Influence of Foliar-Applied Bacillus thuringiensis subsp. tenebrionis and an Early Potato Harvest on Abundance and Overwinter Survival of Colorado Potato Beetles (Coleoptera: Chrysomelidae) in North Carolina

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ABSTRACT
The effects of Bacillus thuringiensis subsp. tenebrionis applications and early harvest of potatoes on reducing the size of overwintering Colorado potato beetle, Leptinotarsa decemlineata (Say), populations in eastern North Carolina were investigated over 3 yr. In large-plot (0.04 ha) experiments, potato beetle densities and defoliation levels in plots that were either treated with B. thuringiensis or left untreated were compared during the season. Similarly, the size of the overwintered potato beetle populations in these plots was compared the following spring. In a simulated harvest experiment, 1st-generation adults (generation that overwinters) were collected from untreated and B. thuringiensis-treated plots at various times and then placed into small field cages containing tubers and dead potato vines. Survival of these beetles was determined the following spring. Additionally, the effects of early harvests of ‘Atlantic’ and ‘Superior’ potatoes on marketable tuber yields were examined. Results suggest that a single foliar application of B. thuringiensis made when the large larval populations reached 1 per stem reduced and delayed the development of potato beetle populations and, more importantly, suppressed defoliation during the critical bloom stage. Although overwinter survival and the size of the overwintered population from plots treated with B. thuringiensis the previous season did not differ with those from untreated plots, overwinter survival was positively correlated with an increase in the period between planting and harvest. These results suggest that beetle populations could be reduced if potatoes are harvested before many 1st-generation adults emerge. To accomplish this while minimizing the potential for yield loss by harvesting too early, fields of the early-maturing variety Superior could be harvested as early as 85—92 d after planting.

KEY WORDS
Leptinotarsa decemlineata, Bacillus thuringiensis subsp. tenebrionis, overwinter, survival
ation adults from potatoes treated with foliar-applied \textit{B. thuringiensis} is delayed by an average of 90–150 cumulative degree-days (4–8 d) (unpublished data) and that the earliest planted commercial potatoes in North Carolina typically are harvested in early to mid-June, it is possible that a combination of foliar-applied \textit{B. thuringiensis} and early harvest could result in a majority of 1st-generation adults emerging after the foliage has been removed. Denied access to foliage, these adults would not be able to survive the winter unless they dispersed to nearby potato fields (Nault et al. 1996). Further, to the extent that foliar-applied \textit{B. thuringiensis}, or any other potato beetle control measure, reduces the number of 1st-generation larvae, the population of 1st-generation adults overwintering in and around the treated field may be reduced.

Our objectives were to test this hypothesis. Specifically, the 3 objectives were as follows: (1) to compare overwinter survival of 1st-generation adults from plots that were either treated with \textit{B. thuringiensis} or left untreated the previous season, (2) to identify how timing of harvest affects overwintering survival of 1st-generation adults, and (3) to determine if a combination of foliar-applied \textit{B. thuringiensis} and an early harvest reduces the size of the overwintering population.

**Materials and Methods**

Large-plot and simulated harvest date experiments were conducted at the North Carolina Department of Agriculture’s Tidewater Research Station located near Rooper, NC, from 1995 to 1997. All plots in these experiments were planted to the potato variety ‘Atlantic’. The commercial formulation of \textit{B. thuringiensis} subsp. tenebrionis (M-TRAK®) was used in all \textit{B. thuringiensis} applications. The active ingredient in M-TRAK is the delta-endotoxin of \textit{B. thuringiensis} subsp. tenebrionis, which is encapsulated in innocuous \textit{Pseudomonas fluorescens}. M-TRAK was applied at a rate of 4.68 liters/ha using a CO$_2$-pressurized, backpack sprayer equipped with 3 hollow cone nozzles per row and delivering 233.3 liters/ha of spray at 276 kPa.

**Large-Plot Experiments.** Field experiments were conducted in 1995–1996 and 1996–1997. On 20 March 1995, 8 plots (0.04 ha each, 20 rows by 21 m) were planted; and on 25 March 1996, 10 plots (0.04 ha each) were planted. All plots were located in fields that had been planted in potatoes each year since 1992 and all were bordered on at least 2 sides by a minimum of 4 rows of corn. Plots were separated by a minimum of 50 m planted to corn or wheat.

In mid-April, 20 plants were selected randomly in each plot and marked by colored flags. These plants were sampled weekly through harvest (early June) for numbers of Colorado potato beetle adults, egg masses, and small (1st and 2nd instars) and large 3rd and 4th instars larvae. Defoliation of each plant was visually estimated on each sample date using a weighted, pre-transformed rating scale that divided 90° into 13 defoliation categories corresponding to arcsine transformation values, each represented as a percentage (e.g., 3–9%) (Little and Hills 1978). The midpoint of each category was used in the analyses (e.g., 6% for the 3–9% defoliation class). A single foliar application of \textit{B. thuringiensis} was made when the number of large larvae first exceeded a mean of 1 per stem. At the time this threshold was reached, each plot was paired with an untreated control having a similar population of potato beetle adults, egg masses, and larvae. One plot within each pair was selected randomly to receive the \textit{B. thuringiensis} treatment. All \textit{B. thuringiensis} applications were made during the 2nd wk of May in both 1995 and 1996. The experimental design involved 2 treatments (\textit{B. thuringiensis} and untreated) arranged in a randomized complete block design replicated 4 times in 1995–1996 and 5 times in 1996–1997.

The number of 1st-generation adults that emerged in June from the untreated and \textit{B. thuringiensis}-treated plots was compared as was the number of overwintered 1st-generation adults that emerged the following spring. In 1995, the emergence and abundance of 1st-generation adults in June was inferred from weekly samples of the tagged potato plants. In 1996, 4 emergence cages were placed randomly within each plot on 31 May to monitor emergence of 1st-generation adults within the potato plot. These cages consisted of a rectangular piece of mosquito netting placed over 2 potato plants extending out from the plants to cover a soil surface area of 1.83 by 3.05 m ($3.58$ m$^2$). The cage was held in place by burying with soil the edges of the netting that extended outside the trapping area. The potato plants within the cage served to support the netting above the soil surface and provide food for the emerging adult beetles. Cages were monitored weekly through 24 June and the emerged, 1st-generation adults were counted and removed.

On 11 April 1996 and 2 April 1997, 4 emergence cages were placed at the interfaces between the previous year’s potato and corn plantings to trap emerging overwintered Colorado potato beetle adults. The cages were positioned such that the longest edge was perpendicular to rows and bisected the potato–corn interface. This was done because Nault et al. (1997) reported that the highest densities of potato beetle adults overwintered in the soil at the edges of commercial potato fields. These cages were monitored weekly through 24 June to record the number of overwintered adults that emerged.

Data were analyzed with a repeated measures analysis of variance (ANOVA) using the PROC GLM procedure of SAS at $P \leq 0.05$ to identify if trends in large larval populations varied through time (i.e., a significant treatment $\times$ observation date interaction) (SAS Institute 1990). Percentage data were transformed using a square root function ($x + 0.01$) and numerical data were transformed using a log$_{10}$ function ($x + 1$) (Steel and Torrie 1980).

**Simulated Harvest Date Experiment.** In 1996, a 2nd field experiment was conducted to estimate the effects of foliar \textit{B. thuringiensis} treatments and early harvest on the production of 1st-generation Colorado potato beetle adults and their ability to successfully overwin-
ter. This experiment included six 0.04-ha plots of potato planted on 25 March. These plots were sampled weekly as described earlier to determine the abundance of Colorado potato beetle adults, egg masses, and small and large larvae. Three plots were treated with a foliar application of <i>B. thuringiensis</i> timed and applied as described previously. The remaining 3 plots served as untreated controls. Four harvest dates (0, 5, 10, and 15 d after the initial emergence of 1st-generation adults from <i>B. thuringiensis</i>-treated plots) were simulated by collecting 150 randomly selected 1st-generation adults from each plot. The adults from each plot were then partitioned into 5 groups of 30 adults per each main plot. Data were simulated using an ANOVA to determine significantly different means using the procedure described above for the Atlantic experiments, except that there were only 2 replicates in 1995.

Data were analyzed using a one-way ANOVA using the PROC GLM procedure of SAS at <i>P</i> ≤ 0.05 to determine significant differences among treatment (harvest date) means.

**Results**

In the large-plot experiment in 1995, peak density of overwintered adults was moderate (0.21 adults per plant [stem]) and occurred in all plots between 8 and 11 May. The initial number of small larvae per plant on 11 May did not differ between plots designated for treatment with <i>B. thuringiensis</i> or as untreated (mean ± SEM = 3.4 ± 0.3 and 3.4 ± 0.8, respectively; <i>F</i> = 0.01; df = 1, 3; <i>P</i> = 0.9402). Over the season, there tended to be more large larvae in untreated plots than in <i>B. thuringiensis</i>-treated plots (i.e., nearly significant treatment × date interaction) (<i>F</i> = 2.99; df = 5, 15; <i>P</i> = 0.0543) (Fig. 1A). Mean percentage defoliation in the untreated plots remained rather low (10%) at the end of the bloom period (30 May) and did not differ significantly from that in the <i>B. thuringiensis</i>-treated plots (2%) (<i>F</i> = 2.79; df = 1, 3; <i>P</i> = 0.1936). At harvest on 5 June, the difference between treatments was larger (18.4 versus 4% for the untreated and <i>B. thuringiensis</i> treatment, respectively), although this difference was not significant (<i>F</i> = 7.88; df = 1, 3; <i>P</i> = 0.0674) (Fig. 1B).

First-generation adults began emerging in the untreated plots between 23 and 30 May, but did not begin to emerge in the <i>B. thuringiensis</i> plots until between 31 May and 5 June (Fig. 1C). Although there were significantly more 1st-generation adults per plant in the untreated than in the <i>B. thuringiensis</i> plots on 30 May (<i>F</i> = 14.23; df = 1, 3; <i>P</i> = 0.0326) and 5 June (<i>F</i> = 22.38; df = 1, 3; <i>P</i> = 0.0179), the cumulative number of overwintered adults emerging into emergence traps in the untreated and <i>B. thuringiensis</i> plots the following spring (1996) did not differ significantly (0.39 ± 0.16/m² and 0.40 ± 0.15/m², respectively; <i>F</i> = 0.00; df = 1, 3; <i>P</i> = 0.9987).

In the experiment begun in 1996, the density of overwintered adults peaked in all plots at 0.76 adults per plant (stem) between 6 and 10 May. The abundance of small larvae on 10 May in plots designated to be treated with <i>B. thuringiensis</i> did not differ from those in plots designated as untreated controls (19.0 ± 0.6 and 17.1 ± 5.3 for untreated and <i>B. thuringiensis</i> plots, respectively; <i>F</i> = 0.16; df = 1, 4; <i>P</i> = 0.7307). As in 1995, there were more large larvae in the untreated plots, with significantly more large larvae in the <i>B. thuringiensis</i>-treated plots (mean ± SEM = 53 ± 7 and 39 ± 7, respectively; <i>F</i> = 5.3 for untreated and <i>B. thuringiensis</i> plots, df = 1, 3; <i>P</i> = 0.067).

**Effect of Harvest Date on Yield Experiment.** All experiments were conducted in commercial fields of Atlantic and Superior potatoes from 1994 to 1996 and from 1994 to 1995, respectively. Experiments were conducted in Pamlico County, NC, in 1994 and in Washington County, NC, in 1995 and 1996. Seed pieces of Atlantic were planted on 17 March 1994, from 10 to 15 March 1995 and from 14 to 23 March 1996, whereas Superior seed was planted on 17 March 1994 and on 10 March 1995. Seed in all tests was planted at 0.3 m within-row spacing and rows were spaced at 0.91 m. Plots (experimental unit) consisted of 2 rows each 9.14 m long in 1994 and single rows each 1.52 m long in 1995 and 1996.

In 1994, the Atlantic experiment had 4 harvest dates (85, 90, 95, and 100 d after planting) that were arranged in a randomized complete block design. These harvest date treatments were replicated in 5 fields and some treatments in some fields were duplicated (i.e., subsamples). In 1995 and 1996, Atlantic experiments also had 4 harvest dates (79, 86, 93, and 100 d after planting) that were arranged in a randomized complete block design; however, these treatments were replicated in 3 and 6 fields in 1995 and 1996, respectively. Unlike the 1994 experiment, each harvest date was replicated 15 times within each field (i.e., subsamples). Designs for the Superior experiments in 1994 and 1995 were nearly identical to those described for the Atlantic experiments, except that there were only 2 replicates in 1995.

Data were analyzed using a one-way ANOVA using the PROC GLM procedure of SAS at <i>P</i> ≤ 0.05 to determine significant differences among treatment (harvest date) means.
than in the *B. thuringiensis* plots (i.e., significant treatment × date interaction) (*F* = 4.14; df = 5, 20; *P* = 0.0099) (Fig. 2A). In contrast to 1995, the abundance of large larvae in the *B. thuringiensis*-treated plots at the end of the bloom stage increased to the same level as in the untreated plots (Fig. 2A). Defoliation in the *B. thuringiensis*-treated plots did not exceed 8% during the bloom period and was significantly lower than in the untreated plots on 28 May (48 versus 5%, respectively; *F* = 12.98; df = 1, 4; *P* = 0.0227) (Fig. 2B). A portion of the defoliation that occurred in the days immediately before harvest was associated with feeding by newly emerged 1st-generation adults, especially in the untreated plots (Figs. 1 and 2, B and C).

First-generation adults began emerging between 29 May and 3 June in untreated plots, but did not begin to emerge until between 3 and 10 June in the *B.
cant in 1995 and 1996 (No. 1 tubers (Fig. 5). These reductions were significant in 1995 and 1996 (F = 11.14; df = 3, 6; P = 0.0073; F = 99.53; df = 3, 11; P = 0.0001), but not in 1994 (F = 1.28; df = 3, 9; P = 0.3401). In 1996, a significant yield reduction in Atlantic potato tubers was observed when fields were harvested before 100 d after planting. In 1994, no difference in U.S. No. 1 tuber yield of Superior potatoes was observed when plants were harvested from 85 to 100 d after planting (F = 1.82; df = 3, 7; P = 0.3364) (Fig. 6). In 1995, U.S. No. 1 yields of Superior were numerically greatest when plants were harvested from 92 to 102 d after planting (F = 0.65; df = 4, 4; P = 0.0533).

Discussion

Our results suggest that a single foliar application of B. thuringiensis made when the large larval populations reached 1 per stem reduced and delayed the development of large larval populations and more importantly, suppressed defoliation during the critical bloom stage. The potato crop is most sensitive to yield reductions resulting from defoliation during the bloom stage and to be quite tolerant of defoliation during the postbloom stage (Hare 1980, Ferro et al. 1983). During 1995, defoliation in the untreated plots remained generally low, reaching only 10% during the bloom stage. Although defoliation during bloom reached only 2% in the B. thuringiensis-treated plots, it did not differ significantly from the untreated control. In 1996, however, when defoliation reached 48% at the end of the bloom stage in the untreated control, it reached only 5% in the B. thuringiensis-treated plots (P = 0.49). Although defoliation increased during the postbloom period each year in both the B. thuringiensis-treated and untreated plots, the final levels of defoliation at harvest remained lower in the B. thuringiensis-treatment (4 and 10% in 1995 and 1996, respectively) than in the untreated control (18 and
56% in 1995 and 1996, respectively). The difference, however, was significant only in 1996.

Defoliation increased during the postbloom period, in part because of feeding by newly emerged, 1st-generation adults. This defoliation was significant in the untreated plots where 1st-generation adults emerged (7 d earlier than in the \textit{B. thuringiensis}-treated plots). However, although the \textit{B. thuringiensis} treatment delayed emergence of 1st-generation adults in June, it did not significantly reduce the total number of 1st-generation adults produced.

In the simulated harvest date study, the overwinter survival of 1st-generation adults increased as the period between planting and harvest increased. This is consistent with the previous findings of Nault et al. (1996) and reflects the increased period during which 1st-generation adults could feed on healthy potato foliage before they were denied further access to foliage by the simulated harvest. These results suggest that harvesting fields (or top killing vines) as early as possible should help to reduce the size of the local overwintering population. In North Carolina, the most commonly grown varieties are the early-maturing Superior and the midseason-maturing Atlantic. Based on our studies, the potential for experiencing a yield loss by harvesting too early would be greater for Atlantic than Superior. Maximum Superior yields were achieved as early as 85—92 d after planting, whereas maximum Atlantic yields were not observed until 90—100 d after planting.

The delayed emergence of 1st-generation adults in June associated with the \textit{B. thuringiensis} treatment did not result in a decrease in overwinter survival. Perhaps the delay in emergence was not long and consistent enough to result in sufficient foliage deprivation to cause elevated overwinter mortality. In any event, it is clear that although early harvest may reduce overwintering success of local Colorado potato beetle pop-
ulations, a single foliar application of \textit{B. thuringiensis} will not delay emergence of first generation adults sufficiently to reduce their overwintering success. On a local level, the effect of early harvest on colonization of a potato crop the following year by overwintered adults may be enhanced through crop rotation (Weisz et al. 1994, Follett et al. 1996).

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