Tobacco Budworm, *Heliothis virescens* (Lepidoptera: Noctuidae): Larval Location and Mortality on *Bacillus thuringiensis*-Treated Cotton[1]

J. L. Jyoti[2], S. Y. Young, D. T. Johnson and R. W. McNew

Department of Entomology and Agricultural Statistics Lab, University of Arkansas, Fayetteville, AR 72701, USA

J. Entomol. Sci. 34(4): 426-434 (October 1999)

Abstract One-d-old *Heliothis virescens* (F.) larvae were placed on *Bacillus thuringiensis* var. *kurstaki* Berliner-treated cotton leaves and terminals in the laboratory. Larval movement, food consumption and mortality were examined at 3, 6, 12, 24 and 48 h. Untreated leaves had a greater percentage of larvae on their under side than on their upper side; this location was not influenced by treatment with *B. thuringiensis.* When *B. thuringiensis* was applied to cotton terminals, first instars moved from *B. thuringiensis*-treated meristems; this movement increased with an increase in rate. *Bacillus thuringiensis* treatment also resulted in increased movement of larvae from the leaves and terminals onto the inner cup surface, although this movement was significantly greater than the untreated control only during the first 12 h after treatment. The leaf area consumed decreased with an increase in *B. thuringiensis* rate, but was not significantly correlated with larval mortality.

Key Words Insecta, *Heliothis virescens,* cotton, *Bacillus thuringiensis.*

The tobacco budworm, *Heliothis virescens* (F.), is one of the most important pests of cotton, *Gossypium hirsutum* L., in the United States, usually occurring in mixed populations with the bollworm, *Helicoverpa zea* (Boddie). Pyrethroids, since their introduction, have been the most effective insecticides against *H. virescens* and *H. zea*. However, *H. virescens* has developed resistance against pyrethroids (Leonard et al. 1987, Luttrell et al. 1987, Plapp and Campanhola 1986). As a result, there has been interest in alternative strategies for *H. zea* and *H. virescens* management including avoidance of pyrethroid use in early season. One alternative pest management strategy incorporates the use of *Bacillus thuringiensis* Berliner sprays alone or in combination with chemical ovicides or larvicides. These mixtures have not killed a high percentage of larvae, especially *H. zea,* and have resulted in low to moderate efficacy (Moar et al. 1994, White et al. 1994, Young et al. 1997).

Gould and Anderson (1991) reported that *H. virescens* avoided *B. thuringiensis* toxins when added to a semisynthetic diet. Benedict et al. (1992), however, reported no significant differences between transgenic *B. thuringiensis* versus non-transgenic cotton lines in the numbers of third-instar *H. virescens* on leaf, stem or flower bud or

[1]Received 31 July 1997; accepted for publication 19 March 1999.

[2]Present Address: Department of Entomology, Cornell University New York State Agricultural Experiment Station Genevea, NY 14456.

Downloaded from https://prime-pdf-watermark.prime-prod.pubfactory.com/ at 2025-07-02 via free access

larval behavior on their corresponding location. Benedict et al. (1992) found a significantly higher plant abandonment and lower survival rate of larvae on some transgenic *B. thuringiensis* than on non-transgenic cotton lines. Jyoti et al. (1996) reported that, within hours of *B. thuringiensis* application on cotton terminals, there was a shift in the location of *H. zea* larvae away from meristematic tissue and squares to expanded leaves, or off the terminal. Studies on *B. thuringiensis* insecticide spray's effects on *H. virescens* larval movement and behavior on cotton plants are lacking. Reported herein are the results of experiments on the effect of *B. thuringiensis* rates in relation to sampling times on movement, mortality, and leaf area consumption of *H. virescens* first instars exposed to excised cotton leaves and terminals.

Materials and Methods

Experiments were conducted on the Arkansas Agricultural Experiment Station, Fayetteville, AR, during 1993. *Heliothis virescens* larvae were from a laboratory colony maintained by the Department of Entomology, University of Arkansas, Fayetteville, AR. Neonates were allowed to feed in groups of 20 to 25 on untreated cotton leaves in 270-ml wax-coated paper cups for 24 h prior to initiating experiments.

Spray table bioassay on cotton leaves. Cotton, 'Deltapine 50; was planted in staggered plantings in 4-L plastic pots in a 7:7:5 mixture of sand, soil, and peat moss and held in the greenhouse at 14:10 (L:D) h. The plants were fertilized and watered as needed.

The experiment was initiated prior to the plants setting fruit (6 to 8 nodes). Fully expanded (50 to 60 cm^2) leaves with a petiole 10 to 14 cm in length were obtained from near the top of plant and pinned in a 5 × 5 pattern on a styrofoam sheet (50 × 50 cm^2).

Bacillus thuringiensis was applied using a motorized spray-table (Luttrell et al. 1987). The spray apparatus was a CO_2-pressurized boom-type sprayer (R & D sprayers, Inc., Riverside, CA) with a one-row boom equipped with two TX-6 hollow-cone nozzles 0.46 m apart and 30.5 cm above the surface of leaves. The apparatus was calibrated to deliver the equivalent of 96 L/ha. Treatments were aqueous suspensions of Javelin® WG (Sandoz Inc., San Diego, CA) at equivalent rates of 0.0, 0.025, 0.1 and 0.5 kg/ha. *Bacillus thuringiensis*-treated leaves were allowed to dry and placed upright individually in a 270-ml wax-coated paper cup containing a moistened filter-paper disk. A single 1-d old larva was placed on the upper surface in the center of each leaf using a camel's hair brush, and the cup was capped. After being capped, all cups containing terminals were placed in large plastic bags (one treatment per bag) to minimize drying of the terminals. The cups were held in an incubator at 29 ± 1°C and a photoperiod of 14:10 (L:D) h (Young et al. 1997).

Each treatment consisted of five replications of 25 leaves each. The leaves were examined at 3, 6, 12, 24, and 48 h after treatment. Larval location (i.e., lower or upper leaf surface), petiole, inner cup surface, and mortality were recorded.

Leaf area consumption by larvae was measured after 48 h using a LI-COR Model 3100 area meter (Lambda Instruments Corporation, Lincoln, NE). The leaf area consumed was the area recorded before larvae were placed on the leaf minus that recorded after larvae had fed for 48 h.

Spray plot bioassay on cotton terminals. Cotton, 'Deltapine 50', was planted in the field 11 May 1993 and managed using standard agronomic practices. Plant density was approximately four plants per foot of row. Treatments were aqueous sus-

Downloaded from https://prime-pdf-watermark.prime-prod.pubfactory.com/ at 2025-07-02 via free access

pensions of Javelin WG (Sandoz Inc., San Diego, CA) applied with a back-pack sprayer calibrated to deliver 96 L/ha at rates of 0.0, 0.025, 0.1, and 0.5 kg/ha of Javelin WG. At application, cotton was in the squaring stage (prior to initiation of bloom). Immediately after spraying, the primary terminal with a 10 to 14-cm stem was removed from each of 25 plants per treatment. The terminal included two to three recently expanded leaves with an area of 30 to 40 cm^2 each and one to three small squares with a diameter of 7 to 9 mm. Terminals were brought into the laboratory in plastic bags, and transferred individually to a 270-ml wax-coated paper cup containing a moistened filter-paper disk. A single 1-d-old larva obtained from the laboratory culture was placed in the center of each terminal using a camel's hair brush. Each treatment consisted of five replications of 25 terminals. The cups were capped and held in an incubator at 29 ± 1°C and a photoperiod of 14:10 (L:D) h. Larval location, i.e., mainstem, squares, meristems, expanded leaves, and petiole or inner cup surface, was recorded at 3, 6, 12, 24, and 48 h after *B. thuringiensis* exposure. Larval mortality also was recorded.

Data analysis. For each sampling time on each replicate of each treatment, cumulative relative frequency for mortality, based on 25 larvae, and relative frequencies for larval locations, based on live larvae at that sampling time, were transformed by the arcsine transformation. For each sampling time, data were analyzed according to a randomized block design using the general linear model (GLM) procedure of the SAS system (SAS Institute 1989). For larval locations, the number of live larvae at the sampling time was used as a weighting factor in a weighted analysis. When the *F* test for treatments was significant ($P < 0.05$), treatments were compared by multiple *t*-tests obtained by the least square means statement of GLM.

Because there was a fixed number of live larvae to be distributed on the leaf or terminal structures and the cup inner surface, the frequencies for these locations from a single cup were dependent. Therefore, to compare two locations, the difference between the transformed values from these two locations was calculated and used as the data in the analysis described above.

Leaf area consumption was subject to analysis of variance for a randomized complete block design, and *B. thuringiensis* treatment means were separated using the 5% least significant difference (LSD). Linear regression analysis was performed to determine the relationship between leaf area consumption and percentage of larval mortality (PROC GLM, SAS Institute 1989).

Results

Spray table bioassay on cotton leaves. The percentage of larvae on leaves in the untreated control was consistently higher on the lower than on the upper surface of leaves (18 to 45% higher) (Fig. 1). In *B. thuringiensis* treatments through 12 h, the percentage of live larvae was higher on the lower leaf surface than on the upper leaf surface, except at 6 h there was not a significant difference between the surfaces at the two highest rates (Fig. 1). At 48 h the percentage of live larvae was higher on the lower than on the upper surface for the mean of all rates, but it did not differ significantly at 24 h. The percentage of larvae was lowest on petiole at all *B. thuringiensis* rates except it did not differ significantly from the upper leaf surface at 24 h.

The percentage of live larvae on the inner cup surface (12.5%) was much less than on the lower (53.2%), or on upper (31.9%) leaf surface (Fig. 1) at 3 h in the control. However, an increase in *B. thuringiensis* rate resulted in an increase in the percent-

Downloaded from https://prime-pdf-watermark.prime-prod.pubfactory.com/ at 2025-07-02 via free access

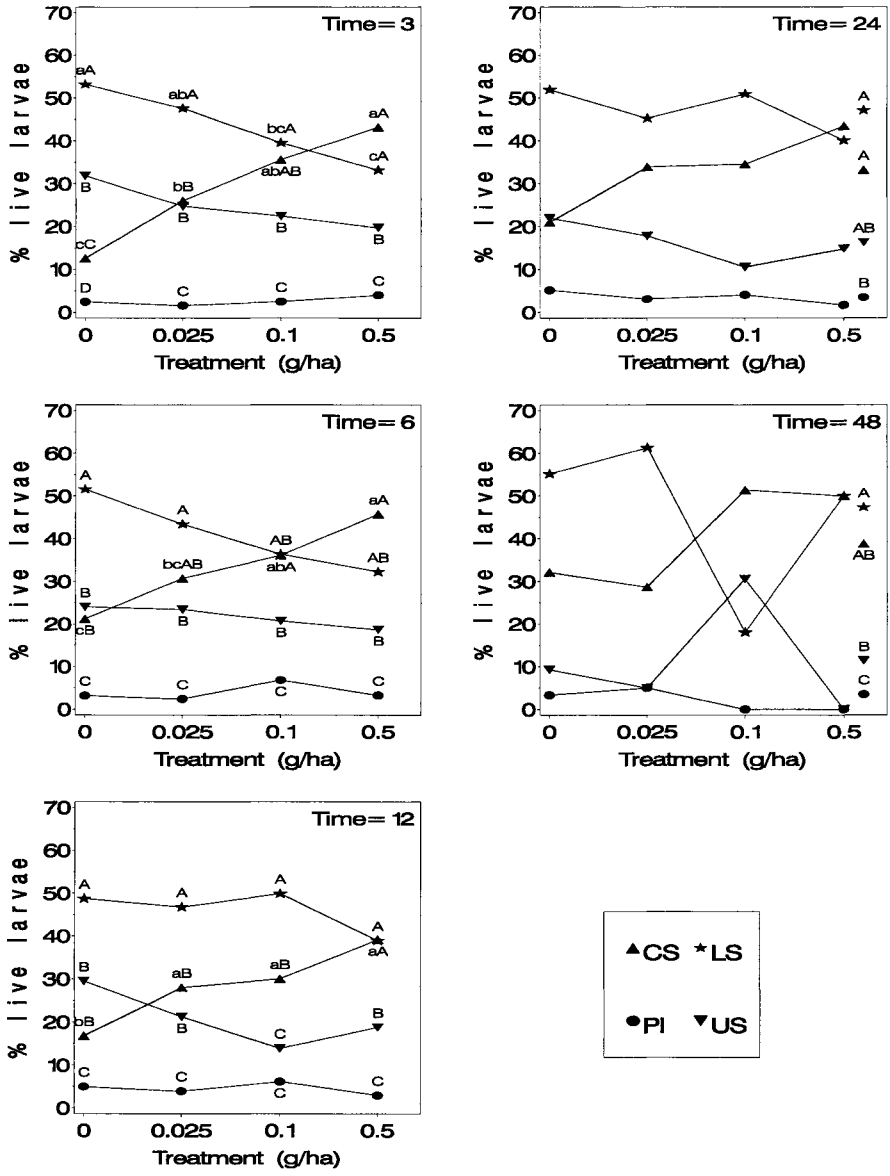

Fig. 1. Mean percentages of live larvae at a series of *B. thuringiensis* (Bt) rates and sampling times on leaf structures. Means were separated by multiple *t* tests (PL. 0.05). Rate means were separated only when the *F* test of no Bt rate effects from the analysis of variance was significant ($P < 0.05$). When the *F* was not significant ($P > 0.05$) for all locations in a time, then locations were compared on their averages across Bt rates. Means in a column sharing a common lower-case letter or in a row sharing a common upper case letter are not significantly different.

Downloaded from https://prime-pdf-watermark.prime-prod.pubfactory.com/ at 2025-07-02 via free access

age of larvae on the cup surface and a decrease on the lower leaf surface. After 3 h the percentage of live larvae on the lower leaf surface decreased at the highest *B. thuringiensis* rates compared to the control. The percentage of live larvae on the inner cup surface increased with the *B. thuringiensis* rate through 12 h. The percentage of live larvae on the upper leaf or petioles did not change significantly with the *B. thuringiensis* rate at any time (Fig. 1).

The percentage of larval mortality did not exceed 16% through the 12 h sampling time and did not differ among *B. thuringiensis* rates (Table 1). Mortality increased rapidly after 12 h, and all *B. thuringiensis* rates caused significantly greater larval mortality than the control at 24 and 48 h. The 0.50 kg/ha *B. thuringiensis* rate at 24 h also caused greater larval mortality (64.8%) than did the lower *B. thuringiensis* rates, but mortality at the three highest *B. thuringiensis* rates did not differ significantly at 48 h (Table 1). At 48 h the mortality in the control (61.4%) and *B. thuringiensis* treatments was unexpectedly high. The leaves in cups were somewhat drier at this time, and may have been a poor quality food source.

Leaf area consumed by larvae at 48 h after treatment decreased significantly with each increase in *B. thuringiensis* rate with 40.9, 29.2, 13.3, and 7.9 cm^2 consumed per surviving larva at 0.0, 0.025, 0.10, and 0.50 kg/ha, respectively. However, leaf area consumption was not significantly correlated with larval mortality ($Y = 38.6 - 0.291x$; $r^2 = 0.191$; $P > 0.05$).

Spray plot bioassay on cotton terminals. The percentage of larvae on the meristematic tissue was influenced much more by *B. thuringiensis* treatment than were the other tissues (Fig. 2). Three h after *B. thuringiensis* treatment of terminals, the percentage of larvae on the meristematic tissue decreased at all rates from that in the untreated control. At the same time there was an increase in larvae on the inner cup surface from 6.0% in the untreated control to 20.7% at 0.5 kg/ha of *B. thuringiensis* (Fig. 2). A trend for larvae on treated meristematic tissues to be lower than on untreated meristematic tissues was observed at all observation times, but this was only significant at 3, 6, and 24 h. In contrast, the percentage of larvae on the inner cup surface generally increased in treated terminals at all observation times. At 48 h only 9.8% of the untreated larvae were on the inner cup surface compared to 70.0% of the 0.5 kg/ha of *B. thuringiensis*-treated larvae. Larval location on other tissues was not

Table 1. Percentage mortality of *Heliothis virescens* larvae at a series of *Bacillus thuringiensis* (Bt) rates in relation to sampling times on cotton leaves

Bt rate (kg/ha)	Time (h)				
	3	6	12	24	48
0.00	2.4aC	4.0aC	8.0aC	26.4cB	61.6bA
0.025	3.2aC	8.0aC	13.6aC	41.6bB	86.4aA
0.10	0.8aC	4.0aC	9.6aC	38.4bB	88.8aA
0.50	3.2aD	5.6aCD	16.0aC	64.8aB	96.8aA

Means within a column (lowercase) or within a row (uppercase) followed by the same letter are not significantly different ($P < 0.05$).

Downloaded from https://prime-pdf-watermark.prime-prod.pubfactory.com/ at 2025-07-02 via free access

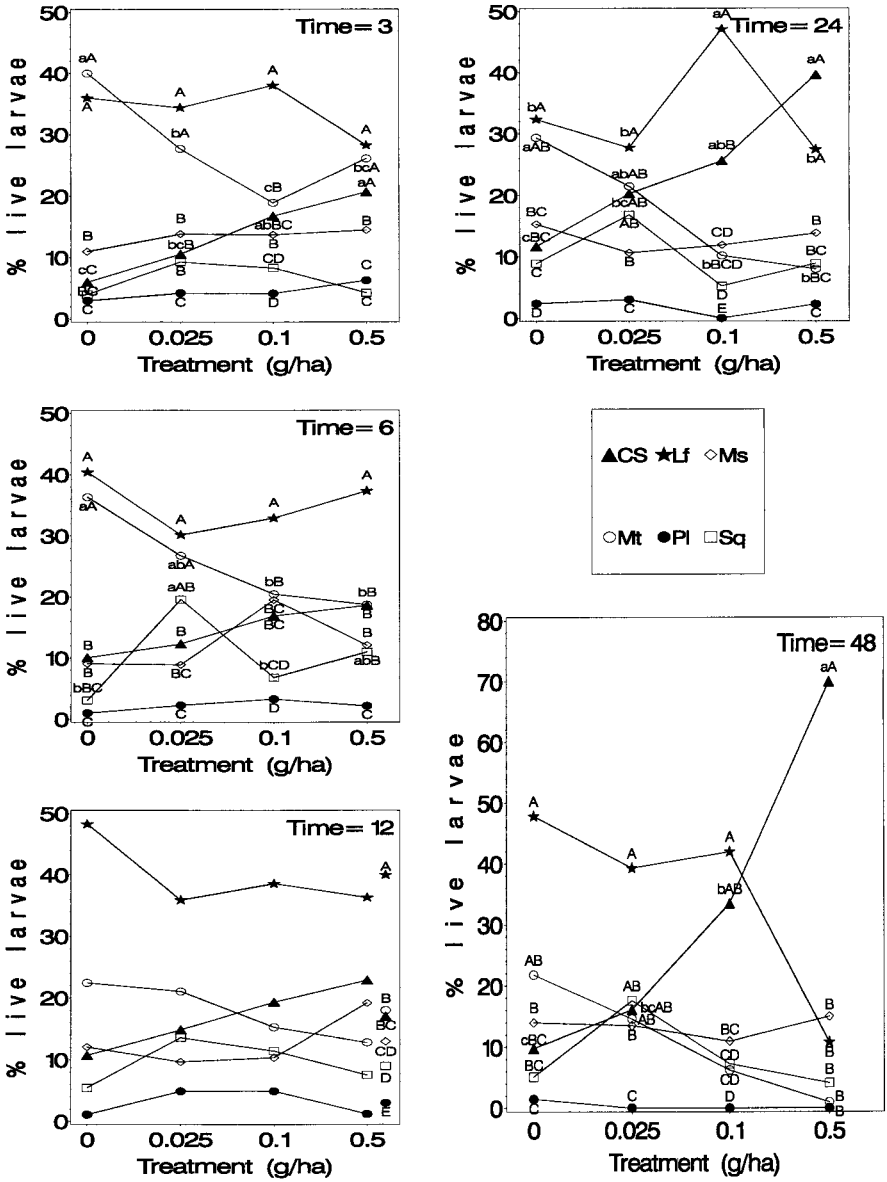

Fig. 2. Mean percentages of live larvae at a series of *B. thuringiensis* (Bt) rates and sampling times on terminal structures or cup inner surface. All mean separations were done by multiple *t* tests ($P < 0.05$). Rate means were separated only when the *F* test of no Bt rate effects from the analysis of variance was significant ($P < 0.05$). When the F was not significant ($P > 0.05$) for all locations in a time, locations were compared on their averages across Bt rates. Means in a column sharing a common lower case letter or in a row sharing a common upper case letter are not significantly different.

Downloaded from https://prime-pdf-watermark.prime-prod.pubfactory.com/ at 2025-07-02 via free access

altered by *B. thuringiensis* treatment at any observation time, except on terminal leaves at 24 h, the percentage of larvae was higher in the 0.10 kg/ha treatment than other treatments.

Although *B. thuringiensis* treatment generally had little effect on the percentage of larvae on terminal leaves, the percentage was as high or higher than on any other tissue at all *B. thuringiensis* rates and observation times (Fig. 2). The percentage of larvae was generally greater on leaves > meristematic tissue > mainstem > square > petioles. The percentage of larvae on the untreated leaves was also much higher than on the inner cup surface at all observation times. In *B. thuringiensis* treatments the percentage of larvae on leaves was higher than on the cup surface through 12 h, but thereafter it was significantly higher only in the 0.1 kg/ha treatment at 24 h (Fig. 2).

Larval mortality was significantly higher on *B. thuringiensis*-treated terminals than on the control at all sampling times, except at 3 h (Table 2) when it did not exceed 6% at any rate. However, it did not differ among *B. thuringiensis* rates until 24 h when it was lower at 0.025 kg/ha than at higher rates. Mortality increased with each increase in *B. thuringiensis* rate at 48 h. There was also a significant increase in mortality within each *B. thuringiensis* rate between 3 and 12 h, except within the control. After 12 h, the percentage mortality increased significantly at all rates at each sampling time.

Discussion

A greater percentage of *H. virescens* larvae was found on the lower than on the upper surface on non-treated leaves, and results were similar to those for *H. zea* larvae on non-treated leaves (Jyoti 1997). First-instar *H. virescens* had previously been reported to feed on the lower epidermis and palisade cells, leaving the upper epidermis intact (Parrott et al. 1983). *Bacillus thuringiensis* application generally did not alter the location of *H. virescens* larvae on the leaf surface in the laboratory assay.

Heliothis virescens larvae on untreated terminals were located predominantly on expanded leaves and meristems. Ramalho et al. (1984) reported that more first-instar *H. virescens* were found on meristems and leaves when plants were predominantly in early fruiting stages, and later moved to squares. Furthermore, Farrar and Bradley (1985) noted that first-instar *H. zea* were most prevalent on the meristems and

Table 2. Percentage mortality of *Heliothis virescens* larvae at a series of *Bacillus thuringiensis* (Bt) rates in relation to sampling times on cotton terminals

Bt rate (kg/ha)	Time (h)				
	3	6	12	24	48
0.00	0.0aC	1.0bC	8.0bC	23.0cB	44.0dA
0.025	6.0aD	10.0aCD	19.0aC	35.0bB	60.0cA
0.10	5.0aD	12.0aCD	22.0aC	45.0aB	74.0bA
0.50	4.0aD	9.0aD	21.0aC	52.0aB	86.0aA

Means within a column (lowercase) or within a row (uppercase) followed by the same letter are not significantly different ($P < 0.05$).

Downloaded from https://prime-pdf-watermark.prime-prod.pubfactory.com/ at 2025-07-02 via free access

squares, the primary feeding sites, and were rarely on the mainstems. These results did not indicate that the higher rates of *B. thuringiensis* resulted in movement of *H. virescens* larvae from meristems to other terminal structures as had been observed for *H. zea* larvae on *B. thuringiensis*-treated terminals (Jyoti et al. 1996). Instead, there appeared to be no movement among terminal tissues as a result of *B. thuringiensis*-treatment of cotton.

Heliothis virescens larval movement from leaves and terminals onto the cup surface increased with *B. thuringiensis* rates as early as 3 h after exposure. The trend for an increase in larval movement onto the cup surface and an increase in mortality with *B. thuringiensis* rates and sampling times also occurred with *H. zea* (Jyoti et al. 1996). This suggests that many of the larvae of both species that moved onto the cup surface had consumed a lethal dose of *B. thuringiensis*. Although larvae in our test had a choice of *B. thuringiensis*-treated or untreated surfaces, these results concur with those of Benedict et al. (1992) on transgenic *B. thuringiensis* cotton. They reported significantly higher plant abandonment and lower survival rates of *H. zea* and *H. virescens* larvae on some transgenic *B. thuringiensis* cotton lines compared with nontransgenic cotton lines. An increase in *B. thuringiensis* rate on leaves generally resulted in reduction in leaf area consumption by *H. virescens* larvae, but this was not significantly correlated with larval mortality. On the other hand, an increase in *B. thuringiensis* rate resulted in significant reductions in leaf area consumptions for *H. zea* larvae that were significantly linear and negatively correlated with larval mortality (Jyoti et al. 1996). These results suggest that larvae which consumed a lethal dose of *B. thuringiensis* fed little prior to death. Others have reported that under their test conditions larvae lethally exposed on transgenic *B. thuringiensis* tobacco plants consumed only a small amount of foliage (3%) before death, while larvae on non-*B. thuringiensis* varieties consumed 12% or more of the foliage (Hoffmann et al. 1992).

Results suggest that behavioral changes of *B. thuringiensis* exposed larvae of *H. virescens* should be considered when evaluating *B. thuringiensis* applications in pest management systems on cotton. The use of *B. thuringiensis* in management systems and its resistance in field populations of insect pests (Tabashnik et al. 1990) emphasize the need to determine the role of behavioral effects of the *B. thuringiensis* toxin on *H. virescens* control on crops. Experiments are needed to study the movement of *B. thuringiensis*-treated *H. virescens* larvae in relation to larval mortality and plant damage and to confirm these results on populations in *B. thuringiensis*-treated cotton fields.

Acknowledgments

We thank P. J. McLeod and T. J. Kring (Department of Entomology, University of Arkansas) for reviewing the earlier drafts of the manuscript. This research was supported in part by grant 89-34195-4378, USDA-CRS. Published with the approval of the director, Arkansas Agricultural Experiment Station.

References Cited

Benedict, J. H., D. W. Altmann, P. F. Umbeck, D. R. Ring and R. R. DeSpain. 1992. Behavior, growth, survival and plant injury by *Heliothis virescens* (F.) (Lepidoptera: Noctuidae) on transgenic *Bt* cottons. J. Econ. Entomol. 85: 589-593.

Farrar, R. R., Jr. and J. R. Bradley, Jr. 1985. Within-plant distribution of *Heliothis* species

Downloaded from https://prime-pdf-watermark.prime-prod.pubfactory.com/ at 2025-07-02 via free access

(Lepidoptera: Noctuidae) eggs and larvae on cotton in North Carolina. Environ. Entomol. 14: 205-209.

Gould, F. and A. Anderson. 1991. Effects of *Bacillus thuringiensis* and HD-73 delta-endotoxin on growth, behavior, and fitness of susceptible and toxin-adapted strains of *Heliothis virescens* (Lepidoptera: Noctuidae). Environ. Entomol. 20: 30-38.

Hoffmann, M. P., F. G. Zalom, L. T. Wilson, J. M. Smilanick, L. D. Malyj, J. Kiser, V. A. Hilder and W. M. Barnes. 1992. Field evaluation of transgenic tobacco containing genes encoding *Bacillus thuringiensis* delta-endotoxin or cowpea trypsin inhibitor: efficacy against *Helicoverpa zea* (Lepidoptera: Noctuidae). J. Econ. Entomol. 85: 2516-2522.

Jyoti, J. L., S. Y. Young, D. T. Johnson and R. W. McNew. 1996. *Helicoverpa zea* (Lepidoptera: Noctuidae): Larval location, mortality, and leaf area consumption on *Bacillus thuringiensis*-treated cotton. Environ. Entomol. 25: 1438-1443.

Leonard, B. R., J. B. Graves, T. C. Sparks and A. M. Pavloff. 1987. Susceptibility of bollworm and tobacco budworm larvae to pyrethroid and organophosphate insecticides, Pp. 320-324. *In* Proceedings, Beltwide Cotton Production Research Conferences, National Cotton Council, Memphis, TN.

Luttrell, R. G., R. T. Roush, A. Ali, J. S. Mink, M. R. Reid and G. L. Snodgrass. 1987. Pyrethroid resistance in field populations of *Heliothis virescens* (Lepidoptera: Noctuidae) in Mississippi in 1986. J. Econ. Entomol. 80: 985-989.

Moar, W., R. McCollum and R. Smith. 1994. Comparative toxicity of *Bacillus thuringiensis* against bollworm/budworm in Alabama cotton, Pp. 1052-3. *In* Proceedings Beltwide Cotton Conferences, National Cotton Council, Memphis, TN.

Parrott, W. L., J. M. Jenkins and J. C. McCarty, Jr. 1983. Feeding behavior of first-stage tobacco budworm (Lepidoptera: Noctuidae) on three cotton cultivars. Ann. Entomol. Soc. Am. 76: 167-170.

Plapp, F. W. and C. Campanhola. 1986. Synergism of pyrethroids by chlordimeform against susceptible and resistant *Heliothis*, Pp. 167-169. *In* Proceedings, Beltwide Cotton Production Research Conferences, Las Vegas., NV.

Ramalho, F. S., J. C. McCarty, Jr., J. N. Jenkins and W. L. Parrott. 1984. Distribution of tobacco budworm (Lepidoptera: Noctuidae) larvae within cotton plants. J. Econ. Entomol. 77: 591-594.

SAS Institute. 1988. SAS/STAT User's guide, release 6.04 ed. SAS Institute, Cary, NC.

Tabashnik, B. E., N. L. Cushing, N. Finson and M. W. Johnson. 1990. Field development of resistance to *Bacillus thuringiensis* in diamondback moth (Lepidoptera: Plutellidae). J. Econ. Entomol. 83: 1671-1676.

White, C. A., B. R. Leonard, E. Burris, J. B. Graves, P. A. Cloy and S. H. Martin. 1994. Control of tobacco budworm and bollworm with *Bacillus thuringiensis* in Northeast Louisiana, Pp. 1054-8. *In* Proceedings Beltwide Cotton Conferences. National Cotton Council, Memphis, TN.

Young, S. Y., T. J. Kring, H. R. Johnson and C. D. Klein. 1997. *Bacillus thuringiensis* alone and in mixtures with chemical insecticides against heliothines and effects on predator densities in cotton. J. Entomol. Sci. 32: 183-191.

Downloaded from https://prime-pdf-watermark.prime-prod.pubfactory.com/ at 2025-07-02 via free access