

Toxicity of Emamectin Benzoate Foliar Dislodgeable Residues to Two Beneficial Insects

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The persistence and residual contact toxicity of foliar dislodgeable emamectin benzoate residues to *Apis mellifera* (L.) and *Diglyphus isaea* (Walker) were studied following applications to foliage at maximum anticipated labeled use rate (0.0168 kg of active ingredient/ha). Bee mortality from contact exposure to dislodgeable alfalfa residues aged for 3, 8, and 24 h was 100, 46, and 3%, respectively. This contact toxicity was directly correlated with the magnitude of dislodgeable residues (9.1, 3.6, and 1.3 ng/cm², respectively), with a dissipation half-life of about 10 h. Similarly, *D. isaea* mortality from contact exposure to dislodgeable residues aged for 0.5, 1.0, 3.5, 35, and 60 h was 75, 65, 50, 20, and 15%, respectively; these residues (5.2, 5.0, 4.5, 1.4, and <0.2 ng/cm², respectively) had a dissipation half-life of about 15 h. Therefore, these beneficial insects are expected to survive and colonize treated crops within relatively short intervals (≤ 24 h) after applications of emamectin benzoate.

Keywords: Avermectin; foliar dislodgeable residues; beneficial insects; residual contact toxicity; emamectin benzoate

INTRODUCTION

Avermectins are a class of macrocyclic lactones produced by the soil actinomycete *Streptomyces avermitilis* (Burg et al., 1979). The natural avermectin product, abamectin, is currently registered in the United States and worldwide as an insecticide/miticide. Emamectin benzoate, or MK-0244 [4''-(*epi*-methylamino)-4''-deoxy-avermectin B₁ benzoate or MAB₁ benzoate], is a semi-synthetic epimethylamino derivative of abamectin and consists of two avermectin homologues, each with a molecular mass of approximately 900 Da. By content specification, these homologues are present in a ratio of $\geq 90\%$ 4''-(*epi*-methylamino)-4''-deoxy-avermectin B_{1a} (MAB_{1a}) benzoate and $\leq 10\%$ of 4''-(*epi*-methylamino)-4''-deoxy-avermectin B_{1b} (MAB_{1b}) benzoate. These homologues differ by only an additional methylene group on the isobutyl side chain of the B_{1a} component (Figure 1). Emamectin benzoate is an effective broad-spectrum lepidopteran insecticide (Dybas et al., 1989; Trumble et al., 1987) and is currently under development by Merck Research Laboratories for use on a number of crops including celery, lettuce, cole crops, and tomatoes.

The fate of emamectin benzoate on lettuce and other crop foliage has been studied, and multiple residual products including 4''-deoxy-4''-*epi*-(*N*-formyl)avermectin B₁ (FAB₁), 4''-deoxy-4''-*epi*-(*N*-formyl-*N*-methyl) aver-

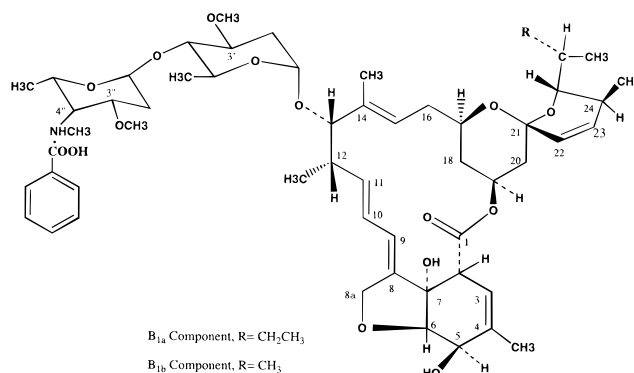


Figure 1. Structure of emamectin (MAB₁) benzoate showing major and minor homologues.

mectin B₁ (MFB₁), 4''-deoxy-4''-*epi*-amino-avermectin B₁ (AB₁), and 8,9-(*Z*)-4''-deoxy-4''-*epi*-methylamino-avermectin B₁ (8,9-ZMAB₁) were found (Crouch and Feely, 1995; Wrzesinski et al., 1996). There is, however, little published information available on the relationship between the persistence of dislodgeable emamectin benzoate residues on crop foliage and the residual toxicity of these surface residues to beneficial insects. Residual toxicity tests were therefore conducted on alfalfa and celery, respectively, with the beneficial hymenopteran insects honey bee [*Apis mellifera* (Linnaeus)] and *Diglyphus isaea* (Walker). The honey bee is perhaps the most widely known and economically important beneficial insect and, in the United States, is distributed in all 50 states (Grout, 1946; Pellet, 1946). Of all the pollinating insects known to man, the honey bee is the most widespread and easily managed. For this reason, in certain parts of the world and in the case of certain crops, agriculture has become dependent on honey bees. In the United States alone, honey bees

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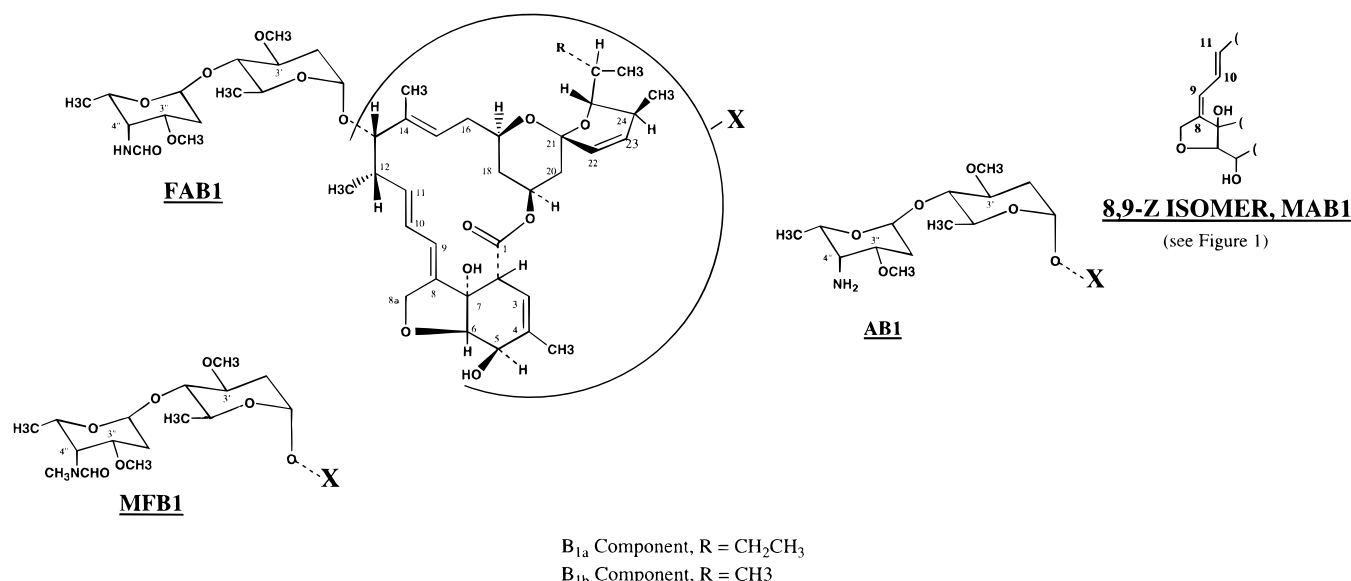


Figure 2. Structure of emamectin benzoate-related avermectin standards.

pollinate about a third of the crop plants (Simpson and Ogorzaly, 1995), with current annual dollar values estimated in the tens of billions. *D. isaea* is a parasitic hymenopteran insect (Eulophidae family) distributed throughout the Palearctic region and, more specifically, the eastern United States and California. Along the eastern U.S. coast it has been released in large numbers against *Agromyza frontella* (Rondani). It is an economically important natural enemy of many Agromyzidae and Lepidoptera (Lyontiidae) species (LaSalle and Parrilla, 1991). By determining the persistence and residual toxicity of dislodgeable emamectin benzoate residues on leaf surfaces to these two beneficial insects, we can better understand the overall environmental safety and ecological selectivity following typical uses. From this understanding, the integration of emamectin benzoate with biological control and other methods of pest management can be achieved on labeled crops.

MATERIALS AND METHODS

Chemicals. The test compound, emamectin benzoate, was prepared at Merck Research Laboratories, Rahway, NJ, with purity of about 97.5%. For applications to alfalfa foliage, it was mixed with an EC formulation blank and diluted with water. For applications to celery foliage, an emamectin benzoate 0.16 EC formulation plus Leaf Act 80A surfactant (Spreader Activator; Crop Protection Services, Tulsa, OK) diluted with water was used. In addition to emamectin benzoate, standards used for the HPLC determination of dislodgeable residues were FAB₁, MFB₁, AB₁, and 8,9-ZMAB₁. These latter standards were also prepared at Merck Research Laboratories in Rahway, NJ, and their structures are shown in Figure 2.

Environmental Conditions. Weather conditions during applications and leaf harvests were monitored at the field sites. Temperature and relative humidity were measured using a hand-held hygrometer, while precipitation was monitored with a rain gauge. In addition, complete weather data including wind speed and direction, solar irradiance, cloud cover, and maximum, minimum, and average daily temperatures were also collected and recorded on a Campbell Scientific CR10 DataLogger.

Foliar Applications. *On Alfalfa.* To provide aged residues for honey bee exposure, a single application of emamectin benzoate at 0.0168 kg of active ingredient (ai)/ha (the maximum anticipated single application rate) was made to mature alfalfa foliage growing in different plots at preharvest intervals

(PHI) of 3, 8, and 24 h. These three plots (~0.84 m²/plot) were arranged in a randomized complete block design, and the applications were made with a CO₂-assisted hand-held boom calibrated to spray a water volume of 32 mL/plot (~380 L/ha). The first application (24 h PHI) was made on September 15, 1993 (2:50 p.m. local time). The second (8 h PHI) and third (3 h PHI) applications were both made on September 16, 1993 at 6:45 and 11:47 a.m. local time, respectively. Control alfalfa growing in replicate plots (~0.84 m²/plot) was sprayed with deionized water, 32 mL/plot, and maintained concurrently with the treated samples. As in treated plots, the control alfalfa was mature at application. Each plot was encircled with plastic sheeting during application to serve as a wind-block and to minimize spray drift. The average hourly solar irradiances, in Easton, MD, on the two application days, September 15 and 16, 1993, were 213.4 and 42.1 W/m², respectively. At 24, 8, and 3 h before harvest, the hourly solar irradiances were 632, 30, and 101.5 W/m², respectively. The lower irradiance values on September 16, 1993, are consistent with the overcast weather conditions during the 8 and 3 h PHIs and at alfalfa leaf harvest. A light precipitation (0.075 cm) occurred at the 3 h PHI, thereby necessitating the use of tarpaulins placed at 30 cm above the canopy. This tarpaulin placement allowed the foliar residues to age for an additional 3 h without rain wash-off; it also further reduced the light reaching the plots. The relative humidities were 60, 94, and 97%, respectively, at the 24, 8, and 3 h PHIs.

On Celery. Two applications of emamectin benzoate at 0.0168 kg of ai/ha per application and ~380 L of H₂O/ha were made at a 7 day interval together with the surfactant Leaf Act 80A at 584 mL/ha per application. Each application was conducted with a calibrated, tractor-mounted, boom sprayer with eight Teejet 8002 (Spraying Systems Co., Wheaton, IL) nozzles. The treated plot consisted of eight beds, and each bed was subdivided into four equal subplots. The subplots were each separated by 6.1 m buffer zones. The nontreated plot consisted of four beds similarly subdivided into four equal subplots separated from each other by about 3 m buffer zones. To prevent spray drift from reaching the nontreated plot, the nontreated plot was located 62.5 m up-wind from the treated plot. Weather conditions in California at the time of the second emamectin benzoate applications on July 14, 1993, were 17.8 °C, 58% relative humidity (RH), and 50% cloud cover. Within 0.5 h of the second application, the morning fog and clouds dissipated and the sky became sunny. The hourly average irradiance on July 14 was 289.4 W/m², and the average ambient temperature and RH were, respectively, 15.6 °C and 84%. The first trace of rainfall after the second application was recorded 7 days later. The celery was at or near commercial maturity at the time of the second applica-

tion. These application rates and spray intervals correspond to maximum anticipated uses and would lead to the maximum anticipated foliar dislodgeable residues of emamectin benzoate on crops.

Dislodgeable Residue Analysis. Alfalfa leaves, with mean single-sided surface areas of about 67 cm²/g, as measured with a vernier caliper, were manually collected at intervals of 3, 8, and 24 h after emamectin benzoate application and analyzed for the presence of foliar dislodgeable residues. For celery, leaf disks were collected at about 1, 2, 4, 6, 24, 72, 168, and 336 h after the second application to determine dislodgeable foliar residues. Following methods described by Payne et al. (1994), samples of the whole alfalfa leaves and celery leaf disks were rinsed twice with 0.002% Triton X-100 aqueous solution and the rinsates, containing MK-0244, FAB₁, MFB₁, AB₁, and 8,9-ZMAB₁, partitioned into ethyl acetate. After solid phase extraction and cleanup on a propylsulfonic (PRS) column, the neutral (MFB₁) and ionizable (MK-0244, AB₁, 8,9-ZMAB₁) residues were derivatized with trifluoroacetic anhydride in the presence of *N*-methylimidazole. The derivatized samples and controls, together with authentic standards, were analyzed by reversed phase HPLC (Chromegabond ODS, 5 μm, 4.6 × 250 mm; mobile phase of 7% MeOH in H₂O, isocratic) with fluorescence detection (Shimadzu Model RF-551). The foliar dislodgeable residue values were validated by concurrent analyses of control alfalfa and celery leaf rinsates fortified with emamectin benzoate and related standards.

Insect Bioassays. *A. mellifera*, *Honey Bee*. Honey bees used in this experiment were obtained from Wildlife International Ltd., Apiary, Easton, MD. Samples of treated and control alfalfa from each plot were harvested at the same time and separately taken to the laboratory, in Nalgene bottles at about 0 °C, where they were chopped and ~15 g added to each test chamber. The test chambers were disposable rolled paper containers measuring approximately 573 cm² (9 cm diameter × 9 cm height). Each chamber was covered with a disposable plastic Petri dish, about 10 cm in diameter, through which a 20 mL glass vial containing deionized water was inserted; each vial opening was covered with gauze to prevent leakage. A sponge was also inserted through the top of the chamber cover, and it was moistened with deionized water to increase humidity within the test chamber. Worker honey bees (1–6 days old) were immobilized with nitrogen and randomly distributed among the test chambers; ~50 per container and 18 chambers per treatment. A total of about 3600 honey bees, including 900 exposed to untreated foliage, were tested. Honey bees were placed on top of the chopped foliage. To facilitate contact exposure to foliar dislodgeable residues, granular sugar was placed at the bottom of each test chamber, such that the bees had to crawl through the treated foliage to feed on the sugar. Except during observation, the test chambers were kept in darkness and maintained at an average temperature and RH of 33.5 °C (range of 33.4–33.6 °C) and 60% (range of 43–74%), respectively, during the 24 h exposure period. At 24 h, mortality was determined by removing honey bees from the test chambers and probing for movement and other signs of life.

D. isaea. Approximately 4-day-old adult parasitoids were obtained from Koppert Inc. (Berkel en Rodenrijs, Netherlands). At 0 (~15 min), 1, 2, 4, 6, 24, and 72 h after the second emamectin benzoate application (July 14, 1993), 1.8 cm diameter celery leaf disks were collected in 500 mL Nalgene bottles by punching a single disk from each of eight leaves collected per subplot in four subplots of the treated and untreated plots. Leaves were collected at random from the upper canopy. Bottles with leaf disks were immediately transferred to the laboratory in a cooler at about 0 °C. From each bottle, four leaf disks were placed in each of two 15 mL glass vials with 10 adults per vial. For the 0, 1, 2, 4, 6, 24, and 72 h post-second-treatment intervals, a total of about 280 adults were tested with no attempt to sex adults; however, historical data from the supplier, Koppert Inc., indicated the sex ratio is typically ~50:50 at pupal eclosion. Vials were held at room temperature (25–28 °C) during insect exposure. Relative humidity inside the vials was estimated at 80–90% since leaf turgor remained high during the exposure period.

Adults were provided with 10% sugar water to maintain survival. Adults exposed to untreated leaf disks were also kept under the same conditions. Mortality was evaluated at 48 h after exposure; adults that could not upright themselves after probing were recorded as dead. *D. isaea* mortality data were subjected to probit analysis (SAS Institute, 1989) after correction for control mortality (Abbott 1925). Mortality data were subsequently compared with the amount of foliar dislodgeable residues on celery.

RESULTS AND DISCUSSION

Biological Activity of Foliar Dislodgeable Residues. *Honey Bee on Alfalfa Leaves*. Nearly all (896 of 900) of the honey bees died after a 24 h exposure to 3-h-old surface residues of emamectin benzoate on alfalfa. On residues that were 8 h old, only 46% (415 of 900) of the honey bees died during the same exposure period. For honey bees exposed to 24-h-old residues, the percent mortality, 3% (29 of 900), was the same as that seen with control foliage samples. Regardless of the age of foliar dislodgeable residues, all surviving honey bees were normal in appearance.

D. isaea on *Celery Leaves*. The corrected percent mortalities of *D. isaea* adults after 48 h exposure to field-applied emamectin benzoate residues field-aged for intervals of approximately 0.5, 1, 3.5, 35, and 60 h were calculated to be 75, 65, 50, 20, and 15%, respectively [probit analysis: $n = 471$, slope = -0.36 , $LT_{50} = 3.6$ (FL = 1.5–10.5), Pearson chi-square = 0.006]. Mortality in *D. isaea* adults exposed to untreated celery leaves was about 15%. Similar to the results with honey bees, all surviving *D. isaea* adults were normal in appearance. Similar studies with 13 other beneficial organisms also indicate that the sensitivity of *D. isaea* was related to the amount of foliar dislodgeable emamectin benzoate residues, requiring about a 36 h aging period after application before complete or nearly complete (≥80%) population survival was observed (D. L. Cox, unpublished data, Merck Research Laboratories).

The graphical relationship between honey bee and *D. isaea* survival and the magnitude of foliar dislodgeable residues is presented in Figure 3. By 24 h, the majority of honey bee and *D. isaea* adults survived the exposures to dislodgeable emamectin benzoate residues.

Magnitude of Dislodgeable Residues. Untreated alfalfa and celery leaf rinsates fortified with emamectin benzoate and the related standards and analyzed by HPLC led to recoveries of about 99% (MK-0244) and 80% (FAB₁, MFB₁, AB₁ and 8,9-ZMAB₁), respectively. Since the foliar dislodgeable residues consisted of MK-0244, FAB₁, MFB₁, AB₁, and 8,9-ZMAB₁ (Payne et al., 1994), the results are expressed as *total residues* (in ng/cm²).

Alfalfa. The emamectin benzoate EC aqueous mixtures applied to the 24, 8, and 3 h PHI plots had measured values approximating 93, 97, and 98%, respectively, of nominal. Foliar dislodgeable total residues after 3, 8, and 24 h aging periods were determined to be 9.1, 3.6, and 1.3 ng/cm², respectively, resulting in a calculated dissipation half-life, by regression analysis, of about 10 h. Thus, a rapid depletion of the foliar dislodgeable residues occurred in spite of cloudy conditions and tarpaulin screens that likely decreased the photolytic depletion rate relative to that in full sunlight.

Celery. Similarly, selected samples of emamectin benzoate aqueous mixtures applied to celery had quantitative values, approximating 90%, or more, of nominal. Upper canopy leaves collected at ~0.25, 0.5, 4, 24, and 72 h after the second emamectin benzoate application

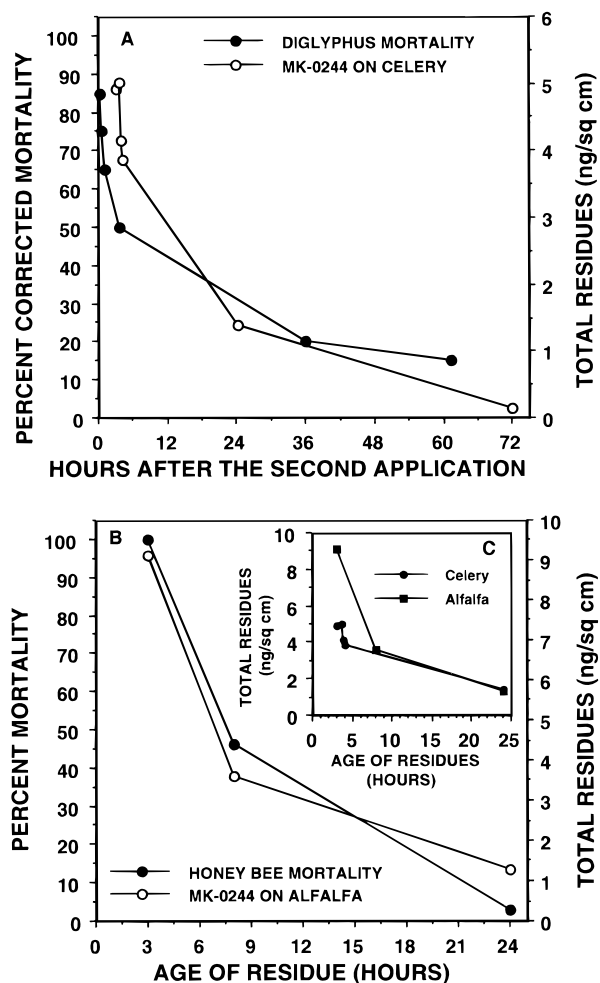


Figure 3. Mortality of *D. isaea* adults on celery (A) and honey bee workers on alfalfa (B) after a 48 or 24 h exposure to field-aged residues of emamectin benzoate (celery, MK-0244, 2×0.0168 kg of ai/ha; alfalfa, MK-0244, 1×0.0168 kg of ai/ha), respectively, compared with the foliar dislodgeable residues of emamectin benzoate. (For celery, mortality and foliar dislodgeable residue values were determined after two applications of emamectin benzoate; for alfalfa, there was only a single application. The treatment-related mortality in treated alfalfa was not corrected for control mortality, which was 3%.) Insert C shows the foliar dislodgeable residues on celery and alfalfa for 0–24 h after treatment.

and analyzed for foliar dislodgeable total residues averaged about 5.2, 5.0, 4.5, 1.4, and <0.2 ng/cm², respectively. No foliar dislodgeable residues were measurable after 72 h, and the dissipation half-life of foliar dislodgeable emamectin benzoate residues on celery was calculated, by regression analysis, to be about 15 h.

Thus, in spite of differences in application rate and frequency (1×0.0168 kg of ai/ha for alfalfa vs 2×0.0168 kg of ai/ha for celery), geographical location, and weather conditions at the time of application (alfalfa, Maryland, cloudy; vs celery, California, sunny), the half-lives of foliar dislodgeable residues on the two crops are comparable ($t_{1/2} \sim 10$ h for alfalfa vs ~ 15 h for celery). These results, presented in Figure 3C, demonstrate that foliar dislodgeable residues of emamectin benzoate are rapidly depleted from crop foliage during the first 24 h.

It has also been reported that foliar dislodgeable residues of the chemically related abamectin rapidly dissipate to levels which are nontoxic to honey bees within 1–2 days following applications (Wislocki et al., 1989). For the rapidly dissipating foliar dislodgeable residues of organophosphates, carbamates, and pyre-

throids, their average half-lives, 2.5 ± 2.8 , 2.3 ± 2.3 , and 4.9 ± 2.3 days, respectively, are also comparable to that of emamectin benzoate (Willis and McDowell, 1987). These results, therefore, indicate that foliar dislodgeable residues of avermectins (e.g., emamectin benzoate and abamectin) have relatively very low persistence on leaf surfaces.

Conclusions. On both alfalfa and celery leaves, a direct relationship was found between a decline in foliar dislodgeable residue levels and reduced toxicity to honey bee and *D. isaea* adults. The results of this study also demonstrate that foliar dislodgeable residues of emamectin benzoate, following maximum anticipated label applications, are rapidly depleted and thus unavailable to insects by contact. This rapid depletion or unavailability of foliar dislodgeable residues indicates that other beneficial arthropods with similar physiologic responses to emamectin benzoate on alfalfa and celery foliage will be exposed to toxic doses for only very brief periods (24 h or less) after application. Since toxicity was shown to be primarily due to the magnitude of foliar dislodgeable residues, it is also probable that similar brief exposures to toxic levels of dislodgeable residues will also apply to other crop foliage besides alfalfa and celery. During these exposures to foliar dislodgeable residues of emamectin benzoate, both honey bee and *D. isaea* adults were held in artificial confinement, thereby maximizing their contacts with the foliar dislodgeable residues. Under unrestricted field conditions, contact with treated foliage would be for much more limited periods of time since the insects will occasionally forage beyond the treated test zone(s). Additionally, these dislodgeable residues on leaves held in confinement were not subjected to the weathering effects of open-field wind, photolysis, irrigation, and rain or to normal watering/irrigation in a glass house. These environmental factors would have resulted in lower insect mortality, relative to the values found in this study. However, despite the artificial confinements created in this study, these beneficial organisms are expected to survive and colonize treated plots within 24 h after foliar treatment with emamectin benzoate. Because emamectin benzoate can penetrate leaf surfaces (Crouch and Feely, 1995) and thus provide residual control of lepidopteran pests, this second-generation avermectin is a good candidate for integration with biological control and other pest management practices that conserve natural enemies.

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