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Comparison of Two Isolates of *Bacillus thuringiensis* in a Field Test on Western Spruce Budworm (Lepidoptera: Tortricidae)[1]

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ABSTRACT Two isolates of *Bacillus thuringiensis* formulated as Thuricide 32LV and SAN-415 32LV were compared for efficacy against western spruce budworm, *Choristoneura occidentalis* Freeman, in Oregon. The products were applied by helicopter at 20 and 30 billion International Units (BIU) in a spray volume of 7.1 liters/ha. The 30 BIU per ha dosage provided better population control than the 20 BIU dosage with both *B. thuringiensis* isolates; however, only the difference between dosages for the SAN-415 32LV formulation was significant. Differences in efficacy between the two *B. thuringiensis* isolates were not significant. The application of *B. thuringiensis* improved foliage protection by 15 to 25% compared with untreated checks.

KEY WORDS Insecta, Microbials, field testing, *Choristoneura occidentalis*

COMMERCIAL SPRAY FORMULATIONS of *Bacillus thuringiensis* Berliner have been used to control a wide variety of lepidopterous forest insect pests (Harper 1974, Morris 1982). Current formulations are based on the HD-1 isolate *B. thuringiensis* subspecies *kurstaki* (Dulmage & Aizawa 1982). Recently, Dubois (1985) isolated a new strain designated as NRD-12. The serological characteristics of this isolate are the same as those for HD-1 (i.e., it belongs to serovar H_{3a3b} subspecies *kurstaki*). Laboratory comparisons indicated that the potency of this new strain is several times greater than HD-1 against the tobacco budworm, *Heliothis virescens* (F.), and the gypsy moth, *Lymantria dispar* (L.) (Dubois 1985). Commercial interest in the development of NRD-12 was further stimulated by the acute sensitivity of beet armyworm, *Spodoptera exigua* (Hübner), to the new isolate (A. T. Bowen, personal communication) and has resulted in production of the formulation SAN-415 32LV by Zoecon, Inc., Palo Alto, Calif.

The new strain, NRD-12, is under consideration as a replacement for the HD-1 isolate; therefore, field efficacy data are needed against forest insect pests including western spruce budworm, *Choristoneura occidentalis* Freeman. Here we report results of a field efficacy trial in Oregon to compare the two *B. thuringiensis* strains applied at two dosages against western spruce budworm larvae.

Materials and Methods

Spray trials were conducted in 1984 on the Ochoco National Forest near Prineville, Oreg., in an area that had moderate to heavy defoliation caused by the western spruce budworm the previous year. Treatments consisted of two strains of *B. thuringiensis* formulated by Zoecon, as Thuricide 32LV and SAN-415 32LV; they were applied at 20 and 30 billion International Units (BIU) per ha.

Experimental Plots. Twenty-five 16-ha plots were arranged in five randomized blocks with each block containing the four treatments and an untreated plot (Fig. 1). Buffer strips of at least 0.33 km separated each plot to avoid contamination by drift. The plots ranged in elevation from 1,340 to 1,740 m. The physiography, soils, and climate are characteristic of the western Blue Mountain Province (Franklin & Dyrness 1973). The tree vegetation consisted primarily of Douglas-fir, *Pseudotsuga menziesii* (Mirbel) Franco, at the lower elevations; grand fir, *Abies grandis* (Douglas) Lindley, at the highest elevations; and a mixture of the two species in between. A scattered overstory of mature ponderosa pine, *Pinus ponderosa* Douglas ex Laws., was common throughout the area. Some of the plots contained a few large western larch, *Larix occidentalis* Nuttall.

Spray Application. A Hiller-Saloy 12J3 turbo helicopter equipped with two model 360A Beecomist rotary atomizers (Beemer Engineering, Fort Washington, Pa.) on a standard spray boom was used to apply the treatments at the rate of 7.1 liters/ha. A foodstuff dye, red no. 40, was added to the tank mixes at the rate of 1.20 g/liter to make spray drop stains visible on white detection cards. The helicopter was operated at an airspeed of 100 km/h, on a 25-m swath width, about 16 m above the forest canopy. Characterization and calibration tests before spray application showed that these spray parameters provided deposits of 20–35 droplets per

[1] The use of trade, firm, or corporation names in this publication is for the information and convenience of the reader. It does not constitute an official endorsement or approval by the USDA of any product or service to the exclusion of others that may be more suitable.

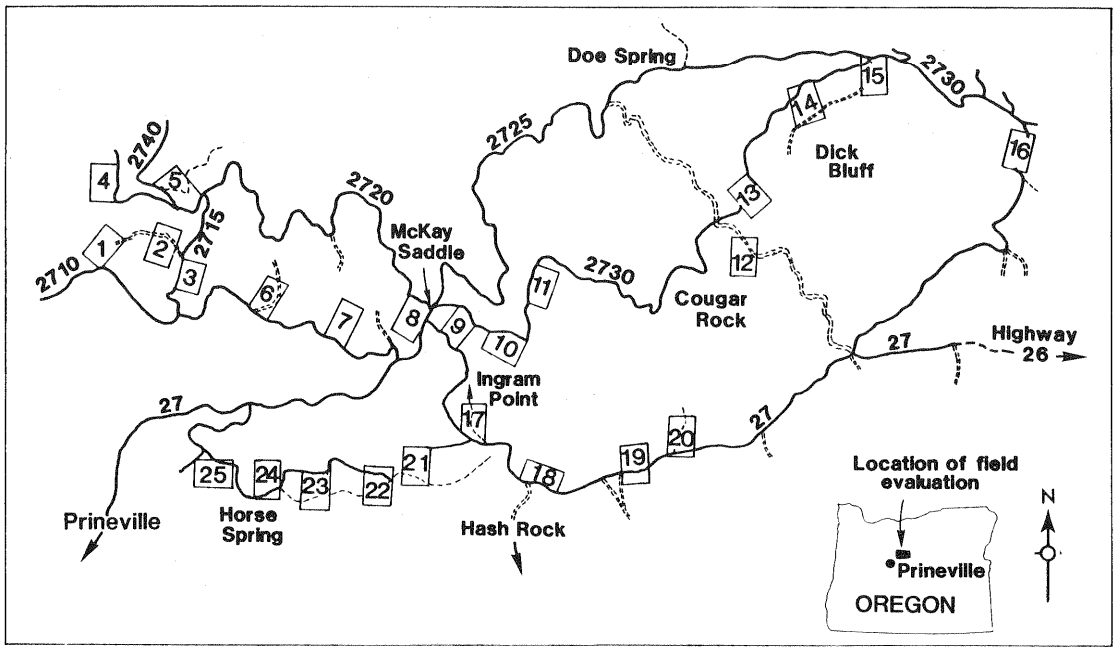

Fig. 1. Plot locations for the 1984 *B. thuringiensis* evaluation, Ochoco National Forest, Prineville, Oreg. Inset: Approximate location of the field evaluation in Oregon.

cm^2 with volume median diameters from 125–150 μm.

The spray application began after the new shoots on both host species had expanded sufficiently to provide adequate surface for impingement of the spray droplets. Timing of application was decided by monitoring accumulated degree-days (DD) at an elevation of 1,524 m on a site adjacent to plot 8. Degree-days can be used as an index to relate the percentage of shoot growth to western spruce budworm development (Ives 1974, Beckwith & Kemp 1984, Dennis et al. 1986). About 29% of the total shoot elongation occurs after 200+ DD (5.5°C threshold) when most western spruce budworm are in the fourth instar (Beckwith & Kemp 1984). Block numbers 1, 3, and 2 were sprayed on 30 June, 1 July, and 2 July, respectively. Blocks 4 and 5 were treated on 7 July and 8 July. The delay was necessary to permit proper expansion of Douglas-fir foliage on the higher elevation plots. Larvae were predominantly in the fourth and fifth instars within 48 h before spraying. The daily order of treatment was randomized to prevent a single treatment always being applied on the first helicopter flight. The SAN-415 32LV at 20 BIU/ha was not applied on block 5 because of a shortage of this formula; Thuricide 32LV at 30 BIU/ha was applied instead.

Spray-Deposit Assessment. White Kromekote cards (10.2 by 12.7 cm) (Champion International, Stamford, Conn.) were used to assess spray deposit on the ground. Twenty cards were distributed in openings across the width of each plot, perpendicular to the direction of spray swath application. The cards were collected about 30 min after spraying and read later by PEST, Inc., Sacramento, Calif., with a Quantimet 900 Image Analyzer (Cambridge Instruments, Buffalo, N.Y.) for sizing and counting of spray–deposit stains (Young et al. 1977).

Although the red dye enhanced the contrast of the deposits on the Kromekote cards, spray residues were not visible on the needles. Therefore, we were unable to count and measure droplet diameters directly on the foliage as originally planned. As an alternative evaluation, 10 needles were removed from a midcrown branch that had been collected immediately (about 30 min) after spraying from 10 grand fir and 10 Douglas-fir trees on each plot. The 100-needle, host–tree samples from each plot were consolidated and mixed thoroughly. Then, two 20-needle subsamples were drawn at random and washed with water to remove spray deposits. Pour plates were prepared on nutrient agar from the wash samples to determine the concentration of viable *B. thuringiensis* spores. Deposits were expressed as the number of viable spores per needle and as the number of viable spores per gram of oven-dried needle weight.

Population Measurements. Larval density was measured 1–2 d before and 16 d after treatment to determine population changes. Samples before spraying consisted of one 45-cm branch from the midcrown from each of 50 Douglas-fir or grand fir trees per plot. Three 45-cm branches per tree were taken in the sample after spraying from the same 50 trees. All western spruce budworm larvae were removed from the branch samples and counted in the field; density was recorded as the mean number of larvae per branch.

Table 1. Spray–deposit assessment from Kromecote cards[a]

Treatment	BIU/ha	No. of plots[c]	MMD[b]		No. of plots	Drops/cm^2	
			Mean	SEM		Mean	SEM
Thuricide 32LV	20	5	144.8	17.3	5	14.1	4.1
Thuricide 32LV	30	5	113.8	8.6	6	20.8	5.0
SAN-415	20	3	175.0	36.7	4	9.0	2.6
SAN-415	30	5	198.2	14.2	5	17.3	3.1

[a] Twenty cards per plot.
[b] MMD—The droplet size diameter that divides the spray mass into equal parts; half of the spray mass is in droplets below the MMD and half of the mass is larger.
[c] High humidity rendered the cards from blocks 2 and 3 unacceptable for MMD determination; however, droplet densities were estimated by ocular counts under a microscope.

Insect Collections. About 100 larvae per plot from both the branch samples before spraying and those 16 d after spraying were collected and reared individually on an artificial diet (Thompson & Peterson 1978) to determine survival and mortality. In addition, 50 larvae per plot were collected for rearing at intervals of 0.25, 1, 2, 3, 5, and 10 d after spraying. These larvae were obtained by dislodging them from randomly selected branches on at least 20 trees in each plot. The individual rearings were maintained in the laboratory until death or adult western spruce budworm emergence. All adult parasites were saved and identified to determine the composition of the parasitoid complex at the time of the collection. Tissues from dead larvae were examined microscopically to confirm the presence of *B. thuringiensis*.

Pupae were collected to determine mortality factors and possible sublethal effects of *B. thuringiensis* ingestion on pupal weight. We intended to collect at least 100 pupae per plot, but some plots did not yield any pupae after 8 work hours of searching. All pupae were individually weighed within 24 h of collection and held until adult emergence, parasite emergence, or death. Sex was determined in the adult stage, or in the pupal stage by the method of Jennings & Houseweart (1978). Only those pupae that produced adults were used in the analysis of pupal weight.

Defoliation. Estimates of feeding damage were based on the examination of two midcrown branches collected from each of the 50 trees used to measure population density. Feeding damage on each of 25 current shoots per branch was rated in one of four defoliation classes: 1, 0–25%; 2, 26–50%; 3, 51–75%; and 4, 76–100% (Grimble & Young 1977). The average index of all 50 trees was used to compute the average defoliation value for each plot. The difference between the plots treated with *B. thuringiensis* and the untreated check plots represented foliage saved.

Statistical Analyses. Larval density data and mortality caused by *B. thuringiensis* in the larval-rearing collections were subjected to conventional analysis of variance after an adjustment was made for unequal block representation. The sums of squares and four degrees of freedom for treatments were partitioned into sets of orthogonal single degree-of-freedom comparisons (t test). Comparisons of interest were between the means; untreated checks versus the four *B. thuringiensis* treatments; the low (20 BIU/ha) versus the high (30 BIU/ha) rates for Thuricide 32LV and for SAN-415 32LV; and Thuricide 32LV (20 + 30 BIU/ha) versus SAN-415 32LV (20 + 30 BIU/ha).

Results and Discussion

Spray–Deposit Assessment. Results of spray–deposit assessment on Kromekote cards are presented in Table 1. All of the cards showed deposit stains, indicating no apparent skips in coverage of the target areas during spraying. Droplet sizes were consistently larger for formulations of SAN-415 32LV ($\bar{x}$ = 175, 198 μm) than for Thuricide 32LV ($\bar{x}$ = 114, 145 μm).

Applications of formulations of Thuricide 32LV provided greater levels of spray deposit on foliage than SAN-415. Spore counts were consistently higher on grand fir than on Douglas-fir (Table 2). The larger grand fir needles, having a two-ranked flat arrangement, may be more efficient collectors of spray deposit than the small Douglas-fir needles arranged in a spiral. Spray deposit, expressed as the number of viable spores per gram of oven-dried weight, was similar on the two host species; however, we did not find any difference in the response of western spruce budworm larvae on Douglas-fir and grand fir.

Population Measurements. Western spruce budworm larval densities before spraying ranged from 15.2 to 22.0 larvae/branch among the four *B. thuringiensis* treatments and the untreated checks (Table 3). Comparisons of paired treatment means (t test) showed that the population was relatively homogeneous. Differences for the contrasts to compare the two dosages of SAN-415 32LV, SAN-415 32LV (20 + 30 BIU/ha) versus Thuricide 32LV (20 + 30 BIU/ha), or for the average density of all four *B. thuringiensis* treatments versus the untreated checks, were not significant. Only the difference between the two dosages of Thuricide 32LV was significant (P = 0.05). The influence of dosage is similar to that found by other researchers. Formulations of Thuricide and Dipel applied aerially to stands of balsam fir, *Abies balsamea* (L.)

Table 2. Analysis of *B. thuringiensis* spray deposits on Douglas-fir (DF) and grand fir (GF) foliage

Treatment	BIU/ha	No. of plots	Tree species	Number of viable spores per needle ($\times 10^3$)[a]		Number of viable spores per gm dry weight ($\times 10^3$)[b]	
				Mean	SEM	Mean	SEM
Thuricide 32LV	20	5	DF	4.1	1.1	486.0	123.0
			GF[c]	7.9	2.9	396.0	134.0
Thuricide 32LV	30	6	DF	3.7	1.1	429.3	127.0
			GF	10.6	3.2	530.5	157.0
SAN-415	20	4	DF	0.5	0.4	50.0	31.7
			GF	5.6	4.7	61.0	28.7
SAN-415	30	5	DF	0.9	0.3	102.4	40.2
			GF	2.5	0.9	121.4	44.4

[a] Sample size of 20 needles repeated two times per treatment.
[b] Needles oven-dried 48 h at 65°C after washing to remove spray deposit. Oven-dried weight averaged 8.9 and 20.6 mg per needle, respectively, for DF and GF.
[c] Data for only four plots.

Miller, in Ontario, Canada, against *C. fumiferana* (Clemens) were most effective at 30 BIU applied in 2.4–4.7 liters/ha. The application of 20 BIU/ha was only marginally effective (Morris 1984).

Population measurements taken 16 d after spraying ranged from a low of 0.7 larvae/branch on plots treated with SAN-415 32LV at 30 BIU/ha to a high of 3.4 larvae/branch for the untreated checks. Comparison of treatment means showed that the difference between the combined *B. thuringiensis* treatments versus the untreated checks was significant ($P = 0.01$). Populations were significantly lower ($P = 0.025$) on the plots treated with SAN-415 32LV at 30 BIU/ha than at 20 BIU/ha, but not between the two dosages of Thuricide 32LV.

Western Spruce Budworm Collections. A total of 11,903 western spruce budworm larvae was collected and reared individually in the laboratory until death or adult moth or parasite emergence. Parasitoids accounted for 26.3% of the reared western spruce budworm larval mortality; 26.0 and 0.3% of the parasitoids were Hymenoptera and Diptera, respectively. The scarcity of dipterans would be expected because many of the larvae were collected before oviposition by this group, or host larvae succumbed to *B. thuringiensis* infection before these parasitoids completed their development.

Table 3. Comparison of western spruce budworm larval densities before and after application of four treatments using *B. thuringiensis*

Treatment	Dose (BIU/ha)	No. plots	Budworm larval density[a]	
			Before spraying	16-d after spraying
Thuricide 32LV	20	5	15.2	1.6
Thuricide 32LV	30	6	22.0	1.2
SAN-415 32LV	20	4	18.6	2.8
SAN-415 32LV	30	5	18.0	0.7
Untreated check		5	21.0	3.4

[a] Average number of spruce budworm larvae per 45-cm branch based on sample of one branch before spraying and three branches after spraying per tree from 50 trees per plot.

Early instar parasites, *Glypta fumiferanae* Viereck and *Apanteles fumiferanae* Viereck, made up 86.5% of the total Hymenoptera. The parasites and their interaction with *B. thuringiensis* were reported by Niwa et al. (1987).

Mortality rates in larvae diagnosed as positive for *B. thuringiensis* infection were less than 4% in samples taken before spraying from all plots (Table 4). Rearings from blocks 1 and 3 before spraying, which were collected before any aerial application, were free of *B. thuringiensis*. Other blocks showed a small incidence of *B. thuringiensis* which probably resulted from contamination of larvae during field handling. Field crews sampled treated and untreated check plots without disinfecting their collecting equipment. Significant mortality amounting to about 38% at 20 BIU/ha and to >46% at 30 BIU/ha, occurred in collections taken within 0.25 d (6 h) after spraying. Mortality reached a peak in the 1-d sample at 20 BIU/ha, and in the 2-d sample at 30 BIU/ha. Both formulations of *B. thuringiensis* produced consistently higher mortality at 30 BIU/ha than at 20 BIU/ha. Longer persistence and increased mortality would be expected at a higher dosage. Single degree-of-freedom comparisons of mortality rates averaged over the seven sample intervals after spraying showed that the difference between the untreated checks versus the four *B. thuringiensis* treatments, and the 20 BIU versus 30 BIU/ha dosages for the SAN 415 32LV formulation, were both highly significant ($P = 0.001$). The difference between dosages of Thuricide 32LV was not significant.

The relation between treatment mean mortality after spraying in the larval rearings (Table 4) and population densities 16 d after spraying (Table 3) was apparently inverse. For example, SAN-415 32LV at 30 BIU/ha produced the highest average rate of mortality (45.5%) and the lowest density (0.7 larvae/branch) after spraying. At 20 BIU/ha, this formulation produced the lowest average mortality (28.5%) after spraying and had the highest larval density (2.8 larvae per branch). This same pattern was exhibited by Thuricide 32LV.

Table 4. Comparison of percentage of mortality of western spruce budworm larvae caused by *B. thuringiensis* infection when fed samples of foliage collected before spraying and at seven intervals after spraying

Treatment	Dosage (BIU/ha)	Sample (d)							
		Before spraying	0.25	1	2	3	5	10	16
Thuricide 32LV	20	2.4 (0.9)[a]	37.2 (6.8)	44.8 (6.6)	39.1 (4.6)	36.1 (4.2)	28.4 (3.4)	17.3 (5.5)	5.7 (1.8)
Thuricide 32LV	30	3.4 (2.3)	46.7 (4.8)	39.7 (6.8)	53.4 (5.7)	39.2 (5.9)	35.8 (4.8)	22.0 (4.6)	18.2 (6.7)
SAN-415 32LV	20	3.8 (3.0)	39.0 (7.3)	51.2 (2.9)	35.1 (7.3)	33.9 (3.0)	20.3 (5.9)	14.0 (8.0)	6.0 (2.9)
SAN-415 32LV	30	0.7 (0.4)	52.2 (5.8)	56.0 (2.8)	62.0 (7.0)	48.5 (6.4)	46.0 (7.9)	36.9 (6.4)	17.0 (6.7)
Untreated check		1.4[b] (0.7)	5.4 (1.4)	5.5 (2.6)	2.4 (2.0)	6.1 (1.4)	1.6 (1.0)	2.8 (2.8)	0.5 (0.5)

[a] Standard error.
[b] Attributed to contamination.

Pupal Weights. Mean pupal weights ranged from 55.9 to 64.8 mg for males and 75.6 to 85.3 mg for females (Table 5). These data do not suggest a significant sublethal effect of the 1984 *B. thuringiensis* treatments because the untreated check plots also produced small pupae. Feeding larvae would have to be continually exposed to a sublethal dose of *B. thuringiensis* to affect pupal weight (Fast & Régnière 1984). Lighter weight pupae found in this and other studies (Beckwith 1983) probably reflect differences in population density, resulting in a competition for available current foliage, differential feeding on old-growth needles, or differences in the gene pool between populations.

Number of pupae collected per unit collecting time decreased with increasing *B. thuringiensis* dosage (Table 5). At the 30 BIU rate, only two pupae were collected despite 4 to 5 times the work hours spent per plot in searching, compared with plots receiving the 20 BIU rate.

Defoliation. Some foliage protection obviously occurred in all *B. thuringiensis* spray plots because their defoliation indices are not as high as those in the checks (Table 6). Defoliation of current year's growth ranged from 30+% to complete; all untreated check plots were essentially completely defoliated of current foliage. Comparison of the mean index of the *B. thuringiensis* treatments with that of the untreated check indicated that application of *B. thuringiensis* apparently saved 15–25% of the current year's foliage. Treating stands with less history of previous defoliation would be preferable, so that 100% defoliation with the related population decline would not have occurred in the untreated check plots.

Block 5 was not included in the mean index because extremely dense larval populations, coupled with delayed shoot elongation, resulted in heavy destruction of new growth before spray date. Asynchrony between the host and western spruce budworm often occurs because termination of larval diapause is primarily a response to ambient temperatures and bud burst is a response to the combination of ambient and soil temperatures (Cleary & Waring 1969). The snowpack in the *B. thuringiensis*-evaluation area was heavy and remained late in 1984, resulting in delayed bud burst, especially in the higher elevation plots. Soil temperatures probably remained low, thus disrupting the normal synchrony between insect and host.

Conclusions. Our results indicate that *B. thuringiensis* is efficacious against the western spruce budworm. Population reduction to <1.5 larvae per 45-cm branch-tip was reached on >75% of the treated plots. This residual population density is accepted as adequate control by the USDA Forest Service, Forest Pest Management, in Oregon and

Table 5. Average pupal weights of pupae collected from treated and check plots during *B. thuringiensis* evaluation, 1984

Treatment	Dosage (BIU/ha)	♂♂			♀♀		
		No.	Weight (mg)		No.	Weight (mg)	
			$\bar{x}$	SEM		$\bar{x}$	SEM
Thuricide 32LV	20	43	55.9	3.2	28	76.6	5.9
Thuricide 32LV	30[a]	—	—	—	—	—	—
SAN-415	20	44	63.8	3.6	66	85.3	3.4
SAN-415	30[a]	—	—	—	—	—	—
Check	—	275	64.8	2.5	303	79.2	3.2

[a] No data because only one or two pupae were found in 8 work hours of searching on each plot.

Table 6. Defoliation index values and percentage of protection of current year's foliage provided by *B. thuringiensis*

Block no.	T-32LV 20 BIU/ha	T-32LV 30 BIU/ha	SAN 415 20 BIU/ha	SAN 415 30 BIU/ha	Untreated check
1	3.17 (21%)	3.46 (14%)	3.27 (18%)	3.67 (8%)	4.00
2	3.81 (4%)	3.70 (7%)	3.68 (8%)	3.31 (17%)	3.98
3	1.91 (52%)	2.35 (41%)	2.94 (26%)	2.77 (30%)	3.96
4	3.10 (22%)	2.93 (27%)	3.37 (16%)	3.72 (7%)	4.00
Mean[a]	3.00 (25%)	3.11 (22%)	3.31 (17%)	3.49 (15%)	3.98

[a] Block 5 not included because trees on all plots were almost completely defoliated before spray application.

Washington.[2] Although other control factors contributed to the reductions, populations on all of the untreated check plots exceeded two larvae per branch after spray.

We found no significant difference in efficacy between the two *B. thuringiensis* formulations under the conditions of these trials. The 30 BIU/ha dosage provided better population control than the 20 BIU/ha dosage with both *B. thuringiensis* strains, although only the difference between dosages for the SAN-415 32LV formulation was significant. Spraying third and fourth instars, rather than fourth and fifth instars, may have been preferable.

Residues of both formulations, especially at the 30 BIU/ha dosage, remained active for several days after spraying under our test conditions (Beckwith & Stelzer 1987). The prolonged activity of *B. thuringiensis* that we observed on foliage may reflect an improvement in the ultraviolet stability of the formulations. Conditions after spray application were characterized by continuous clear, dry weather during the period of evaluation.

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