Effect of *Bacillus thuringiensis* Application Interval on European Corn Borer (Lepidoptera: Pyralidae) Control in Sweet Corn[1]

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ABSTRACT Ground-applied treatments of two commercial *Bacillus thuringiensis* subsp. *kurstaki* formulations (MVP and Dipel ES) and tank-mixes with a pyrethroid (Ambush 2E) were evaluated for control of European corn borer, *Ostrinia nubilalis* (Hübner), larvae in sweet corn. Treatments were applied at average intervals of 3.4, 5, 7, and 10 days to determine field persistence. Manual infestations of first-instar *O. nubilalis* were used to augment natural populations. During both years, there were no significant interactions between application interval and treatment for all dependent variables tested, including late instars per ear, percent marketability, yield, and predator density. Regardless of application interval, MVP provided greater larval control than Dipel ES. However, the decline in efficacy of the encapsulated MVP formulation occurred at the same rate as that of the non-encapsulated Dipel ES formulation over the 3.4 to 10-d intervals. Tank-mixes of *B. thuringiensis* + low-rate permethrin provided no additional control compared with low-rate permethrin alone. Given the infestation levels present in this test, neither *B. thuringiensis* formulation provided control sufficient to maintain current processor standards of 5-10% infested ears at harvest.

KEY WORDS *Ostrinia nubilalis*, microbial control, microbial persistence

The presence of insects in sweet corn, *Zea mays* L., is a major concern for the processing industry. Insects, parts of insects, and damaged kernels can be transported through the processing facility and contaminate final product. Consequently, vegetable processors set extremely low tolerance levels for insect pests to met consumer expectations for insect-free food (Pimentel et al. 1977, Shelton 1986, Grafius et al. 1990).

In Minnesota, European corn borer, *Ostrinia nubilalis* (Hübner), is the most economically important insect pest of sweet corn. Producers usually apply two to four applications of a pyrethroid or organophosphate insecticide between first silk and harvest to manage *O. nubilalis* (Noetzel et al. 1985, Gingera et al. 1993).

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Although most synthetic insecticides, particularly the pyrethroids, provide good control of *O. nubilalis* (e.g., Bartels and Hutchison 1993, 1994), these materials are being scrutinized more closely for potential negative effects on non-target organisms (e.g., Jepson 1989, Somerville and Walker 1990).

One biorational alternative to synthetic insecticide is the bacterium *Bacillus thuringiensis* Berliner. Early commercial formulations of *B. thuringiensis* have been evaluated for *O. nubilalis* control in sweet corn with variable and generally unacceptable results compared with synthetic insecticides (Hudon 1962, 1963). Although laboratory research has verified that *B. thuringiensis* is highly toxic to *O. nubilalis* larvae (Beegle et al. 1981a, Mohd-Shalleh and Lewis 1982), the bacterium loses biological activity quickly under field conditions (Ignoffo et al. 1974, Pinnock et al. 1974, Lynch et al. 1980, Beegle et al. 1981b). Poor field performance is usually attributed to inactivation by ultraviolet light (Ignoffo et al. 1977), but exposure to other abiotic influences such as leaf temperature and vapor pressure deficit also affect pathogenicity (Leong et al. 1980).

Efforts to improve field persistence have focused on a variety of encapsulation technologies. Two popular formulations include the use of cornstarch granules to encapsulate *B. thuringiensis* spores and the protein crystal with an ultraviolet protectant (Dunkle and Shasha 1988, 1989), and encapsulation of the *B. thuringiensis* crystal within another bacterium (Gelernter 1990). Mycogen (San Diego, CA) developed the MVP formulation using the MCap delivery system, which encapsulates *B. thuringiensis* subsp. *kurstaki* δ-endotoxin within *Pseudomonas florescens*. A genetically engineered strain of *P. florescens* is used which contains the gene coding for the production of the δ-endotoxin. After the δ-endotoxin is produced, *P. fluorescens* are killed and treated to form a protective microcapsule composed of the cell matrix. In contrast to most *B. thuringiensis* products, spores are not present in the MVP formulation (Gelernter 1990).

The objective of this study was to evaluate the use of *B. thuringiensis* subsp. *kurstaki* as a viable alternative for *O. nubilalis* management in midwestern sweet corn. The performance of MVP was compared with a traditional *B. thuringiensis* formulation, Dipel ES (Abbott Laboratories, North Chicago, IL), a synthetic insecticide, Ambush 2E ([permethrin] Zeneca, Wilimington, DE), and combinations of *B. thuringiensis* with reduced rates of Ambush 2E. To determine persistence in the field, each treatment was evaluated at four application intervals.

Materials and Methods

Evaluation of *B. thuringiensis* formulations and application interval was conducted during 1990 and 1991 at the Southern Experiment Station, Waseca, MN. 'Jubilee' sweet corn was mechanically planted with a four-row planter into 76-cm rows on a Nicollet clay loam (Aquic Hapludoll, fine loamy, mixed mesic). The crop was managed using standard agricultural practices for dryland production in the region (Hutchison et al. 1991, Rosen and Munter 1992). Four-row plots, 8.5 m in length, with two buffer rows on each side, were separated by 1.2 m alleys on each end.

Manual infestations of *O. nubilalis* first instars were made to ensure uniform pest pressure across treatments. Larvae were obtained from a colony maintained in the Department of Entomology, University of Minnesota, St. Paul. The colony originated from collections in Goodhue Co. in southeastern Minnesota during 1989. The colony was screened extensively for pathogens and reared through 5 and 17 generations before use in 1990 and 1991, respectively. Before infesting field plots, neonate larvae were mixed with ground, dry corn grits and poured in 0.5-liter bottles. Each bottle was sub-sampled to verify that it contained the desired infestation density and a uniform mix. Bottles with larval counts outside 2.5 standard deviations of the mean were remixed. Larvae were then transported to the field in coolers and applied to the axis of the primary ear leaf of each plant using a Davis inoculator (BioServ, Frenchtown, NJ). All infestations were made between 2 to 4 hr after mixing in the laboratory.

During both years, treatments were applied using a modified 6-row Hagie high-clearance sprayer pressurized with CO_2. Applications were made a rate of 327 liters/hectare at 2.3 kg/cm^2 and 1.2 km/h. In 1990, materials were applied with three nozzles per row (one over the top and one drop nozzle on each side). In 1991, only two drop nozzles per row were used to apply materials to the ear zone. Applications were scheduled to be made at 3, 5, 7 and 10 day intervals until harvest. However, some application dates were missed because of rain; final application intervals averaged 3.4 (8 applications), 5.0 (6 applications), 7.0 (4 applications), and 10.0 (3 applications) days for both years. Treatments for both years are summarized in Table 1. An untreated check was included to determine larval survival, but was not included in the factorial analysis.

1990. Sweet corn was planted 7 June. Experimental plots were arranged in a randomized complete block design and replicated four times. Plants were at the R1 (early silk) growth stage (Ritchie et al. 1992) on 4 August and the first *O. nubilalis* infestation was made 6 August with 25 larvae per ear. A second infestation of 25 larvae per ear was made 14 August. All plots were treated with insecticides for the first time 8 August, two days after the first infestation.

To estimate the impact of each interval and treatment combination on the natural enemy complex, counts of predators in the ear zone were taken from 10 plants per plot in replicates 1 and 2 on 29 August. The ear zone included all surfaces one leaf above and one leaf below the primary ear. Numbers of generalist predators were recorded by family and life stage.

Evaluation of larval infestation and ear marketability was made 4 September by hand-harvesting 20 ears from each plot (80 ears per treatment). Larval instar and location within the ear were recorded. Instar determination was made by visual comparison of the head capsule width with larval specimens of known instar. Yield evaluations were completed 5 September by mechanically harvesting 9 m of row from each plot and processing the samples through commercial husking and cutting equipment at the Southern Experiment Station.

1991. Sweet corn was planted 29 May. Plants were at the R1 (early silk) growth stage (Ritchie et al. 1992) 23 July and the first insecticide applications were made 26 July. In contrast to the 1990 study, treatments were arranged in a split-plot design with application intervals as main plots and treatments as

Table 1. Treatments applied during 1990 and 1991 to 'Jubilee' sweet corn, Waseca, MN.

Treatment*	Rate (AI/ha)	Year
MVP	0.079 kg endotoxin/ha**	1990, 1991
Dipel ES	39.5 BIU/ha†	1990, 1991
Ambush 2E	0.17 kg	1990, 1991
MVP +	0.079 kg endotoxin/ha**	1991
Ambush 2E	0.056 kg	
Dipel ES +	39.5 BIU/ha†	1991
Ambush 2E	0.056 kg	
Ambush 2E	0.056 kg	1991

* For both years, all treatments were applied at average application intervals of 3.4, 5, 7, and 10-d.
** Equivalent to 5.8 liters of product per ha; formulation averaged 13.5 g endotoxin/liter.
† Equivalent to 2.3 liters of product per ha; formulation averaged 17,600 IU/mg and 16.9 BIU/liter.

subplots. This design was employed primarily for logistical reasons, but also to improve the precision in testing differences among treatment means. Also in 1991, manual infestations were delayed until after the first insecticide application of each treatment had been made to improve control of larvae. To better simulate a natural oviposition window, the number of infestations was increased from two to three, but the number of larvae per infestation was reduced from an average of 25 to 10 larvae per ear. Larval infestations were made 29 July, 1 August, and 5 August.

On 22 August, predator abundance was measured in the ear zone of 10 plants per plot for all treatments (40 plants per treatment). Larval infestations and ear marketability were determined 23 August by hand-harvesting 25 ears per plot (100 ears per treatment). Larval instar and location within the ear were recorded. Yield was determined 26 Aug by mechanically harvesting 12 m of row and processing the corn through commercial husking and cutting equipment. In addition to recording harvest weights, counts were taken on insect contaminants present in the final cut-corn product. Before counts were taken, cut-corn samples were transferred to the Green-Giant processing facility at Le Sueur, MN, and processed through standard froth washer and shaker table equipment.

Data Analysis. Before analysis, data were tested for homogeneity of variances using Levene's test (Levene 1960). To correct for unequal variances, all 1991 data were transformed using $\sqrt{(x + 0.5)}$; percentage data for both years

were transformed using arscine. Data for 1991 were analyzed as a split-plot design. However, because the main-plot errors were less than the sub-plot errors, they were considered estimates of the same σ^2 (Steel and Torrie 1960). Data for each year were, therefore, analyzed as a two-factorial analysis of variance (ANOVA). The Ryan-Einot-Gabriel-Welsch Multiple F-test ($P = 0.05$) was used to distinguish treatment and application interval differences (SAS 1988). The persistence of MVP and Dipel ES in relation to application interval was compared using the continuous-by-class effects in the general linear model (GLM) procedure (SAS 1988). This model tests for a significant linear effect and homogeneity of the slopes. Larval contaminant and damaged kernel data from 1991 were analyzed using Pearson's correlation coefficient (SAS 1988).

Results and Discussion

For both years, no significant interaction ($P > 0.05$) was observed between application interval and treatment for *O. nubilalis* late instars per ear, percent marketability, cut-corn yield or total predator density (Table 2). All treatments were consistent each year in relative performance across the application intervals tested. Dominant predator species found during both years included two coccinellids (*Hippodamia convergens* Guérin-Méneville and *Coleomegilla maculata* [De Geer]), an anthocorid (*Orius insidiosus* [Say]), chrysopids, nabids, and spiders.

In 1990, there were significantly fewer late instars in the tip, side, or butt of ears for the 3.4-d application interval compared with the 7.0-d interval (Fig. 1 A). Application interval had no effect on percent marketable ears (defined as no late instars in the tip, side, or butt) (Fig. 2A), or predator density (Fig. 3 A).

MVP and Ambush 2E did not differ significantly in effectiveness and had fewer late instars in the tip, side, or butt of ears than Dipel ES (Fig. 4 A). MVP and Ambush 2E also provided more marketable ears than Dipel ES (Fig. 5 A). Predators were more numerous in the *B. thuringiensis* treated plots than in the Ambush 2E treated plots (Fig. 6A).

In 1991, compared with the 5, 7, and 10-d application interval, the 3.4-d interval had fewer late instars in the tip, side, or butt of ears. There were also fewer late instars in the tip, side, or butt of ears with the 5-d application interval than in the 10-d interval (Fig. 1 B). The 3.4-d interval had a higher percentage of marketable ears than 5, 7, and 10-d intervals (Fig. 2 B). Predators were more abundant in the 10-d interval than in all three shorter intervals (Fig. 3 B). The significant application interval effect for cut-corn weight (Table 2) resulted from a yield reduction in the 3.4-d interval plots. This finding is opposite from the expected result of increased yield because of fewer larvae and probably resulted from the split-plot design. In the 3.4-d interval plots, the soil was compacted by more frequent passes with the high-clearance sprayer, as compared with the randomized complete block design in 1990, in which all plots received the same amount of wheel traffic. This compaction was probably responsible for the yield reduction.

Ambush 2E-significantly reduced at-harvest infestations of late instars in the tip, side, or butt of ears over all other treatments (Fig. 4 B). Ambush 2E also provided more marketable ears than all other treatments (Fig. 5 B). All

Table 2. Analysis of variance results (P values) for *O. nubilalis* larval parameters, cut corn yield, and predator density in response to application interval (INT) and treatment (TRT) 1990 - 1991, Waseca, MN.

Dependent Variable	1990			1991		
	INT	TRT	INT × TRT	INT	TRT	INT × TRT
Late-instars/ear*	0.03	0.0001	NS	0.0001	0.0001	NS
% Marketable ears**	NS	0.0004	NS	0.0001	0.0001	NS
Cut corn wt./9 m-row	NS	NS	NS	0.017	NS	NS
Predators/10 plants	NS	0.0005	NS	0.0001	0.0001	NS

NS = non-significant (P > 0.05).
* Late-instars includes 3-5 present in tip, side or butt of ears only.
** Marketability based on percentage of ears with no larvae or damage in tip, side or butt of the ear.

tank-mix treatments significantly reduced larval numbers compared with MVP or Dipel ES alone, for late instars in the tip, side, or butt of ears (Fig. 4 B). Tank-mix treatments also resulted in more marketable ears (Fig. 5 B). However, neither of the tank-mix treatments provided significantly greater larval control than low-rate Ambush 2E alone (Fig. 4 B and 5 B). The MVP formulation had significantly fewer larvae compared with Dipel ES for late instars in the tip, side, or butt of ears (Fig. 4 B). Both *B. thuringiensis* treatments resulted in higher predator numbers than any treatment with Ambush 2E (Fig. 6 B).

Efficacy data for late instars was used to evaluate differences in persistence between MVP and Dipel ES, relative to application interval. Preliminary analysis of variance (GLM) indicated no significant differences between years when analyzing only the *B. thuringiensis* treatments. Therefore, data from both years were pooled. A significant linear effect (P = 0.001) was detected for percent control of late instars in the tip, side, or butt for both MVP and Dipel ES. However, no difference was detected between the slopes of the two lines (P = 0.95) (Fig. 7), suggesting that the decline in percent control, relative to application interval, was similar for encapsulated MVP and non-encapsulated Dipel ES. Despite the similarity in persistence between the formulations however, overall control by MVP at each spray interval was significantly greater than that of Dipel ES.

Correlations between at-harvest larval densities and the incidence of larval or larval + damaged kernel contaminants were highly significant (P = 0.006 and P = 0.003, respectively). However, the relationship, based on 1991 data, was highly variable resulting in low correlation coefficients. Values of r were only 0.52 and 0.57, respectively. Although most of the larval contaminants occurred at infestation densities exceeding 0.5 larvae per ear, 7.7% (2/26) of the samples

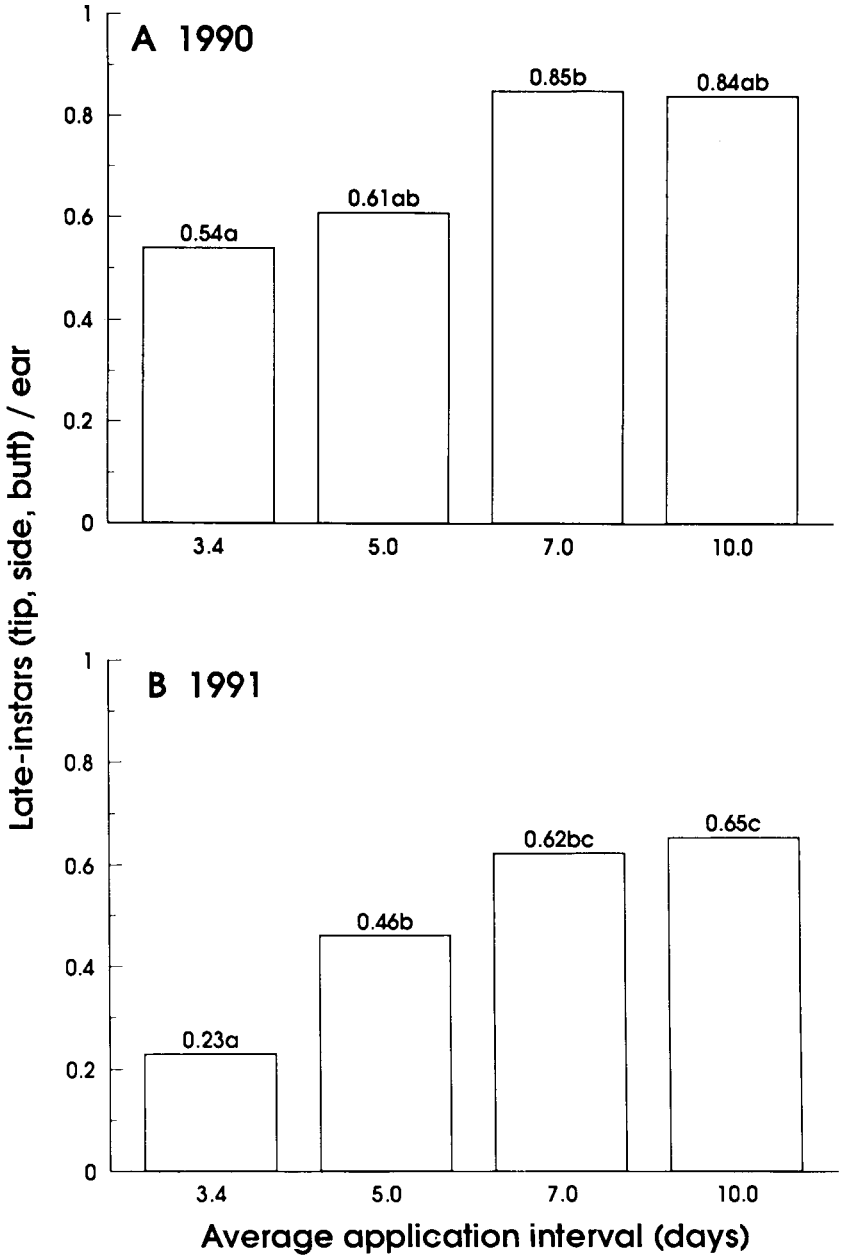

Fig. 1. Mean number of *O. nubilalis* late instars (tip, side, or butt) in relation to application interval, Waseca, MN 1990 (A) and 1991 (B.) Means followed by the same letter are not significantly different, REGWF test ($P = 0.05$).

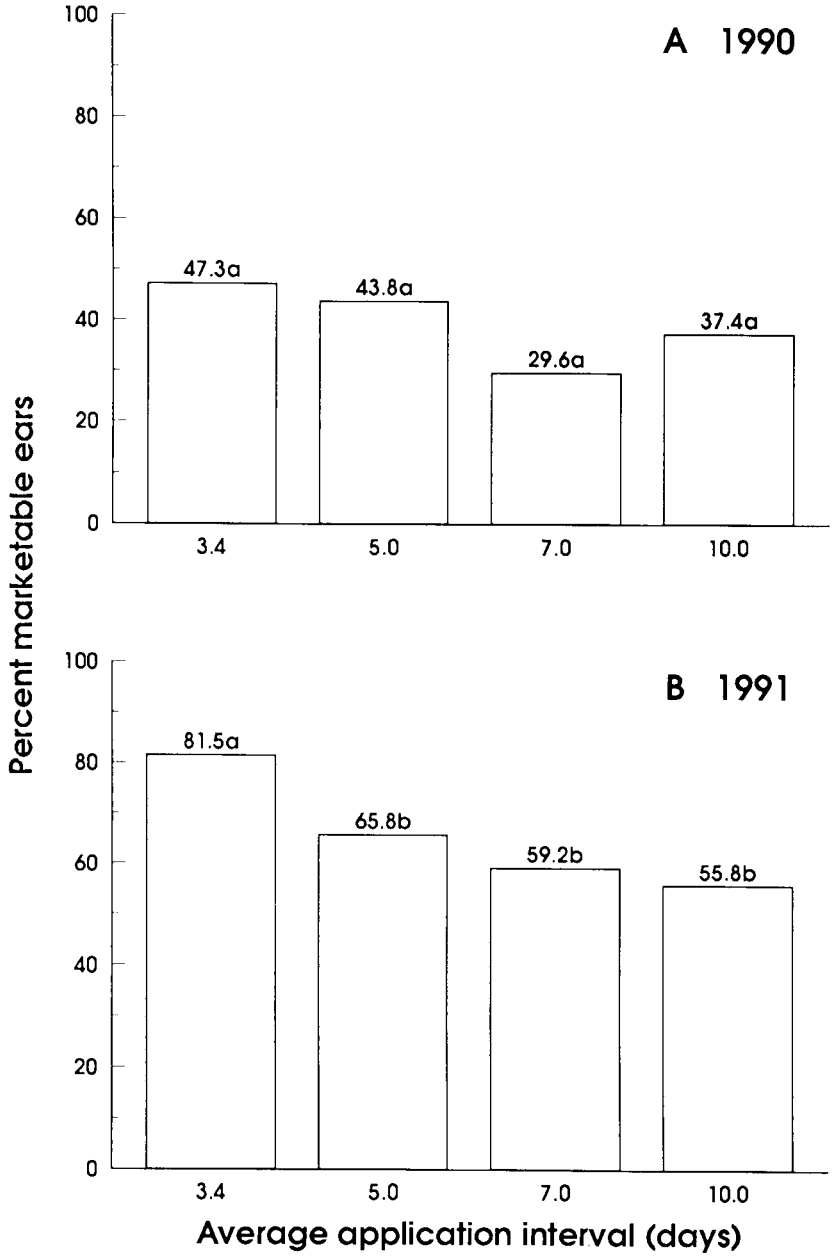

Fig. 2. Mean percent marketable ears (defined as no late instars in the tip, side or butt) in relation to application interval, Waseca, MN 1990 (A) and 1991 (B). Means followed by the same letter are not significantly different, REGWF test ($P = 0.05$).

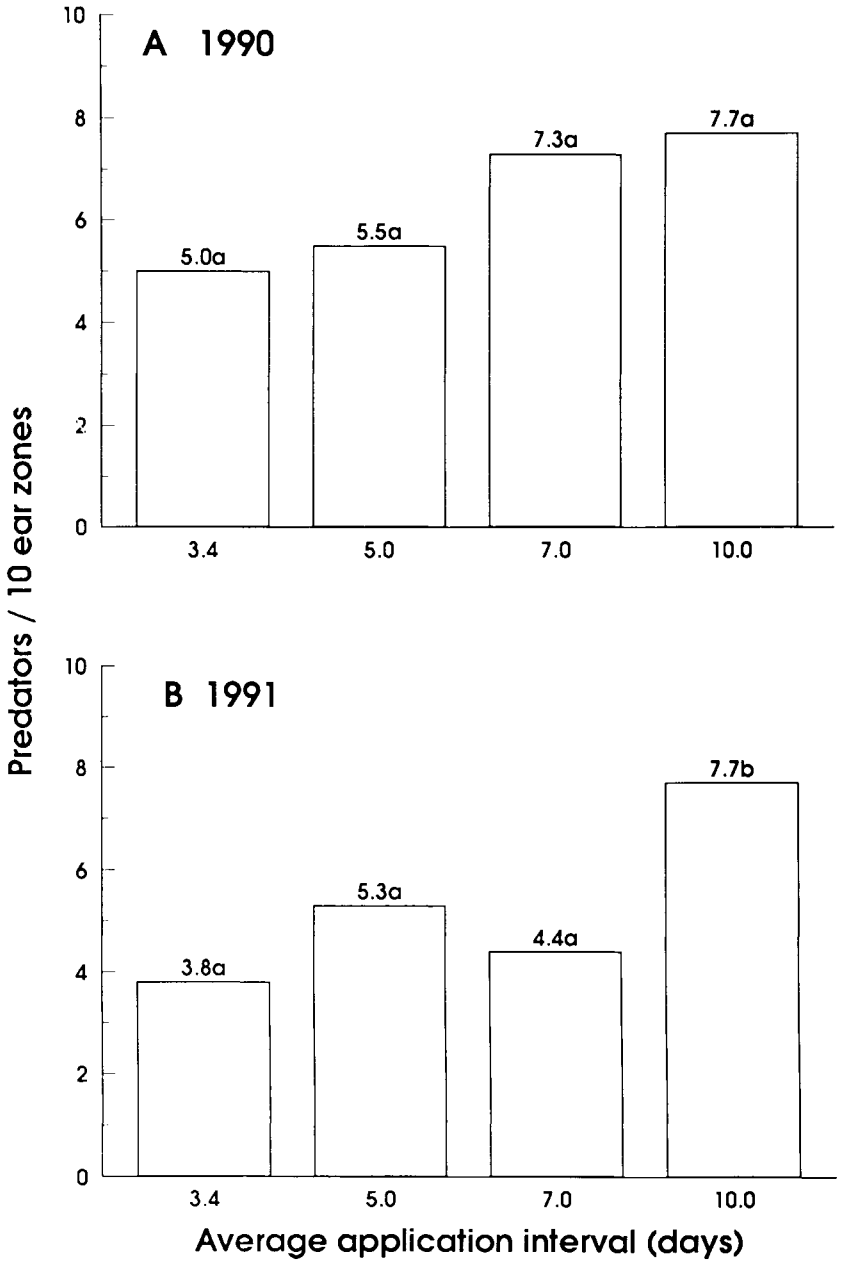

Fig. 3. Mean total predator density in relation to application interval, Waseca, MN 1990 (A) and 1991 (B). Means followed by the same letter are not significantly different, REGWF test ($P = 0.05$).

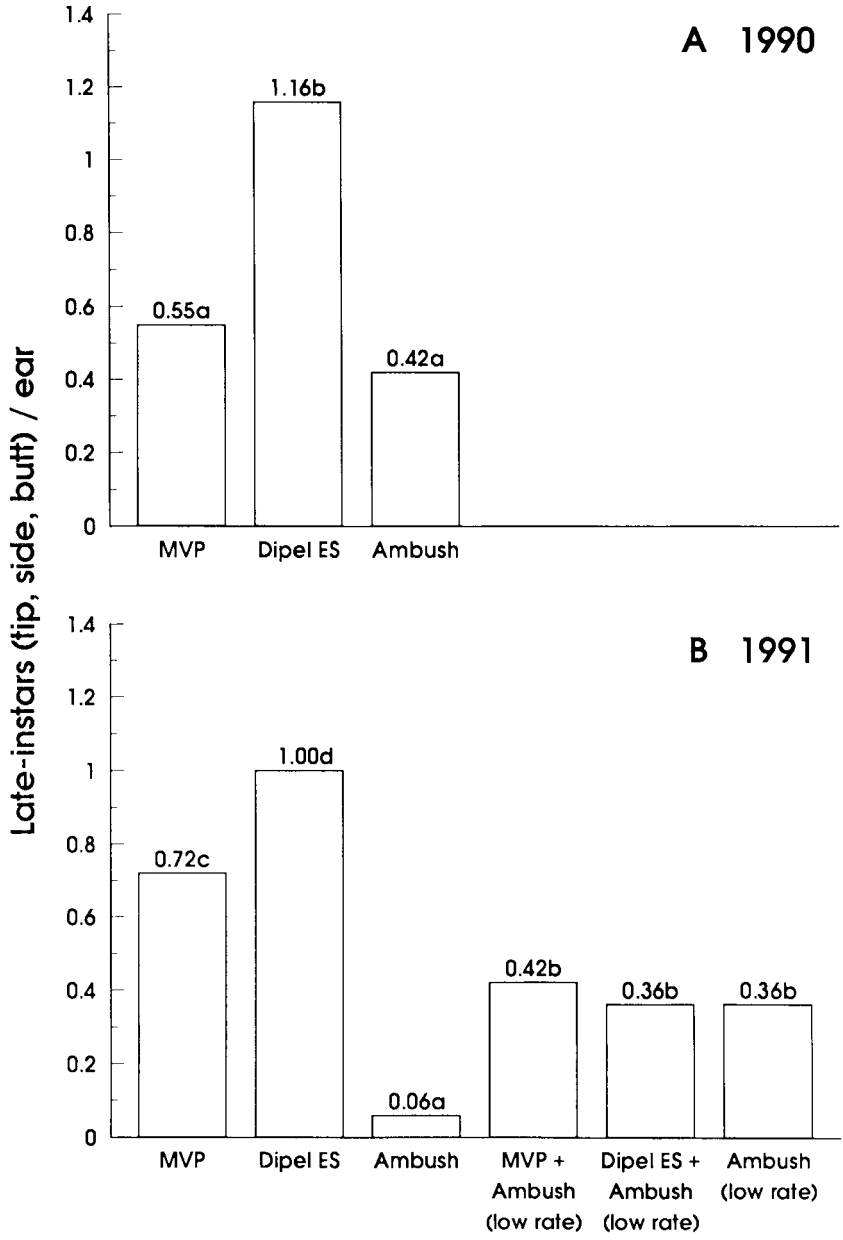

Fig. 4. Mean number of *O. nubilalis* late instars (tip, side, or butt) in relation to treatment, Waseca, MN 1990 (A) and 1991 (B). Means followed by the same letter are not significantly different, REGWF test ($P = 0.05$).

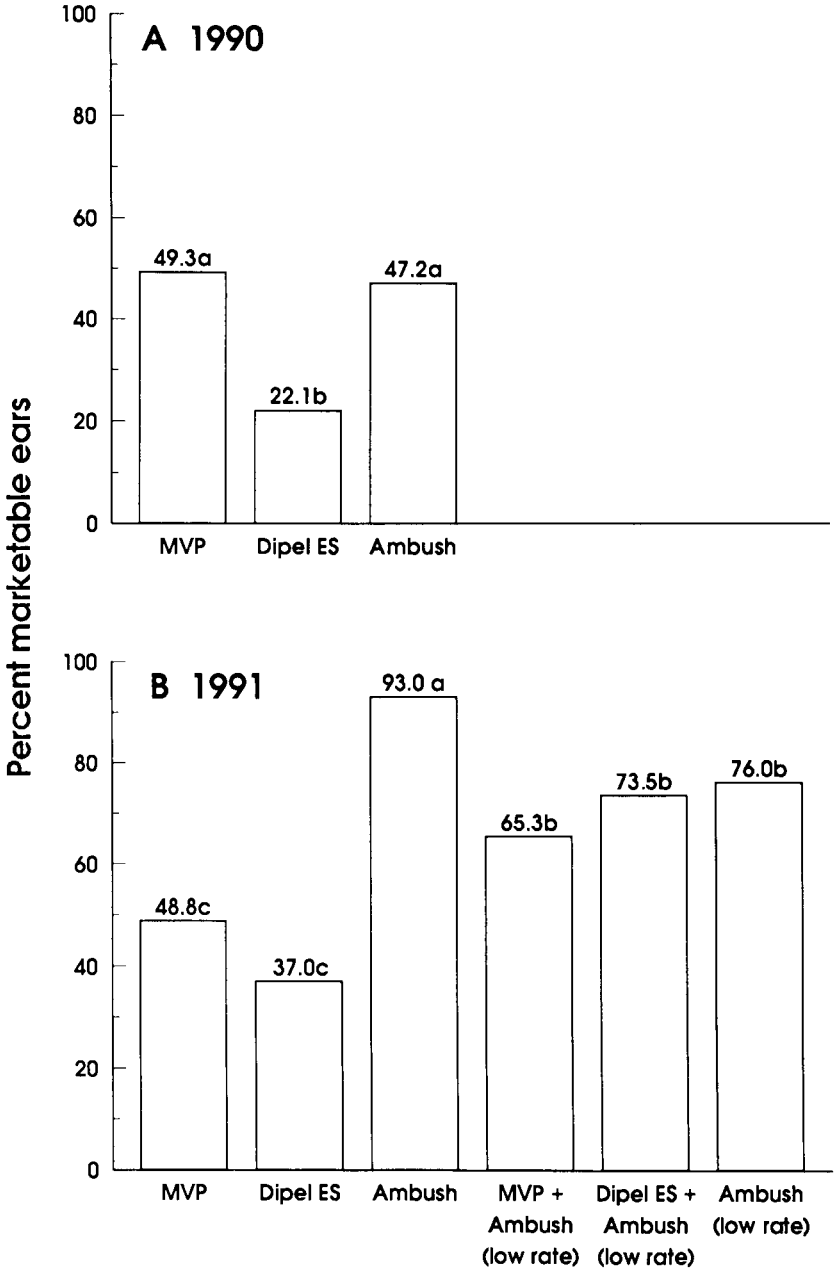

Fig. 5. Mean percent marketable ears (defined as no late-instars in the tip, side or butt) in relation to treatment, Waseca, MN 1990 (A) and 1991 (B). Means followed by the same letter are not significantly different, REGWF test ($P = 0.05$).

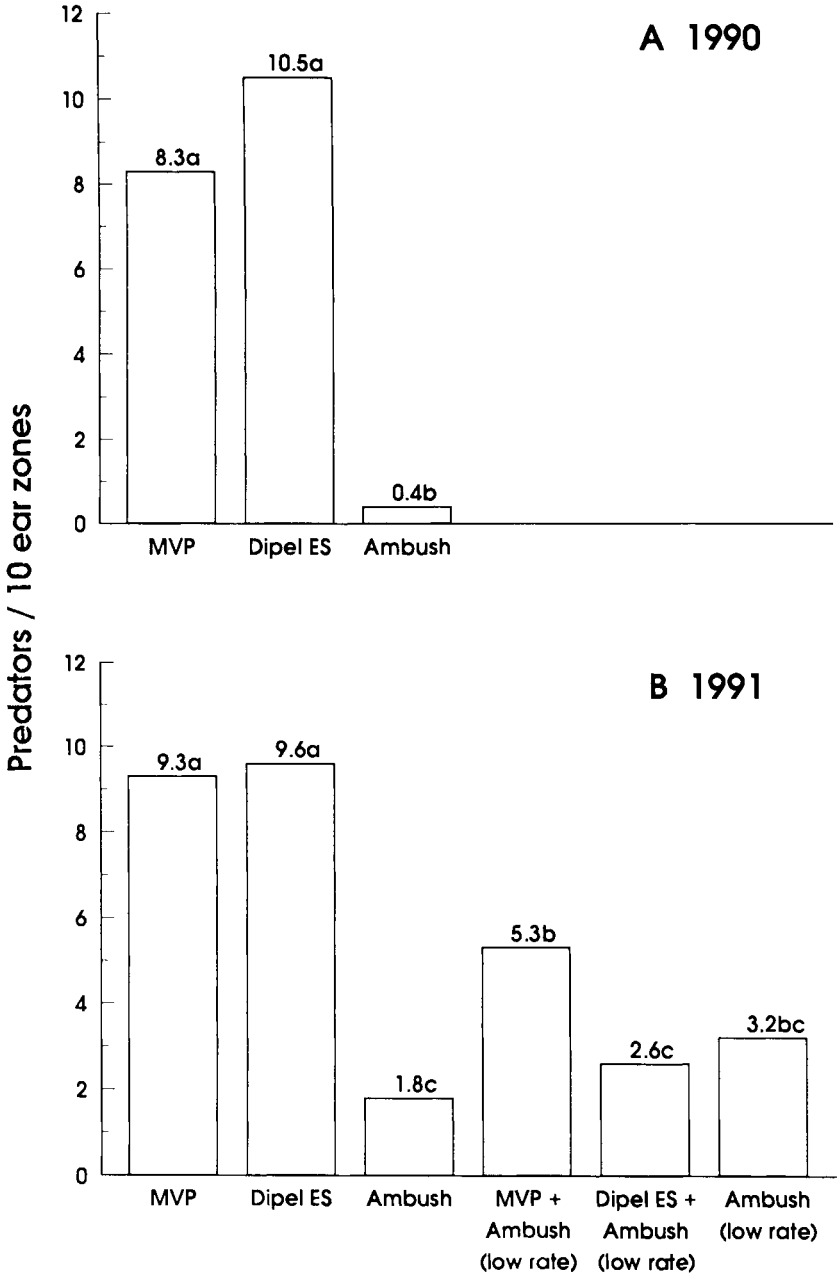

Fig. 6. Mean total predator density in relation to application interval, Waseca, MN 1990 (A) and 1991 (B). Means followed by the same letter are not significantly different, REGWF test ($P = 0.05$).

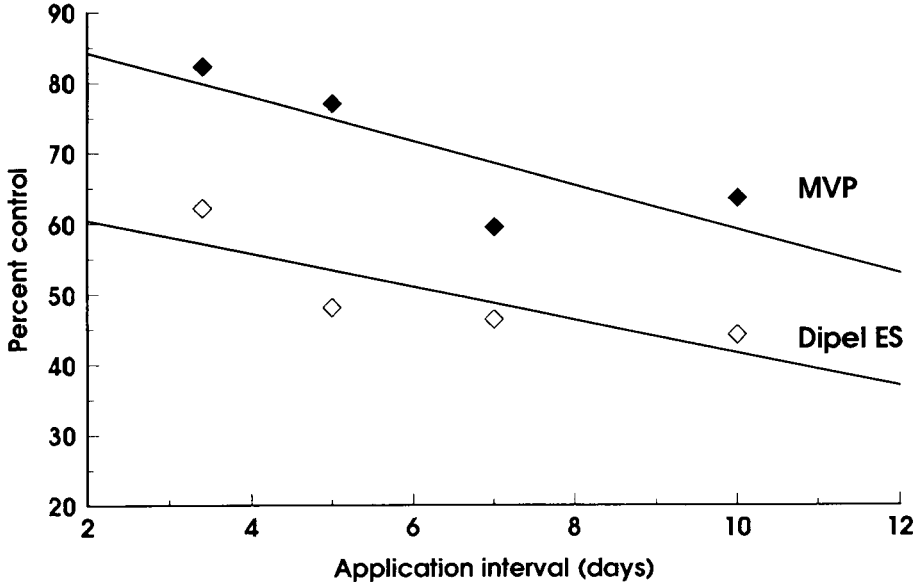

Fig. 7. Mean percent control of *O. nubilalis* late instars by MVP (y = 90.45 - 3.12x, R^2 = 0.68) and Dipel ES (y = 65.06 = 2.34x, R^2 = 0.67) in relation to application interval, Waseca, MN 1990 & 1991.

contained larval contaminants for field densities ranging from 0.025 to 0.075 larvae per ear. While an incidence of 0.04 larvae per kg cut-corn seems low, this is equivalent to one larva per 24.9 kg cut-corn product, and a much higher incidence than the current acceptable maximum of one larva per 454 kg cut-corn product (G. R. K, unpublished data). Likewise, 27% (7/26) of the samples harbored damaged kernels and/or larval contaminants for field densities averaging ≤ 0.20 larvae per ear. Damaged kernels, with either obvious feeding holes or brown discoloration, are nearly as damaging to final product as larval contaminants. However, larval contaminants alone result in high volumes of complaints annually to sweet corn processing companies (G. R. K., unpublished data).

Under the high *O. nubilalis* infestation levels present during both years, neither *B. thuringiensis* formulation provided control sufficient for meeting vegetable processor standards of at-harvest ear infestations of 5-10% (Gingera et al. 1993). Infestations >15% usually cause increased processing costs, or rejection of infested fields. These results support the findings of previous ground-application studies including those of Hudon (1962) in Canada, and more recent studies in Minnesota, where MVP provided a wide range of larval control (44 to 84%) at ≈7-d intervals (e.g., Bartels and Hutchison 1993, 1994). However, both MVP and Dipel ES, combined with low rates of permethrin,

provided more consistent control of natural infestations of *O. nubilalis* in an aerial application study (Bartels and Hutchison 1995). Specifically, the *B. thuringiensis* plus low-rate permethrin treatments provided control equal to that of permethrin or methyl parathion applied at labeled rates.

Although more research should be done to elucidate the relationship between at-harvest larval densities and actual probability of insect contamination in final product, results from this study suggest that significant levels of larval or damaged kernel contaminants can result from relatively low field infestations (i.e., 5-10% of ears infested). Thus, any alternative pest management systems in processing sweet corn must continue to provide consistently high levels of insect control.

As the negative effects of conventional insecticides continue to be investigated, and environmental costs of pesticides are included in action threshold equations (e.g., Kovach et al. 1992, Higley and Wintersteen 1992), *B. thuringiensis* products may receive more consideration as a sweet corn insecticide alternative by the processing industry. Although *B. thuringiensis* and native natural enemies may not provide adequate ear protection from *O. nubilalis,* the results from this study indicate that *B. thuringiensis* is an effective complementary tactic that could be successfully integrated with other biologically-based control strategies (e.g., Frisbie and Smith 1989).

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