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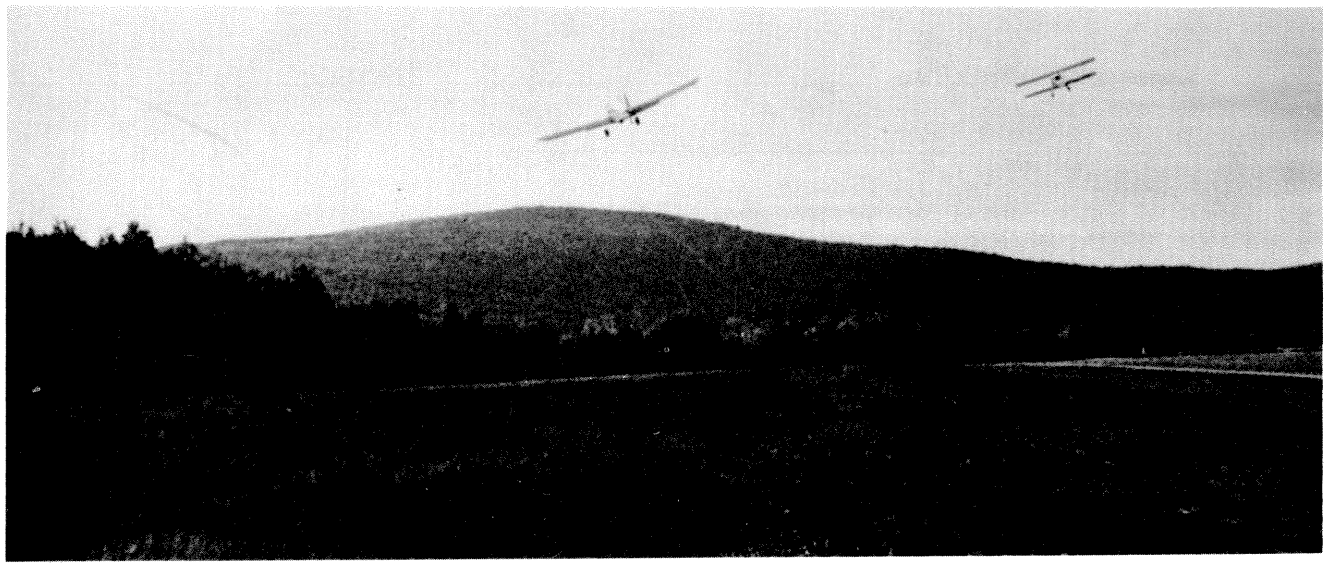
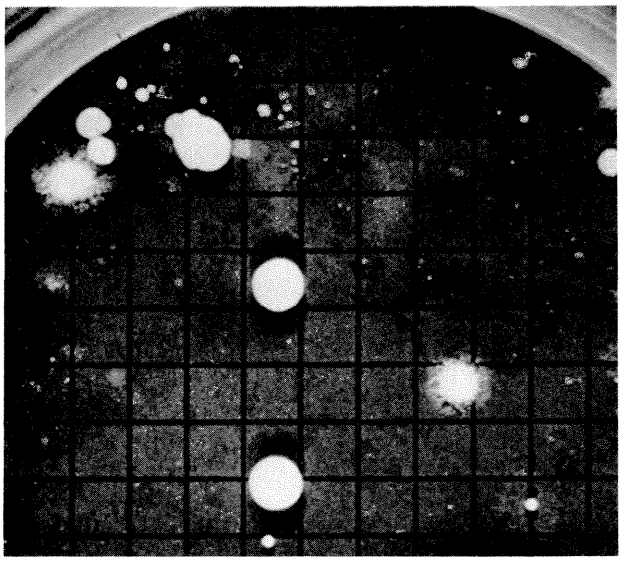
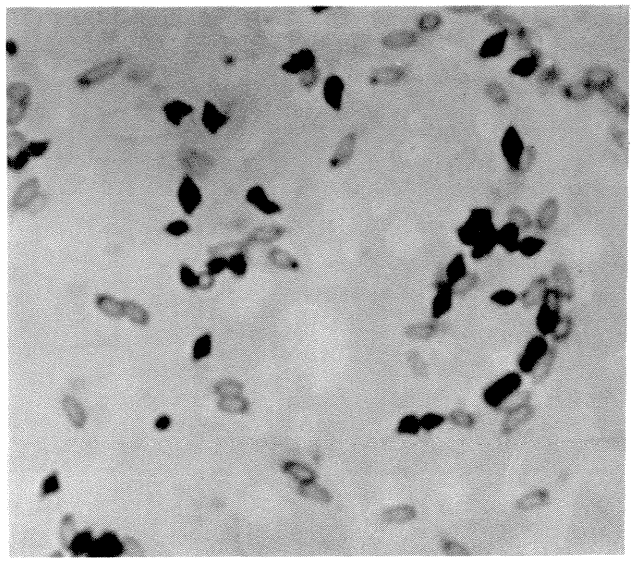
Spruce Budworm Core *B.t.* Test—1980

Combined Summary







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Abstract

Two commercial preparations of *Bacillus thuringiensis* (*B.t.*) were aerially applied in 1980 to populations of spruce budworm (*Choristoneura fumiferana* (Clem.)) in Arizona, Maine, New Hampshire, and Wisconsin. Operations were conducted under the auspices of CANUSA-East with standardized procedures for spray application and population monitoring. The 8 Billion International Units (BIU) Dipel® and Thuricide® formulations are effective and similar for spruce budworm control when applied under favorable conditions. This paper compares results between locations and critiques the experimental design, spray operations, and data collection procedures.

A major objective of the Canada-United States (CANUSA) Spruce Budworms program is the development of *Bacillus thuringiensis* (*B.t.*) for control of spruce budworm (*Choristoneura fumiferana* (Clem.)). *B.t.* formulations are registered for spruce budworm control in the United States and Canada, but operator acceptance has been disappointing, primarily due to varying results. Several formulations and dosages have been applied under a wide range of conditions, evaluated by different methods and, not surprisingly, yielded inconsistent results (Morris 1981). The national and international nature of the CANUSA Program provided an opportunity for a fully coordinated cooperative evaluation. We established uniform work plans for testing in four geographic locations to obtain general conclusions regarding the efficacy of the *B.t.* treatments. This report is an evaluation of the core *B.t.* test and provides an overview of the results.

Core Concept

The test was designed to apply and evaluate two *B.t.* formulations and to allow reasonable population control comparisons within and between four different geographic locations. Similar operation and sampling procedures were prescribed at each location because of prior erratic results when using *B.t.* against the spruce budworm.

The specific objectives of the core test were:

1. To determine population reductions due to treatments in the year of application.
2. To determine the degree of foliage protection due to treatments in the year of application.
3. To determine if and why differences occurred in treatment responses between locations where possible.

The core test was conducted in Maine, New Hampshire, Wisconsin, and Arizona. Each principal investigator[1] (Dimond et al. 1981, Reardon et al., 1982) detailed the results of each core test.

[1] Swier, S. R. 1980 Core *B.t.* field efficacy test. Canada/United States Spruce Budworms Program. Unpublished report. 1981.

Population Characteristics

The desired conditions, as stated in the core test study plan, were vigorous insect populations capable of causing obvious damage on relatively undamaged balsam fir (*Abies balsamea* (L.) Mill.) in the East and Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) in the West. Final selections of populations were left to the principal investigators. These ideal test conditions could not be found in Maine and New Hampshire because population levels were higher than desired and tree conditions were poor due to previous defoliation. Prespray populations for the four core test areas are listed in Table 1.

Sampling Design and Methods

At each location, 15 blocks were designated for core study sampling. Five blocks were assigned randomly to each of the two treatments and the check. At all locations but Arizona, 10 clusters of three trees were located in each block. In Arizona, where there was an extra treatment, a line of trees in each block was used in place of clusters. The number of trees per

Table 1. Prespray and postspray conditions encountered in the four core test areas

Item	Maine	New Hampshire	Wisconsin	Arizona
Maximum number of days from prespray sample to spray	8	6	2	?
Larvae/branch prespray	35	30	14	15
Area/branch, prespray (M^2)	.15	.15	.10	.05
Buds/branch, prespray	156	66	37	60
Buds/branch, 14 day, postspray	86	51	20	?
Percent natural mortality (prespray, 14 days after)	60	85	64	50
Tree condition	Noticeable damage due to prior defoliation.	Noticeable prior defoliation damage. Frost damage in 1980.	No evident prior defoliation damage.	?
Check defoliation	91.6 ± 3.1[a]	95.1 ± 3.1	91.6 ± 2.6	81.8 ± 7.6

[a] 84.5 ± 7.5 with plot 9 included.

line and branches per tree differed from block to block and period to period.

Spray operations were to be timed so one block of each treatment and a check block would be treated as a group, and have a common date (within 48 hours) for prespray larval evaluation. This procedure was to ensure that similar numbers of blocks in the checks and in each treatment spanned similar periods of larval development. In New Hampshire and Wisconsin, this was achieved by doing all spraying within 24 hours. In Arizona and Maine, the check block and treatment block evaluations were made over different periods of time.

At all locations, Millipore® filters placed in an opening near each tree were used to evaluate spray deposit. The filters were tacked to small wooden or cardboard blocks and placed in the openings just before spraying. They were picked up 20 to 30 minutes after spraying, placed in labeled envelopes, and returned to the laboratory. Only in Wisconsin was this done on the check plots to detect drift.

For population sampling, two midcrown branches, 38 to 45 cm, were taken from opposite sides of each tree for the prespray larval sample at all locations but Arizona. Four branches were sampled postspray for larvae, one from each cardinal direction, at midcrown on the same trees. The length, width, number of buds, and number of larvae were recorded in a laboratory.

Prespray sampling was to have been carried out within 48 hours of spraying. Actual time is shown in Table 1. Postspray larval samples were to be taken at 14 days and at first adult emergence. Wisconsin added a 24-day sample, which is included in the analysis. Arizona used a weekly sampling interval for postspray counts.

Defoliation samples were taken from the trees after first adult emergence when feeding had ceased. A modification of Fette's bud defoliation evaluation method (McGugan 1954) suggested by Lidstone was used. Arizona modified this further by changing the definitions of the lowest and highest defoliation classes. Up to 25 buds on each of four branches per tree were evaluated for defoliation.

New Hampshire followed the core plan of collecting up to 100 pupae at first adult emergence and again a week later. These pupae were sexed and reared, and parasitism and non-emergence were noted. Wisconsin sexed a different number of pupae than they reared, but the data are usable though prepupae may have been included in the sample. From Maine, only the sex ratios could be used, and in Arizona only larvae were collected and reared, so the data from Arizona were not analyzed. Pupal collections were taken to determine if significant *B.t.* kill occurred in pupae, if pupal parasitism was adversely affected, and if sex ratios were affected by *B.t.* treatment. The pupal collections failed to answer these questions.

Equipment and Ground Operations

To standardize the mixing and application of the *B.t.* formulations throughout the core test, all materials were mixed using equivalent ground equipment and applied with small fixed-wing aircraft. An observation aircraft was used in all operations. A single application of the two *B.t.* formulations was applied at 8 Billion International Units (BIU) per acre in 1 gallon of finished spray (Table 2).

To assure adequate mixing of materials at each site, tank mix samples of the formulations were taken and shipped to three independent locations for BIU determinations. A separate report on these analyses is being prepared.

Calibration of the fixed-wing aircraft specified for the test was done a month before operations to facilitate on-site prespray calibrations of aircraft actually used in the core tests. Aircraft specifications are listed in Table 3.

Weather and Operational Problems

The individual tests were affected by equipment and weather problems, which caused trouble in interpreting the data. The problems encountered are given by core location with general comments on their possible effects on operations:

Arizona. Because of a series of unforeseen delays which postponed the application of the test material, the insects had developed beyond the specified stage. The larger larvae treated were less susceptible to *B.t.*, and thus the anticipated effects of treatments were reduced.

Maine. When insect development was right for treatment, rainy cool weather delayed and extended application. Rain occurred on both days core treatments were applied and continued sporadically for several days after application. With cool wet weather, generally poor tree conditions, delayed spraying, and higher insect populations than anticipated, the treatment effects probably were not realized fully.

New Hampshire. A severe killing frost occurred in scattered areas 3 days before treatment. The frost, the effects of which were not noticeable at spray time, killed a large number of buds and insects in some plots. This presented difficulties in distinguishing between the effects of treatment and frost.

Wisconsin. This was the only core site that did not have any serious weather or operational difficulties. Timing of application occurred as specified. No rain occurred within several days of treatment.

Results

The core study was designed to eliminate or to control as closely as possible all procedural and population differences between locations, and to permit similar analyses and direct comparisons. We also attempted to minimize the number of factors to be considered in explaining different results between locations. Unfortunately, there were unanticipated difficulties that we have

Table 2. Test parameters used

Parameter	Maine	New Hampshire	Wisconsin	Arizona
Tree species	Balsam fir	Balsam fir	Balsam fir	Douglas-fir
B.t. treatment	Thuricide 16B® —diluted 1:1 with water + 3 fl oz Chevron spray sticker Dipel 4L® —diluted 3:1 with water + 3 fl oz Chevron sticker			
Block size (acres)	100	50	50	50-100
Sampling points	10-3 tree clusters	Same as Maine	Same as Maine	Line sample
Branch size	45-cm branches	45-cm branches	38-cm branches	38-cm branches
Budworm sample timing	Prespray—2 branches 14 day—4 branches 1st adult emergence—4 branches Pupal—100 pupae	Same as Maine	Prespray—2 branches 14 day—4 branches 24 day—4 branches 1st adult emergence—4 branches Pupal—100 pupae	Prespray, 12 day, 19 day, and 26 day
Defoliation sampling	25 new shoots from 4 branches	Same as Maine	Same as Maine	Same as Maine
Dosage	8 BIU/acre	8 BIU/acre	8 BIU/acre	8 BIU/acre
Rate	1 gal/acre	1 gal/acre	1 gal/acre	1 gal/acre
Replicates	5	5	5	5
Treatment timing	90% bud burst, peak IV instar	Same as Maine	80%, III and IV instar larvae	Late

Table 3. Application data

Item	Maine	New Hampshire	Wisconsin	Arizona
Aircraft	Thrush 600 h.p. Stearman 450 h.p.	Same as Maine	Thrush 600 h.p. Air Tractor 600 h.p.	Turbo-Thrush
Nozzling	Thrush 50—8004 tee jet Stearman 50—8004 tee jet	Same as Maine	Thrush 52—8004 tee jet Air Tractor 59—8004 tee jet	28 full jet 8W
Pump pressure	45 psi	Same as Maine	Same as Maine	Same as Maine
Swath width	100 ft.	Same as Maine	75 ft.	—
Mixing equipment	2—275 gal Tanks, 1-1/2″ Pump, Meter, #30 mesh Screen	F.S. Spray Trailer, 2—300 gal Mixing Tanks 400 gal Water Tank, 2 Pumps and Meters, #30 mesh Screen	Same as Maine	F.S. Regional Trailer, 2—250 gal Tanks, Pumps, Meters

tried to accommodate and interpret in the analyses.

An analysis of variance (ANOVA) on the larval counts shows that treatment effects were not significantly different from the checks in Maine, but they were significantly different in New Hampshire and Wisconsin (Table 6, Appendix). To make population reductions due to *B.t.* comparable between areas with different natural mortalities, Abbott's formula was applied to the larval counts. The Arizona data could not be analyzed in the ANOVA model with the other locations; however, the population reduction estimates are included in Table 4 on the presumption that there were treatment effects.

We had expected to find the number of buds per branch and perhaps area per branch useful in evaluating population reduction. The data showed a 50 percent decrease in buds per branch when comparing the prespray and 14 day count in Maine and in Wisconsin (Table 1). This change was not related to a change in branch size. In Wisconsin, there was no significant change in branch area, and though branch area did change in Maine, the magnitude of the change was negligible in comparison to the change in buds (Table 6, Appendix). The implications of this phenomenon are discussed later. Because of these changes, larvae per branch were used to evaluate population reduction at all locations.

Treatments in all areas had a significant effect on bud defoliation (Table 6, Appendix). Defoliation was similar on the check blocks in all locations, and Table 5 shows both actual defoliation levels and net percentage of foliage saved—check block defoliation less the treatment defoliation percent. In Maine, the protection in the Dipel® treated blocks was significantly better than that in the check blocks, but protection in the Thuricide® treated plots was not. In New Hampshire, the Dipel treatment was not different from the control, but the Thuricide treatment was significantly better

Table 4. Net population reductions[a] and standard errors of estimate over the period from prespray to the postspray sample indicated

Location and treatment	Postspray sample			
	14 days	19 days	24 days	First adult emergence
Maine:				
Dipel 4L	− 19.0 ± 49.3	—	—	− 5.0 ± 45.4
Thuricide 16B	− 52.4 ± 63.8	—	—	6.0 ± 40.2
New Hampshire:				
Dipel 4L	72.9 ± 8.6	—	—	79.3 ± 8.8
Thuricide 16B	68.3 ± 8.3	—	—	82.7 ± 5.1
Wisconsin:				
Dipel 4L	72.6 ± 4.1	—	58.6 ± 4.5	43.2 ± 16.4
Thuricide 16B	84.2 ± 3.2	—	52.1 ± 7.2	− 2.0 ± 29.8
Arizona:				
Dipel 4L	30.6 ± 8.1	43.6 ± 12.3	—	32.1 ± 15.0
Thuricide 16B	26.4 ± 9.8	43.2 ± 12.8	—	37.6 ± 13.8
Thuricide 32B	55.9 ± 14.4	66.8 ± 10.3	—	67.9 ± 9.0

[a] Abbott's correction for natural mortality was used.

Table 5. Net defoliation prevented by treatment[a] and resultant defoliation levels, with standard errors, by treatment and location

Location	Dipel 4L	Thuricide 16B	Thuricide 32B
	RESULTANT DEFOLIATION		
Maine	57.2 ± 4.6	80.0 ± 3.8	—
New Hampshire	91.2 ± 3.1	76.7 ± 5.6	—
Wisconsin	46.9 ± 12.1	48.4 ± 11.3	—
Arizona	75.5 ± 4.5	68.9 ± 6.2	22.2 ± 11.6
	NET FOLIAGE SAVED		
Maine	34.4 ± 4.5	11.7 ± 3.4	—
New Hampshire	3.9 ± 4.4	18.4 ± 6.4	—
Wisconsin	44.7 ± 12.4	43.2 ± 11.6	—
Arizona	14.7 ± 7.7	17.1 ± 10.2	65.5 ± 4.7

[a] Net defoliation prevented was computed by subtracting defoliation on the treated blocks from defoliation on the checks.

than the control at the .066 level. Swier,[1] using a different analysis, determined that Thuricide was significantly better than Dipel or the check treatment ($P < .05$). Foliage protection was not evident in Arizona, but in Wisconsin, Dipel and Thuricide were significantly and equally effective.

The Millipore filters were used to verify misses on the spray blocks and drift on the check blocks. Correlation coefficients of colony counts with defoliation and population reduction were computed using the spray-block figures. Only chance relationships could be found. An ANOVA was run on the colony counts for each block, and significant differences were found between locations at the .001 level, but not between treatments. The average counts per square centimeter for both treatments combined were:

Maine	25.8 ± 4.3
New Hampshire	18.8 ± 5.4
Wisconsin	41.3 ± 3.9
Arizona	15.5 ± 3.8

Upon analysis, the pupal data were so highly variable that no conclusions could be drawn on *B.t.* effects on parasitism or sex ratio.

Critique of Operations

Under good conditions such as those encountered in Wisconsin, *B.t.* at 8 BIU can account for high population reductions. Comparison of Thuricide 16B and 32B results from Arizona shows that significant increases in *B.t.* induced mortality can be achieved by increases in dose rates using one application. The acceptable levels of foliage protection and population reduction in Wisconsin where operational, timing, and weather conditions were good during and subsequent to treatment as contrasted with the unacceptable levels of effect in Maine where weather conditions were poor, lead us to conclude that the effects of one application at 8 BIU can be markedly influenced by these factors. Intermediate results in New Hampshire further substantiate this.

The previously discussed colony counts show the deposits for each location in terms of bacterial colonies per square centimeter. The count for Maine probably exceeds the amount available to the budworm larvae because of rain after spraying. The high, medium, and low values for Wisconsin, New Hampshire, and Arizona, respectively, correspond roughly with the population reduction figures in Table 4.

Within each location, colony counts by spray block were compared with 14-day population reduction and defoliation estimates. None of the eight correlation coefficients were significant at the .05 level, probably because (1) there were few blocks in each test; (2) coverage was fairly similar within each location; and (3) at some locations, branches were cut back to prescribed lengths before being bagged. This bagging procedure would reduce any correlation between larval counts and branch area and would be reflected in deposit-population reduction correlations. Data for unsprayed blocks were not used because only Wisconsin collected the data and inclusion of the checks would have partly duplicated the "treatment effect" tests made in the ANOVA.

These results are typical in that correlations of deposit data with population reduction and defoliation estimates, computed using tree or cluster observations, are small and only occasionally statistically significant. Coefficients of variation, which are useful dimensionless measures of precision, were computed from the same figures used in the correlations for the Wisconsin data. We obtained 85 percent coefficient of variation for 14-day larval survival, 45 percent for foliage protected, and 24 percent for colony counts. These figures clearly indicate that measurement of larval survival has the greatest potential for improvement if the objective is to relate spray deposit and population reduction by cluster. This does not mean that deposit assessment should not be improved, that card location is adequate, or that card evaluations are in any way satisfactory. It does indicate that, whatever the card problems may be, the difficulties in estimating population reduction are even more serious.

Population reduction can be evaluated directly by comparing counts of larvae per branch during prespray and postspray sampling periods, or the counts can be adjusted by buds or area per branch. Adjustment can reduce bias caused by the sampling of different sized branches during prespray versus postspray, if branch area or buds on a branch do not change over the sampling interval. If they do change, the adjustment introduces bias rather than correcting for it. It was noted that buds per branch decreased by 50 percent in Maine and Wisconsin with no indication that the counting and evaluation procedures or branch size changed over time. Another reason for using adjustments to obtain population reduction estimates is that, if the area (or bud count) on a branch is correlated with larval counts, a more precise (smaller variance) estimate can be calculated. Because there was no correlation in any location, branch area adjustment is pointless. Although bud and area adjustments may introduce bias into population reduction estimates, larvae per unit area or per bud may be appropriate measures of population density at fixed points in time.

The disappearing buds have serious implications for branch sampling. If bud counts change because crews select different kinds of branches for prespray than postspray, which seems to be the most logical explanation, then this sampling bias could have as much, or perhaps even more, effect on the larval counts. Because the larval counts are expected to change over time in any case, this bias would not be readily detected. An unsuspected bias factor of two is a serious matter.

There is good reason for recording counts of live and dead larvae separately for postspray sampling. If live larvae only are counted, the tacit assumption is that all dead larvae on the branches were killed by the agent. If live and dead larvae are counted together, the assumption is being made that none of the dead larvae died as a result of the agent. With neural toxins, larvae generally go into spasms upon contact and dislodge themselves, so all dead larvae on the branches can be presumed to have succumbed as a result of the storage and handling of the branch samples. With biological agents, however, dead larvae may have been predominantly killed by the agent. It is not practical to autopsy each dead larvae found, and it may not be clear until after the data are recorded what the dominant mortality factor was. Separate recording of live and dead larvae seems to be the safest method for maintaining flexibility in analyzing the data.

Natural mortality was accounted for by using Abbott's (1925) formula. This results in a better approximation of the true effect of the treatment. Abbott's adjustment assumes only that the natural mortality would occur at the same rate in the treated areas as the check areas in the absence of treatment. This ought to be assured by correct study design and proper randomization. The formula adjusts total mortality by calculated natural mortality, thus giving an estimate of mortality due to treatment. Because there were distinct differences in check mortality among the different areas, Abbott's formula was required to unbias comparisons between the areas. The adjustment does add a new source of variability into the corrected estimates. The impact of this variability can be lessened by restricting the period of observation to the period of activity of the agent as closely as possible. For *B.t.*, 14 days after spraying would appear to be appropriate for the second sample to assess the effect of this agent. It must be borne in mind, however, that significant *B.t.* mortality can occur after 14 days and therefore an additional sampling time close to pupation may be advisable. The prespray sample should be taken as near the spray date as practicable.

The efficiency of the sampling design was evaluated for three locations in the core test to determine how larval and defoliation sampling might have been done more effectively. The evaluation sets the sampling error on the spray blocks at the level obtained in the core test. The standard error of estimate for gross population reduction with various numbers of blocks, assuming 20 larvae per branch is:

Percent reduction	*Number of replicates (blocks) per treatment*							
	1	*2*	*3*	*4*	*5*	*6*	*8*	*10*
90	5.6	4.0	3.2	2.8	2.5	2.3	2.0	1.8
80	11.2	7.9	6.4	5.6	5.0	4.6	4.0	3.5

Five spray blocks per treatment (replicates), as used in the core design, yield a standard error of ± 2.5 percent at 90 percent, and ± 5 percent at 80 percent population reduction.

The appropriate sampling intensity for the prespray and postspray larval sampling effort depends on the population reduction anticipated. Twice the intensity is appropriate for 75 percent reduction, whereas three times the intensity is best for 89 percent reduction. These results follow from empirical heterogeneity of variance relations. Because the postspray work more critically effects the precision of the population reduction estimates and requires more total effort, it is reasonable to optimize postspray work and adjust prespray sampling accordingly.

The variability between branches, trees, and clusters was computed for the larval counts, using $\ln(x + 1)$, where x is the number of larvae on a branch. The criterion for comparison was time needed to carry out sampling on a block. This criterion does not account for larval counts or other costs that might be considered important in other instances.

The time to take a branch, once the pole pruner is up, is assumed to take 1 minute, and getting the pole up and down, bagging the branches, and moving to the next tree takes 3.5 minutes per tree. Time to move from one cluster to the next is taken to average 10 minutes. These figures represent the judgment of experienced people and are used to compute sampling times for different cluster-tree-branch sampling schemes. Under the 10 cluster, 3 tree, 4 branch scheme (10, 3, 4), used in the core tests, a block was sampled in 5 hours and 25 minutes. With the variability taken from the core work, less time is required by a 12, 3, 2 scheme, which took 5 hours and 18 minutes.

The 12, 3, 2 scheme might be preferable, because it involves fewer

total branches, but this consideration was not part of the criterion. On the other hand, a 12, 3, 1 prespray sample would take 4 hours and 25 minutes compared to 4 hours and 42 minutes for the 10, 3, 2 scheme. The main finding here is that either sampling plan closely optimizes man-hours in the field with the figures we used.

An evaluation of the efficiency of the defoliation work was made also. The arcsine transformation was applied directly to the midclass defoliation rating for each bud. The time for classifying each bud was set at 1.2 seconds in evaluating cost, and the trees in the 12, 3, 2 postspray larval sampling scheme were considered. The problem was to select branches and buds to minimize sampling time on a block and keep the precision of the block defoliation estimates as high as it was in the core work, given the components of variation between buds, between branches, etc computed from the bud defoliation data. The result was that only one branch with nine buds needed to be evaluated if 12 clusters of 3 trees were sampled. This took 4 hours and 48 minutes. If the 10 cluster of 3 trees were used, then the optimal number of branches and buds is 4 and 25, respectively, as in the core work. However, the time per block is then 6 hours and 25 minutes, an increase of 2 hours. There is a great deal of variability between clusters, compared to within clusters, and any reduction in cluster numbers must be paid for dearly in terms of increased subsampling to make up for the loss in precision.

Conclusions

The principal investigators at the core locations were able and conscientious, and responded well to adverse circumstances. These core tests show that it is difficult to maintain:

1. sufficient uniformity for sound comparisons between locations,
2. compatability with the needs of different studies at each location, and
3. latitude adequate for individual cooperators to respond to exceptional situations.

If we were to rewrite the work plan for this test, the following changes would be made. No pupal collections for parasite or sex ratio work would be specified. The Millipore filters would be used to detect drift and misses, and would not be linked to the larval sampling points. Separate live and dead larval counts would be required. No postspray bud counts would be taken. The greatest source of variability within a location was between spray blocks. This variability would be changed little by altering the number of clusters, trees, or branches allocated to larval sampling within a location. However, fewer buds and fewer branches per tree would be used in the defoliation evaluation work. We would ensure random selection of any midcrown branch on the prespray or postspray samples, and only one postspray sample at 14 days would be specified.

The apparent sensitivity to weather and application timing remains a most serious impediment to *B.t.* acceptance. Improved equipment and formulation, multiple application, and higher dosages could decrease this sensitivity. The results from Arizona with 32 BIU and recent work (personal communication) in Canada by O. N. Morris, and in Maine by H. Trial, University of Maine, indicate that increased dosage may be a feasible solution. Testing dosages above 8 BIU appears to be the next logical step for work similar to the 1980 core test. Comparable tests are needed at several locations, first because evaluation of the success (that is, evaluation of the combined locations) depends upon encountering various adverse conditions, and second because a few instances such as when 8 BIU fails to perform and higher doses succeed, might be construed as exceptional cases, attributable to the erratic behavior of *B.t.*

For operational success, we feel that spray formulation and multiple application depend on questions that need study under more controlled conditions than those in the field. These questions involve drop impingement on tree foliage, availability of active material at feeding sites, larval feeding behavior and consumption rates by instar, and as a function of temperature, susceptability of surviving larva to a second exposure to *B.t.*, environmental degradation of material, and on and on. These questions are common to other biological agents against other forest defoliation insects, and the solution transcends the *B.t.*-spruce budworm connection. Work is underway in these areas.

Acknowledgments

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Appendix A

Analysis of Variance Summaries

Analysis of variance has been used to determine the presence or absence of effects on larvae, buds, branch area, and defoliation. If an effect did not prove to be significant (for example, spray effects in Maine) or was of no interest (for example, population changes over time), nothing further was done. When an effect of interest was significant (for example, time effects on buds per branch in Wisconsin), the magnitude and interpretation of the effects were incorporated in the report.

The tables give mean squared errors (MSE) and degrees of freedom (df), from which a full ANOVA table can be derived. The models are hierarchical (block, cluster) and crossed with time, except for defoliation. The basic sample unit is the cluster. The dependent variable corresponds to averages per branch transformed as follows: ln(larva + 1), ln(buds + 1), ln(l*w/100 + 1), where l and w are the length and width, in cm, of the branches, and arcsine-root, in radians, of the mean bud defoliation percent divided by 100.

Table 6. MSE and df for ANOVA of effects on buds, branch area, larvae, and defoliation.

Source	df	Maine	New Hampshire	df	Wisconsin
		BUD COUNTS			
Treatment	2	26.38*	9.335*	2	4.594
Block	12	4.788	2.598	12	2.992
Period	2	182.8***	3.481	3	19.65***
Treatment × Period	4	5.312	0.9424	6	0.7431
Block × Period	24	2.408	2.156	36	0.6474
Cluster	135	0.7791	0.3600	135	0.4988
Error	570	0.2707	0.0837	855	0.0828
		BRANCH AREA			
Treatment	2	0.0991	0.2970	2	0.3427
Block	12	0.0749	0.1879	12	0.3115
Period	2	0.8903***	0.2026	3	0.5786
Treatment × Period	4	0.0832	0.0065	6	0.2724
Block × Period	24	0.1369	0.0673	36	0.2519
Cluster	135	0.0647	0.0460	135	0.0770
Error	570	0.0232	0.0127	855	0.0185
		LARVAE			
Treatment	2	3.258*	30.21*	2	18.59**
Block	12	1.233	5.271	12	2.637
Period	2	209.8***	350.1***	3	183.2***
Treatment × Period	4	2.286*	5.873*	6	7.478***
Block × Period	24	3.360	1.350	36	0.6394
Cluster	135	0.3891	0.4001	135	0.4138
Error	570	0.1203	0.1014	855	0.0734
		BUD DEFOLIATION			
Treatment	2	1.6693*	1.0954*	—	6.0145**
Block	12	0.2675***	0.2298	—	0.6633***
Cluster	35	0.02387	0.04423	—	0.06111

* Significant at the 0.05 level.
** Significant at the 0.01 level.
*** Significant at the 0.001 level.

Appendix B

Data Summaries

The following summaries of larvae and bud counts and defoliation ratings are derived from the data on which the analyses in the report are based.

Table 7. Numbers of spruce budworm per branch, prespray, by location and treatment.

Location	Treatment	Block (Replicate)				
		1	2	3	4	5
Maine	Dipel 4L	19.45	22.03	56.87	34.49	30.37
	Thuricide 16B	24.10	32.90	26.43	60.03	42.3
	Check	40.70	9.32	25.98	45.38	55.98
New Hampshire	Dipel 4L	41.35	20.30	31.52	31.58	28.31
	Thuricide 16B	32.40	28.82	33.22	17.19	32.53
	Check	41.75	11.75	33.42	30.12	39.25
Wisconsin	Dipel 4L	18.36	13.78	8.87	23.63	20.57
	Thuricide 16B	16.35	6.43	9.27	13.15	8.20
	Check	16.53	9.75	15.51	10.58	10.38
Arizona	Dipel 41	11.07	14.90	14.53	16.70	14.16
	Thuricide 16B	30.00	10.80	17.37	10.60	7.43
	Thuricide 32B	9.00	8.67	36.80	5.50	7.52
	Check	18.13	12.33	14.93	15.00	24.73

Table 8. Number of spruce budworm per branch, by sample, location, and treatment

Sample	Location	Treatment	Block (Replicate)				
			1	2	3	4	5
14 day	Maine	Dipel 4L	20.08	12.48	21.82	9.86	14.38
		Thuricide 16B	23.14	27.20	24.64	20.06	19.60
		Check	14.75	18.94[a]	19.21	13.91	4.98
14 day	New Hampshire	Dipel 4L	0.13	2.67	0.42	1.32	1.73
		Thuricide 16B	1.51	1.78	1.81	.42	1.39
		Check	9.92	2.38	6.09	2.94	2.30
14 day	Wisconsin	Dipel 4L	2.23	0.70	0.95	3.22	1.23
		Thuricide 16B	1.00	.34	.30	.22	1.03
		Check	5.09	2.52	6.22	3.62	4.92
24 day	Wisconsin	Dipel 4L	0.84	1.00	0.53	1.31	0.82
		Thuricide 16B	.39	.17	.52	1.28	.90
		Check	1.96	.78	2.42	1.21	1.63
12 day	Arizona	Dipel 4L	3.63	5.20	5.30	4.93	4.07
		Thuricide 16B	9.73	5.20	4.63	3.07	3.60
		Thuricide 32B	5.07	2.50	3.17	.90	2.30
		Check	8.73	7.20	5.10	9.40	9.40

[a] Drift is suspected.

Table 9. Number of spruce budworm per branch at first adult emergence, by location and treatment.

Location	Treatment	Block (Replicate)				
		1	2	3	4	5
Maine	Dipel 4L	7.75	5.00	2.78	2.44	3.14
	Thuricide 16 B	6.48	6.08	3.66	1.59	3.71
	Check	6.69	7.01[a]	2.80	2.73	2.63
New Hampshire	Dipel 4L	0.26	3.63	0.37	0.18	0.24
	Thuricide 16B	1.06	.88	.62	.21	.91
	Check	10.59	2.64	5.16	1.89	2.77
Wisconsin	Dipel 4L	0.17	0.12	0.19	0.37	0.26
	Thuricide 16B	.10	.05	.40	.48	.22
	Check	.16	.13	.63	.31	.21
Arizona	Dipel 4L	1.33	2.25	1.47	1.27	0.90
	Thuricide 16B	2.65	1.82	.97	1.08	.57
	Thuricide 32B	1.08	.67	.58	.32	.58
	Check	2.73	3.20	2.60	2.42	1.75

[a] Drift is suspected.

Table 10. Percentage of bud defoliation estimates, by location and treatment.

Location	Treatment	Block (Replicate)				
		1	2	3	4	5
Maine	Dipel 4L	58.70	40.99	66.27	54.46	65.57
	Thuricide 16B	74.08	91.84	74.85	85.97	73.01
	Check	93.71	56.07[a]	92.25	83.02	97.56
New Hampshire	Dipel 4L	92.07	86.25	99.17	96.17	82.49
	Thuricide 16B	87.86	56.36	83.84	73.46	82.19
	Check	99.92	99.31	98.63	83.24	94.50
Wisconsin	Dipel 4L	71.49	19.53	15.53	64.72	63.15
	Thuricide 16B	64.51	17.00	34.39	81.90	44.36
	Check	97.39	84.81	93.95	95.41	86.22
Arizona	Dipel 4L	67.0	71.0	62.3	67.1	68.3
	Thuricide 16B	85.7	46.2	69.1	54.5	68.1
	Thuricide 32B	92.0	41.3	49.0	62.4	53.2
	Check	94.6	55.2	90.8	74.7	93.8

[a] Drift is suspected.

Table 11. Number of buds per branch, prespray by collection period and location with standard errors.

Collection period	Maine	New Hampshire	Wisconsin
Prespray	156.4 ± 9.3	66.0 ± 5.5	37.4 ± 2.2
14 Day	86.2 ± 7.1	50.7 ± 4.4	19.1 ± 1.3
First Adult Emergence	34.7 ± 4.4	51.6 ± 4.6	18.8 ± 1.7

Walton, Gerald S.; Lewis, Franklin B. **Spruce budworm core *B.t.* test—1980, combined summary.** Broomall, PA: Northeast. For. Exp. Stn.; 1982; USDA For. Serv. Res. Pap. NE-506. 12 p.

Two commercial preparations of *B.t.* were applied aerially under the auspices of CANUSA-East. This paper compares results and critiques the experimental design, spray operation, and data collection procedures.

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