

Managing Colorado Potato Beetles (Coleoptera: Chrysomelidae) and European Corn Borers (Lepidoptera: Pyralidae) in Potato with Foliar Applications of *Bacillus thuringiensis* Berliner¹

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Abstract Application timing and rate combinations of *Bacillus thuringiensis* Berliner used for protecting Irish potato, *Solanum tuberosum* L., from defoliation by the Colorado potato beetle, *Leptinotarsa decemlineata* (Say), and stem injury by the European corn borer, *Ostrinia nubilalis* (Hübner), were investigated. Significant reductions in defoliation levels during bloom, populations of first-generation potato beetle adults, and the percentage of stems injured by corn borers at the end of the season in *B. thuringiensis*-treated potato were considered criteria for a successful management strategy. Although Colorado potato beetle infestations were managed effectively with *B. thuringiensis*, European corn borer populations were not reduced to a commercially acceptable level. In each of 3 yrs, neither an increase in the number of applications (from 1 to 4) nor an increase in rate (from 0.9 to 3.8 liters/ha) of *B. thuringiensis* subsp. *kurstaki* improved the level of corn borer control. In contrast, results indicated that 1 application of *B. thuringiensis* subsp. *tenebrionis*, timed when there was >1 large potato beetle larva per stem, using a 4.7 liters/ha rate protected the potato crop during the bloom stage. However, this strategy may not be sufficient to prevent significant levels of defoliation by first-generation potato beetle adults during post-bloom or reduce the size of this population, which will infest next season's crop. For this reason, the *B. thuringiensis* subsp. *tenebrionis* timing and rate regimen described above may be most effective in fields where the overwintering potato beetle population is predicted to be low to moderate (e.g., <1 adult per 5 stems), whereas two applications may be most effective in fields where densities are greater.

Key Words *Leptinotarsa decemlineata*, *Ostrinia nubilalis*, Irish potatoes, *Bacillus thuringiensis*, biointensive pest management

The Colorado potato beetle, *Leptinotarsa decemlineata* (Say), and European corn borer, *Ostrinia nubilalis* (Hübner), are important pests of Irish potato, *Solanum tuberosum* L., in the Mid-Atlantic U.S. Both pests complete their first generation on potato and this is normally the only generation that requires control. A smaller second generation of potato beetles may occur in potato, but it typically is not completed before the crop is harvested in June to early-July. Similarly, a second generation of corn borers rarely develops in potato because other hosts (e.g., corn) are more attractive to the emerging first-generation adults. Most growers use conventional

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insecticides that have broad-spectrum activity to manage potato beetle and corn borer infestations.

Some growers limit their use of broad-spectrum materials in favor of the selective *Bacillus thuringiensis* Berliner-based foliar products for controlling potato beetles; whereas, an even smaller percentage relies exclusively on *B. thuringiensis* for growing organically-certified potatoes. Despite the overall infrequent use of *B. thuringiensis* sprays for managing the potato beetle, pest management specialists in the Mid-Atlantic U.S. encourage growers to use them in rotation with conventional products within and between years as part of an insecticide resistance management strategy (Nault 1997).

Growers have not widely adopted the use of *B. thuringiensis* products for managing potato beetles because applications must be timed precisely using ground equipment. In addition, they are expensive and limited in supply. The recommendation for optimizing the performance of *B. thuringiensis* was to use multiple applications with the first timed when eggs began hatching and small larvae were the most predominant lifestage present (Zehnder and Gelernter 1989, Zehnder et al. 1992, Ghidui and Zehnder 1993, Bystrak et al. 1994). This management approach was recommended because large larvae have to consume more *B. thuringiensis* toxin to receive a lethal dose than do small larvae (Ferro and Lyon 1991). However, this information was based on results from research in which older, less effective formulations of *B. thuringiensis* (e.g., M-One, Mycogen Corp., San Diego, CA) were used. Since that time, formulations of *B. thuringiensis* have improved substantially (Ferro et al. 1993). For example, research by Nault et al. (2000) showed that third-instar Colorado potato beetles remaining on plants after a *B. thuringiensis* subsp. *tenebrionis* application were unlikely to feed, and fourth instars consumed only about half as much foliage as those fed untreated foliage. Moreover, many late instars subjected to *B. thuringiensis* subsp. *tenebrionis*-treated foliage do not survive to adulthood (Ferro et al. 1997), many (58 to 83%) of which die during the larval stage (Nault et al. 2000). Based on this information, fewer applications may be required to effectively manage the potato beetle and would consequently reduce production costs.

A procedure for optimizing the use of *B. thuringiensis* subsp. *kurstaki* foliar sprays for managing European corn borer in potato has not been developed. European corn borers feed briefly on the leaves before boring into the mid-rib of the leaf, leaf axil or stem (Jones et al. 1939). Nault and Kennedy (1996) showed that these larvae exit and reenter a stem an average of 4.7 times during their lifetime. Because these larvae are not likely to feed on leaves between the time they exit and reenter the stem, ingestion of a lethal dose of *B. thuringiensis* residue is unlikely. Perhaps, the most effective approach for managing corn borers would be to initiate applications of *B. thuringiensis* early in the infestation episode, when larvae are hatching and spending time on foliage. Nault and Kennedy (1996) showed that one application of a conventional insecticide (e.g., methamidophos or carbofuran) timed when 10 to 20% of the stems were infested with neonates provided excellent control. The objective of this research was to identify an optimal field application strategy for protecting the potato crop from defoliation by the Colorado potato beetle and stem injury by the European corn borer using *B. thuringiensis*-based foliar products.

Materials and Methods

Field sites. Experiments were conducted at North Carolina State University's Vernon James Tidewater Research Station near Plymouth, NC, in 1994, 1995, and

1997 and at Virginia Polytechnic Institute and State University's Eastern Shore Agricultural Research and Extension Center near Painter, VA, in 1998 and 1999. The potato cultivars 'Atlantic' and 'Superior' were used in the Colorado potato beetle and European corn borer experiments, respectively. Fertilization, weed and disease management for all experiments were followed according to current recommendations for the Mid-Atlantic region (VCE 2000).

Colorado potato beetle. Potato seed pieces were planted on 23 March 1994 and 16 March 1995. Plots were 2 and 3 rows each 6.1 m long and flanked by one untreated row in 1994 and 1995, respectively. Plants were spaced approximately 30.5 cm apart in rows spaced 0.97 m apart. In 1994, 3 application timing thresholds and 3 rates of *B. thuringiensis* subsp. *tenebrionis* delta-endotoxin encapsulated in innocuous *Pseudomonas fluorescens* Migula (M-Trak, Mycogen Corp., San Diego, CA) were examined for Colorado potato beetle control. The rates examined were 2.3, 4.7, and 7.0 liters/ha or 70.2, 140.4, and 210.5 g (pure Cry3A toxin)/ha, respectively. Application timing thresholds were as follows: (1) one application when 30 to 50% of egg masses were hatching; (2) one application made when density of large larvae (third and fourth instars) reached >1/stem; and (3) two applications 7 d apart initiated when 10 to 30% of egg masses were hatching. The experiment was designed as a 3 (timing threshold) × 3 (application rate) factorial plus an untreated control arranged in a randomized complete block each replicated 4 times. In 1995, the experimental treatments and design were similar, but consisted of an additional rate of *B. thuringiensis* subsp. *tenebrionis*, 1.2 liters/ha (35.1 g [pure Cry3A toxin]/ha, respectively). To determine percentage egg mass hatch, 20 egg masses from the first cohort of egg masses laid in the experimental area were tagged and monitored twice per week. A summary of the number and dates of applications is included in Table 1.

Ten stems from each plot were chosen randomly and the number of potato beetle adults and larvae were recorded each week from late April through early June. Percentage defoliation was estimated visually for each plot (see Little and Hills 1978). Plots were divided into 4 equal sections and each section was given a defoliation rating. The mean percentage defoliation for each plot then was used in the data

Table 1. The number and dates of *B. thuringiensis* subsp. *tenebrionis* applications used in 1994 and 1995 experiments in North Carolina

Application timing threshold	1994		1995	
	Number of applications	Dates	Number of applications	Dates
1. One application at 30 or 50% egg mass hatch	1	2 May	1	8 May
2. One application at >1 large larva/stem	1	6 May	1	15 May
3. Two applications spaced 7 days apart with the first timed at 10 to 30% egg mass hatch	2	2 and 9 May	2	1 and 8 May

analyses. Data collection was terminated shortly after first-generation potato beetle adults began to emerge in early June, which coincided with harvest.

Rows between plots except those adjacent to the untreated control were sprayed once with *B. thuringiensis* subsp. *tenebrionis* at a rate of either 1.2 liters/ha (2 May 1994) or 2.3 liters/ha (8 May 1995) to minimize movement of late-instar potato beetles into plots. European corn borer was not managed in these experiments because populations never exceeded the threshold of 20% infested stems for the cultivar Atlantic (Nault and Kennedy 1996).

European corn borer. Potato seed pieces were planted on 25 March 1997, 30 March 1998 and 8 April 1999. Each plot consisted of a single 9.1-m long row flanked by one untreated row. Plants were spaced approximately 30.5 cm apart in rows spaced 0.9 m apart. In 1997, 3 application timing strategies (1, 2 and 3 applications) and 2 rates of *B. thuringiensis* subsp. *kurstaki* delta-endotoxin encapsulated in innocuous *P. floescens* Migula (MVP II, Mycogen Corp., San Diego, CA) were examined for European corn borer management. The rates evaluated were 2.3 and 4.7 liters/ha or 65.5 and 131.0 g (pure CryIA[c] toxin)/ha, respectively. In 1998 and 1999, 3 application-timing strategies (1, 2 and 3 applications in 1998 and 2, 3, and 4 in 1999) and 3 rates of *B. thuringiensis* subsp. *kurstaki* delta-endotoxin encapsulated in innocuous *P. floescens* Migula (Match, Mycogen Corp., San Diego, CA) were examined. Rates examined were 2.3, 4.7 and 7.0 liters/ha or 37.4, 74.9 and 112.3 g (pure CryIA[c] + CryIC toxins)/ha, respectively. In each experiment, the first application was timed when neonates were observed penetrating plants early in the infestation episode, and additional applications were made at 5-day intervals. Initial applications occurred on 16 May 1997, 20 May 1998 and 18 May 1999 when the percentage of entry/exit holes per stem was 8, 25 and 26%, respectively. No stems were infested within 2 to 3 d of these initial applications. All experiments were arranged as factorials with main plot factors consisting of *B. thuringiensis* application timing and rate. Treatments were randomized in a complete block design and included an untreated control and a standard insecticide, carbofuran [Furadan 4F, FMC, Philadelphia, PA], replicated 4 times. Carbofuran was applied one time on the initial spray dates listed above.

At the end of the first generation (18 June 1997, 11 June 1998, and 16 June 1999), 25 stems from each plot were chosen randomly and the number of stems injured by European corn borer was recorded. In 1997, imidacloprid [Admire 2F, Bayer, Kansas City, MO] was used at planting at a rate of 1.3 liters/ha to manage Colorado potato beetles, whereas *B. thuringiensis* subsp. *tenebrionis* [Novodor, Abbott Laboratories, North Chicago, IL] was used at a 4.7 liters/ha rate in 1998 and 1999 (spray dates: 22 May and 1 June 1998 and 1 and 8 June 1999). Also, on 1 June 1998 and 8 June 1999, dimethoate [Dimethoate 4EC, Helena, Memphis, TN] was used at rate of 1.2 liters/ha to control potato leafhoppers, *Empoasca fabae* (Harris). Imidacloprid, *B. thuringiensis* subsp. *tenebrionis* and dimethoate were chosen because our research with these products shows no adverse effects on European corn borer (B. A. N., unpubl. data).

Applications of M-Trak and MVP II were made using a CO₂-pressurized backpack sprayer equipped with a 3-nozzle (D3 disk/25 core hollow cone), single-row boom calibrated to deliver 233 liters of spray per ha at a pressure of 275.7 kPa. One nozzle was directed above the row and the others were on drop pipes directed laterally into the canopy. Applications of Match, Novodor and Dimethoate were made using the same apparatus, spray volume, and pressure, except that the backpack sprayer was propane-pressurized. Imidacloprid was applied in furrow with a CO₂-pressurized

sprayer, mounted on the planter, that delivered 60.6 liters/ha at pressure mentioned above.

Statistical analyses. The effect that insecticide timing and rate had on Colorado potato beetle and European corn borer control was determined using a two-way analysis of variance on certain sampling dates (PROC GLM; SAS Institute 1990). These analyses initially excluded the untreated control, and all treatment means were compared using a Fisher's protected-LSD at $P \leq 0.05$. For the potato beetle experiments, differences in population size of large larvae throughout the season in various treatments were compared using a repeated-measures analysis of variance (PROC GLM; $P \leq 0.05$). Data also were analyzed by a one-way analysis of variance, including the untreated control, to determine differences within each main effect (treatment or rate) after all levels of the main effect were pooled across the other main effect. For the corn borer experiments, data from all *B. thuringiensis* treatment combinations were pooled, and this mean was compared with either the untreated control mean or both the untreated and standard means. Percentage data were transformed using a $\log_{10}$ function ($x + 1$) (Steel and Torrie 1980), but untransformed data are presented.

Results

Colorado potato beetle management. In both years, the size of populations of large larvae, the final percentage of defoliation and the number of first-generation adults that emerged before harvest in *B. thuringiensis*-treated plots were not affected significantly by an application timing $\times$ rate interaction ($P > 0.05$). Therefore, results from each main effect are discussed separately.

Colonization by overwintered Colorado potato beetle adults into the test site peaked at slightly over 1 adult per 10 stems (0.11 adult/plant [=stem]) from 1 through 15 May 1994 (Fig. 1A and B). In 1995, overwintered adult density peaked at 1 adult per 4 stems (0.25 adult/plant [=stem]) from 30 April through 8 May (Fig. 1C and D). In both years, the initial number of small larvae early in the season did not differ significantly between plots designated for treatment or to remain untreated (1 May 1994: mean $\pm$ SEM = 5.1 ± 1.5 and 4.6 ± 2.2 , respectively; $P = 0.7899$; 30 April 1995: mean $\pm$ SEM = 1.3 ± 0.5 and 0.3 ± 0.2 , respectively; $P = 0.0841$).

Over both seasons, there tended to be more large larvae in untreated plots than in *B. thuringiensis*-treated ones (Fig. 2A-D). The size of populations of large larvae among treated plots was significantly affected by the time in which applications were made (1994: $F = 3.6$; df = 10, 150; $P = 0.0003$; 1995: $F = 12.3$; df = 14, 266; $P = 0.0001$). In both years, after all applications had been made, there were fewer large larvae in plots that were either treated twice or only once when there was >1 large larva/stem than in plots only treated once at either 30% or 50% egg hatch (Fig. 2A and C). The size of the population of large larvae among treated plots was influenced by the rate of insecticide in 1995 ($F = 6.8$; df = 21, 259; $P = 0.0001$), but not in 1994 ($P = 0.5958$). In 1995, large larval abundance during the bloom stage was negatively correlated with increasing rates of *B. thuringiensis* (Fig. 2D).

Defoliation in plots treated with *B. thuringiensis* was significantly lower than defoliation in untreated plots at the end of bloom on 31 May 1994 ($F = 50.2$; df = 1, 3; $P = 0.0058$) and on 30 May 1995 ($F = 52.5$; df = 1, 3; $P = 0.0054$) (Fig. 3A-D). Levels of defoliation in *B. thuringiensis*-treated plots at the end of bloom did not exceed 1% in 1994 and from 2 to 16% in 1995. Although defoliation increased in all plots in early June as a result of feeding by newly-emerged, first-generation adults, defoliation

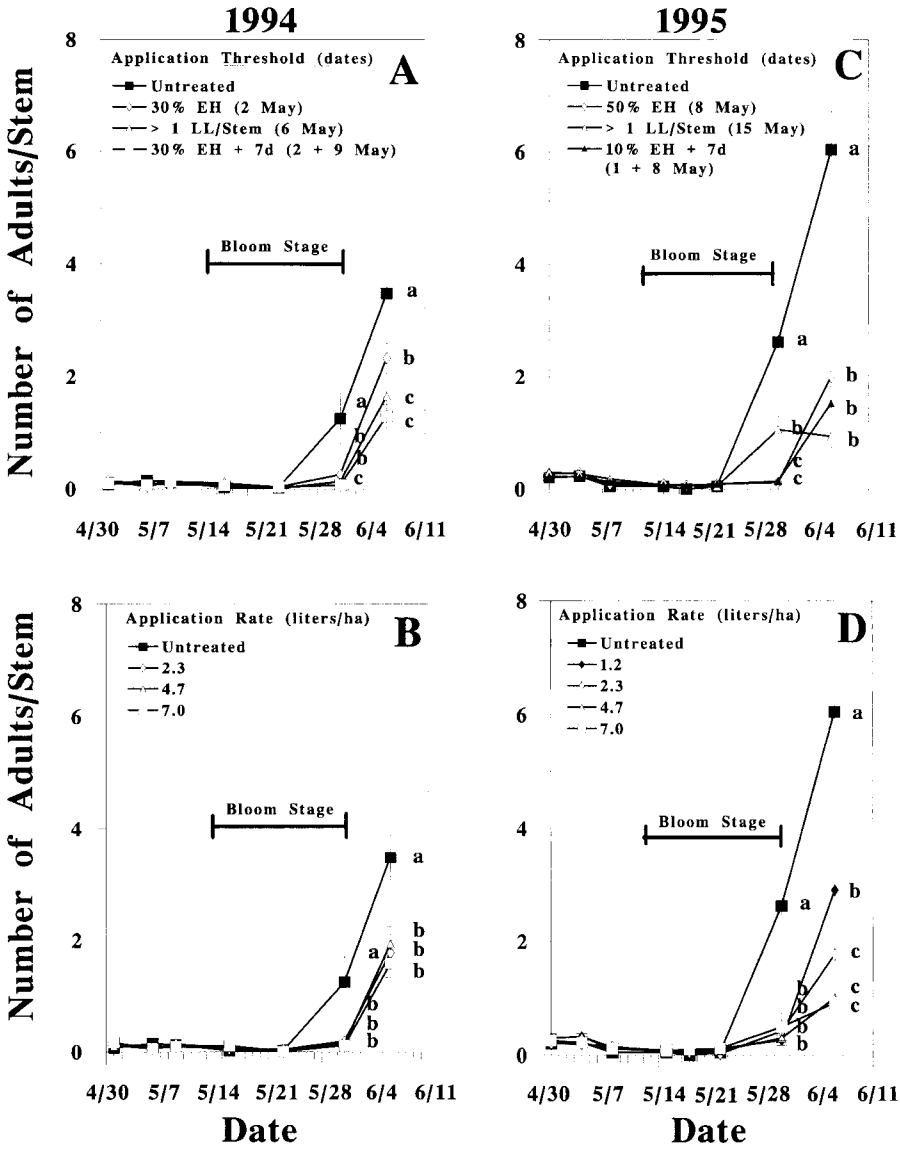


Fig. 1. Mean ($\pm$ SEM) number of *L. decemlineata* adults per potato stem in small plots that were either untreated or treated with *B. thuringiensis* subsp. *tenebrionis* using various timing thresholds (A and C) and rates (B and D) in 1994 and 1995. Number of adults in treated plots was compared among treatments at the end of the bloom stage on 31 and 30 May 1994 and 1995, respectively, and again immediately prior to harvest on 6 June 1994 and 1995. Means followed by the same letter on a specific date are not significantly different ($P > 0.05$; Fisher's Protected LSD).

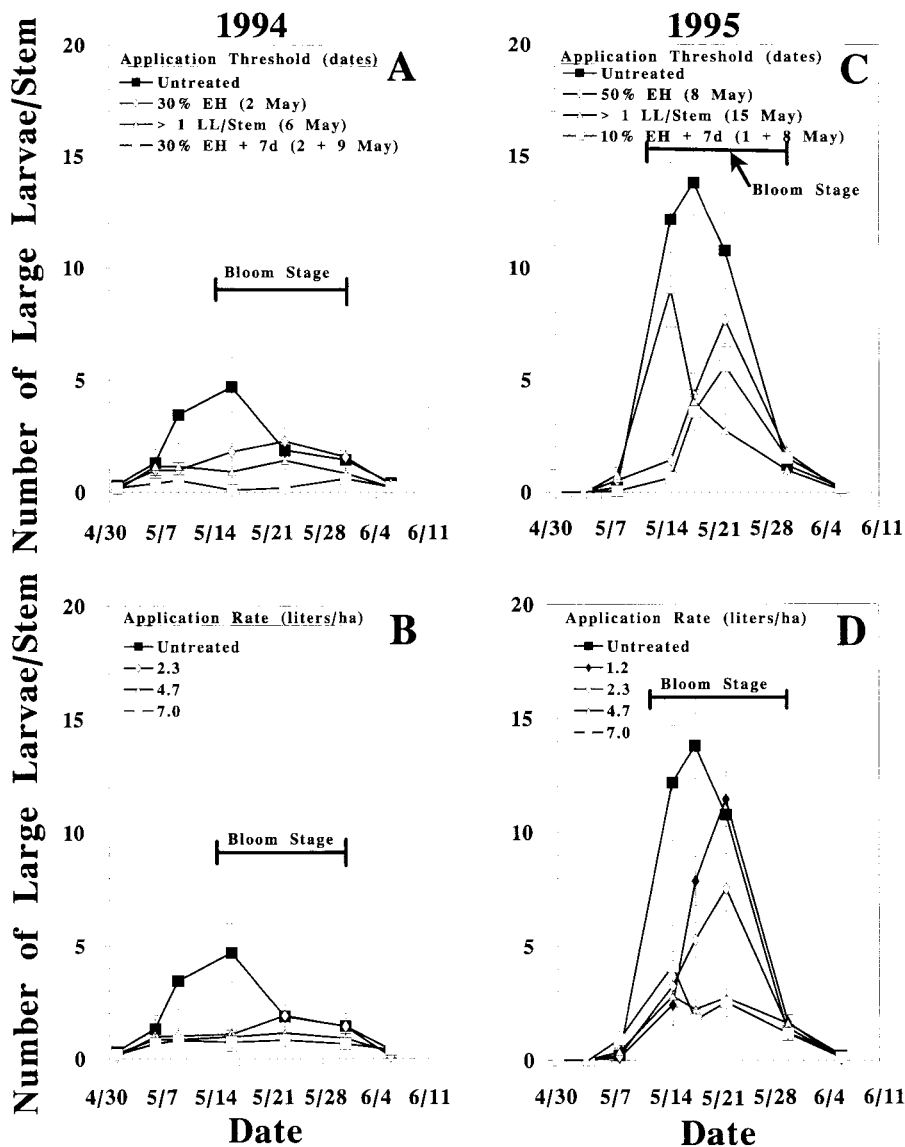


Fig. 2. Mean ($\pm$ SEM) number of large *L. decemlineata* larvae per potato stem in small plots that were either untreated or treated with *B. thuringiensis* subsp. *tenebrionis* using various timing thresholds (A and C) and rates (B and D) in 1994 and 1995.

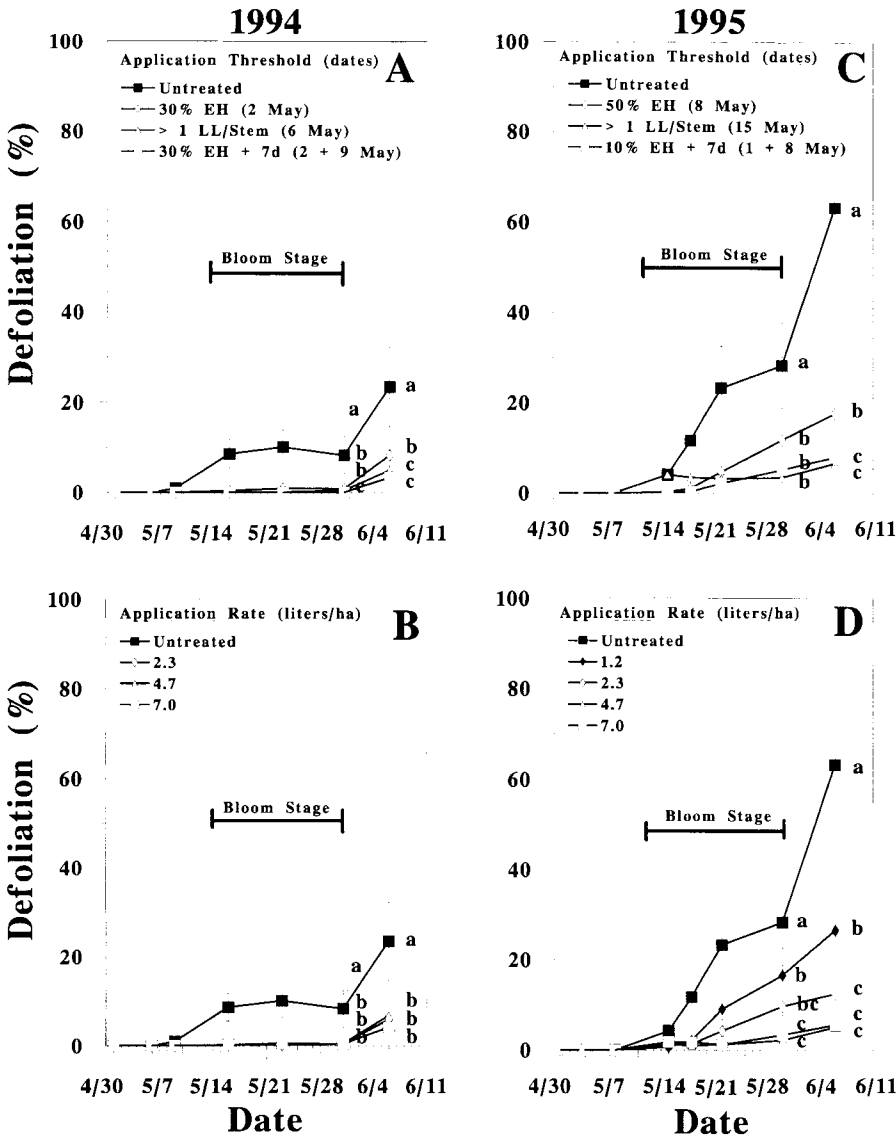


Fig. 3. Mean ($\pm$ SEM) percentage defoliation per plant by *L. decemlineata* in small plots that were either untreated or treated with *B. thuringiensis* subsp. *tenebrionis* using various timing thresholds (A and C) and rates (B and D) in 1994 and 1995. Percentage defoliation among treatments was compared at the end of the bloom stage on 31 and 30 May 1994 and 1995, respectively, and again immediately prior to harvest on 6 June 1994 and 1995. Means followed by the same letter on a specific date are not significantly different ($P > 0.05$; Fisher's Protected LSD).

levels in treated plots continued to be significantly lower than those in untreated ones in 1994 (6 June: $F = 1031.3$; $df = 1, 3$; $P = 0.0001$) and were nearly significant in 1995 (6 June: $F = 8.5$; $df = 1, 3$; $P = 0.062$) (Fig. 3A-D). The timing of *B. thuringiensis* applications significantly affected the mean defoliation levels observed in 1994 (31 May $F = 5.5$; $df = 2, 24$; $P = 0.0107$; and 6 June: $F = 9.4$; $df = 2, 24$; $P = 0.001$) and in 1995 (6 June: $F = 4.6$; $df = 2, 30$; $P = 0.0186$). The lowest levels of defoliation occurred in plots treated either twice or once when there was >1 large larva/stem (Fig. 3A and C). The rate of *B. thuringiensis* did not affect levels of defoliation on either 31 May or 6 June 1994 ($P = 0.9464$ and $P = 0.0946$, respectively) (Fig. 3B); however, in 1995, the lowest levels of defoliation occurred in plots treated with either the 4.7 or 7.0 liters/ha rates (Fig. 3D).

More first-generation adults emerged from untreated plots than from plots treated with *B. thuringiensis* in 1994 (31 May: $F = 16.6$; $df = 1, 3$; $P = 0.0267$; and 6 June: $F = 29.2$; $df = 1, 3$; $P = 0.0124$) and in 1995 (30 May: $F = 127.3$; $df = 1, 3$; $P = 0.0015$; and 6 June: $F = 25.5$; $df = 1, 3$; $P = 0.0150$) (Fig. 1A-D). On 31 May and 6 June 1994, significantly fewer first-generation adults emerged from plots that were sprayed either twice or once, when there was >1 large larva/stem, than those that emerged from plots treated only once at 30% egg mass hatch (31 May: $F = 4.6$; $df = 2, 24$; $P = 0.0211$; 6 June: $F = 7.2$; $df = 2, 24$; $P = 0.0036$) (Fig. 1A). On 30 May 1995, fewer first-generation adults emerged from plots that were either treated twice or once when 50% of the egg masses hatched than from those that were treated once when there was >1 large larva/stem ($F = 15.4$; $df = 2, 30$; $P = 0.0001$); however, on 6 June adult density did not differ significantly among the various timing treatments ($P = 0.2156$) (Fig. 1C). The rate of *B. thuringiensis* did not affect the numbers of first-generation adults that emerged from plots in 1994 (Fig. 1B); however, significantly fewer first-generation adults emerged from plots treated with the higher rates of *B. thuringiensis* than from plots treated with the lowest rate in 1995 (6 June: $F = 7.5$; $df = 3, 30$; $P = 0.0007$) (Fig. 1D).

Overall, results from both experiments indicated that either one application of *B. thuringiensis* timed at a threshold of >1 large larva/stem or two applications (first timed at 30 to 50% egg hatch) timed 7 days apart at a rate of 4.7 to 7.0 liters/ha provided the best protection of the potato crop during the bloom stage.

European corn borer. The level of European corn borer control was not affected either by the application timing, rate, or timing $\times$ rate interaction of *B. thuringiensis* ($P > 0.05$). Therefore, the following discussion will compare pooled *B. thuringiensis* treatment means with untreated control means, standard means, or both.

Mean percentage of injured stems by corn borer was lower in *B. thuringiensis*-treated plots than in untreated ones (Table 2). However, these differences were significant only in 1998 ($F = 10.2$; $df = 1, 3$; $P = 0.0495$), approached significance in 1997 ($F = 9.4$; $df = 1, 3$; $P = 0.0549$) and were not significant in 1999 ($P = 0.2245$). The percentage of injured stems was significantly lower in carbofuran-treated plots than in either the *B. thuringiensis*-treated or untreated plots in 1997 ($F = 19.30$; $df = 2, 6$; $P = 0.0024$), 1998 ($F = 11.86$; $df = 2, 6$; $P = 0.0082$), and 1999 ($F = 7.6$; $df = 2, 6$; $P = 0.0227$) (Table 2).

Discussion

Defoliation by Colorado potato beetles was effectively managed by using one spray, timed when there was >1 large larva/stem and coincided with the appearance

Table 2. Mean ($\pm$ SEM) percentage of potato stems injured by *O. nubilalis* at the end of the season after plots were either treated with *B. thuringiensis* subsp. *kurstaki*, carbofuran or were not treated. Means for the *B. thuringiensis* treatments represent pooled data from all timing $\times$ rate combinations

Treatment	Mean percentage ($\pm$ SEM) of injured stems in June
<i>1997 Experiment</i>	
Carbofuran	1.0 $\pm$ 1.0
<i>B. thuringiensis</i>	7.7 $\pm$ 1.3
Untreated	16.0 $\pm$ 2.8
<i>1998 Experiment</i>	
Carbofuran	7.0 $\pm$ 2.1
<i>B. thuringiensis</i>	29.8 $\pm$ 2.1
Untreated	55.0 $\pm$ 13.2
<i>1999 Experiment</i>	
Carbofuran	15.3 $\pm$ 6.5
<i>B. thuringiensis</i>	38.7 $\pm$ 6.5
Untreated	58.0 $\pm$ 12.5

of many early third instars, at a rate of 4.7 liters/ha. This application timing $\times$ rate combination provided commercially acceptable protection of the potato crop during the bloom stage (defoliation = 2%) when the peak overwintered adult density was at or below 0.25 adults/stem. Effective use of a single *B. thuringiensis* subsp. *tenebrionis* application will reduce the overall cost of using this biopesticide compared with past regimens, which targeted neonates with repeated applications (Zehnder and Gelerner 1989, Zehnder et al. 1992, Ghidui and Zehnder 1993). A reduction in cost, protection of foliage during bloom, a similar application-timing regimen as conventional insecticides, and minimized selection pressure on potato beetle populations could increase the use of this biopesticide by growers.

Although significant levels of defoliation during bloom can be avoided with one well-timed application of *B. thuringiensis*, the potential exists for the development of a damaging first-generation adult population that could cause serious injury to the crop during the post-bloom stage before harvest. If the crop is harvested after many first-generation adults have the opportunity to feed for several days, they are likely to successfully overwinter (Nault et al. 1996) within and near these fields and could cause problems the following year. However, this situation could be avoided as long as the crop is rotated ≥ 0.2 to 0.5 km from previous year's field (Weisz et al. 1994, Follett et al. 1996, Speese and Sterrett 1998). Based on this information, the *B. thuringiensis* timing and rate regimen mentioned above may be most effective in fields where the overwintering potato beetle population is predicted to be low to moderate

(e.g., <1 adult per 5 stems). In fields where the overwintering population is ≥ 1 adult per 4 stems, two applications in which the first still targets large larvae may be most effective.

Although European corn borer infestations were reduced using *B. thuringiensis*, the level of control was not commercially acceptable. An optimal *B. thuringiensis* application timing x rate strategy was not identified. Perhaps, in our study, the initial timing of the *B. thuringiensis* application was too late to be effective. For example, the mean percentages of corn borer infested stems before application were 8, 25 and 26% in 1997, 1998 and 1999, respectively. At the end of the season, the mean percentages of injured stems in *B. thuringiensis*-treated plots had increased by only 0, 20 and 50%, respectively; whereas, corn borer injury in the untreated plots had increased by 100, 120 and 123%, respectively. The neonates that were established at the time of application in the *B. thuringiensis*-treated plots may never have encountered a lethal dose of the toxin on the foliage. Although larvae will make several tunnels during their development (Nault and Kennedy 1996), they are unlikely to feed on foliage between the time they exit and reenter the inside of the stem. If this was the case, *B. thuringiensis* applications should be initiated earlier, when corn borer egg masses are first observed in the field, rather than when neonates are first observed.

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