg/ha) |
| GP | 0 | 48.0 ± 2.0 b, 1 | 0 ± 0 b, 2 | 0 ± 0 a, 2 | 0 ± 0 a, 2 |
| BM | 0 | 41.7 ± 8.3 b, 1 | 0 ± 0 b, 2 | 0 ± 0 a, 2 | 0 ± 0 a, 2 |
| CM | 0 | 67.8 ± 11.5 a, 1 | 0 ± 0 b, 2 | 0 ± 0 a, 2 | 0 ± 0 a, 2 |
| CN | 0 | 81.7 ± 6.0 a, 1 | 47.2 ± 5.7 a, 2 | 5.0 ± 2.2 a, 3 | 0 ± 0 a, 3 |
| GP | 24 | 82.0 ± 3.7 b, 1 | 41.9 ± 11.3 b, 2 | 10.0 ± 1.7 b, 3 | 0 ± 0 a, 3 |
| BM | 24 | 81.7 ± 4.8 b, 1 | 13.3 ± 4.9 c, 2 | 3.3 ± 2.1 b, c, 3 | 0 ± 0 a, 3 |
| CM | 24 | 100 ± 0 a, 1 | 3.3 ± 3.3 c, 2 | 0 ± 0 c, 2 | 0 ± 0 a, 2 |
| CN | 24 | 100 ± 0 a, 1 | 100 ± 0 a, 1 | 20.0 ± 1.7 a, 2 | 0 ± 0 a, 3 |
| GP | 48 | 95.0 ± 2.2 a, 1 | 49.8 ± 8.5 b, 2 | 41.7 ± 7.2 b, 2 | 0 ± 0 b, 3 |
| BM | 48 | 92.0 ± 4.9 a, 1 | 40.0 ± 10.9 b, 2 | 0 ± 0 c, 3 | 0 ± 0 b, 3 |
| CM | 48 | 100 ± 0 a, 1 | 3.3 ± 3.3 c, 2 | 1.7 ± 1.7 c, 2 | 0 ± 0 b, 2 |
| CN | 48 | 100 ± 0 a, 1 | 100 ± 0 a, 1 | 80.0 ± 2.6 a, 2 | 20.0 ± 1.7 a, 3 |

[1] Mortality is corrected for control mortality using Abbott's formula (1925). Values within a column followed by the same lower case letter are not significantly different (LSD; $P >$ 0.05) between species for a single treatment for a single treatment period (0, 24, 48 HAT). Values within a row followed by the same number are not significantly different (LSD; $P >$ 0.05) between insecticides for a single species.

[2] With Orchex®.

residue on the surface upper leaves treated with malathion ULV at 0.85 kg (AI)/ha for 0 h after treatment was 2.5056 μg/cm$^2$, exposure to residues of malathion at this rate for this time regime resulted in low survival for all insects. Residues of this insecticide at 24 h after treatment were highly toxic for all insect species except *C. nigriceps*. Exposure to residues of malathion at the lower rate resulted in high survival for *C. nigriceps*, moderate survival for *G. punctipes*, and low survival for *B. mellitor* and *C. marginiventris* at 48 h after treatment. Generally, residues of fipronil were less toxic to *C. nigriceps* than to the other three beneficial insects. Residues of this insecticide were less toxic to *G. punctipes* than to *B. mellitor* and *C. marginiventris* at 24 h after treatment. Fipronil residues were highly toxic to *C. marginiventris* through 48 h after treatment. Exposure to residues of cyfluthrin resulted in equal survival for *G. punctipes* and *B. mellitor* and greater survival for *C. nigriceps* and *C. marginiventris* in comparison to the other two natural enemies at 0 and 24 h after treatment. Cyfluthrin was not very toxic to any of the insect species at 48 h after treatment. The general trend for the four natural enemy species from the most toxic to the least toxic insecticide was malathion at the high rate, malathion at the low rate, fipronil, and cyfluthrin.

## DISCUSSION

High levels of toxicity of malathion to natural enemies in a wide range of insect families have been well documented in both topical and residual toxicity tests (Burke 1959, Bartlett 1963, 1964, Elsey and Cheatham 1976, Lingren et al. 1972, Niemczyk and Flessel 1975, Wilkinson et al. 1975, Powell et al. 1986, King et al. 1993, Ruberson et al. 1993, Easwaramoorthy and Bai 1994, Mani 1994). England et al. (1997) conducted a study similar to our residual toxicity study, which compared the impact of malathion ULV on the boll weevil and selected natural enemies. Malathion ULV was equally toxic to boll weevil adults, *C. marginiventris* adults, *Orius insidiosus* (Say) adults, and *Hippodamia convergens* Guérin-Meneville adults. Our study corroborates their finding that residues of malathion were highly toxic to *C. marginiventris*. Residual studies conducted by Elzen et al. (1998) using plants treated with malathion ULV and fipronil in the laboratory resulted in lower mortality for natural enemies for malathion ULV than found in this study, but similar mortality for natural enemies with fipronil in comparison to our study. The differences observed for malathion between the two tests may be due to differences in application methodology. We can conclude, however, that natural enemies are very susceptible to malathion ULV in topical and residual toxicity tests.

Other researchers have reported that aerial applications of malathion ULV adversely affect natural enemy populations in cotton fields. Aerial applications of azinphosmethyl, oxamyl, endosulfan, fipronil, and malathion ULV were evaluated for the impact of the insecticides on beneficial arthropods in cotton by Sparks et al. (1997b). Immediate suppression of beneficial arthropod populations occurred after the second application of these insecticides. These authors concluded that all of these insecticides were equally likely to cause disruptions of beneficial arthropods in a boll weevil eradication program. Leggett (1992) also reported that predators were reduced for two weeks when malathion ULV was applied aerially. Since it is unlikely that any boll weevil insecticide currently being used or considered for use against the boll weevil will not disrupt natural enemy populations, establishment of refuges for natural enemies may be a good approach to conserve these natural enemies. *Grindelia squarrosa* (Pursh) Dunal var. *nuda* and *Datura stramonium* L., plants which harbor alternate hosts of *B. mellitor*, grow during the cotton season in Texas (Tillman 1985) and could possibly be planted or preserved to provide refuges for this parasitoid. Tobacco can be planted to provide an excellent refuge for *C. nigriceps* (Tillman unpublished data). Alfalfa interplanted in a mixed-culture experiment has been shown to serve as a refuge for *Geocoris* spp. (Tamaki and Weeks 1972).

Even though natural enemies are generally susceptible to malathion, some are relatively tolerant and resistant. The predators *H. convergens*, *Nabis americoferus* Carayon, and *Chrysoperla carnea* Stephens have been reported to be less vulnerable to malathion than their aphid host (Hamilton and Kieckhefer 1969). Zeleny (1965) reported that *Coccinella septempunctata* L. also has greater tolerance to malathion than two aphid host species. In contrast, the predator, *C. carnea*, and the parasitoids, *Praon abjectum* (Haliday) and *Lysiphlebus fabarum* (Marshall) were less tolerant to malathion than the aphid host. Baker et al. (1995) determined that two field strains of *Bracon hebetor* Say were more resistant to malathion than a laboratory strain. Our data have shown that the parasitoid *C. nigriceps* is more tolerant to malathion than the other three beneficial insects. Field strains of *C. nigriceps* should be collected and compared to a long-standing laboratory colony of this insect to determine if this parasitoid has developed resistance to malathion in the field.

Parker and Huffman (1997) reported that cyfluthrin was one of the more effective insecticides in reducing boll weevil damage compared with azinphosmethyl, fipronil, oxamyl, endosulfan, and methyl parathion. Our residual studies indicate that cyfluthrin is not as toxic as fipronil and malathion for any of the five insects studied. In the laboratory, insects that were exposed to cyfluthrin stopped feeding and could barely move, but it generally took them longer to die. Under field conditions, insects exposed to cyfluthrin would probably die quickly or be easy prey for predators such as fireants. The sublethal effects of cyfluthrin need to be studied in the laboratory and mortality under field conditions should be examined to fully understand the effect of this insecticide on pest and natural enemy species.

*C. nigriceps* is somewhat tolerant to fipronil and cyfluthrin and less so to malathion, and *C. marginiventris* is somewhat tolerant of cyfluthrin. Using the less toxic insecticides to conserve these natural enemies could be very important in an integrated pest management program designed to control the tobacco budworm and the beet armyworm. Field population studies need to be conducted in order to fully evaluate the effect of these insecticides on parasitoid populations and on cotton production.

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