Field Testing of Two Isolates of *Bacillus thuringiensis* Against the Western Spruce Budworm

(A Progress Report)

R. Beckwith, M. Stelzer, and B. Hostetler

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Acknowledgment

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Introduction

Bacillus thuringiensis (*B.t.*) is being considered for broader-scale use against lepidopterous forest pests throughout North America because of its selectivity, high degree of environmental safety, and general public acceptance. A new strain of *B.t.* (SAN 415) may eventually replace Thuricide 32LV in commercial formulations because it has been found to be more effective against the gypsy moth, spruce budworm, and several agricultural lepidopterous pests in the Eastern United States. To date, however, the new strain has not been field tested in Western North America. This report covers the 1984 field test comparing the two strains against the western spruce budworm, *Choristoneura occidentalis*, in north-central Oregon.

Objective

The primary objective was to compare the efficacy of two commercial strains of *B.t.* applied by helicopter at two dosages under normal field conditions.

Methods

General

The field evaluation was a cooperative effort between Forest Pest Management, R-6, and Research Work Unit 2204 of the Pacific Northwest Forest and Range Experiment Station. It was conducted entirely on the Prineville Ranger District of the Ochoco National Forest. The study site was located east and west of McKay Saddle about 43 K (26.7 miles) northeast of Prineville, Oregon (Fig. 1).

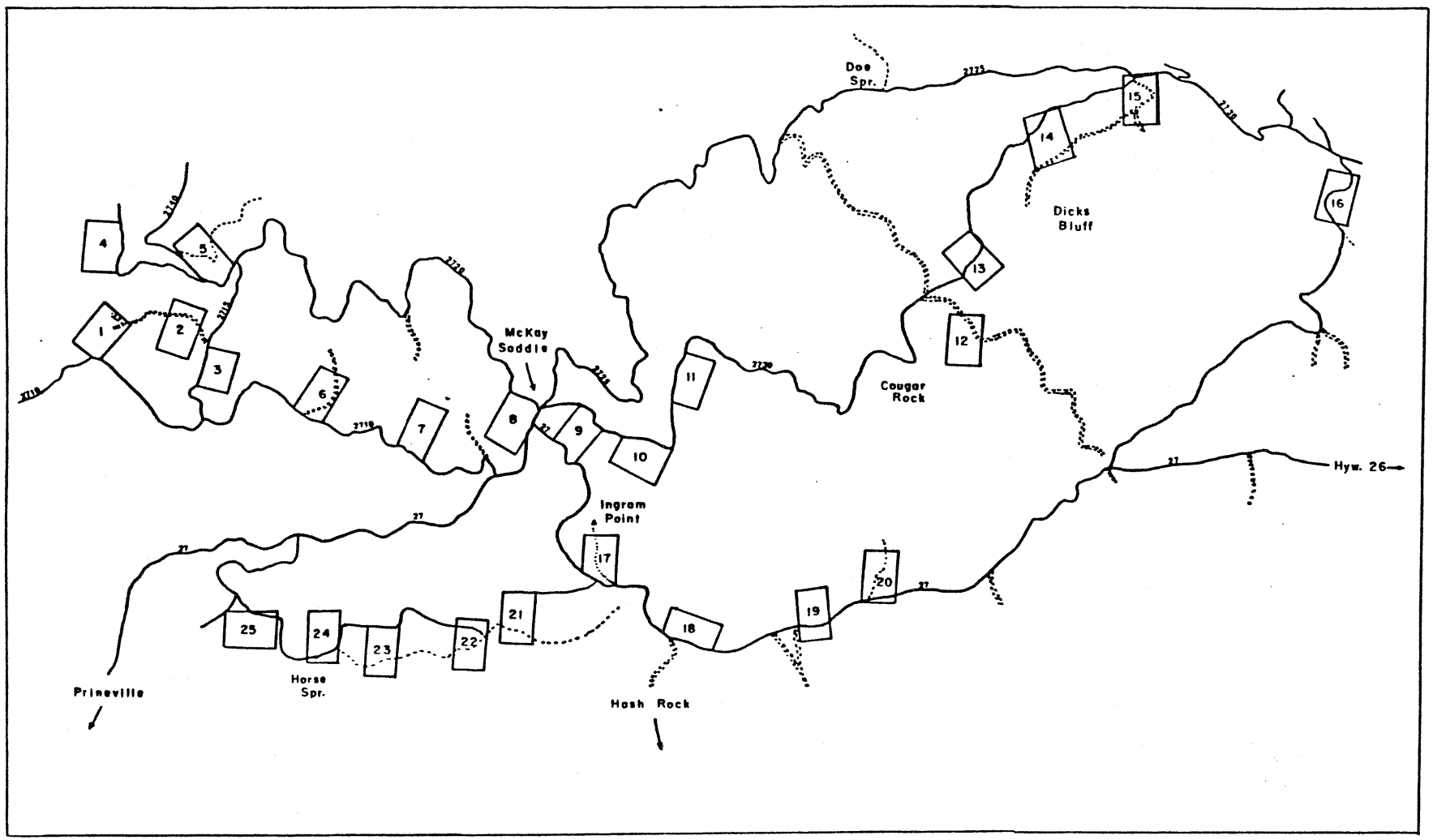

Figure 1. Plot locations for the 1984 Bacillus thuringiensis evaluation, Ochoco National Forest, Prineville, Oregon.

Twenty-five 16-hectare (40 acre) plots were selected within the area to be included in the B.t. evaluation. A buffer strip of at least 0.33 K (0.2 miles) separated each plot. The plot elevations ranged from 1341 m (4400') to 1737 m (5700') (Table 1). The physiography, soils, and climate are characteristic of the western Blue Mountain Provenance (Franklin and Dyrness 1973). The tree vegetation consisted primarily of Douglas-fir, Pseudotsuga menziesii (Mirb.) Franco at the lower elevations; grand fir, Abies grandis (Dougl.) Lindl., at the highest elevations; and, a mixture of the two species in between. A scattered overstory of tall ponderosa pine, Pinus ponderosa Dougl. ex Loud., was common throughout the area. Some of the plots contained a few large western larch, Larix occidentalis Nutt.

Generally, flat or slightly sloping ground was chosen for plot location to facilitate sampling. The exposure varied, with all cardinal directions represented; however, north-facing slopes were predominant. Most of the plots were relatively open to allow thorough coverage by the spray droplets. Also, dense stands reduce the number of available trees for sampling because their high live crowns are beyond the reach of standard sampling equipment.

The 25 test plots were laid out in 5 randomized blocks; each block consisted of all treatments and a check. A single block was treated on any one day so that all treatments were under the same basic environmental conditions. The spray was applied at the rate of 7.1 liters per hectare (96 oz. per acre) using a Hiller-Soloy 12J3 turbo helicopter equipped with two model 360A Beecomist rotary atomizers on a standard spray boom. The two B.t. strains (SAN 415, Thuricide 32LV) were applied at 20 BIU and 30 BIU per hectare (8 BIU and 12 BIU per acre). The daily order of plot treatment was randomized to eliminate a single treatment always being applied on the first

Table 1. Description and treatment of 16 hectare plots used in the 1984 *Bacillus thuringiensis* evaluation.

Block	Plot	Location T (S)	Location R (E)	Location S	Elevation (m)	Treatment	Dosage (BIU/ha)	Spray date
	1	12	17	16	1494	T-32LV	20	6/30/84
	2	12	17	15	1494	T-32LV	30	"
1	3	12	17	15	1500	SAN-415	20	"
	4	12	17	9	1402	check	--	--
	5	12	17	10	1341	SAN-415	30	"
	6	12	17	14	1615	check	--	--
	7	12	17	24	1585	T-32LV	30	7/2/84
2	8	12	17	24	1548	T-32LV	20	"
	9	12	18	19	1560	SAN-415	20	"
	10	12	18	19	1548	SAN-415	30	"
	11	12	18	17/18	1560	SAN-415	30	7/1/84
	12	12	18	16	1463	SAN-415	20	"
3	13	12	18	9/10	1402	T-32LV	30	"
	14	12	18	10	1378	check	--	--
	15	12	18	2/3	1402	T-32LV	20	"
	16	12	18	12	1524	SAN-415	30	7/7/84
	17	12	18	19/30	1731	T-32LV	20	"
4	18	12	18	29/30	1737	T-32LV	30	"
	19	12	18	29	1707	SAN-415	20	"
	20	12	18	28	1737	check	--	--
	21	12	17	25	1707	T-32LV	30	7/8/84
	22	12	17	25	1646	SAN-415	30	"
5	23	12	17	26	1628	T-32LV	30	"
	24	12	17	26	1579	check	--	--
	25	12	17	27	1539	T-32LV	20	"

helicopter flight (Table 2). An observation helicopter was used to guide the spray helicopter to the proper plots and to check on spray coverage. Boom samples were taken of the insecticide mixture at the end of each flight to check the potency of the active ingredient, if necessary; these samples are being held under refrigeration until needed.

The spray helicopter was calibrated just prior to the first day of spraying to meet the following parameters:

1. Air speed -- 100 kilometers (60 miles) per hour
2. Swath width -- 25^{+} meters (80 ft.)
3. Flight altitude -- 16 m (50^{+} ft.) above canopy
4. V.M.D. -- 125 - 150 u

The spray application began after the new shoots on both host species had expanded sufficiently for adequate impingement of the spray droplets. The proper timing was determined by following accumulated degree-days at the 1524 m (5000 ft.) elevation adjacent to plot 8. Degree-days can be directly related to the percentage of shoot growth and insect development (Beckwith and Kemp 1984).

Blocks 1-3 were sprayed on June 30 - July 2; Blocks 4 and 5 were completed on July 7 and 8. The 5-day delay between Blocks 3 and 4 was required to allow proper expansion, especially on Douglas-fir, on the higher elevation plots. The larvae in all plots were primarily in the 4th and 5th instars at the time of spray application (Table 3).

Kromekote cards were laid in a line perpendicular to the intended spray swath just prior to spraying to determine coverage and assess spray droplet deposition. These were removed after allowing about 30 minutes for drying following spraying.

Table 2. Treatment assignments, plot size, and meteorological conditions at the time of spraying with B.t.

Block	Plot	Treatment[1]	Dosage (BIU/ha)	Plot size (ha)[2]	Spray Date	Hour	Temp. °C	Humidity %	Wind
	1	T-32LV	20	15	6/30/84	0537	2.8	99	calm
	2	T-32LV	30	13	"	0622	2.2	92	calm
1	3	SAN	20	16	"	0720	2.8	99	calm
	4	check		--	--	--	--	--	--
	5	SAN	30	16	"	0830	7.2	78	calm
	6	check		--	--	--	--	--	--
	7	T-32LV	30	20	7/2/84	0541	8.3	85	3-4 N
2	8	T-32LV	20	17	"	0518	7.2	87	calm
	9	SAN	20	17	"	0655	9.4	82	calm
	10	SAN	30	17	"	0608	7.8	86	0-1 N
	11	SAN	30	17	7/1/84	0554	6.7	80	0-2 SW
	12	SAN	20	17	"	0531	6.1	77	5 S
3	13	T-32LV	30	21	"	0718	8.3	84	calm
	14	check		--	--	--	--	--	--
	15	T-32LV	20	16	"	0616	5.0	86	0-2 NE
	16	SAN	30	19	7/7/84	0648	3.3	74	calm
	17	T-32LV	20	17	"	0728	7.2	58	0-2 N
4	18	T-32LV	30	19	"	0809	9.4	55	0-2 NW
	19	SAN	20	16	"				
	20	check		--	--	--	--	--	--
	21	T-32LV	30	19	7/8/84	0601	5.0	70	calm
	22	SAN	30	16	"	0644	9.4	70	calm
5	23	T-32LV	30	19	"	0708	8.9	72	calm
	24	check		--	--	--	--	--	--
	25	T-32LV	20	18	"	0534	8.3	76	calm

[1]Treated at 7.1 liters per ha.

[2]Rounded to nearest ha.

Table 3. Western spruce budworm instar distribution within 48 hours prior to spraying with *B.t.*

Plot No.	Instar Distribution (%)			
	3rd	4th	5th	6th
1	1	53	46	--
2	--	36	64	--
3	3	36	61	--
4	6	31	63	--
5	--	46	54	--
11	6	73	21	--
12	6	62	32	--
13	--	29	69	2
14	--	19	72	9
15	1	66	33	--
16	--	8	69	23
17	2	35	62	1
18	11	44	44	1
19	2	35	63	--
20	2	55	41	2
21	2	49	49	--
22	--	38	60	2
25	--	27	73	--

Sampling

Prespray foliage samples were obtained within 48 hours before spraying and again 16 days after the spray application to determine larval population changes. One 45-cm (about 18 in.) branch from each of 50 trees per plot was used for the prespray sample. Three 45 cm branches from each of 50 trees per plot were used in the postspray sample because of the normal population reduction over time and the effects of the treatment. All western spruce budworm were removed from the branch samples and counted in the field. A number of larvae were saved and put into rearing for determination of mortality factors (for details, see Appendix A).

Branch samples were also taken at periodic intervals (.25, 1, 2, 3, 5, 10 days); the new growth was fed to laboratory stock to determine changes in *B.t.* mortality over time. Fourteen- and twenty-day samples were each made on one block of plots. This bioassay procedure showed the degradation of the bacillus when exposed to the weather for varying lengths of time.

Samples of larvae were collected before (100 per plot) and at 0.25, 1, 2, 3, 5, 10 (50 per plot), and 16 days (100 per plot) after spray application to determine all mortality factors. These larvae were obtained by rapping random branches from at least 20 trees in each plot. The larvae were reared individually in no. 1006 disposable petri dishes on insect diet (Thompson and Peterson 1978) until adult emergence or death occurred. Dead larvae were examined microscopically to determine the cause.

A pupal collection was made to determine mortality factors and the possible sublethal effects of *B.t.* ingestion. The goal was to collect about 100^{+} pupae per plot; however, some plots did not yield any pupae after 8 man-hours of searching. All pupae were weighed and individually reared until adult emergence; the adults were separated by sex.

Defoliation estimates were obtained from two mid-crown branches on each of 50 trees following 90% pupation. The budworm feeding damage on 25 shoots per branch was rated in one of four defoliation classes (1 = 0-25%, 2 = 26-50%, 3 = 51-75%, 4 = 76-100%). The average of all 50 trees was used to compute the average defoliaton value for each plot. The difference between the treated plots and the check represents foliage saved.

The details of all methods can be found in Appendix A. Some slight changes were made in the field because of conditions or time constraints.

Results and Discussion

Population density

Average prespray larval densities ranged from 3.56 - 32.00 larvae per branch and from 5.33 - 29.00 larvae per branch on grand fir and Douglas-fir, respectively (Table 4). Densities were consistently higher on grand fir and were of sufficient magnitude on both species to cause unacceptable defoliation if left untreated. Both of the *B.t.* formulations were more effective at 30 BIU/ha than at 20 BIU/ha and produced greater larval mortality on grand fir than on Douglas-fir (Table 5). The difference in budworm mortality on the two hosts may be explained, in part, by the fact that grand fir bud break and shoot elongation occurred several days before foliage development on Douglas-fir. Also, the wider needles and horizontal orientation of grand fir foliage may be better for spray impingement than Douglas-fir foliage.

A stated objective of the 1983 USDA, Forest Service, R-6 operational control program conducted in Oregon with chemical insecticides was to reduce budworm populations to or below an average of 1.5 larvae per 45-cm branch.[1/] Our 1984 *B.t.* results show that budworm populations fell below this threshold on grand fir in 79 percent of the plots and on Douglas-fir in 60 percent of the plots (Table 6). The proportion of plots with acceptable control was greater at 30 BIU/ha (86.4 percent) than at 20 BIU/ha (47.1 percent). The budworm populations on only 11.1 percent of the checkplots were below this threshold where the trees were separated by species. However, if the trees are pooled in each plot, all the checkplots have an average of two or more larvae per branch and 75 percent of the sprayed plots were near or below the 1.5 larvae threshold (Fig. 2).

[1/] Insect sampling plan for 1983 western spruce budworm suppression project in eastern Oregon. Work plan. Forest Pest Management, Pacific Northwest Region, Portland, Oregon. 5 pp.

Table 4. Reduction of spruce budworm larval populations following aerial applications of four treatments using *Bacillus thuringiensis*. Prineville - 1984.

Treatment	Plot	Prespray[a] DF	Prespray[a] GF	Postspray[a] DF	Postspray[a] GF	% Reduction DF	% Reduction GF
Thuricide 32LV	1	12.25(4)	24.70(46)	5.92	3.17	51.7	87.2
(20 BIU/ha)	8	15.47(19)	21.90(31)	0.54	0.52	96.5	97.6
	15	7.52(50)	-	1.47	-	80.4	-
	17	17.83(24)	25.35(26)	3.19	1.17	82.1	95.4
	25	5.33(18)	3.56(32)	0.50	0.20	90.6	94.4
	Average	11.68	18.88	2.32	1.27	80.2	93.6
Thuricide 32LV	2	29.00(7)	24.14(43)	3.38	1.36	88.3	94.4
(30 BIU/ha)	7	15.00(12)	28.11(38)	3.72	0.90	75.2	96.8
	13	17.91(45)	19.00(5)	1.39	1.47	92.2	92.3
	18	18.58(19)	30.22(31)	0.88	0.49	95.3	98.4
	21	18.88(8)	13.60(42)	0.21	0.13	98.9	99.0
	23	14.59(34)	19.50(16)	0.61	0.02	95.8	99.9
	Average	18.99	22.43	1.70	0.73	91.0	96.8
SAN-415	3	23.07(15)	32.00(35)	2.87	2.88	88.4	91.5
(20 BIU/ha)	9	18.57(21)	19.97(29)	1.08	0.43	94.2	97.8
	12	15.00(34)	16.31(16)	4.90	4.23	67.3	74.1
	19	18.15(13)	22.32(37)	3.51	3.44	80.7	84.6
	Average	18.70	22.65	3.09	2.74	82.6	87.0
SAN-415	5	20.13(31)	18.74(19)	1.33	0.60	93.4	96.8
(30 BIU/ha)	10	20.33(9)	32.24(41)	0.67	0.24	96.7	99.3
	11	16.04(27)	21.43(23)	1.78	0.30	88.9	98.6
	16	17.60(10)	15.75(40)	0.50	0.23	97.2	98.5
	22	8.48(25)	6.12(25)	0.32	0.13	96.4	97.9
	Average	16.52	18.86	0.92	0.30	94.5	98.2
Check	4	20.46(24)	28.69(26)	3.51	3.03	82.8	89.4
	6	18.76(25)	31.04(25)	3.53	1.93	81.2	93.8
	14	22.56(50)	-	2.09	-	90.7	-
	20	24.08(24)	21.88(26)	7.10	7.09	70.5	67.6
	24	9.78(23)	6.78(27)	3.10	1.22	68.3	82.0
	Average	19.13	22.10	3.87	3.32	78.7	83.2

[a]Average number of spruce budworm larvae per branch on a sample size of 1 prespray and 3 postspray branches per tree from 50 trees per plot.

Table 5. Prespray and postspray budworm larval density and postspray percent population reductions on grand fir and Douglas-fir trees following aerial applications of *Bacillus thuringiensis*. Prineville - 1984.

Treatment	Dosage (BIU/ha)	Population density ($\bar{x}\pm SE$)[a]				% Population reduction		Corrected % reduction[b]	
		Prespray		16 days postspray					
		Douglas-fir	Grand fir	Douglas-fir	Grand fir	Douglas-fir	Grand fir	Douglas-fir	Grand fir
Thuricide 32LV	20	11.7±2.4	18.9±5.2	2.3±1.0	1.3±0.7	80.3	93.2	7.9	54.4
"	30	19.0±2.1	22.4±2.5	1.7±0.6	0.7±0.2	91.0	96.9	57.9	79.2
SAN-415	20	18.7±1.7	22.6±3.4	3.1±0.8	2.7±0.8	83.4	88.0	22.4	19.5
"	30	16.5±2.1	18.9±4.2	0.9±0.3	0.3±0.1	94.6	98.4	74.8	89.3
Untreated		19.1±2.5	22.1±5.5	3.9±0.8	3.3±1.3	79.6	85.1		

[a]Average number of spruce budworm larvae per branch based on a sample size of 1 prespray and 3 postspray branches per tree from 50 trees per plot. Thuricide 32LV at 20 BIU per ha, SAN-415 at 30 BIU per ha, and the untreated control were replicated on 5 plots, SAN-415 at 20 BIU per ha on 4 plots, and Thuricide 32LV at 30 BIU per ha on 6 plots.

[b]Abbott's formulae (1925).

Table 6. Number of plots classified as acceptable or nonacceptable control of western spruce budworm populations.

Treatment	BIU/ ha	Number of plots			
		Douglas-fir		Grand fir	
		Acceptable[a]	Nonacceptable[b]	Acceptable[a]	Nonacceptable[b]
Thuricide 32LV	20	3	2	3	1
Thuricide 32LV	30	4	2	6	0
SAN-415	20	1	3	1	3
SAN-415	30	4	1	5	0
Check		0	5	1	3

[a]Acceptable = 1.5 or less larvae/45-cm branch

[b]Nonacceptable = More than 1.5 larvae/45-cm branch

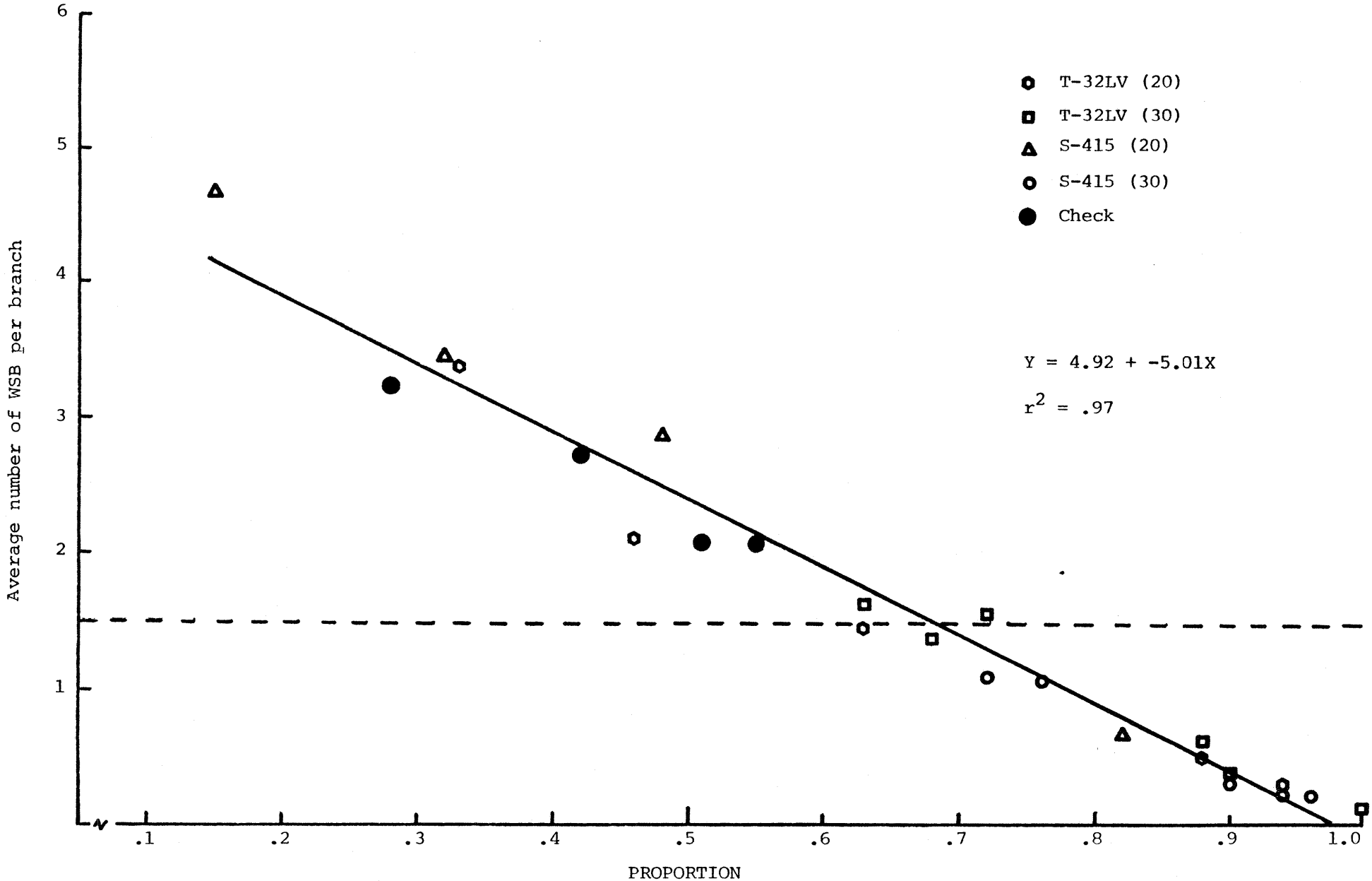

Figure 2. Regression of the average number of western spruce budworm per 45-cm branch based on the proportion of 1.5 larvae or less per branch by plot.

Larval rearings

Prespray and postspray collections of budworm larvae were reared individually on an artificial diet (Thompson and Peterson 1978) to identify and evaluate the mortality factors affecting survival. A total of 11,903 budworm larvae were reared from various sampling intervals until death or adult emergence (Table 7).

Infection rates of *B.t.*, as determined by microscopic diagnosis in the postspray samples at 0.25 days from treated plots, ranged from 37-47 percent with the mortality rate greater at 30 BIU/ha than at 20 BIU/ha. A comparison of Thuricide 32LV and SAN-415 shows almost an identical response. Mortality increased after spraying as more larvae continued to ingest active spray residues. The mortality of the 20 and 30 BIU/ha dosages increased for 1 and 2 days, respectively, and then gradually decreased through time; however, the heavier dosage of both strains remained consistently higher (Table 8).

In most cases, the incidence of *B.t.* mortality in the prespray collections and in the postspray collections from the checkplots was less than 5 percent. The infection source is attributed to contamination by the collecting crews and their equipment or during laboratory feeding as opposed to a native *B.t.* strain present in the environment. *B.t.* mortality did not occur in prespray rearings from Blocks 1 and 3 which were collected before the initiation of any spraying.

Table 7. Number of individually reared western spruce budworm larvae collected prespray and at seven postspray intervals.

Treatment	# Plots	SAMPLE INTERVAL							
		Prespray	.25d	1d	2d	3d	5d	10d	16d
Thuricide 32LV (20 BIU/ha)	5	472	245	248	248	249	239	238	346
Thuricide 32LV (30 BIU/ha)	6	596	295	299	295	295	295	295	485
SAN-415 (20 BIU/ha)	4	417	198	199	199	197	195	195	380
SAN-415 (30 BIU/ha)	5	537	250	248	250	249	250	246	285
Control	5	502	243	241	245	246	248	247	496
Total	25	2524	1231	1235	1237	1236	1227	1221	1992
								(Σ = 11,903)	

Table 8. Incidence of *B.t.* in western spruce budworm larvae collected prespray and at seven postspray intervals.

Treatment	BIU/ha	SAMPLE INTERVAL							
		Prespray	.25d	1d	2d	3d	5d	10d	16d
Thuricide 32LV	20	2.42 (0.87)[1]	37.16 (6.83)	44.84 (6.56)	39.14 (4.58)	36.08 (4.18)	28.42 (3.40)	16.86 (5.46)	5.70 (1.80)
Thuricide 32LV	30	3.37 (2.26)	46.73 (4.83)	39.73 (6.78)	53.42 (5.72)	39.22 (5.88)	35.82 (4.84)	22.05 (4.62)	18.20 (6.67)
SAN-415	20	3.85 (2.96)	38.95 (7.31)	51.25 (2.93)	35.08 (7.32)	33.88 (2.95)	20.30 (5.92)	10.02 (8.03)	6.00 (2.91)
SAN-415	30	0.74 (0.37)	46.32 (5.84)	56.02 (2.84)	62.00 (6.96)	48.52 (6.35)	46.00 (7.90)	36.86 (6.36)	17.04 (6.72)
Control		1.42 (0.71)	5.44 (1.43)	5.48 (2.63)	2.86 (1.97)	6.06 (1.40)	1.60 (0.98)	2.80 (2.80)	0.54 (0.54)

[1]Standard errors are in parentheses.

Mortality from parasitoids in the 11,903 reared budworm averaged 26.3 percent; Hymenoptera and Diptera represented 26.0 percent and 0.3 percent, respectively. The following parasitoids were recovered from the sample collections:

Hymenoptera

- *Glypta fumiferanae*
- *Apanteles fumiferana*
- *Enytus montanus*
- *Meteonus trachynotus*
- *Phytodietus fumiferanae*
- *Itoplectis guadricingulatus*
- *Phaeogenes haniolus*
- *Mesochorus tachypus* (secondary)

Diptera

- *Madremyia saundersii*
- *Ceromasia auricaudata*
- *Phorocera incrassata*

The early instar parasites, *Glypta fumiferanae* and *Apanteles fumiferanae*, comprised 66.6 percent and 19.9 percent of the total Hymenoptera recovered; the remaining 13.5 percent included the other six species. *Mesochorus tachypus* was a secondary parasite that was recovered from *Apanteles fumiferanae* and *Enytus montanus* cocoons. The Dipterous parasites represented only a small fraction of total parasitism. The apparent scarcity of dipterans would be expected as most of the larval collections were made before normal parasitization by this group or the larvae died from *B.t.* before parasitoid emergence.

The rates of parasitism dropped sharply on the treated plots immediately (0.25 day) postspray (Table 9); the rates remained at low levels until the collections made at 10 and 16 days where they approached the levels observed in the untreated check areas. This increase in the treated areas would suggest that exposure to B.t. affected the behavior of parasitized budworm larvae in the earlier collections so that they did not occur in representative numbers. The increase would not occur if the parasitized larvae were more susceptible to B.t. mortality.

Mortality rates caused by unknown factors were similar for the collections from treated and checkplots (Table 10). The exact cause of death was not identified except that no identifiable pathogens were involved. Possibly, the larvae were injured during handling; many of those that reached the pupal stage appeared to have "dried up."

The emergence of adults was affected by the B.t. treatment. The average reductions for the seven postspray sample intervals were 30.9, 37.0, 19.1, and 39.7 percent, respectively, for the T-32LV (20), T-32LV (30), SAN-415 (20), and SAN-415 (30) treatments (Table 11). There was a greater spread in survival between the low and high dosage of SAN-415 than T-32LV. This is basically the same situation that occurred in the foliage bioassay results and supports the idea that the SAN-415 formulation must reach a threshold to achieve adequate efficacy.

Table 9. Incidence of Hymenopterous parasites in western spruce budworm larvae collected prespray and at seven postspray intervals.

Treatment	BIU/ha	SAMPLE INTERVAL							
		Prespray	.25d	1d	2d	3d	5d	10d	16d
Thuricide 32LV	20	36.04 (6.02)[1]	18.14 (4.87)	16.94 (4.29)	20.94 (6.55)	14.92 (4.61)	27.88 (3.82)	31.56 (4.59)	29.06 (2.11)
Thuricide 32LV	30	31.30 (1.85)	17.33 (2.83)	19.08 (3.71)	14.82 (2.44)	17.38 (3.44)	17.85 (3.26)	25.43 (2.69)	28.72 (4.22)
SAN-415	20	30.00 (4.42)	11.08 (2.64)	13.08 (1.35)	11.00 (3.00)	13.72 (1.02)	19.00 (2.30)	27.70 (3.77)	37.62 (3.56)
SAN-415	30	32.72 (5.03)	7.90 (2.41)	12.08 (2.91)	10.40 (3.19)	8.84 (0.81)	14.00 (2.68)	23.26 (3.78)	26.52 (8.06)
Control		32.66 (2.24)	35.68 (6.53)	29.40 (6.52)	27.50 (6.52)	24.76 (5.86)	25.70 (5.22)	33.60 (5.53)	36.78 (4.10)

[1]Standard errors are in parentheses.

Table 10. Incidence of unknown mortality (non-pathogenic) in western spruce budworm larvae collected prespray and at seven postspray intervals.

Treatment	BIU/ha	SAMPLE INTERVAL							
		Prespray	.25d	1d	2d	3d	5d	10d	16d
Thuricide 32LV	20	20.96 (2.30)[1]	8.50 (2.65)	11.26 (3.67)	8.04 (1.37)	9.66 (2.34)	7.14 (0.82)	15.06 (2.27)	19.30 (4.11)
Thuricide 32LV	30	21.07 (1.91)	10.53 (3.09)	10.73 (3.52)	7.10 (1.25)	6.77 (2.01)	10.55 (2.43)	15.27 (1.58)	16.15 (2.10)
SAN-415	20	26.75 (2.31)	6.55 (1.74)	6.52 (2.05)	9.00 (2.58)	15.15 (2.28)	10.28 (1.52)	16.28 (4.23)	11.92 (1.80)
SAN-415	30	20.40 (2.67)	7.28 (2.54)	5.26 (1.39)	4.40 (1.33)	5.62 (0.75)	8.80 (2.56)	14.30 (2.59)	15.52 (1.97)
Control		23.96 (2.25)	12.72 (1.92)	15.42 (1.22)	17.98 (3.15)	11.48 (3.51)	14.94 (3.27)	14.18 (2.83)	7.96 (2.20)

[1]Standard errors are in parentheses.

Table 11. Percent mortality and adult emergence in rearings of spruce budworm larvae[a] collected prespray[b] and postspray[c] following aerial application of four *Bacillus thuringiensis* (*B.t.*) treatments. Prineville - 1984.

Treatment	BIU/ ha	B.t.		Hym. parasites		Other		Adult emergence		% emergence red. due to *B.t.*
		Prespray	Postspray	Prespray	Postspray	Prespray	Postspray	Prespray	Postspray	
Thuricide 32LV	20	2.42	29.74	36.04	22.78	20.96	11.28	35.82	36.08	30.9
Thuricide 32LV	30	3.37	36.45	31.30	20.09	21.07	11.01	43.62	32.33	37.0
SAN-415	20	3.85	27.92	30.00	19.03	26.75	10.81	42.52	41.56	19.1
SAN-415	30	0.74	44.68	32.72	14.71	20.40	8.74	46.18	30.95	39.7
Control		1.42	3.54	32.66	30.49	23.96	13.52	41.56	51.36	

[a]See Table 7 for the number of larvae in each rearing.

[b]Prespray collection taken 24-72 h before spraying.

[c]Postspray collections averaged for 7 sample intervals, i.e., 0.25, 1, 2, 3, 5, 10, and 16 days after spraying.

Foliage bioassay

Bacillus thuringiensis-caused mortality, as determined in the foliage bioassay study, ranged from 33-64 percent at day .25 (spray date) to 6-35 percent at day 10 (Fig. 3). Each point on the graph represents the average mortality for Blocks 1-4; Block 5 did not have enough new foliage to conduct a bioassay because of very dense larval populations that destroyed most of the new foliage before spray application. The 30 BIU's per hectare of both strains produced the greatest initial mortality and maintained the mortality at a relatively high level over the first 10 days. The 20 BIU's per hectare of SAN-415 is not as effective as the low dosage of Thuricide 32LV. Although there is a difference between the high dosage of the two strains, it may not be significant. There is a larger spread in mortality caused by the two dosages of SAN-415 than between the two dosages of T-32LV. Possibly there is a threshold between 20 and 30 BIU's that must be reached before the SAN-415 can achieve adequate efficacy.

Both *B.t.* strains maintained longer efficacy on the foliage than has been previously reported (Pinnock, et al. 1974; Leong, et al. 1980; Beegle, et al. 1981). In the past, most activity has disappeared by 3 days and has been attributed to ultraviolet degradation of the bacillus. Our 1984 field evaluation did not show this rapid degradation; in fact, *B.t.* mortality varied from 3-26 percent in laboratory stock fed foliage collected 20 days following spraying on Block 4. Recontamination of the foliage by dying larvae is partly responsible for this apparent longevity of the *B.t.* The unusually good weather may also be a factor; it did not rain for 5 weeks after the spray application. The washing of foliage by heavy rains may contribute more to poor control by *B.t.* than degradation of the bacillus by ultraviolet rays.

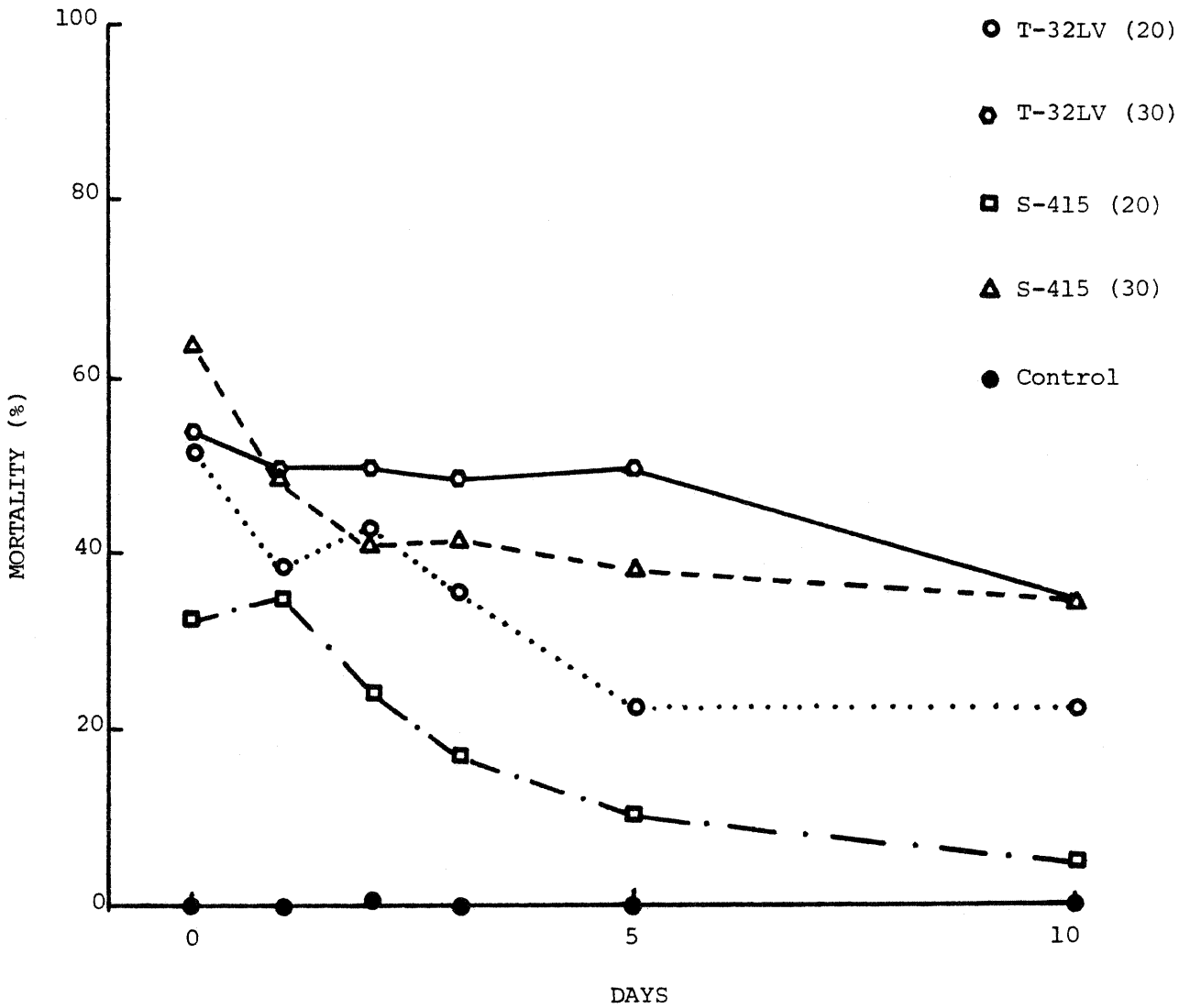

Figure 3. Percent mortality by B.t. treatment in a foliage bioassay test, 1984. Laboratory stock were fed treated and untreated foliage collected at intervals over a 10-day period after spraying.

Pupal weights

All live pupae were individually weighed the same day they were collected or kept at 35°C until the next day. The data do not suggest a significant sublethal effect of the treatment (Table 12); however, a statistical analysis has not been completed. Possibly the males from the T-32LV treatment are lighter than those from the SAN-415 treatment or checkplots. The populations in the check areas produced significantly lighter pupae than past experience in other parts of Oregon and Washington. The difference, however, may be the result of differences in population densities, competition for available foliage, forced feeding on old-growth needles for longer times, or differences in the gene pool between populations.

The high dosages of both *B.t.* formulations are not included in Table 12 because only 1 or 2 pupae were collected despite spending 4 to 5 times the man-hours of searching. In fact, the search time in the low dosage plots was much greater than in the checkplots, indicating a definite population reduction because of the *B.t.* treatment. Pupae obtained in the checkplots represented 76 percent of all pupae collected and weighed.

Table 12. Average pupal weights of pupae collected from treated and checkplots during *B.t.* evaluation. 1984.

Treatment	Dosage (BIU/ha)	Males			Females		
		No.	$\bar{x}$ (mg)	S.E.	No.	$\bar{x}$ (mg)	S.E.
Thuricide 32LV	20	43	55.9	3.25	28	76.6	5.86
Thuricide 32LV	30[1]	--	--	--	--	--	--
SAN-415	20	44	63.8	3.65	66	85.3	3.43
SAN-415	30[1]	--	--	--	--	--	--
Check	--	267	64.3	2.49	302	79.2	3.17

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

Defoliation

It is obvious that some foliage protection occurred in all B.t. spray plots (except Block 5) as their defoliation indices are not as high as those in the checks (Table 13). Defoliation ranged from 30^{+} percent to complete; all checkplots were essentially completely defoliated. By comparing the mean index of the treatment against that of the checkplot, it appears that the application of B.t. saved from 15 percent to 25 percent of the foliage. However, this type of comparison is not entirely valid because of the differences in prespray larval populations between the plots (Table 4). These differences also prevent a valid comparison between SAN-415 and T-32LV or between the 20 BIU/ha and 30 BIU/ha dosages.

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 budworm often occurs because: (a) termination of budworm diapause is primarily a response to ambient temperatures, and (b) bud burst is a response to the combination of ambient and soil temperatures (Cleary and Waring 1969).

Table 13. Defoliation index values and percent foliage protection provided by four treatments with *Bacillus thuringiensis*.

Block No.	T-32LV 20 BIU/ha	T-32LV 30 BIU/ha	SAN 415 20 BIU/ha	SAN 415 30 BIU/ha	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[1]	3.00 (25%)	3.11 (22%)	3.31 (17%)	3.49 (15%)	3.98

[1]Block 5 not included as the trees on all plots were nearly completely defoliated before spray application.

Spray deposit

White Kromekote cards, 10.2 by 12.7 cm (4 by 5 in.), were used to assess spray deposit at ground level. The cards were distributed in openings across the width of the plot, perpendicular to the direction of spray swath application. After spraying, the cards were collected after drying about 30 minutes. PEST, Inc., Sacramento, CA, read the cards using a Quantimet 720 Image Analyzer. All of the cards contained some spray deposit stains indicating no apparent skips occurred in coverage of the plots. Results of the spray deposit assessment are presented in Table 14.

A foodstuff dye, Red No. 40, was added to the tank mixes at the rate of 120g per liter (1 lb. per gal.). Although the dye enhanced the contrast of the deposits on the Kromekote cards, it did not permit visual detection of spray residues on the needles. Therefore, we were not able to count and measure droplet diameters on the foliage as we had originally planned. As an alternative evaluation, we removed 10 needles from a midcrown branch that had been collected immediately (ca. 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, thoroughly mixed, and then two 20-needle subsamples were drawn at random for washing with water to remove the deposit. Pour plates will be prepared on nutrient agar from the wash samples to determine the concentration of viable spores (*B.t.*). Deposits were expressed as the number of viable spores per needle and as the number of viable spores per gram of needle (oven-dry weight).

Table 14. Spray deposit assessment obtained from Kromecote cards.[a]

Treatment	BIU/ha	Plot	Spray deposit MMD[b]	Spray deposit Drops/cm^2
Thuricide 32LV	20	1	205	30.1
		8	153	11.0
		15	142	9.4
		17	104	12.8
		25	120	7.1
	Mean		144.8	14.1
Thuricide 32LV	30	2	--[c]	39.9
		7	148	11.8
		13	103	32.1
		18	109	17.4
		21	105	13.9
		23	104	9.5
	Mean		113.8	20.8
SAN-415	20	3	--[c]	12.5
		9	185	13.8
		12	233	2.9
		19	107	6.7
	Mean		175.0	9.0
SAN-415	30	5	172	13.0
		10	220	11.0
		11	225	15.2
		16	218	18.5
		22	156	28.7
	Mean		198.2	17.3

[a]20 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 plots 2 and 3 unacceptable for MMD determination; however, droplet densities were estimated by ocular counts under the microscope.

Counts of spores recovered from the foliage varied from 100 to 7,600 per needle on Douglas-fir and from 200 to 23,600 per needle on grand fir (Table 15). Spore counts were consistently higher on grand fir than on Douglas-fir. The larger-sized grand fir needles having a two-ranked flat arrangement may be more efficient collectors of spray deposit than the spiral arrangement of the smaller-sized Douglas-fir needles. Spray deposit, expressed as the number of viable spores per gram of oven-dry weight, was similar on the two host species.

Table 15. Analysis of B.t. spray deposits on Douglas-fir and grand fir foliage.

Treatment	Plot	# viable spores per needle[a] Thousands		# viable spores per gram dry weight[b] Thousands	
		DF	GF	DF	GF
Thuricide 32LV (20)	1	7.6	14.3	840	645
	8	4.4	10.0	560	545
	15	5.0	no sample	595	no sample
	17	2.5	6.6	310	360
	25	1.2	0.7	125	34
	Average	4.14	7.9	486	396
Thuricide 32LV (30)	2	6.3	23.6	690	1040
	7	3.4	4.1	425	188
	13	7.5	13.5	830	620
	18	1.2	4.6	130	220
	21	0.2	3.7	26	200
	23	3.7	14.2	475	915
	Average	3.7	10.6	446	530
SAN-415 (20)	3	1.6	19.5	144	122
	9	0.3	0.5	32	12
	12	0.1	2.0	11	98
	19	0.1	0.2	13	12
	Average	0.5	5.5	50	61
SAN-415 (30)	5	0.7	5.2	78	230
	10	2.0	4.0	255	225
	11	.4	.4	38	16
	16	.3	1.4	37	76
	22	.9	1.4	104	60
	Average	0.9	2.48	102	121

[a] Sample size of 20 needles repeated two times per treatment.

[b] Needles ovendried 48 h at 65°C after washing to remove spray deposit. Ovendried weight averaged 8.9 and 20.6 mg per needle, respectively, for Douglas-fir and grand fir.

Problems

The 1984 aerial *B.t.* test on the western spruce budworm was conducted with a minimum of problems. The weather prior to spraying was characterized by cool temperatures resulting in a delay of shoot expansion; this forced a 5-day delay in spraying the higher elevation blocks to allow adequate new growth for spray impingement. The delay, coupled with high-density larval populations, eliminated Block 5 from the foliage bioassay study because of the lack of current growth. The cold weather also delayed final plot selection at the higher elevations because of snow drifts on north-facing slopes. The weather after spraying was nearly perfect with no rain for at least 5 weeks.

A partially plugged boom line prevented the proper flow of the *B.t.* mixture through the rotary nozzle on the first plot sprayed. The problem was corrected at the heliport before the plot was completed. Based on ground reports, the spray pilot apparently had difficulty with proper application on plot 19 on the fourth day of spraying. The difficulty could have been caused by a number of factors. Either the pilots could not locate the plot corners from the air; or, the plot was aerial marked incorrectly; or, the spray swaths were applied in the wrong direction after locating the plot boundary. The plot was resprayed to cover the section that was missed, creating a shortage in one formulation (SAN-415). The end result was eliminating one dosage of SAN-415 from Block 5.

Conclusions

Our results indicate that *Bacillus thuringiensis* is a viable pesticide to use against the western spruce budworm. Population reduction to below 1.5 larvae per 45-cm branch-tip was reached on about 75 percent of the treated plots (Fig. 2). This residual population density is acceptable as adequate control by Forest Pest Management, R-6. Although other control factors had a part in the reduction, none of the checkplots were below two larvae per branch. Abbott's formula is often used to correct for natural controls in computing the effectiveness of an insecticide (Abbott 1925); however, we strongly believe its use is not applicable for evaluating insecticidal control in the field. Abbott's formula masks the control actually achieved by the material. Also, the ecological conditions in the spray plots may be completely different than in the checkplots.

Statistical analyses of the data have not been completed; however, there appears to be little difference between the two *B.t.* formulations. The 30 BIU/ha dosage gives better control than the 20 BIU/ha dosage in both *B.t.* strains. Both *B.t.* strains maintained longer efficacy on the foliage than has been reported in the past (Fig. 3). The longer *B.t.* life could be the result of an improved formulation and/or excellent weather after spraying. Possibly, the severity and timing of rainstorms are more important in the success of *B.t.* control than the apparent degradation of the active material by ultraviolet rays.

APPENDIX A

Methods

The field evaluation will be conducted on the Ochoco National Forest north and east of Prineville, Oregon. This general site was selected because of the newness of the infestation, the potential population density of the budworm in 1984, the presence of two major host species, and the availability of adequate field laboratory facilities. Specific sites and spray plots will be located and selected from maps, aerial photos, and by field examination and conferences with Ranger District personnel. The terrain must be suitable to both spray application and budworm population sampling; relatively flat areas containing open-grown stands of moderate height (7-14 m) trees would be ideal for the evaluation. It is difficult to conduct the required sampling within the proper timeframe in steep terrain or dense stands.

Twenty-five 16-32 hectare (40-80 acre) plots will be required. The size of the plot may be determined by the amount of the new B.t. strain that can be supplied by Zoecon. A 16-h plot is ample for our needs. The elevation, exposure, and stand characteristics will probably vary in order to find enough suitable plots. Each plot will be separated by at least a .2 mile buffer strip from adjacent plots. Test plots will be established in five blocks containing five plots each (Appendix I). Within each block, the treatment (4) and control (1) will be assigned randomly to the plots. Five replicates will be established for each of the following treatments:

1.	8 BIU	32LV	96 oz/acre	HD-1
2.	12 BIU	32LV	96 oz/acre	HD-1
3.	8 BIU	32LV	96 oz/acre	NRD-12
4.	12 BIU	32LV	96 oz/acre	NRD-12
5.	Untreated Control			

Mixing of materials will be under the direction of the entomologists. The aerial application will be made by the proper equipment as specified in the contract. Rotary nozzles will be required to produce the proper droplet size (vmd 125-150 um). Spray application will be made in June after the shoots have expanded and at least 80% of the larvae have reached the fourth instar (Beckwith and Kemp 1984).

Population Density

Population densities of western spruce budworm will be obtained on all plots (25) before and after B.t. application. Density values will be computed on 50 randomly selected trees per plot; 25 trees of both Douglas-fir and grand fir will be used if both species are present to determine possible differences between species. Needle anatomy and branch configuration could cause differences in impingement and retention of the spray droplets thus affecting the results. The prespray sample will be obtained within 72 hours before spray application and will consist of one 45-cm branch tip obtained at midcrown per tree. The postspray sample will be taken at the initiation of pupation. It will consist of three 45-cm branch tips per tree; the threefold branch sample is because of the normal survivorship over time and the reduction in population by B.t.

The branch samples will be obtained by a pole-pruner with a basket attached below the cutting head to prevent larval loss. Any branch not caught in the basket will be disregarded. The excised sample will be measured in the field as to length and width. The measurements will be recorded on a slip of paper which will be placed with the branch sample into a fine-mesh nylon bag which will be tightly closed by a wire "twist-em." Each plot will be coded to prevent a mixup during processing at the field laboratory.

The bags will be processed at the laboratory the same day the samples are collected. The number of current shoots will be counted so that budworm density can be expressed on the basis of 100 buds as well as on a per branch tip or per m^2 of foliage. All western spruce budworm will be counted and numbers recorded on the proper field data sheet (Appendix 2).

For each plot, 5 larvae will be randomly selected from each of the first 20 bags examined in the prespray sample. The larvae will be reared individually on standard artificial diet in Lok-Tight petri dishes until adult emergence to determine natural mortality factors. All larvae will be reared in the postspray sample.

Larval Rearings

Determination of the rates of mortality caused by infection by *B.t.*, parasites, and other causes will be based on the results of rearing budworm larvae collected at various times postspray. The larvae will be obtained from beating lower crown branches on open-grown host trees. A total of 50 larvae taken from 20 or more trees/plot will be collected into individual petri dishes (#1006, Falcon plastic) provisioned with artificial diet. All 25 plots (20 treated, 5 control) will be sampled at 0-2 hr, 1-, 2-, 3-, 5-, and 7-days postspray. Rearings will be maintained at the field laboratory until death or adult emergence. Presence of *B.t.* infections will be verified by microscopic examinations or plating or nutrient agar. Survivors reaching the pupal stage will be weighed and sexed to provide data on possible sublethal responses to exposure to *B.t.* These data will permit us to examine the response patterns of the specific mortality factors over time, e.g., does infection by *B.t.* buildup gradually during the first 3-7 days postspray or are the majority of the larvae infected within 24 hrs postspray (indicating a very short residual life of spray deposits).

Foliage Bioassay

To determine the residual life (degradation rate) of B.t. spray deposits, midcrown foliage samples will be obtained by 9 AM on each sample day beginning with the 1st sample on the spray date (0-2 hr.). Subsequent samples will be collected on day 1-, 2-, 3-, 5-, and 7-days postspray. A single midcrown branch having current shoots will be obtained from 10 trees/plot at each exposure period. Five to ten current shoots from each branch will be clipped into a 7-oz plastic cup and stocked with 10 budworm larvae (2nd instar) taken from a disease-free laboratory colony (COC-FS-01) initiated at the Forestry Sciences Laboratory, Corvallis, in 1979. The cups will be examined after 7 days exposure to record mortality and verify infection caused by B.t. Persistence of B.t. is calculated at percent of original activity remaining (% OAR) based upon 100% at day 1 of posttreatment (Ignoffo and Batzer 1971). Results will be used to plot degradation rates of spray residues for each treatment (Y) over time (X).

Deposit Assessment on Foliage

Foliage samples for deposit assessment will be collected immediately postspray from all B.t.-treated plots. The samples will consist of a single midcrown branch from each of 50 randomly selected trees/plot; 25 trees of both Douglas-fir and grand fir will be used if both species are present to determine possible differences between species. Laboratory studies will be completed by May to determine the optimum procedure for handling foliage samples, i.e., storage in paper vs. plastic bags, at room temperature vs. walk-in refrigerator, that best maintains the integrity of the spray deposits. This is needed because examination of the foliage will have to be delayed for at least 1 month after the samples are collected.

A subsample of 20 needles will be removed from each branch sample and examined at 30-40X magnification to count and record the number of spray droplets. In addition, a random selection will be taken from those needles having spray deposits for the measurement of droplet diameters. All of the droplets on a given needle will be measured to the nearest .025 um, with selection of needles stopped after at least five droplets have been recorded for the branch.

Graphs will be prepared to examine the relationships between the 21-day postspray larval mortality rate (dependent variable) and the droplet density (independent variable).

Pupal Collection

Lower crown foliage will be searched to collect budworm pupae after about 50 percent of the population has reached this staged of development. We will attempt to obtain 100 pupae/plot, but sampling time will be limited to 2 person-hours/plot. Pupae will be weighed, sexed, and placed in rearing to determine survival rates to adult and to quantify mortality factors. A comparison of pupal weights and sex ratios between collections from treated and check plots may show effects of larval exposure to sublethal dosages of *B.t.*

Defoliation

Foliage samples for the evaluation of feeding damage will be collected after pupation exceeds 90 percent. Assessment of defoliation will be made by examining 25 current shoots on each of 4 midcrown branch samples per tree from 50 trees/plot. The budworm-feeding damage on each shoot will be rated in one of four defoliation classes: 1=0-25%, 2=26-50%, 3=51-75%, 4=76-100%. These data will be used to compute the average defoliaton (%) value for each plot.

Analysis

The overall experimental layout is a complete block design comparing two *B.t.* isolates (HD-1, NRD-12) applied at two dose levels (8, 12 BIV per ha). The four treatments and a control will be randomized within each block; the blocks will be replicated five times. The prespray and postspray densities (WSB per m^2 foliage and/or per 100 buds) will be tested by ANOVA as follows:

Source of variation	df
Blocks	4
Treatments	4
Error	16
Total	24

Tukey's *w* procedure (Steel and Torrie 1960) will be used to test differences between individual treatment means at the .05 probability level.

Rearing budworm larvae (100/plot) at various postspray periods will provide data on survival and mortality rates due to *B.t.* infections and other factors. The data at each sample period will be tested by ANOVA using the same model as the population density data.

Data from foliage bioassays (% OAR values) to determine degradation rates of *B.t.* spray deposits will be evaluated only on the 8 BIU/acre dose level plots and controls. Analysis by ANOVA with time (sampling intervals) as a split plot factor will be used according to the following model:

Source of variation	df	
Blocks	4	
Treatments	2	
Control vs. treatment		1
HD-1 vs. NRD-12		1
Error a	8	
Times	5	
HD-1 x Time	5	
NRD-12 x Time	5	
Error b	60	
Total	89	

Differences between individual treatment means will also be tested by Tukey's *w* procedure at the .05 probability level.

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