# STABLIZATION AND APPLICATION OF BT

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## Introduction

This research report is concerned with: 1). A field application program using encapsulated Bt. The objectives of that program involved spray physics and the delineation of the critical parameters inherent in the delivery of Bt sprays to the microhabitat of the spruce budworm. The field application work was carried out jointly with Dr. Alam Sundaram and the Forest Pest Management Institute (Canada). Most of the data from the field studies will be published in a separate paper with Dr. Sundaram. 2). A laboratory program concerned with stabilization studies on pure Bt and BtI crystals. The laboratory studies on pure Bt and Bti crystals were carried out at the University of Georgia with the objective of providing a base-line system by which environmental parameters adverse to Bt (BtI) could be determined, quantified and controlled.

The application of pesticide sprays in forestry and agriculture, appears to be a simple procedure. It is widely assumed that each of the spray droplets in a spray cloud falls by gravity, over a time period determined by its mass (size), and that the downwind travel of droplets is directly related to that size (i.e., drift is directly related to droplet size). The final assumption of such simplistic concepts of pesticide sprays is that droplets can (fall) into the foliage system and kill target pests in their microhabitats. None of the above assumptions are correct, nor have any experimental proof. They are widely accepted. That widespread acceptance has had a devastating effect on pest management and ecosystem accountability. It is the single most significant cause of the present problems that beset the pest management industry. It provides no basis for survival of the industry into the 21st Century. Major changes, critical to the pest management industry may depend on the emerging science of pesticide spray physics.

The spray application of Bt has used the conventional wisdom of chemical insecticide application. Bt is a particulate toxin, has no contact activity, and requires substantially different spray methodology from chemical systems. The successful use of Bt in pest management requires delivery of a toxic dose to the target insect in a highly specific manner. That toxic dose must be injested by the insect within a limited time frame. That time frame is determined by feeding habits of the insect and by the stability (effective presence) of Bt on the foliage within the microhabitat of the target insect. The mechanism of action of Bt, and particularly the effects of sub-lethal doses of Bt, requires that the "window of opportunity" with Bt be substantially greater than that required of most chemical insecticides. Optimum systems may require that Bt remain stable on the foliage for several weeks. Toxic levels of Bt must be provided on foliage injested during a limited feeding cycle. Substantial residual action of Bt on foliage is important.

There is no general agreement as to the relative stability of Bt (as crystals) or Bt (as spores) on foliage, in the presence of UV light, foliage bactericides, and foliage pH. The second phase of the research reported herein was concerned with the stability of purified Bt and BtI crystals and the development of formulations to stabilize these biological insecticides. Purified Bt crystals were used to provide a base-line system for the study. Purified BtI crystals were used in the study because of the speed and simplicity of its bioassay.

## Experimental Data and Conclusions

In 1981, the physics of spray delivery to a spruce forest were studied in a 15-hectare plot near Searchmont, Ontario. In 1982, two 50-hectare plots near Marathon, Ontario were studied. The 1981 research involved the use of encapsulated droplets of vegetable oil as a tracer system. The 1982 research was carried out by application of encapsulated droplets of Bt as a tracer system. During the 1981 test, wind velocity and turbulence was close to zero. In the 1981 test the wind velocity was 13-17 Km/hr. The combined data confirm that spray droplets larger than the range of 100 microns have substantially zero probability of delivery to the microhabitat of the spruce budworm. The delivery optimum (as a function of mass and number) is in the range of 15-50 microns. In general, typical Bt sprays used by present field application methods are not more than 5% efficient in delivery to the spruce budworm. To the extent that commercial Bt sprays are unstable on the foliage, the "window of opportunity" for spruce budworm control by Bt is very limited.

The data in Tables I and II, when combined with the physics of particulate distribution in sprays, and the feeding habits of spruce budworms, provide a basis for prediction that present field methods for spruce budworm control by Bt will continue to be marginal until brought into conformity with applicable physical principles. The quantitative analytical data available from the 1981 and 1982 research are not different from analytical data available from the fluorescent particle spray droplet tracer system (1969), the dry liquids tracer system (1978), the reversibly soluble pigment tracer system (1978), the fluorescent pigment system (1978-79), and by other research in spray physics which has been publsihed. In contrast, no quantitative data exist to support the widespread, widely accepted conventional wisdom that pesticide spray droplets of "any" size can fall into foliage, and can penetrate to target microhabitats.

TABLE 1
1982 SIZE RANGE AND DROPLET VOLUME DISTRIBUTION: FOLIAR DATA

| Drop size range (um) | Average drop size (um) | Frequency distribution % | Cumulative frequency distribution % |
|---|---|---|---|
| 2 - 5 | 3.5 | 0.00 | 0.00 |
| 6 - 10 | 8.0 | 0.33 | 0.33 |
| 11 - 20 | 15.5 | 7.81 | 8.14 |
| 21 - 30 | 25.5 | 23.51 | 31.65 |
| 31 - 40 | 35.5 | 24.48 | 56.13 |
| 41 - 50 | 45.5 | 13.83 | 69.96 |
| 51 - 75 | 63.0 | 9.00 | 78.96 |
| 76 - 100 | 88.0 | 15.15 | 94.11 |
| 101 - 125 | 105.5 | 5.89 | 100.00 |

VMD = 38u

TABLE 2
1982 SIZE RANGE AND DROPLET NUMBER DISTRIBUTION FOLIAR DATA

| Drop size range (um) | Average drop size (um) | Frequency distribution % | Cumulative frequency distribution % |
|---|---|---|---|
| 2 - 5 | 3.5 | 3.22 | 3.22 |
| 6 - 10 | 8.0 | 14.17 | 17.39 |
| 11 - 20 | 15.5 | 46.56 | 63.94 |
| 21 - 30 | 25.5 | 23.76 | 87.70 |
| 31 - 40 | 35.5 | 8.18 | 95.88 |
| 41 - 50 | 45.5 | 2.38 | 98.26 |
| 51 - 75 | 63.0 | 0.90 | 99.16 |
| 76 - 100 | 88.0 | 0.71 | 99.87 |
| 101 - 125 | 105.5 | 0.13 | 100.00 |

NMD = 13u
Dmax = 110u
Dmin = 3u

The stabilization and formulation studies were approached on the basis of use of purified crystal preparations. The crystals were isolated by renografin gradients, followed by suspension in deionized water. The fluorescence studies were made with crystals solubilized with dithiothreitol. Quartz cuvettes were used to allow study of the absorption and fluorescence emission of the solubilized preparations. In order to determine the effect of a standardized (hard) UV source, crystals were suspended in a quartz cuvette and irradiated for specific periods of time. Aliquots of the suspension were then solubilized and the fluorescence emission obtained. Other aliquots of the crystals were used to determine biological activity. The biological activity studies were limited to BtI because of the ease and speed of the bioassay procedure. At present we are starting bioassay work with cabbage looper (*T. ni*), and with cotton boll worm (*H. zea*). The fluorescence data obtained were not different with Bt (HD-2) crystals and BtI crystals. The fluorescence spectra correspond to protein tryptophan (class B proteins) in which the tryptophan exists in a non-polar environment.

Under the experimental conditions used, BtI and Bt cyrstals are photolytically unstable and their tryptophan fluorescence decreases relative to the time of irradiation. In the case of BtI, that decrease in tryptophan fluorescence is directly related to decrease in biological activity. If tryptophan fluorescence can be correlated with biological activity, then it could be a basis for analytical quality control, by spectroscopy. The tryptophan absorption spectra show no significant differences before and after irradiation.

Encapsulated formulations of BtI crystals with UV-Chek® (Ferro Chemical Co.) were prepared and subjected to irradiation using the same UV source under standardized conditions. Under these conditions, unprotected BtI crystals lost all biological activity after 6 hours. At present we have found no loss in biological activity of encapsulated, protected, samples irradiated continuously over a period of 20 days. In control studies, all adjuvant components of the formulation were shown to have no adverse effect on the mosquito larvae.

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## SPRAY SWATH PATTERNS OF SMALL AIRCRAFT AND VERTICAL DISTRIBUTION OF MICROBIAL SPRAY DEPOSITS

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### Introduction

Each year in Northeastern United States over 500,000 acres of oak forests are aerially sprayed to prevent massive defoliation by the gypsy moth. In Pennsylvania alone 400,000 acres were proposed for treatment in 1983 with commercial preparation of *Bacillus thuringiensis* (Bt).

Preparations of Bt must be consumed by the pest insect to be effective, and cannot be absorbed through the insect's integument like most of the organic insecticides. Therefore, it is important that the Bt spray deposit be distributed evenly over the feeding surface and in sufficient quantity to be lethal. Consequently, the more efficient and precise the application