Bt

Field and laboratory investigations of *Bacillus thuringiensis* as a control agent for gypsy moth, *Porthetria dispar* (L.)

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Introduction

SUCCESSFUL control of the gypsy moth, *Porthetria dispar* (L.), has been a goal of entomologists in the northeastern United States for many years. When DDT came into use, and more efficient aerial-spraying methods were developed after World War II, this goal was apparently reached. However, a few years ago there arose a vehement public reaction against the use of chemical insecticides. This spurred new interest in the use of biotic methods for combatting insects, and much research is now centered on insect diseases and their possible uses in insect control.

Because disease is a primary factor in the population dynamics of the gypsy moth (Campbell 1963), disease-causing agents are biologically feasible as an alternative to DDT. Commercial preparations of a microbial insecticide, *Bacillus thuringiensis* var. *thuringiensis* Berliner (referred to from here on as *Bt*), have been available since the late 1950s for control of leaf-feeding lepidopterous pests.

To explore the possibilities of using *Bt* as a means to control the gypsy moth, a series of field and laboratory tests were begun in 1961 by the Northeastern Forest Experiment Station at its Forest Insect Laboratory at New Haven, Conn. This paper is a report on the techniques used in those tests.

Preliminary laboratory and field tests were conducted with a wettable powder formulation of *Bt* against the gypsy moth in 1961. Though the results were inconclusive in terms of practical control, considerable information and experience were gained in handling and evaluating this type of insecticidal material.

The necessity for adequate pre-testing of the material to be used in the field, and the necessity for special techniques for the proper assessment of spray deposit, became very apparent during the 1961 field test conducted in New York.[1] In addition, the development of special methods for evaluating the effects of the microbial insecticide was clearly demonstrated by this test.[2]

A larger-scale field test, using a *Bt* powder plus Lovo 192[3] as a sticker-anti-evaporant, was conducted in 1962. The powder was specially formulated without a wetting agent. This test, also carried out in New York State, was encouraging too; but the results were erratic, and operational difficulties again were encountered in mixing, storing, and applying the spray. Yet more information was obtained about the field techniques needed to properly evaluate the distribution and effects of the spray. Moreover, valuable information was obtained in corollary laboratory tests of the effects of *Bt* on the gypsy-moth larva.[4]

The development in late 1962 of a new emulsifiable liquid *Bt* concentrate appeared to alleviate the difficulties of mixing and applying the powder preparation, which had seriously limited the practical effectiveness of the 1961 and 1962 tests. This new formulation was field-tested in New York in 1963 with much better results.[5]

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[1] Lewis, F. B., D. P. Connola, and R. C. Sweet. FIELD TEST OF A BACILLUS THURINGIENSIS SPORE-CRYSTAL CONCENTRATE AGAINST THE GYPSY MOTH IN NEW YORK. U. S. Forest Serv. NE Forest Exp. Sta., N. Y. State Museum and Science Service, and N. Y. State Conservation Dep. Unpublished report, 19 pp., 1961.

[2] Connola, D. P., F. B. Lewis, and J. L. McDonough. EXPERIMENTAL FIELD TECHNIQUES USED TO EVALUATE GYPSY MOTH CONTROL IN NEW YORK. (In press.)

[3] Regular trademark Fisons, Ltd., distributed by Stauffer Chemical Co. Mention of a particular commercial product should not be construed as an endorsement by the U. S. Department of Agriculture or the Forest Service or the New York State Museum and Science Service.

[4] Lewis, F. B., D. P. Connola, and J. L. McDonough. AERIAL APPLICATION OF A BACILLUS THURINGIENSIS SPORE-CRYSTAL CONCENTRATE IN FUEL OIL AND WATER FORMULATIONS FOR GYPSY MOTH CONTROL—NEW YORK STATE, 1962. U. S. Forest Serv. NE Forest Exp. Sta. Unpublished report, 54 pp., 1962.

[5] Lewis, F. B., D. P. Connola, J. L. McDonough, and others. PILOT TEST OF A BACILLUS THURINGIENSIS LIQUID CONCENTRATE APPLIED BY AIRCRAFT FOR CONTROL OF THE GYPSY MOTH—NEW YORK STATE, 1963. U. S. Forest Serv. NE Forest Exp. Sta. Unpublished report, 24 pp., 1963.

The Nature of Bt

Before discussing these field and laboratory experiments with *Bt*, it is apropos to review briefly the available information about the nature and mode of action of the active agent in the materials used in the tests.

Bt belongs to the aerobic, spore-forming, gram-positive bacterial genus *Bacillus*. *Bt* and five other species and several varieties form a distinct group within the genus, based on classical biochemical tests and the formation of a parasporal body (crystal) at the time of sporulation (Heimpel 1962). A serological typing system has been reported (DeBarjac and Bonnefoi 1962). All of these crystal-producing organisms are pathogenic for insects, principally the Lepidoptera, and as far as is known do not cause disease reactions of any sort in higher animals. In the United States, only one crystalliferous bacillus, *Bt*, is produced in commercial quantities; this is the active agent in the preparations used in the experiments reported here.

The life cycle of *Bt*, which is characteristic of the crystalliferous spore-forming group, will be used as an illustration. The cycle of the bacillus is essentially divided into two main stages: (1) the actively multiplying stage (the vegetative cell) and (2) the resistant dormant stage (the spore). The vegetative cell is not heat-resistant and requires a moist environment that contains suitable nutrients for growth and multiplication. During the multiplication of the vegetative cell, metabolic byproducts are excreted. Many of these are toxic to insect larvae. In the case of *Bt*, one of the byproducts (Phospholipase C) is known to be toxic to insects (Heimpel 1955; Bonnefoi and Beguin 1959).

In the restricted environment of the insect gut, as well as in a tube of nutrient medium, the active vegetative growth of the bacillus slows and eventually ceases because of exhaustion of available nutrients and adverse changes in the immediate environment. When these nutrients are exhausted, the vegetative cell undergoes a process that will enable the organism to survive under unfavorable circumstances. This process involves a shortening and thickening of the vegetative rod, and concurrently an

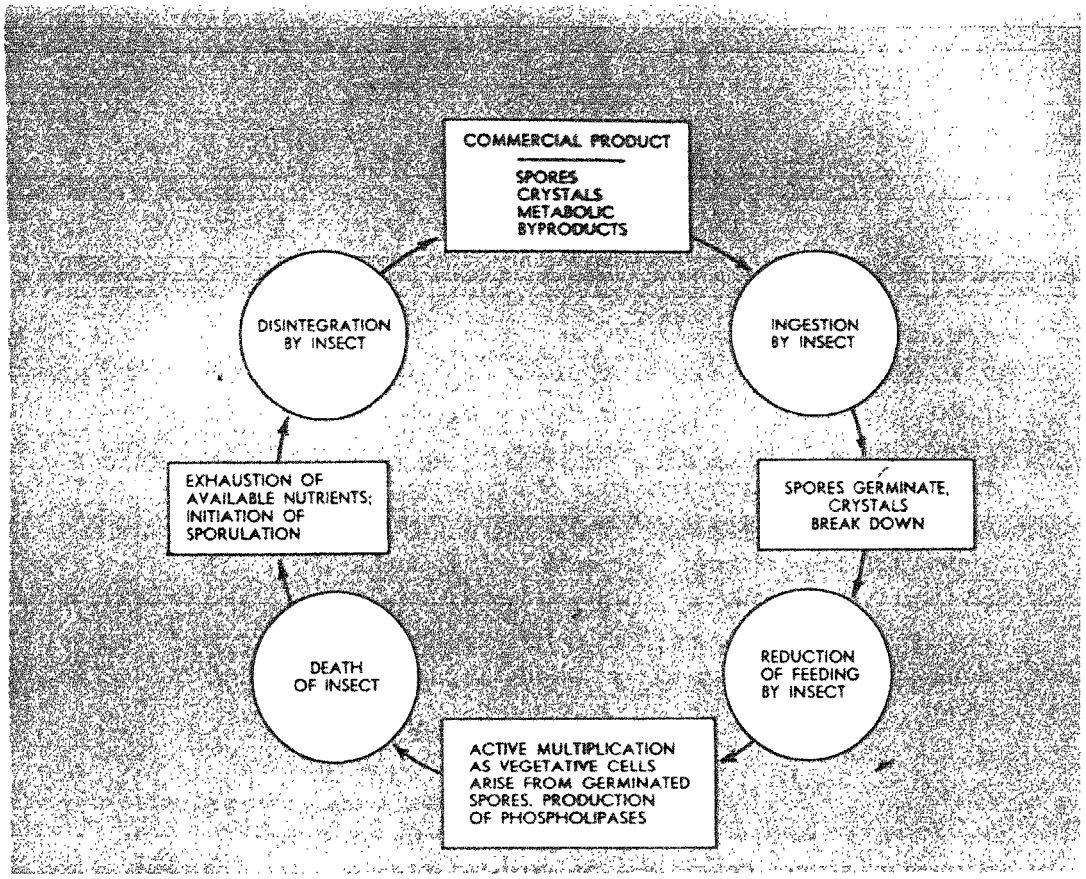

Figure 1.—A diagram of the life cycle of *Bt* as it would occur in a gypsy moth after ingestion of a commercial product applied for control purposes.

ovoid spore is produced in the rod along with, in the case of *Bt*, a diamond-shaped parasporal body or crystal.

Upon completion of this process, called sporulation, only the spore and crystal remain. Both the spore and crystal are heat-resistant and can remain in a dry environment for long periods of time without losing their viability or toxicity. In addition to the production of spore and crystal at the time of sporulation, complex soluble byproducts are also produced. One of these was first shown to be toxic to fly larvae (McConnell and Richards 1959).

Commercial *Bt* preparations are concentrates of the spores, crystals, and metabolic byproducts. The diagram (fig. 1) outlines

the life cycle of the organism as it would occur in a pest insect (gypsy moth) after ingestion of a commercial product applied for control purposes.

Several toxic principles are involved in killing the gypsy moth: the crystal, the so-called "fly factor," and phospholipases. Some information is available about the mode of action of the crystal (Angus 1956; Heimpel and Angus 1959; Bonnefoi and Beguin 1959), the fly factor (McConnell and Richards 1959), and Phospholipases (Heimpel 1955).

The diagram illustrates, in general, the known or suspected pathogenic effect of *Bt* and its life cycle in the gut of a gypsy moth larva. The larva ingests the spores, crystals, and metabolic byproducts contained in the commercial preparation. Within the insect's gut, the spores germinate and give rise to the vegetative cell (fig. 2). At the same time the crystal is dissolved or broken down under the influence of alkaline pH or enzymatic action that gives rise to a toxin. The toxin exerts a paralytic effect, causing in some insects a total and immediate paralysis and in

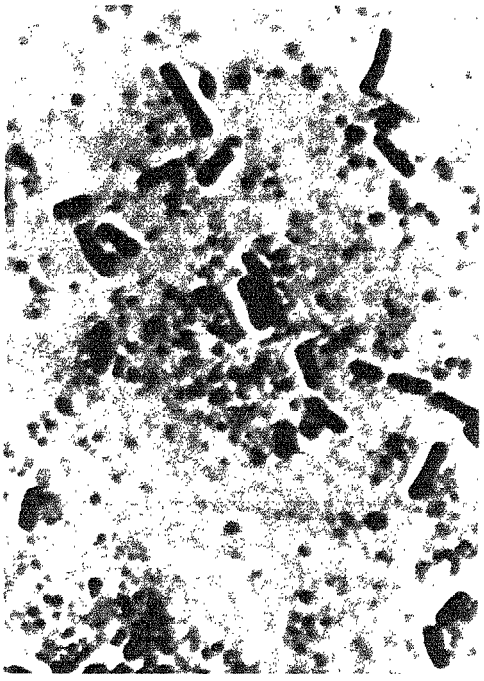

Figure 2.—Actively growing vegetative cells of *Bt*.

others only a gut paralysis in which the larva slowly desiccates (fig. 3). In the gypsy moth, total paralysis does not occur, and gut paralysis is only partial, which permits the affected larva to feed sporadically for several days. In addition to the direct paralytic effect of the crystal, toxic activity can occur because of the ingested metabolic byproducts contained in the commercial preparation. The mechanisms and significance of this activity in the gypsy moth are at present unknown.

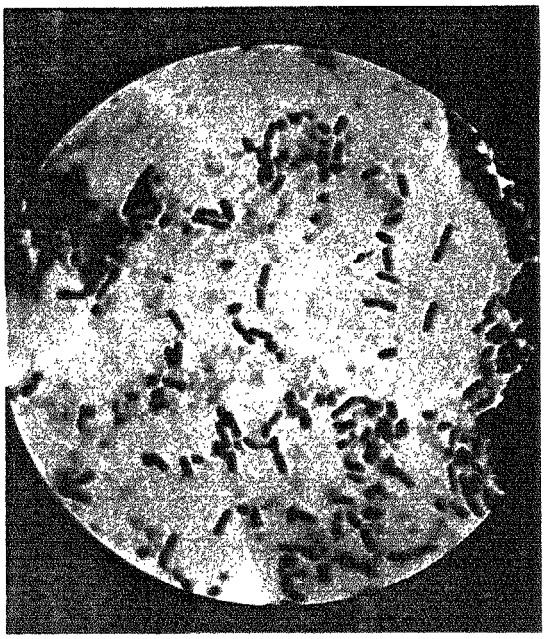

Figure 3.—Free spores and crystals in gut smear of *Bt*-killed gypsy moth larvae.

The events described occur rapidly after ingestion. However, in the gypsy moth, which is not immediately paralyzed by the ingested toxins, active multiplication of vegetative cells arising from ingested spores goes on until after the larva dies. Death of the gypsy-moth larva occurs 4 to 6 days after ingestion of the *Bt* material. The vegetative cells continue to multiply for 3 or more days in the cadaver until the nutrients are exhausted, whereupon the vegetative cells sporulate and give rise finally to only spores and crystals in the larval remains.

Materials and Methods

1961 Experiment

Field layout.—Seven woodlots were selected for spraying and three others were designated as checks by personnel from the New York State Conservation Department and the New York State Museum and Science Service. All selected woodlots had an estimated 300 to 2,000 gypsy-moth egg masses per acre, no known disease history, and indications of a rising population. An attempt was made to obtain predominantly oak woodlots. The plots were 25 to 200 acres in size and were located near Saratoga Springs, N. Y.

Treatments.—The *Bt* preparation[6] was applied by a Stearman biplane at three dosages (1/4 pound, 1 pound, and 2 pounds in 2 gallons of water per acre) with tung oil as a sticker. The tung oil mix, supplied by Pesticide Chemical Research Division, Agricultural Research Service, contained an emulsifier that was not sufficient when the spray was first prepared at the airport. The addition of 1-percent experimental 9D-207 emulsifier (Rohm and Haas) corrected the situation and apparently gave an excellent suspension. One plot received the finished spray without the added emulsifier.

Each of the three treatments had two replicates. In addition, a seventh plot received 1/4 pound *Bt* plus 48 x 10^9 polyhedra per acre of *Borrelinavirus reprimens* Holmes, which causes the nuclear polyhedral virus disease of the gypsy moth, commonly called gypsy-moth wilt disease.

Evaluation procedure.—Five 0.1-acre subplots were established in each of the 10 experimental plots. Four types of samples were taken in each of these subplots.

- Three sets of five sleeve cages, each containing 25 healthy larvae each were placed on foliated branches within reach of the ground in each subplot. One set was placed immediately after spraying, another 4 days after spraying, and the last 8

[6] Thuricide wettable powder with a spore count of 60 x 10^9 per gram; product of Biofern Corp., Wasco, Calif.; distributed by Stauffer Chemical Co., New York.

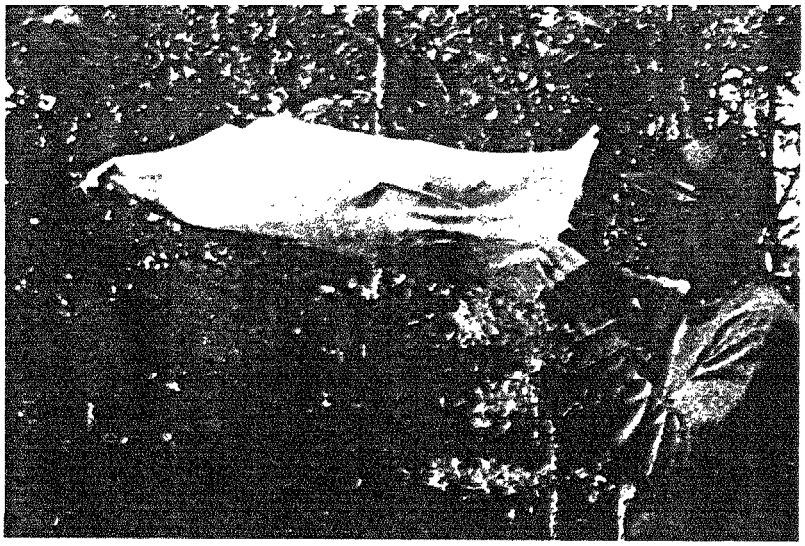

Figure 4.—Sleeve cage used in the 1961 field test.

days after spraying. The sleeve cages were examined every other day for 16 days and then removed. Dead larvae were collected from the cages as found and mailed to the Forest Insect Laboratory at New Haven, Conn., or transported by car to the Bacteriology Department, University of Connecticut, Storrs, for examination as to cause of death (fig. 4).

- One cloth drop tray (3 x 3 feet) was placed in each subplot. Dead larvae in these trays were sent to New Haven or Storrs for examination. In addition, 10 living larvae from each subplot were collected 2, 4, and 8 days after spraying, sent to Storrs, and serially plated on trypticase soy agar (TSA) to determine the amount of *Bt* spores and cells present in the insect.
- Leaf samples were collected in each subplot approximately 2, 4, and 8 days after spraying. One lot was sent to the Bioferm Corporation for a viable spore count; a second lot was sent to the Insect Pathology Pioneering Research Laboratory, Agricultural Research Service, Beltsville, Md. for a similar count.
- Before- and after-treatment egg-mass counts were made in all plots. In one plot the counts were made in the 0.1-acre subplots. In all other plots, the egg-mass counts were made along string lines between the subplots.

Laboratory methods.—No laboratory tests preceded the 1961 field test. However, after the field test a series of experiments were conducted to determine the effectiveness of the tung oil sticker and the effects of the 9D-207 emulsifier on the activity of the experimental material. The same commercial preparation of *Bt* was used in these tests as in the field test. Three formulations were used: (1) *Bt* powder in water alone; (2) *Bt* in water with tung oil added; and (3) *Bt* in water with tung oil and 9D-207 emulsifier added. The tung oil and emulsifier were added at the same concentrations as used in the field.

1962 Experiment

Experimental areas.—Twenty-seven woodlots, averaging 73 acres, were used in the 1962 experiment. All woodlots were located in eastern New York and were predominantly oak. The initial insect populations in these plots were 150 to 2,000 egg masses per acre, except for one plot, which approximated 8,500. There was no known disease incidence in any of the experimental areas in 1961. Twenty-four plots were treated in three series of eight each, and three were used as checks (one in each series).

Treatments.—Based on the results of the 1961 experiment, three *Bt* concentrations again were chosen: 1, 2, and 4 pounds per acre. In addition, more plots were treated with the combination of the bacillus and the polyhedral virus. The dosages used were as follows:

- 4 pounds Thuricide[7] in 4 gallons of diluent per acre.
- 2 pounds Thuricide in 2 gallons of diluent per acre.
- 2 pounds Thuricide plus 50 x 10^9 polyhedra in 2 gallons of diluent per acre.
- 1 pound Thuricide plus 50 x 10^9 polyhedra in 2 gallons of diluent per acre.

The four dosages were applied in two diluents: (1) No. 2 fuel oil and (2) water with 6 ounces of Lovo 192 per gallon of

[7] Thuricide 65 B, product of Bioferm Corp., distributed by Stauffer Chemical Co., New York.

spray. The eight treatments were replicated three times, each replicate constituting a series. Each series was completed before the next was begun. The sprays were applied by two Stearman biplanes, one of which applied the oil sprays and the other the water sprays. The treatments were begun when oak leaf expansion was nearly complete, and the gypsy-moth larvae were in the second and third instars.

Evaluation procedure.—In 1962, certain modifications were made in previously used evaluation procedures. Seven methods were used, as follows:

1. Spray-deposit data obtained in 1961 had two major shortcomings: samples were taken from understory foliage and thus were not representative of the spray distribution; and considerable time elapsed between sampling and the analysis of deposit, which permitted both spore death and germination. In 1962, foliage was pole-pruned from the lower half of the crowns of treated oaks, and petri dishes containing TSA were placed below openings in the canopy at each of the five subplots in two of the three series. To eliminate the time lapse between sample collections and deposit analysis, a field laboratory[8] was established in Saratoga Springs, N. Y. This laboratory greatly facilitated the evaluation procedures.
2. Mortality data obtained from the sleeve cages in 1961 was inadequate because the cages were placed on understory foliage, which did not represent the spray deposit; and the cages collapsed during the rain. To compensate for these deficiencies, a rearing station was established after treatment in the central subplot of each experimental plot. Five 12 x 12 x 6-inch screened cages constructed of waterproof plywood were placed on a rack at the Station—one cage for each of the five subplots in the plot. Each cage was supplied with 25 healthy larvae and foliage pole-pruned from the corresponding subplot. Foliage was replaced every other day. Dead larvae were removed from the cages and taken to the field laboratory for microscopic diag-

[8] Facility provided by the N. Y. State Conservation Department at its Saratoga Spa laboratories.

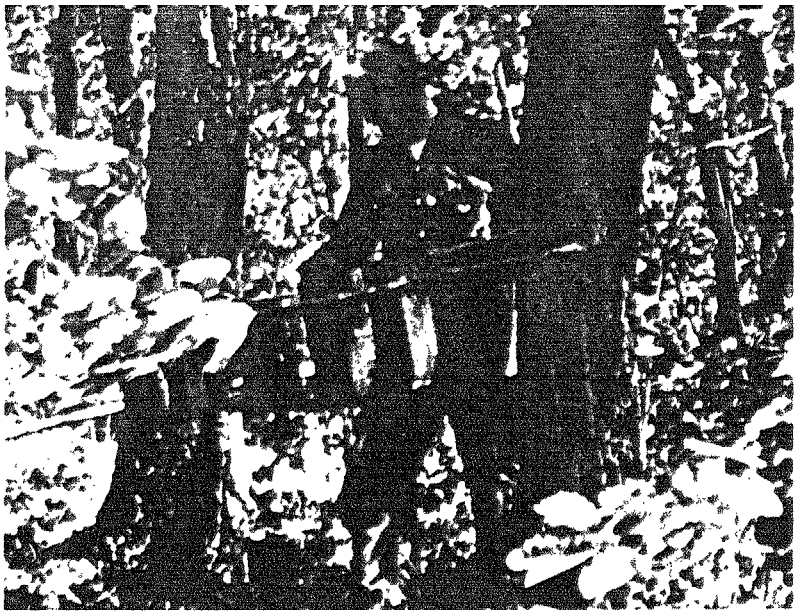

Figure 5.—Rearing station and *Bt* mortality cages used in 1962 experiment.

nosis of cause of death. Approximately 3,500 larvae were examined (fig. 5).

3. Frass samples were collected in cheesecloth dropping cloths suspended in hammock fashion under oak trees in each subplot of certain plots. This frass was collected at intervals, dried, and weighed after debris and non-gypsy-moth frass had been removed. The frass was also segregated according to the third, fourth, and fifth to sixth instars, and the pellets were counted (fig. 6).

4. Ten-minute counts of living larvae were made by researchers as they walked slowly along the string-line that connected the five subplots. This method of counting was used only when the larvae were third instar or larger.

5. Defoliation estimates were made at the completion of the larval feeding period in each subplot. No defoliation readings were made before spraying.

6. Before- and after-spray egg-mass counts were made at the same places in the treated plots in the 1962 experiment in contrast

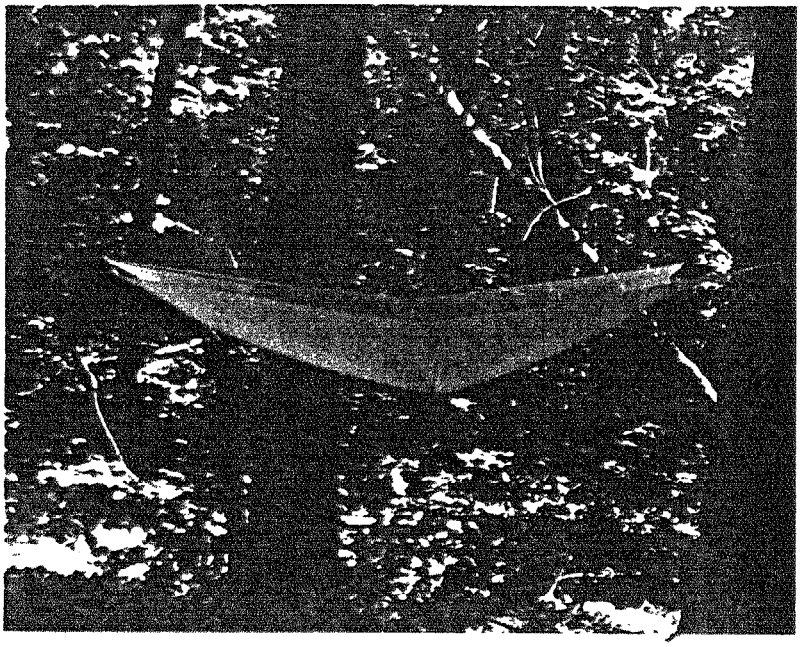

Figure 6.—One type of drop tray used in the field tests.

to before-spraying egg-mass counts taken in different locations from after-spraying egg-mass counts in the 1961 experiment. This was done to minimize the wide variation in population numbers that normally occurs in different parts of each woodlot and to provide a better basis for measuring control.

7. The water-based and oil-based formulations of the active material were pre-tested at Beltsville, Md., and New Haven, Conn., for physical characteristics and biological effectiveness of the proposed field dosages.

Laboratory procedures.—Because the 1961 field test indicated the need for concurrent laboratory tests, a field laboratory was set up to provide facilities for the laboratory phase of the 1962 experiment. This phase was primarily for the purpose of examining spray deposit and dead larvae from the cages for the cause of death.

Two methods were used to check spray deposit on experimental

plots: (1) leaf collections before spraying, immediately after spraying, and 8 days after spraying; and (2) exposure during spraying at each of the five sampling subplots of a 10-cm. petri dish containing TSA.

In the first method, oak leaves were collected by pole pruner in each subplot and placed in plastic bags. The bags were then returned to the field laboratory immediately after collection, and 4 grams of leaf material were weighed out and washed with agitation for 15 minutes in 200 ml. of phosphate-buffered distilled water. The washing produced what we considered to be a 2×10^{-2} dilution. Two other dilutions—2×10^{-3} and 2×10^{-4}—were made from this original dilution. These dilutions were heat-shocked at 65°C. for 30 minutes and plated (0.5 ml. per plate) on TSA. Finally, the plates were incubated at 37°C. for 20 hours before the colonies were counted. The counts were recorded as spores per gram of leaf material.

In the second method, one petri dish was placed below an opening in each subplot and the lid was removed immediately before spraying began. At the conclusion of spraying, the lids were replaced and taped, and the petri dishes were returned to the field laboratory. The plates were incubated for 24 hours at 37°C.; then the colonies were counted. The colonies represented droplets and not individual spores.

All dead larvae found in the cages in the plots were returned to the field laboratory in sterile tin pill boxes. Here the larvae were macerated, smeared on microscope slides, stained with Buffalo black-Safranin O, and examined for the presence of spores, crystals, and vegetative cells of *Bt.*

Additional laboratory analyses were made of the viable spore concentration of the finished sprays applied to each plot. Aliquots of the finished spray were removed from the tank in the airplane and taken immediately to the field laboratory. Tenfold dilutions of an aliquot were made, plated out on TSA, incubated at 37°C. for 24 hours, after which the colonies were counted. The concentration of the viable spores determined in this manner in the finished spray was compared with the theoretical concentration calculated from the manufacturer's label count.

1963 Experiment

Experimental areas.—Eleven woodlots, primarily composed of oaks, were selected for the 1963 experiment. They were located in the Duanesburg-Schenectady area of New York State and ranged from 30 to 100 acres in size. One woodlot was composed of three segments, which were treated the same but assessed separately. The initial insect population in the plots ranged from 2,200 to 10,000 egg masses per acre. The higher initial populations were permitted in order to have a better comparison between treated and untreated areas, although the hazard of higher natural disease was greater than in the previous tests.

Treatments.—The treatments used in 1963 were based on the results of the 1962 experiment, No oil formulations were used, and the active ingredient, assessed by biological activity, was applied in water formulations only. The formulations used were: (1) 1 gallon of Thuricide 90T Flowable[9] in 1 gallon of water; (2) ½ gallon of 90T in 1½ gallons, and (3) ½ gallon of 90T plus 15×10^9 *B. reprimens* polyhedra in 1½ gallons. All applications were made at the rate of 2 gallons of finished spray per acre. There were three replications of each dosage. Each replicate was referred to as series I, II, and III; and each series was completed before the next was started. All treatments were applied when oak leaf expansion was complete and the larvae were second instar or larger. Application was made with a U. S. Department of Agriculture Piper Super Cub 135.

Evaluation procedures.—Experience with caged larvae in the 1961 and 1962 tests indicated that the data provided by this technique did not correlate satisfactorily with the final results of the experiments. Therefore the use of caged larvae was discontinued.

Additional modifications were made in the evaluation procedures used in the 1963 experiment as a result of experience during the 1962 test. The major changes were in the elimination of techniques that did not provide useful information and in the

[9] Product of Bioferm Corp., Wasco, Calif. distributed by Stauffer Chemical Corp., N. Y.

increased concentration on pre-test evaluation of the material. The field evaluation techniques used in 1963 were as follows:

- The use of cheesecloth dropping cloths for the collection of frass proved to be very useful in the 1962 experiment; therefore they were employed in the 1963 experiment. A dropping cloth was established in each subplot before spraying and was maintained until feeding activity of the larvae ceased. Collections were made two or three times a week and were handled in the same manner as in 1962. In 1963, dead larvae were picked up in the cheesecloth hammocks and from leaves in each subplot and were returned to the field laboratory for microscopic examination. These examinations provided sufficient information on the course and cause of mortality in the treated plots, and gave evidence of a change in biological activity of the *Bt* material (fig. 7).

- Ten-minute counts of living larvae proved to be useful in assessing the trend of the population after spraying in 1962. Larval counts were made in 1963 in the same manner as in 1962.

- Defoliation estimates were made in all subplots before spraying and at intervals until larval feeding ceased. In each subplot estimates were made of (1) oak defoliation only, and (2) defoliation of all tree species considered together.

- The number of egg masses per acre after spraying was specified as the primary criterion of control in 1963. However, to minimize the amount of labor and time used on the final egg-mass counts, a preliminary egg-mass count was made in all plots immediately after egg deposition. Plots that showed 50 egg masses per acre or less were examined in greater detail after leaf fall, and all plots that had more than 50 egg masses per acre were eliminated from further examination.

Laboratory procedure.—Experience in the 1962 test indicated that detailed before-spraying checks must be made of the biological activity of the active *Bt* ingredient, of the physical characteristics of the concentrate, and of the concentration of viable spores and crystals in the bulk concentrate. Further, during the actual

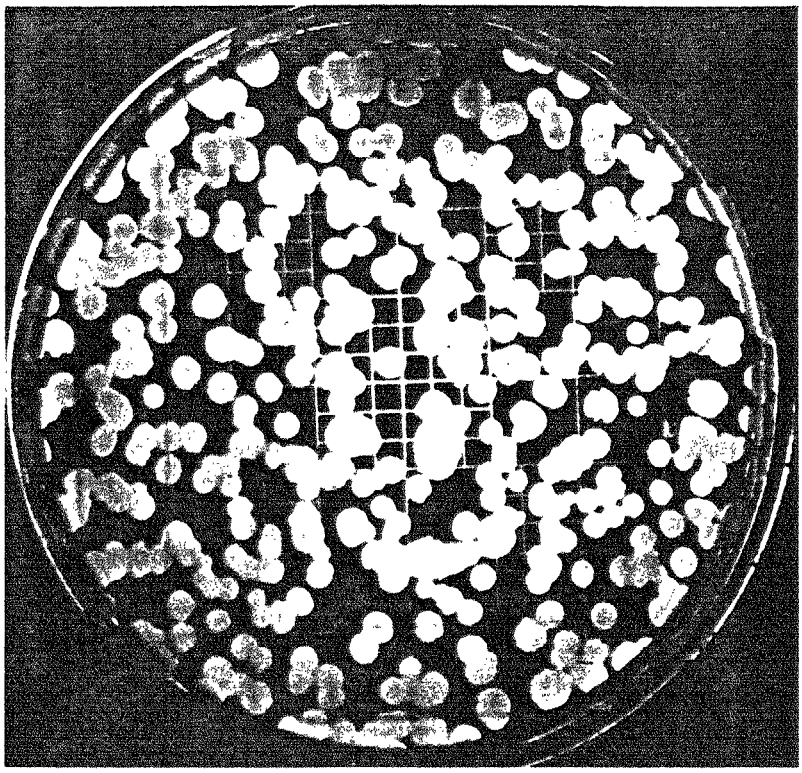

Figure 7.—*Bt* colony growth from spray droplets caught on agar-filled petri dish exposed during spraying.

spraying operations, checks must be made of the concentration of viable spores and crystals in the finished spray mixes. As a result, a large number of checks were made before and during the spray operation. All pre-testing of the 1963 material was done on production samples shipped in bulk as they would be for a control operation.

Prespray laboratory tests.—Biological-activity determinations were conducted with the proposed field dosages from the emulsifiable concentrate, and the new material was compared with the powder formulation used in 1962 for biological equivalence. The sticking qualities of the new material were tested under 10 inches of simulated rain. Since a fluorescent tracer was to be used for

spray deposit assessment, the biological compatibility of the tracer and the *Bt* concentrate was examined.

The physical characteristics of the new material, such as mixability and flowability, were tested at the Beltsville Forest Insect Laboratory. The aircraft to be used in applying the sprays were pre-checked for flowability of the sprays and were calibrated to deliver 2 gallons of spray per acre.

To assure that no settling had occurred in the drums of concentrate shipped to the airfield, aliquots were taken from the top, middle, and bottom third of each drum. These aliquots were diluted with 10-fold dilutions of phosphate-buffered distilled water. The dilutions were heat-shocked and plated on TSA as described previously. The viable spore concentration in each drum layer was obtained from dilution plates and compared with the labeled concentration. Crystal concentrations in each level of each drum were determined by Breed smears stained with Buffalo black.

Laboratory tests during spraying.—To guard against any loss of viable spores or crystals during the mixing and loading operations, a sample of the finished spray was taken from the spray tank of the aircraft. This aliquot was subjected to the microbiological procedures described previously for viable spore and crystal determinations. The actual concentrations of the finished sprays were compared to the theoretical concentrations based on the spore and crystal counts made of the drum concentrates used to formulate the finished sprays.

Results and Discussion

Field Operations

Field mixing and application.—In the 1961 test, all formulations were hand-mixed at the airport in 50-gallon drums. A circulating pump kept the finished spray in suspension. The material for each experimental plot was mixed just before application after a telephone call from field observers on the plots indicating that spraying conditions were suitable. The first plot sprayed did not receive the tung-oil sticker nor the added 9D-207 emulsifier because the emulsion was unstable in the aircraft. All other plots received the full amount of tung oil and additional 9D-207 emulsifier. During the application of the finished spray to the experimental plots, considerable trouble was encountered with clogging of the boom and nozzles on the aircraft due to settling of the *Bt* agglomerates and debris.

In summary, three major problems occurred (principally in mixing and application) in the 1961 test: (1) poor communication between the observation crews at the plots, the spray aircraft, and the airport due to lack of radios; (2) inadequate mixing and storage facilities at the airport; and (3) clogging of the spray in the plane's boom and nozzle system.

These problems were corrected in all but one important aspect during the larger field test in 1962. Radios were used in the 1962 experiment, which speeded up communications between the field plots, spray planes, and the airport. Mixing and loading facilities were improved so that the mechanical mixing, recirculation, and metering replaced the hand paddle and circulating pump used in 1961.

The physical characteristics of the oil-*Bt* concentrate and the water-Lovo 192-*Bt* concentrate still gave considerable difficulty in the field, contrary to data accumulated in pre-spray tests. The problem of keeping the *Bt* material in suspension in both the oil and water sprays was not overcome. Clogging of the aircrafts' booms and nozzles did not occur, but the *Bt* particulate material would not stay in suspension in the mixing tanks or the airplane tanks. This was particularly true of the oil suspension. As a

result, less than one-half of the specified amount of material in water-*Bt* suspensions and as little as one-thirtieth the desired concentration of the oil suspensions was transferred into the planes (table 1).

Table 1.—*Specified and estimated dosages applied to experimental Bt plots, 1962*

Specified dosage per acre	Estimated dosage of *Bt* per acre in—	
	Oil	Water[1]
Pounds	*Pounds*	*Pounds*
4	0.32	1.40
2	.16	.70
1	.08	.35

[1] Calculated from agar plate counts of viable spores in finished sprays.

The principal difficulty was discovered at the end of the first day's spraying. In cleaning the 660-gallon tank used to mix the oil-*Bt* suspension, a large amount of sediment was discovered at the bottom. A 50-gallon drum of the oil-*Bt* concentrate then was opened (the *Bt* concentrate that was diluted with oil was compounded with 4 pounds of *Bt* powder concentrate, ball-milled with 1 gallon of No. 2 fuel oil); and a 1-to-2-inch-thick layer of sediment was found hard packed on the bottom. It became apparent that the *Bt* powder had settled out of suspension during shipment. This sediment could not be re-suspended by rolling or tipping the drums.

The discovery of the sediment in the mixing tank and drum made it necessary to make additional checks on the delivered concentrates.

The operational problems encountered in 1961 and 1962 made any further aerial testing of *Bt* powder formulations of doubtful value until the announcement of a new liquid emulsifiable concentrate in late 1962.

Extensive pre-testing of production samples in bulk containers indicated that the physical characteristics of the new liquid formulation were excellent and would, in fact, eliminate the opera-

tional problems. These predictions were amply fulfilled during the field operations of the 1963 test. No difficulties were encountered in the mixing and application of this new emulsifiable concentrate. However, a new problem relating to the biological activity of the material arose. This is discussed in the section on Supplementary Laboratory Studies.

Control Effects

Primary criteria.—As indicated previously, the primary criteria used to judge the effectiveness of the experimental *Bt* materials were the percentage of egg-mass reduction and the number of residual egg masses per acre.

Table 2 shows the results of the 1961 experiment. In this test, two treatments (plots 3 and 7) gave significant egg-mass reductions. The only real difference between the treatment of plot 3 and all other plots was in the formulation of the spray applied to it. Plot 3 was sprayed before it was noticed that the emulsifier in the tung oil was not sufficient to produce a stable

Table 2.—*Results of Bt spraying experiment in New York State, 1961*

Plot No.	Dosage	Egg masses per acre before spraying	Egg masses per acre after spraying	Reduction
	Pounds	*Number*	*Number*	*Percent*
1	2	350	510	—
2	1	1,400	25,350	—
3	[1]1/4	1,500	40	97.3
4	2	400	575	—
5	1	1,200	10,760	—
[2]6	—	1,400	7,772	—
7	[3]1/4	1,200	219	81.75
8	1/4	1,120	2,440	—
[2]9	—	[4]1,200	99	91.75
[2]10	—	2,000	[5]1,252	37.40

[1] Spray without emulsifier.
[2] Check plot.
[3] *Bt* plus virus.
[4] Heavy winter kill reduced this figure.
[5] Very heavy polyhedrosis occurred in this plot.

emulsion. Thus plot 3 received the *Bt* material essentially in water alone, with little or none of the tung oil. All other plots received a stable emulsion including the tung-oil sticker.

Later laboratory tests showed that the tung oil and the added emulsifier had an inhibitory effect on the biological activity of the *Bt* material. Thus plot 3, which received the *Bt* without the sticker and added emulsifier, showed the lethal activity of the *Bt* more fully. Plots 1 and 4, which received 2 pounds of *Bt* per acre, had little change in egg masses per acre because the full effect of the material was blunted by the additives, but sufficient control was obtained to keep the populations stable. On the other hand, plots 2 and 5, which received 1 pound of *Bt* per acre, had a large buildup of egg masses per acre because the deleterious effect of the additives nullified the action of the *Bt*. Although only plot 7 received the combination of *Bt* and the nuclear polyhedral-virus disease organism, the large reduction in egg masses gave hope that this combination treatment would be more effective than *Bt* alone.

Table 3 presents the composite data for the 1962 experiment. Only plot 11 showed an egg-mass survival count low enough to be considered satisfactory, and only plots 6 and 11 showed significantly large egg-mass reductions.

The average reduction in egg masses per acre in the three check plots was 1.1 percent; and only 11 of the treated plots showed an egg-mass reduction greater than this. As far as treatment is concerned, there was no consistency in these reductions except in the plots treated with 4 pounds of Thuricide in 4 gallons of water per acre. The six plots that showed greater reductions than the checks received oil formulations, and the five others received water formulations. Six of the 11 plots received the combination treatment of the *Bt* plus polyhedral virus, and five received only the *Bt*.

The results of the 1962 experiment in terms both of egg-mass reduction and residual egg masses per acre showed that the 4 pounds of active material in 4 gallons of water gave the best results. The second best treatment was 2 pounds of active ingredient in 2 gallons of No. 2 fuel oil. The third best treatment

Table 3.—*Results of Bt field tests in New York State, 1962*

Plot No.	Dosage, per acre[1]	Initial egg masses per acre	Final egg masses per acre	10-minute larval count	Average frass per day per hammock	Average pellets per day per hammock	Cage mortality due to *Bt*	Defoliation
	Pounds	*No.*	*No.*	*No.*	*Gm.*	*No.*	*Percent*	*Percent*
1	2 (oil)	424	156	2.0	0.03	28.9	5	8
2	2 + (water)	290	190	3.5	.12	106.6	3	5
3	Check	960	498	158.3	.08	74.3	0	15
4	2 (water)	650	570	32.5	.49	218.4	54	13
5	2 + (water)	1,850	720	6.2	.06	59.9	12	15
6	4 (water)	1,350	310	4.4	.05	39.5	52	7
7	4 (oil)	360	840	44.6	.64	644.4	10	26
8	1 + (oil)	570	240	12.0	.07	64.1	14	12
9	1 + (oil)	410	794	135.6	.24	151.5	6	28
10	2 + (oil)	190	140	22.5	.12	89.7	6	5
11	4 (water)	520	26	.6	.01	10.9	78	13
12	Check	1,130	1,512	31.4	.57	403.0	0	20
13	2 (water)	1,200	1,490	23.6	.45	334.1	84	25
14	1 + (water)	350	1,400	54.5	.60	265.1	11	13
15	1 + (water)	616	310	5.1	.05	40.3	56	5
16	2 + (oil)	1,044	808	15.2	.46	375.1	10	24
17	1 + (oil)	496	8,738	190.0	—	—	8	26
18	4 (water)	626	1,528	13.7	—	—	66	7
19	2 + (water)	968	4,184	247.00	.47	325.0	28	26
20	2 (water)	1,248	29,274	410.0	—	—	1	45
21	4 (oil)	1,648	26,352	393.0	—	—	36	47
22	2 + (water)	546	312	6.1	.08	49.0	37	5
23	1 + (water)	428	7,176	28.0	1.01	457.4	62	32
24	Check	320	284	2.4	—	—	0	14
25	2 + (oil)	269	90	.4	—	—	4	5
26	2 (oil)	228	632	14.2	.03	34.0	22	5
27	4 (oil)	8,500	5,624	93.1	1.86	1,295.0	24	40

[1] + indicates addition of polyhedra of *Borrelinavirus reprimens* Holmes.

Table 4.—*Results of Bt field test in New York State, 1963*

Plot No.	Dosage, per acre[1]	Initial egg masses per acre[2]	Postspray		Egg-mass Change per acre	10-minute larval count	Average frass per day per hammock	Final oak defoliation
			Preliminary egg masses per acre	Final egg masses per acre				
	Gallon	*No.*	*No.*	*No.*	*Percent*	*No.*	*Gm.*	*Percent*
1	½	3,622	48	174+	98.6	6.3	0.06	9.2
2	½+	1,787	744	—	58.4	10.8	.38	12.6
3	1	4,674	5,136	—	+109.9	26.4	.37	17.4
4	½+	5,082	792	—	84.4	20.1	.31	11.2
5	½+	4,269	15,554	—	+364.4	149.8	2.60	73.0
6	½	3,173	144	170+	95.5	12.0	.10	11.6
7A	1	6,605	3,000	—	54.6	46.9	.43	11.8
7B	1	8,082	1,704	—	78.9	20.8	.40	13.0
7C	1	3,469	752	—	78.3	59.3	.16	12.4
8	½	2,560	26	65+	98.9	2.0	.03	7.6
9	1	5,423	2,844	—	47.56	21.8	.49	17.2
10	[3]	4,703	504	796	83.1	6.9	.08	9.8
11	[3]	2,286	10,730	5,650	+247.0	—	—	50.0

[1] + indicates addition of polyhedra of *Borrelinavirus reprimens* Holmes.
[2] Corrected for winter kill.
[3] Check plot.

was 2 pounds of active ingredient plus 50 billion polyhedra in 2 gallons of water. The egg-mass reductions in the latter two treatments were very close, but neither brought about a residual egg-mass count low enough for practical control.

The composite data of the 1963 Thuricide 90T test are shown in table 4. As was indicated in the section on Materials and Methods, a modification of the egg-mass sampling was made in 1963. Thus there are two columns in the table for egg-mass counts after treatment. Only three of the treated plots (plots 1, 6, and 8) showed, in the preliminary egg-mass tally, a residual egg-mass number below or near the arbitrary limit of 50. Therefore only these plots plus the check plots were examined in greater detail for the final egg-mass count. None of these plots showed 50 or less residual egg masses per acre in the final tally; so in these terms satisfactory control was not obtained. However, the reduction in egg-mass numbers in these plots was over 95 percent.

Although the specified degree of control was not achieved in this test, there was a consistent pattern in the result. The ½ gallon of 90T in 1½ gallons of water per acre was the best treatment in all series. The higher concentration (1 gallon 90T in 1 gallon water) gave significant reductions in egg masses, but consistently lower reductions than the lighter concentration gave. The probable reason for this was revealed in the laboratory tests.

Secondary criteria.—The secondary criteria used in these experiments were (1) to assess the effects of the treatments during the development of the larval stage of the insect and (2) to account, insofar as possible, for some mortality effects not attributable to the treatments.

One secondary criterion was larval mortality in sleeve cages. The use and limitations of the cages used in 1961 have been described. Regardless of the limitations, the sleeve cages showed that the residual effects of the *Bt* treatments lasted up to 8 days. This suggested that the principal mortality due to the active material—as well as the effectiveness—was limited to 1 week or less. Table 5 gives the pertinent data on cage mortality in the

Table 5.—*Percentage of larval kill in sleeve cages after Bt treatment, in New York, 1961*

Plot	Dosage	Mortality		
		Immediately after *Bt* treatment	4 days after *Bt* treatment	8 days after *Bt* treatment
	Pounds	*Percent*		
E-3	¼	29	12	5
E-10	[1]	0	1	2
E-1	2	77	56	27
E-2	1	34	19	27
E-5	1	30	23	20
E-8	¼	25	10	5
E-9	[1]	0	1	1
[2] E-4	2	18	22	17
[3] E-7	¼+	[4]40 (63)	21 (43)	10 (20)
E-6	[1]	9	9	[5]13

[1] Check plot.
[2] Larvae mostly fourth instar, gusty winds, heavy rain 8 hours after application.
[3] Heavy rain 8 hours after application.
[4] The figures in parenthesis indicate mortality due to *Bt* and virus combined.
[5] E-6 may have been contaminated by spray from E-5. These plots were relatively close together and nozzle clogging over E-5 caused lengthy spraying in gusty winds.

1961 experiment. There was no apparent correlation between cage mortality and egg-mass reduction (table 2).

In the 1962 experiment, a modification of the cage-mortality technique was attempted (see Material and Methods section). The major portion of the manual labor involved was used in maintaining these cages. As can be seen from table 3, the mortality due to *Bt* in the cages was relatively low; and table 6 shows that there was no correlation of the data obtained by the caging technique and the egg-mass data. Therefore, this method of treatment evaluation appears to be inefficient in terms of manpower required and validity of the data obtained.

In the 1963 experiment no caged larvae were used. Dead larvae were picked up from drop cloths and foliage, and were microscopically examined for cause of death. Although this technique was not used as a criterion of treatment effectiveness, the examination of these larvae gave information on the cause of death. The operation of other mortality factors, such as virus or insect

parasites, could thus be accounted for. An important finding in the examination of these larvae was the reduced number of multiplying vegetative cells of *Bt* over those noted in previous experiments. In addition, many abnormal and "ghost" cells were observed. These observations gave rise to the hypothesis that the new formulation was, in some way, inhibiting feeding of the larvae or changing the normal physiology of the *Bt*.

The use of drop trays in the 1961 experiment proved to be inconclusive in evaluating the trend in effects of the treatment or the final result. However, one very striking effect was revealed

Table 6.—*Correlation data from different methods of evaluation, 1962*

Method	Correlation with egg-mass reduction[1]	Ranked values	Significance
Caged larvae	0.121	27	—
10-minute larval count	.803	27	**
Frass weight	.727	21	**
Frass number	.649	21	**
Spore deposit, on leaf	.241	27	—

[1] Calculated from equation $r = 1 - \frac{6d^2}{n(n^2 - 1)}$

**Statistically significant at the 1-percent level.

by the drop trays. This was the quick and apparently high degree of kill among spring and fall cankerworms (*Paleacrita vernata* (Peck) and *Alsophila pometaria* Harr., respectively) in all plots regardless of dosage. Cankerworms seemed to be nearly eliminated within 4 days after spray application. The effectiveness of *Bt* preparations against spring and fall canker worms in these studies agrees with that reported previously by Jacques (1961), Quinton and Doane (1962), and Thompson (1962).

More drop trays were used in the 1962 experiment. Gypsy-moth frass was collected and measured in terms of dry weight and number of pellets. Table 6 shows that both of these techniques

Table 7.—*Correlation of data from different methods of evaluation, 1963*

Method	Correlation with residual egg masses per acre	Correlation with percentage egg-mass reduction
Percentage egg-mass reduction	0.931**	1.000
Residual egg mass/acre	1.000	.931**
10-minute larval count	.868**	.833**
Prespray frass (wt.)	.826**	.931**
Postspray frass (wt.)	.881**	.881**
Final defoliation	.853**	.917**
Deposit (average colonies/plate)	[1]+.310, [2]—.330	[1]+.154, [2]—.153
Deposit (total viable spores/plate)	[1]—.264, [2]+.189	[1]—.125, [2]+.126
Deposit (average viable spores/drop)	[1]—.399, [2]+.400	[1]—.258, [2]+.259

[1] Ranked highest deposit to lowest deposit.
[2] Ranked lowest deposit to highest deposit.

correlated strongly with the egg-mass data and also gave information on the trend of mortality.

Drop trays were used in the 1963 experiment, and again the data obtained from them correlated highly with the egg-mass data.

Another secondary criterion of evaluation, not used in 1961, was the 10-minute larval count. Although the behavior of gypsy-moth larvae is greatly influenced by their density, stand conditions, and physical factors of the environment, the larval-count data correlated highly with the primary criteria of evaluation (tables 6 and 7). The details of this technique are described elsewhere (see footnote 2).

The apparent foliage protection afforded the treated plots in 1961 suggested the use of defoliation readings in future tests. Thus defoliation estimates were made in all 1962 plots after treatment. Because these readings reflected feeding by all defoliators before spraying, their value was somewhat questionable; and in 1963, defoliation estimates were made before spraying as well as at the conclusion of the gypsy-moth larval feeding. Table 7 shows that the correlation between final defoliation and the primary criteria of evaluation was strong.

In the evaluation of any field test of an insecticidal material, particularly of an experimental nature, the spray distribution and deposit must be measured. In all three experiments, several spray-coverage and deposit techniques were used. These methods proved useful only in evaluating spray coverage; they did not give useful information on actual spray deposit. Data in tables 6 and 7 show that there was no correlation between spray deposit and the final result of the experiments (fig. 8).

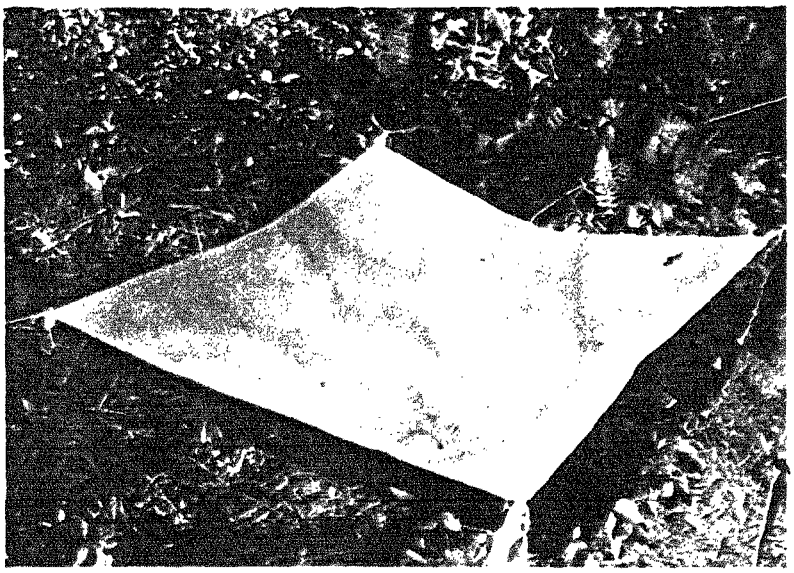

Figure 8.—A second type of drop tray used in field tests.

Supplementary Laboratory Studies

In the course of the three field tests described here, a number of supplementary studies were conducted at the field laboratory in Saratoga Springs, N. Y. and at the Forest Insect Laboratory in New Haven, Conn. The information gained in these studies was important in interpreting the results of the field tests.

In view of the work of Angus (1956) on the effect of gut pH on the activity of the crystal, and the work of Kushner and Harvey (1962) on the antibiotic effect of foliage, several experiments were conducted to gain information on these points.

Heimpel and Angus (1959) classified the action of crystalliferous bacilli on insects according to the speed and type of paralysis that occurs. It was of some importance to determine the general category of the gypsy moth. Tests were conducted on the feeding behavior of these larvae when challenged with *Bt.*

Moreover, additional tests were conducted on the effects of the components of the liquid concentrate on the germination of the *Bt* spore and the metabolism of the resulting vegetative cell. Other tests were conducted on the effect of the components on the feeding activity of the gypsy-moth larvae and any toxic effects that they may possess.

Methods and Results

Gut pH determinations.—Determinations of midgut pH were made on a Photovolt Model 110 pH Meter equipped with a Beckman single-drop electrode. Midguts of fed and unfed fourth, fifth, and sixth instar gypsy-moth larvae were surgically removed; the contents were then placed in the cup of the electrode; and pH readings were made immediately. All test larvae were reared on oak foliage, and at least 25 specimens were tested for each average reading.

Gut pH measurements of the alimentary tract of gypsy-moth larvae indicated that the alkalinity of the gut is low; thus this species should not be as sensitive to *Bt* as insects that have a higher gut pH. However, microscopic examination of guts of

Table 8.—*pH measurements of the alimentary tract and hemolymph of gypsy moth larvae*

Instar	Activity	Crop	Foregut	Midgut	Hindgut	Hemolymph
IV	Feeding	6.94	7.25	7.41	7.10	6.45
IV	Resting	6.71	7.54	8.63	6.35	6.18
V	Feeding	—	7.86	8.22	6.32	6.24
V	Resting	6.75	7.87	7.61	6.67	6.33
VI	Feeding	6.81	8.05	8.69	7.05	6.31
VI	Resting	7.21	7.72	8.50	6.58	6.40
Mean	—	6.96	7.72	8.30	6.67	6.32

gypsy-moth larvae that had ingested *Bt* showed that the crystals had dissolved. Therefore, although the midgut pH is low, it is either sufficiently high to dissolve the crystal, or proteolytic enzyme action is also involved. Table 8 gives the average pH values for the major regions of the gut and of the hemolymph.

Antibiotic effect of foliage.—Water extracts were made of the foliage of all host plants found in the experimental plots. *Bt*-seeded agar plates were challenged with these extracts to determine if antibiosis occurred.

None of the water extracts of foliage that the gypsy moth larvae were feeding on showed any inhibitory effect against *Bt*. The extracts tested were white oak, red oak, black oak, chestnut oak, hickory, white and yellow birch, American beech, and witch-hazel.

Gypsy moth larval gut presses.—Tests were made to determine if antibiotic modification occurred during ingestion and digestion by the larvae or if any antibiotic principle existed in the gut of the larvae. Gut presses of larvae feeding on host material found in the experimental plots were made. The larval guts were removed surgically, and slit longitudinally; and the contents of the dorsal and ventral halves of the gut were absorbed on clean filter paper strips. *Bt*-seeded agar plates were challenged with these filter paper strips to determine the presence of any antibiotic element.

Gut presses prepared from larvae feeding on the above-men-

tioned trees did not exhibit any inhibition against *Bt.* Thus the gut of the gypsy-moth larvae does not contain any antibacterial activity, nor does it modify the activity of ingested leaf material.

Feeding activity of gypsy-moth larvae.—Two lots of 25 larvae each were challenged with a strong dose of wettable *Bt* powder. The larvae were kept under constant observation for 8 days to determine the amount of feeding and general activity of the larvae. Twenty-five larvae were fed untreated foliage to provide a comparison.

Table 9 presents in synoptic form the results of the test in which gypsy-moth larvae were challenged with *Bt.* These observations indicate that the ingestion of *Bt* strongly inhibits the feeding activity of the third instar gypsy-moth larvae. No repellent effect was noted. The 25 check larvae completed development to pupae and emerged as healthy moths.

Table 9.—*Feeding behavior of Instar III gypsy moth larvae fed a strong dosage of Bt*

No. days from start of test	Feeding behavior
1	Check larvae feeding actively; oak seedling nearly defoliated. Larvae on treated foliage feeding very slowly, seedling only slightly defoliated.
3	Check larvae given new oak seedling. Treated larvae appear normal, but feeding markedly reduced.
4	Check larvae feeding heavily, growing normally. Treated larvae ceased feeding. One dead larva (polyhedrosis).
5	Check larvae feeding heavily, seedling changed. Eight treated larvae dead; six appear normal but not feeding. Original treated seedlings only lightly defoliated, and still edible.
7	Check larvae feeding normally; seedling changed. Three more treated larvae dead; remaining three alive; very sporadic feeding.
8	Check larvae still feeding normally, seedling changed. Last three treated larvae dead.

Sporulation of the bacillus in Bt-killed larvae.—To determine if sporulation and/or crystal formation occurred in the remains of *Bt*-killed gypsy-moth larvae, a time series of dead larvae were placed on clean microscope slides. Aliquots of these larvae were examined daily to determine if sporulation and crystal formation had occurred.

The cadavers of the larvae killed in the above test were examined at intervals for spore and crystal production. As indicated in table 10, the bacillus started sporulation between the second and third day after death of the insect, and all cells completed sporulation by the sixth day. Crystal formation was not inhibited in any way (fig. 3).

Table 10.—*Evidence of sporulation and crystal formation of Bt in dead gypsy moth larvae*

Days from death	Vegetative cells	Spores	Crystals
0	Present	Not present	Not present
1	Present	Not present	Not present
3	Sporulating	Present	Present
4	Sporulating	Present	Present
5	Sporulating	Present	Present
6	Absent	Many present, free	Many present

Gross fractionation of emulsifiable Bt concentrate.—The emulsifiable concentrate used in the 1963 field experiment was fractionated by centrifuging into the following components: (1) oil layer, (2) spore-crystal layer, (3) washed spores and crystals, and (4) spore-crystal washings. In addition to the four fractions, two dilutions of the whole concentrate and a laboratory preparation of *Bt* isolated from the concentrate were fed to individual fourth to fifth instar gypsy-moth larvae; 0.1 ml. of each of the test materials was placed on oak-leaf sections and presented to each larva, after which daily observations were made on the feeding activity of the larva. All dead larvae were examined for cause of death.

The results of feeding fourth and fifth instar gypsy-moth larvae gross fractions of the 90T concentrate used in the 1963 field test are presented in table 11. As the commercial concentrate containing stabilizers was diluted, or the spore and crystal fraction was washed, the larvae tended to feed more. The laboratory preparation that contained no additives inhibited feeding least.

Table 11.—*Percentage of gypsy moth larvae feeding on fractions of 90T Flowable and a laboratory preparation of the same strain of Bt*

Test fraction	Larvae feeding
	Percent
Commercial concentrate	11
Spore and crystal washings	30
Commercial concentrate (Dilute 1:1 with water)	40
Washed spores and crystals	48
Commercial concentrate (Dilute 1:4 with water)	55
Laboratory preparation of spores and crystals	75

Effect of emulsifiable Bt concentrate on Bt metabolism.—Through the courtesy of the Bioferm Corporation, the nature and amount of the stabilizing ingredients used in the 90T emulsifiable concentrate were made available for testing. The effects of the five ingredients on spore viability and vegetative-cell metabolism were tested individually and in all possible combinations. The various ingredients and combinations were placed in test tubes and seeded with 5×10^6 viable *Bt* spores. These tube cultures were maintained at room temperature for 55 days; then 0.1 ml. aliquots were removed from each tube, appropriately diluted, heat-shocked at 60°C. for 30 minutes, and plated on TSA by the drop-plate technique. Breed smears were prepared from the appropriate dilution for total spore and crystal determinations. Spore and crystal counts were made at the following time intervals (in days): 0, 1, 2, 3, 4, 6, 8, 10, 13, 15, 17, 20, 24, 27, 34, 41, 49, and 55.

Physiological studies were made of the vegetative cells that arose from the spores in contact with the ingredients. The following tests were conducted on days 0, 6, 13, 20, 27, 34, 41, 49, and 55: motility, colony form, gram stain, catalase production, gelatin proteolysis, litmus milk reaction, fermentation of glucose and lactose.

Table 12 summarizes the essential findings of these tests. Only changes in viable spore count, catalase production, proteolysis, and carbohydrate utilization are listed. In order to interpret the table, the normal reactions of *Bt* are: (1) no change in the number of viable spores should occur; (2) the vegetative cell should produce significant quantities of catalase; (3) proteolysis of gelatin should occur, and glucose and lactose should be utilized with the production of acid or alkali, respectively.

Table 12.—*Results of tests with components of 90T Flowable on Bt spore viability and vegetative cell metabolism*

Ingredients	Reactions that showed significant changes				
	Viable spore count	Catalase production	Gelatin proteolysis	Glucose utilization	Lactose utilization
NaCl	Slight drop	Positive	Positive	Acid	Alkaline
Triton, xylene, diesel oil	No germination after 27 days	Negative after 27 days	Negative after 27 days	No reaction after 13 days	No reaction after 13 days
Triton, diesel oil	No germination after 27 days	Negative after 27 days	Negative after 27 days	No reaction after 13 days	No reaction after 13 days
Triton	No change	Positive	Positive	Acid	Alkaline
Propionic acid	Slight drop	Positive	Positive	Acid	Alkaline
Water	No change	Positive	Positive	Acid	Alkaline

NaCl, Triton, xylene	No change	Positive	Reduced	Acid	Alkaline
NaCl, Triton, diesel oil	No germination after 27 days	Negative after 41 days	Negative after 41 days	Negative after 20 days	Negative after 13 days
NaCl, Triton	No change	Positive	Positive	Acid	Alkaline
NaCl, propionic acid	1-log drop	Positive	Positive	Acid	Alkaline
Triton, propionic acid	No change	Positive	Positive	No reaction after 20 days	No reaction after 20 days
NaCl, Triton, propionic acid	2-log drop	Positive	No reaction after 27 days	No reaction after 20 days	Alkaline
NaCl, Triton, xylene, diesel oil	No germination after 32 days	No reaction after 41 days	Positive	No reaction after 34 days	No reaction after 34 days
NaCl, Triton, xylene, propionic acid	1-log drop	Positive	Positive	Acid	Alkaline
NaCl, Triton, diesel oil, propionic acid	No germination after 23 days	No reaction after 20 days	Positive	No reaction after 20 days	No reaction after 20 days
Triton, xylene, propionic acid	No change	Positive	Positive	Acid	Alkaline
Triton, diesel oil, propionic acid	No germination after 25 days	No reaction after 41 days	No reaction after 41 days	No reaction after 20 days	No reaction after 20 days
NaCl, Triton, xylene, diesel oil, propionic acid	No germination after 23 days	No reaction after 20 days	Positive	No reaction after 20 days	No reaction after 20 days
Commercial 90T	No change	Positive	Positive	Acid	Alkaline

Conclusions

The conclusions that can be derived from the results of the 3 years' tests with *Bt* for control of the gypsy moth can be divided into general and specific categories as follows:

General Conclusions

- *Bt* appears to be practical as a biotic control agent for the gypsy moth. The three field tests exhibited progressively greater reductions of the insect population, and the 1963 test showed consistent results with the most effective dosage. Acceptable reductions were achieved in the 1963 test, and the residual population numbers were very nearly acceptable for practical control.
- The absolute necessity for thorough pre-testing (of both physical and biological properties) of any commercial *Bt* preparation against the target insect is amply demonstrated by the experiences in the three field tests.
- Thorough checking of the concentrations of spores and crystals in commercial concentrates and in the finished sprays must be done; also checks on the mixability and flowability of the finished sprays must be continued until reasonably uniform products are available from the manufacturer.
- Systematic field checks must be made on the target insect population after spray application to distinguish between *Bt* effects and the effects of natural mortality factors.
- It must be realized that the lethal effect of *Bt* applications—particularly against relatively insensitive species such as the gypsy moth—may not be immediate, and that the full effect of the treatment may take a week or more to be noticed.
- Foliage protection due to reduced larval feeding may be accomplished with or without a significant reduction in the larval population.
- Field evaluation techniques had to be developed to properly assess the effect of *Bt* against the gypsy moth. This probably will be true for other insect species.

Specific Conclusions

- It can be concluded from the 1961 and 1962 tests that the data obtained from caging larvae are not valid for estimating the true effects of a *Bt* treatment. However, such data may provide ancillary information about the relative rate of mortality.
- The fluorescent dye-tracer technique is the easiest and quickest technique for discerning spray-distribution patterns, including the detection of drift; whereas the petri-dish technique appears to be the best one for estimating spray deposit quantitatively (fig. 9).
- There is no apparent enhancement of the effect of *Bt* against the gypsy moth when the nuclear polyhedral virus is included in the spray in relatively small amounts.
- Because of the relatively short life of *Bt* sprays in the field, it is necessary to treat all of the infested area that contains sig-

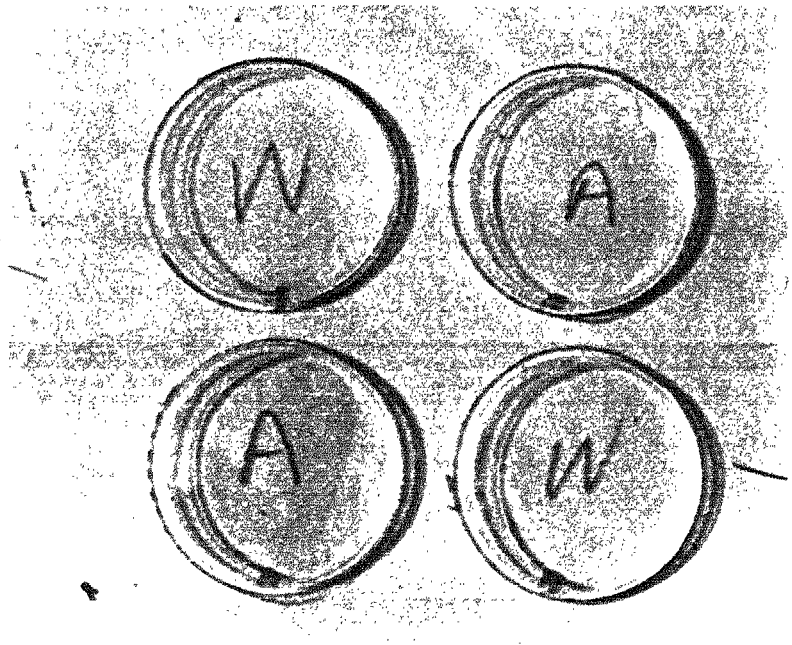

Figure 9.—Set-up for obtaining *Bt* deposit (A = agar-filled petri dish, W = sterile water-filled petri dish).

nificant numbers of the insect to prevent re-invasion by larvae from contiguous untreated areas.

- It is necessary to apply *Bt* sprays when host foliage is nearly fully expanded and before significant defoliation occurs in order to achieve full deposit and maximum effectiveness of the material.
- Care must be taken in field evaluations of *Bt* materials when unexpected results occur. The active ingredients must be evaluated and the additives must be assessed for their possible role in producing unexpected results.

■

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