CESSNA AG-TRUCK CALIBRATION TRIALS FOR THE DISPERSAL OF AQUEOUS FORMULATIONS OF *Bacillus thuringienses kurstaki* IN NEWFOUNDLAND, 1979

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ABSTRACT

Three different water formulations of *Bacillus thuringiensis* (Thuricide 16B, Thuricide 24B, and Novabac N45B), to be sprayed at the rate of 20BlV/5.85 ℓ/ha (8BlV/80 US Fl oz/ac), were tested for rate of flow and droplet spectrum characteristics using a Cessna Ag-truck fitted with twenty No. 8004 and twenty No. 8006 Spraying Systems nozzles. Results of four calibration tests indicated that all formulations flowed through the system, but produced larger spray droplets than the recommended mass median diameter of 90 μm. Problem areas in formulations, assessment of spray deposits, and equipment calibration are discussed.

RÉSUMÉ

Au moyen d'un Cessna Ag-truck equipe de 20 buses n° 8004 et de 20 buses n° 8006 Spraying Systems, on a étudié le débit de pulvérisation et le spectre des gouttelettes de trois préparations aqueuses de *Bacillus thuringiensis* (Thuricide 16B, Thuricide 24B et Novabac N45B) destinées à être pulvérisées à raison de 20 x 10^9 U.I. dans, 5.85 ℓ/ha (8 x 10^9 U.I. dans 80 onces liquides U.S. et à l'acre). D'après les étalonnages, les préparations ont bien coulé mais le diamètre médian de leurs gouttelettes dépassait les 90 μm recommandés. Les A. discutent des difficultés concernant les préparations, l'évaluation des dépôts et l'étalonnage du matériel.

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INTRODUCTION

A series of calibration trials was initiated by N. Carter[1], Nfld. For. Ser., and O. Morris,[2] F.P.M.I., D.O.E., Can., to provide information on emission flow rates, spray break up, and spray droplet dispersal of various experimental and operational *Bacillus thuringiensis* Berliner formulations. These were scheduled for use in the 1979 spruce budworm spray program in Newfoundland. Four Cessna Ag-Truck aircraft,[3] were contracted by the Newfoundland Forest Service to undertake the spray operation. Rate of low calibration of the spray equipment, droplet spectrum analysis, and drop numbers and volume analyses were necessitated because of: A) the experimental nature of the *B.t.* formulations, and B) the recommendation of the formulation manufactureres to use 8004 and 8006 Spraying Systems Teejet nozzles. The latter were recommended as suitable for the production of a fine ultra-low-volume (ULV) spray droplet spectrum, with mass median diameter (MMD) drop size of 90 microns (µm).

The work described herein was carried out during the month of June, 1979 under field conditions at Gander and Bay D'Espoir airports, Newfoundland, Canada.

The study was a cooperative research program between the Forestry Branch of the Dept. of Forest Resources and Lands, St. John's, Nfld., and the Forest Pest Management Institute, D.O.E., Sault Ste. Marie, Ont. Canada.

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MATERIALS AND METHODS

Aircraft and Spray Equipment:

The four Cessna Ag-trucks were equipped with internal tanks capable of holding 757.1 litres [200 US gallons(USG)] of spray formulation. Two of the machines were equipped with a hydraulic motor-driven pump system that conducted the spray formulation into two external booms mounted below and behind the trailing edge of the wings. The remaining two Ag-trucks were fitted with wind-driven fan pumps. Each aircraft was fitted with 20 number 8004 and twenty number 8006 Spraying Systems Teejet nozzles alternately spaced and pointing downwards at approximately 90° to the undersurface of the wing. The nominal spray emission rate (H_2O) was 83.2 L/min (22 USG/min.) at 40 p.s.i.

Spray Materials

The four *Bacillus thuringiensis* Berliner var. "Kurstaki" concentrate formulations selected for the calibration trials were as follows:

1) Thuricide 16 B 2) Thuricide 24 Ba 3) Thuricide 24 BC	Sandoz Inc. (Crop Protection Div.) 480 Camino Del Rio. South San Diego, Calif. U.S.A. 92108
4) Novabac 45 Bactospeine cream 45 BIU/U.S. gal. Serotype 3a 3b.	Biochem Products. A.G. Lange-Gasse 33 4010 Basel, Switzerland

The three Thuricide products are essentially the same insomuch as the Thuricide 24 Ba was prepared by concentrating 16B to provide a biological activity rating of 24 BIU/USG Thuricide 24 Bc was obtained by dilution of a Thuricide 32B to provide a 24 BIU/USG activity rating. Both Thuricide 24 B concentrates were physically identical except in methodology of production. Both materials were provided free of charge for the trials.

The fourth *B.t.* concentrate "Novabac 45" is marketed by American Cyanamid of Canada as a new high concentrate (45 BIU/USG) emulsion. This concentrate has a thick mayonnaise-like consistency and thus requires care in formulating with water. This material was also provided free of charge for the trial.

Expected field application rate of active ingredient was set at 19.8 BIU/ha (8.0 BIU/ac) formulated in 9.353 L/ha (1 USG/ac) of aqueous solution <u>or</u> the lowest flowable volume of liquid formulation compatible with the spray equipment on the Cessna Ag-Trucks. To facilitate selection of the lowest flowable formulation a simple test tube technique was devised for field use to determine ease of mixing, sequence of mixing, and stability of the final mix.

By calculation, the minimal volumes of experimental *B.t.* concentrate required per hectare (acres) would be as follows:

B.t. Concentrate	Fluid Volume Litres/hectare	Fluid Volume (U.S. oz/ac)
Thuricide 16B	4.68	64.0
Thuricide 24B	3.12	42.7
Novabac 45B	1.66	22.8

Since these materials have an extremely high viscosity (600^+ cp), they cannot be used directly as concentrated spray formulations, and therefore must be diluted with an appropriate carrier, in this case water. Previous studies (Randall, 1976) on the flowability of formulations of Thuricide 16B using AU 3000 Micronair equipment on Cessna Ag-Trucks, indicated that the best 16B/H_2O formulation containing a total activity rating of 19.8 BIU/ha was composed of 4.68 ℓ/ha (64 U.S. fluid oz/ac) of Thuricide 16B and 1.17 ℓ/ha (16 U.S. oz/ac) of H_2O to give an emission volume of 5.85 ℓ/ha (80 U.S. oz/ac). By comparison the recommended formulation for a dosage rate of 9.35 ℓ/ha (1 USG/ac) would be 4.68 ℓ/ha (64 U.S. oz/ac) of Thuricide 16B and 4.68 ℓ/ha (64 U.S. oz/ac) of H_2O (50/50 mixture). Details of the various formulations tested and methodology are presented in Table 1 Appendix A.

Sampling Units

Samples of deposited spray droplets were collected on Kromekote cards for drop number counts and size, and on glass slides for colorimetric determination of deposit. Each sampling unit was comprised of two 10.5 x 20 x 0.9 mm aluminum plates hinged together with book binding tape for retaining the Kromekote card (100 cm^2) and two 5.1 x 7.5 cm glass microscope slides. The latter were hinged together with Cellotape to provide a flexible hinge and surface for coding. Drops of silicone plastic on the corners of each slide served as plate separators when the glass slides were folded in the closed position.

Calibration lay-out and Spraying Procedure

All calibration trials were carried out on paved airport runways thus providing a flat, obstacle-free surface for the sampling units and a cross-wind layout in the longest dimension of the runway. A double layout of sampling position comprised of two lines (X and Y) was established on the runway to adjust for wind-reversals that may occur during the preparation or course of the trials.

The two sampling lines (X and Y) were 600 meters) long with the zero marker mid-point across the center and at right angles to both lines. In all trials the sampling units were spaced 5 meters apart from the zero position for the first 100 meters and 10 meters apart for the remainder of the layout. All sampling units were spaced a minimum of two meters from the boundary of the runway to eliminate any wind turbulence effects created by border vegetation. All units were placed with the Kromekote cards facing the zero position and coded according to line and position number. Cards located upwind of the zero position were designated as minus, e.g., (T1-5X etc.) as compared to the downwind layout, e.g., (T1 5X), (T1 10X). Two bright orange flight markers were spaced 100 meters on either side of the runway at the zero position to provide a line-of-site reference flight path for the pilot. Aircraft spray height across the layout was set at 6 m (20 ft) to reduce the effect of wake turbulence on droplet impaction.

Direct ground to air radio communications were unavailable for these trials, thus after take-off, the pilot was committed to prior ground briefing without recourse to inflight corrections or modification of plans. This is evident in Trial 3 as a high drop/cm^2 deposit count on spray cards X5, X10, X15, X20, as a result of a 180° wind shift 30 seconds after the aircraft had passed over the zero position of the card layout. Details of the calibration trials are summarized in Table II Appendix A.

Rate of flow Calibration

Rate of flow calibration of the spray equipment was undertaken using aircraft CF-LDZ equipped with a hydraulic power "take-off" pump system. Static ground tests with H_2O as the test liquid at 40 p.s.i. spray emission pressures using Spraying System Teejet nozzles provided a flow rate of approximately 83.2 L/min (22 USG/min.). This approximated the calculated volume (20 USG/min.) for these nozzles at that particular pressure setting. The standard "static" procedure for rate of flow calibration consisted of adding sufficient water in the tank to allow for normal spray emission of liquid at 40 p.s.i. through all nozzles of the system until a drop in pressure occurs that signals the "empty tank position" of spray fluid in the tank. A known quantity of fluid is then added to the spray tank and the procedure repeated and timed to determine the rate of flow/min at 40 p.s.i. The above system, however, is accurate with water, but is untidy to use at airports with actual spray formulations.

To overcome this problem, and to conserve the experimental materials, a spray collector system was designed and developed (Fig. 1), to provide the data with a minimal loss of material. A water standard, and each *B.t.* formulation were pumped through the spray system for a period of 1 min., and the liquid collected and weighted. A settling period of approximately 12 hours was required for the *B.t.* formulations to arrive at a stable volume equivalent of bubble free liquid *B.t.* formulation. An equivalent volume of water was weighed and the rate of flow/min calculated on a weight equivalent basis. From this data an approximation of the specific gravity of the *B.t.* formulation could also be obtained. Details of the collector system and methods are presented in Appendix B.

DATA AND RESULTS

(a) Spray Deposit Assessment

Data on total deposit recovery (drops/cm^2) and volume in litres/hectare from the four *B.t.* calibration trials are recorded in Table II (Appendix A) and illustrated in the test in Figs. 2 and 3 for total deposit and comparative stain deposit distribution, and size respectively.

A comparison of the spray deposit density (litres/hectare) recovered from each of the four trials, shows that trials T-1

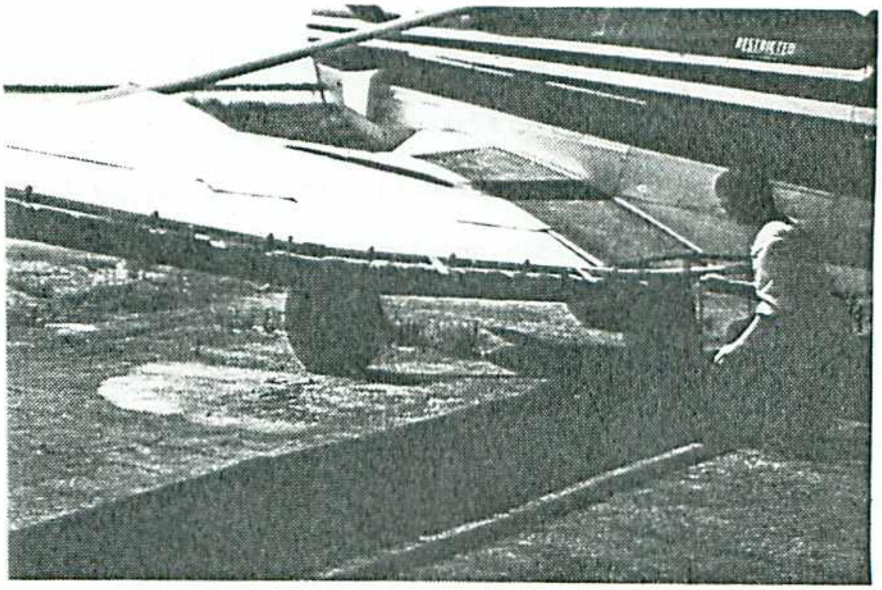


Fig. 1. Development of spray collector unit for rate of flow calibration of *B.t.* formulations.

and T-3 which were undertaken during favorable conditions (wind less than 7 kph [4 mph] provided a realistic spray deposit and drop stain image. Trials T-2 and T-4 were undertaken under adverse conditions and therefore are virtually useless in providing data on droplet spectrum characterisitcs and formulation/ nozzle efficacy. In T-2 a helicopter crossed the spray cloud and disrupted the normal descent path of the falling spray droplets. T-4 was undertaken under adverse wind conditions (greater than 7 kph) thus the spray droplets impacted on the Kromekote cards at sufficient velocity to create elongated stains and multiple droplets due to bounce impaction.

A reappraisal of spray deposit assessment from T-1 and T-3 in terms of volume deposits i.e., (L/ha or oz/ac) by calculation of stain diameter and spread factor vs true drop size, however, was impossible due to the inherent quality of the *B.t.* formulation and the absorbent characteristics of the Kromekote sampling surface. This is illustrated in Fig. 4a, which shows a photograph of the actual spray deposit and drop stain size distribution from sampling surface T1-5X. Enlargement of the same surface area taken from an oblique angle, however, shows the spherical shape of the actual spray droplets as shown in Fig. 4b. Examination of all Thruicide 16B deposit samples indicated that the pattern of the largest drops were hemispherical with gradations to complete spheres at the smallest drop sizes.

Studies by Dr. A. Drummond, of the National Aeronautics Establishment of NRC, Ottawa, on stain/drop sizes of *B.t.* formulations have shown that with the exception of extremely large drops, (1000 μ) it was impossible to create the normal series of smaller sizes required for spread factor analysis (Drummond, 1979). Assessment of volume deposits by mathematical means therefore cannot be undertaken by the usual means.

(b) Colorimetric assessment

The various spray deposits from the *B.t.* trials are recorded in Table I Appendix A. Problems were encountered in colorimetric assessment of the Erio Acid Red XB dye tracer in the spray deposits recovered from the glass slides. It would appear that the absorbance measurements were increased due to suspended *B.t.* and dust particles. This problem was solved by centrifugation of the samples. Further details are presented in Appendix B.

DISCUSSION AND CONCLUSIONS

The calibration trials were planned to ensure optimum use of aerial spray equipment and *B.t.* formulations in terms of spray droplet coverage and drop specturm characteristics using number 8004 and 8006 Spraying Systems Teejet nozzles. The trials with Cessna Ag-truck CF-LDZ were scheduled to be carried out at Gander Airport using the four *B.t.* formulations. This objective was only partially achieved within this study, due to adverse calibration weather and the rapid development of the spruce budworm larvae. Two of the crosswind trials (T-1 with Thuricide 16B and T-2 with Novabac 45B) were conducted at Gander, and the remaining Thuricide 24B trials (T-3 and T-4) were undertaken at the operational spray site of Bay D'Espoir in order to complete the experimental/operational program before pupation of the larvae occurred. Two Thuricide 24Ba calibration trials (T-3 and T-4) were carried out at the Bay D'Espoir test site in order to provide a complete set of drop deposit data on this material. Thuricide 24Bc was not tested unfortunately, due to a shortage of material and operational demands to complete the remaining spray program within the accepted biological timing period.

Trials T-2 and T-4 were undertaken under less than acceptable weather conditions and should therefore be considered

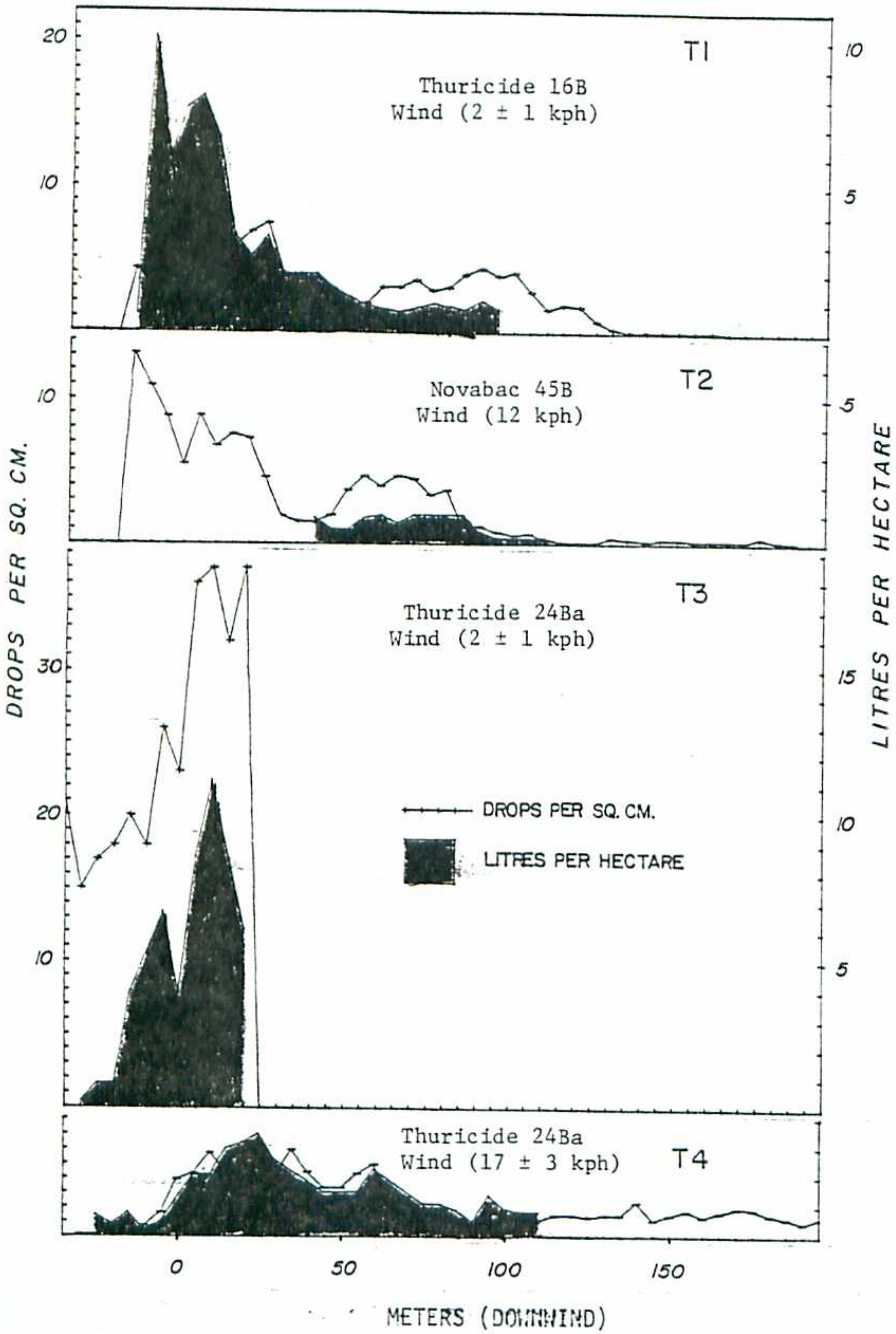
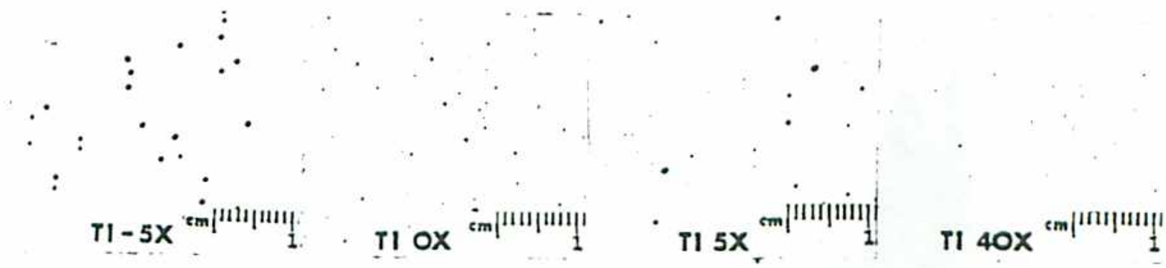
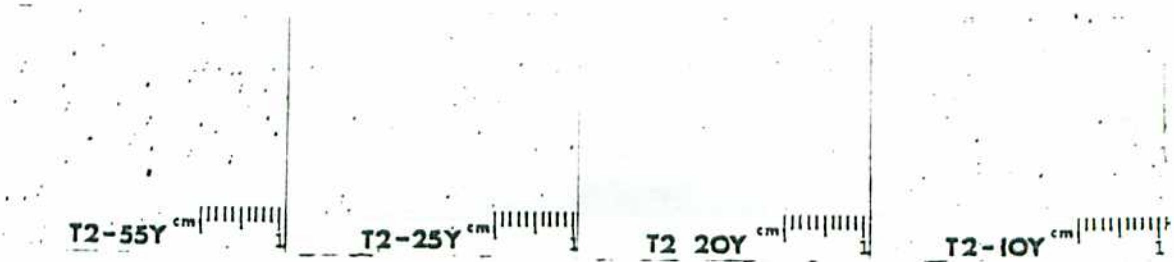


Fig. 2. Spray deposit recovery data from *B.t.* calibration trials.

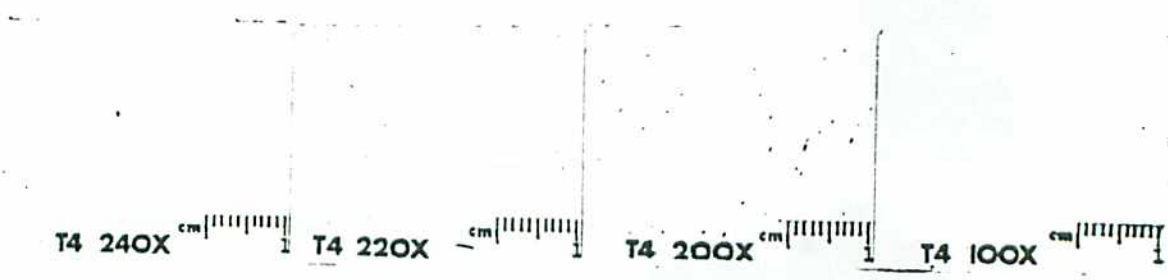


Thuricide 16B/H_2O (64: 16)



Novabac 45B/H_2O (22.8: 57.2)

Thuricide 24Ba/H_2O (42.7: 37.3)



Thuricide 24Ba/H_2O (42.7: 37.3)

Fig. 3. Spray deposit and stain diameters of *B.t.* spray droplets produced by 8004 and 8006 Spraying Systems Teejet nozzles at 40 p.s.i. fluid pressure.

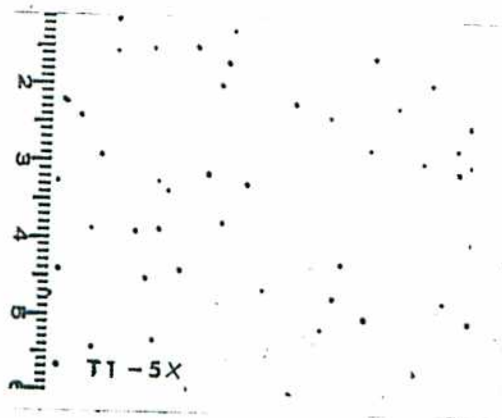


Fig. 4a. Photographic reproduction of *B.t.* spray droplet deposit using Thuricide 16B/water formulation

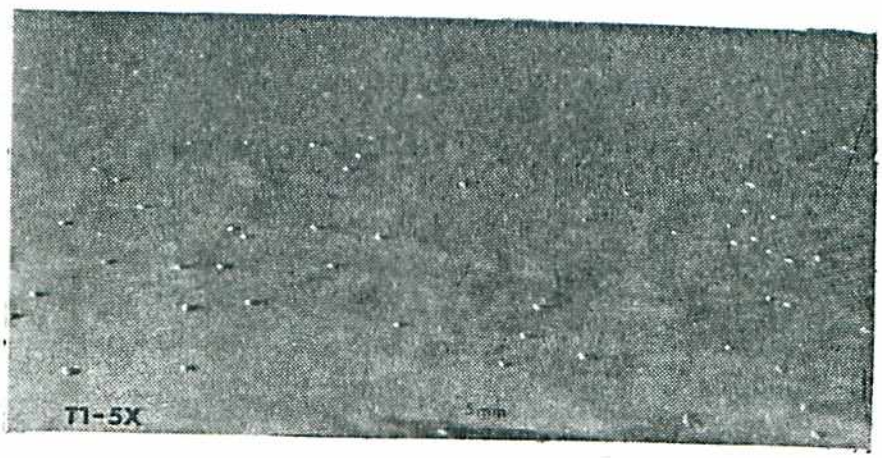


Fig. 4b. Photographic enlargement of same area taken from an oblique angle to show the spherical shape of spray droplets (6 months after spray deposition).

as preliminary runs to establish basic "ad hoc" data on the behavior of the *B.t.* formulation through the boom and Teejet (8004/8006) system. Time did not permit repeat trials, or modifications of the system to achieve the desired droplet spectrum objectives. Sufficient data, however, were generated to define problem areas and to suggest the direction for future research endeavours.

Analysis of all drop deposit data from the four calibration trials strongly suggested that water formulations of *Bacillus thuringiensis* can be emitted through 8004 and 8006 Teejet nozzles provided that the spray formulation is made using water free of extraneous matter. Plugging of both nozzle sizes usually occurred at the terminal ends of the booms, and in all cases plugging was due to impurities such as plant debris present in the field water supply.

A visual analysis of the spray deposit stains from T-1 (Thuricide 16B) and T-3 (Thuricide 24Ba) Fig. 3 suggests that two distinct droplet spectra i.e., large drops and extremely fine droplets were produced. Volume emission at 40 p.s.i. through an 8006 and an 8004 Teejet nozzle is approximately 2.27 ℓ/ha (76.8 U.S. oz/min.) and 1.15 ℓ/ha (51.2 U.S. oz/min.), thus accounting for the production of two separate drop spectrum patterns. The original objective of a mass median diameter drop size of 90 microns (μm) would therefore be difficult to achieve under such conditions especially where the largest stain size is of the order of 1000 (μm). Studies undertaken by

Haliburton (1976) on the relationship of deposit volumes versus drop diameter and density deposit in terms of drops/cm^2 indicated that a total of 90 drops/cm^2 would be required of a uniform 100 micron drop size to have a deposit coverage of 4.68 ℓ/ha (0.5 USG/ac). By contrast 26 drops/cm^2 of a 150 micron drop size would be required for the same volume coverage. It becomes readily apparent that the objective of a mass median diameter of 90 (μm) would be impossible using the boom and nozzle system as fitted to the spray aircraft.

The fact that some *B.t.* formulations produce spherical deposits that do not form stain diameters seriously affects data retrieval. The ability of the flying spot counter to calculate deposit volumes by digital image analysis of normal dyed oil deposit stains on Kromekote cards would not apply under these circumstances and therefore difficulties would arise in accurately assessing operational spray programs utilizing such *B.t.* formulations.

Since aerial application technology is a particular type of tactic that employs aircraft in order to disseminate spray liquids, it is imperative that the performance of the spray formulation and the aerial spray equipment operate at optimum effectiveness in order to achieve maximum impact at the target site.

To achieve this objective, the following recommendations are suggested.

RECOMMENDATIONS

1. Calibration of aerial spray equipment, formulation, and aircraft type should be undertaken on the aircraft scheduled for use on the experimental or operational spray program, and, well in advance of the program spray date to allow sufficient time for reappraisal and modifications if necessary.
2. The *B.t.* concentrate materials should be formulated and tested in a laboratory for compatibility with aerial spray equipment, aircraft, and application tactics in order to provide the type of spray coverage suggested for the program.
3. A change in formulation or equipment will necessitate a recalibration program.
4. Since aerial application technology is the end of the line tactic in controlling the insect pest, sufficient time and resources should be allocated to this important stage to ensure that the pesticide reaches the target at maximum droplet density in order to achieve maximum cost/benefit from the toxic agent.
5. A standard *B.t.* formulation should be established to provide a base line for future research on spray deposit/efficacy, studies and droplet spectrum characteristics. This would provide a reference point for *B.t.* efficacy in much the same way that the 10% DDT/fuel oil formulation served as a standard in chemical insecticide studies.
6. The current system of spray deposit retrieval using Kromekote cards and glass slides, while acceptable for oil formulated

chemical pesticides, is not fully satisfactory for *B.t.* formulations. This area requires further studies to provide a reliable system of spray deposit data retrieval.

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APPENDIX A

APPENDIX A

Table I

Summary of Calibration Data

	Bt. Calibration Trial No.			
	T-1	T-2	T-3	T-4
Date	14/6/79	14/6/79	18/6/79	19/6/79
Location	Gander	Gander	Bay D'Espoir	Bay D'Espoir
Time (hrs.)	18:30	21:15	18:45	19:30
Active Ingredient	Thuricide 16B	Novabac N45	Thuricide 24Ba	Thruicide 24 Ba
Solvent	H_2O	H_2O	H_2O	H_2O
Formulation Ratio	80/20	30/70	54/46	54/46
Emission Rate (ℓ/min.)	50.4	50.4	46.9	46.9
Specific gravity (approx.)	1.16	1.02	1.05	1.05
<u>Spray Aircraft</u>				
Type (Cessna)	Ag-truck	Ag-truck	Ag-truck	Ag-truck
Registration	CF-LDZ	CF-LDZ	CF-LDZ	CF-LDZ
Speed (kph)	176	176	176	176
<u>Spray Equipment</u>	B & N	B & N	B & N	B & N
Spray Nozzles	8004/8006	8004/8006	8004/8006	8004/8006
No. of units	20/20	20/20	20/20	20/20
Spray Height (M)	6	9-15	6	6-9
Spray Pressure (psi)	40	40	40	40
<u>Weather Data</u>				
Wind speed (kph)	2 ± 1	12	2 ± 1	17 ± 3
Air Temp. Dry (F°)	48°	46.5	60.5	59
Wet (F°)	47°	45	60	55
R.H. %	96	90	98(Rain)	80
Cloud Cover	8/10	8/10	10/10 Fog	10/10 Fog

APPENDIX A.

TABLE II

Cessna Ag-Truck Calibration Trials (Gander and Bay D'Espoir, Nfld. 1979)
(*Bacillus thuringiensis* formulations)

Sample Position Number meters (down wind)		Spray Deposit Data											
		T-1 (Thuricide 16B)			T-2 (Novabac 45B)			T-3 (Thuricide 24B)			T-4 Thuricide (24B)		
		Drops/ cm^2	Volume (L/ha) A	Volume (L/ha) B	Drops/ cm^2	Volume (L/ha) A	Volume (L/ha) B	Drops/ cm^2	Volume (L/ha) A	Volume (L/ha) B	Drops/ cm^2	Volume (L/ha) A	Volume (L/ha) B
	X-35	0	-	-	0	-	-	21	-	-	-	-	-
	X-30	0	-	-	0	-	-	15	0.1	0.2	0.1	-	-
	X-25	0	-	-	0	-	-	17	0.4	0.8	0.1	0.2	0.7
	X-20	0	-	-	0	-	-	18	0.5	0.8	0.3	0.0	0.4
	X-15	4.3	0.4	0.6	13.1	↑		20	2.3	3.9	0.2	0.3	0.8
	X-10	3.1	5.9	10.3	10.9	|		18	7.4	22.8?	0.7	0.0	0.2
	X-5	6.6	3.4	6.0	8.8	|		26	4.4	6.7	1.6	0.2	0.5
Flight Track	X 0	11.4	4.1	7.7	5.5	|		23	1.7	3.9	3.9	0.8	10.2?
	X 5	10.0	4.5	8.1	8.9	|		36	5.5	8.6	4.3	0.8	2.1
	X 10	5.6	3.9	6.7	6.8	?		37	6.8	11.2	5.7	0.8	2.1
	X 15	5.9	2.3	3.3	7.6	|		32	3.9	16.5?	4.5	1.5	3.0
Wind direction	X 20	6.9	1.8	2.6	7.3	|		37	4.0	6.1	3.9	1.4	3.2
	X 25	7.5	1.6	3.3	4.6	|		0	0.0	-	5.0	1.7	3.5
	X 30	4.0	1.2	1.9	1.9	|		0	0.0	-	4.1	1.7	2.6
	X 35	3.9	1.2	2.0	1.5	↓		0			5.9	0.9	14.8?
	X 40	3.3	1.1	2.0	1.3	0.4	0.9	0			4.4	0.9	1.9
	X 45	1.9	0.9	1.5	2.0	0.5	0.5	0			3.3	0.9	1.5
	X 50	1.9	0.7	1.2	3.7	0.4	0.5	-			3.3	0.5	16.2?
	X 55	2.0	0.6	0.9	4.7	0.6	0.9	(wind reversal)			4.3	0.6	1.5
	X 60	3.1	0.4	0.8	4.0	0.7	1.0				4.9	1.1	2.3
	X 65	3.1	0.4	0.7	4.7	0.5	0.7				2.1	0.7	10.3?
	X 70	3.6	0.4	7.4?	4.5	0.7	1.0				2.7	0.5	8.9?
	X 75	2.9	0.5	1.0	3.4	0.8	11.5?				1.7	0.5	1.1
	X 80	3.1	0.5	0.9	3.7	0.7	10.9?				1.5	0.4	1.1
	X 85	4.3	0.5	0.8	1.2	0.7	1.0	-			1.2	0.1	0.9
	X 90	4.4	0.7	1.1	1.2	0.4	0.4	-			0.9	0.2	0.5
	X 95	3.9	0.5	0.8	0.8	0.4	0.2	-			0.9	0.2	1.4
	X100	4.1	-	-	0.6	0.2	0.2	-			1.3	0.3	0.9
	X105	2.3	-	-	0.7	0.3	6.1?	-			1.2	0.3	0.8
	X110	1.6	-	-	0.3	0.2	0.2	-			1.1	0.4	0.8
	X115	1.9			0.1	0.2	10.7?	-			1.4	0.2	8.8?
	X120	1.3			0.1	0.3	11.0?	-			1.4	-	-
	X125	0.8			0.1	0.0	-	-			1.3	-	-
	X130	0.3			0.4	-	-				1.4		
	X135	0.1			0.3	-	-				1.4		
	X140	0.1			0.2						2.3		
	X145	0.1			0.3						1.1		
	X150	0.1			0.3						1.4		
	X155	0.1			0.2						1.7		
	X160	0.1			0.2						1.3		
	X165	0.0			0.2						1.6		
	X170	0.0			0.2						1.9		
	X175	-			0.4						1.8		
	X180	-			0.2						1.4		
	X185	-			0.1						1.2		
	X190	-									0.9		
	X195	-									1.2		

A. Deposit samples eluted with water and subjected to colorimetric analysis.
B. Deposit samples eluted with water then centrifuged before colorimetric analysis.
?. Aberrant readings believed due to bubbles in the flow cell of the spectrophotometer (excluded from calculations).

APPENDIX B

Methodology and Techniques

1. Test tube technique for formulation compatibility and stability.
2. Fig. 5. Stability of *B.t.* formulations 0, 2, 4, 6, and 8 hours after mixing.
3. Fig. 6. Stability of *B.t.* formulations 1, 2, 3, 4 and 20 days after mixing.
4. Colorimetric determination of volume deposits.
   - Fig. 6. Rate of flow of Thuricide 16 B formulation through spraying systems nozzles numbers 8004 and 8006.
   - Fig. 7. Close up view of spray fan from 8006 and 8004 nozzles.
5. Development of Spray Collector unit for rate of flow determination of spray equipment.
   - Fig. 8. Construction of the spray collector unit (Cessna Ag-truck) for rate of flow calibration of *B.t.* formulations.
   - Fig. 9. Rate of flow calibration of Thuricide 16 B formulation through Spraying Systems 8006 and 8004 Teejet nozzles at 40 p.s.i.
   - Fig.10. Close up of terminal end of boom showing spray fans from 8006 and 8004 nozzles.
   - Fig.11. Close up view of collector tube showing arrangement of tube openings and nozzles.
   - Fig.12. "Ad. hoc." field arrangement for weight/volume measurement of *B.t.* formulations during rate of flow calibration trials.

TEST TUBE TECHNIQUE FOR STABILITY DETERMINATION OF *B.t.* FORMULATIONS

A rapid "ad hoc" field/laboratory tehcnique for the determination of the ease of mixing and stability of *B.t.* formulations was developed to provide initial information in methodology and ease of formulating the three different *B.t.* concentrates for experimental use. This step was deemed necessary because of the limited supply of *B.t.* concentrate material and the fact that it may be necessary to store formulations after mixing over a period of hours due to adverse weather conditions.

Three formulations for each of the experimental *B.t.* materials were set up on the basis of 19.8 BIU/ha (8.0 BIU/acre) as shown in the following Table 1.

Table 1. Experimental Formulations of *B.t.*

	Mix No.	Mixing proportions of *B.t.* conc./H_2O (US oz)	Fluid (oz/ac)	Volumes L/ha
	1	64 + 16	80	5.85
Thuricide 16B	2	64 + 64	128	9.35
	3	64 + 192	256	18.71
	1	43 + 21	64	4.68
Thuricide 24B	2	43 + 37	80	5.85
(a) and (b)	3	43 + 85	128	9.35
	1	22.8 + 41.2	64	4.68
Novabac 45B	2	22.8 + 57	80	5.85
	3	22.8 + 105	128	9.35

The materials were mixed in 10 cm test tubes in two series,

A. water was added to the *B.t.* concentrate.

B. the *B.t.* concentrate was added to the water.

Results from these preliminary trials indicated that mixing of the two ingredients was improved by the addition of the *B.t.* concentrate to the water. By controlling the sequence of mixing i.e., rotating the tube from the vertical to the horizontal position (90°) and back again an index of "ease of formulation" could be established on a comparative basis by the number of rotations required for adequate mixing. Surprisingly the mayonnaise-like Novabac 45B exhibited the greatest ease of mixing (1 rotation) at all concentrations tested. Thuricide 16B exhibited the least at the lowest concentration of 64/16 *B.t.*/ water proportions. All *B.t.* concentrate materials were miscible at the concentrations tested.

Stability of each formulation was observed in the laboratory at regular 2 hour intervals during a 12 hr period and daily thereafter. The results are shown in the following sequence in Figs. 5 and 6 respectively. Precipitates appeared in the highest Thuricide 16B formulation within a short period of time and showed distinct layering of component parts. These unfortunately have not been identified. The least amount of precipitation occurred in the more concentrated formulations.

Thuricide 16B | Thuricide 24Ba | Novabac 45B

Time (hours) B C D A B C A B C

0 hrs

2 hrs

4 hrs

6 hrs

8 hrs

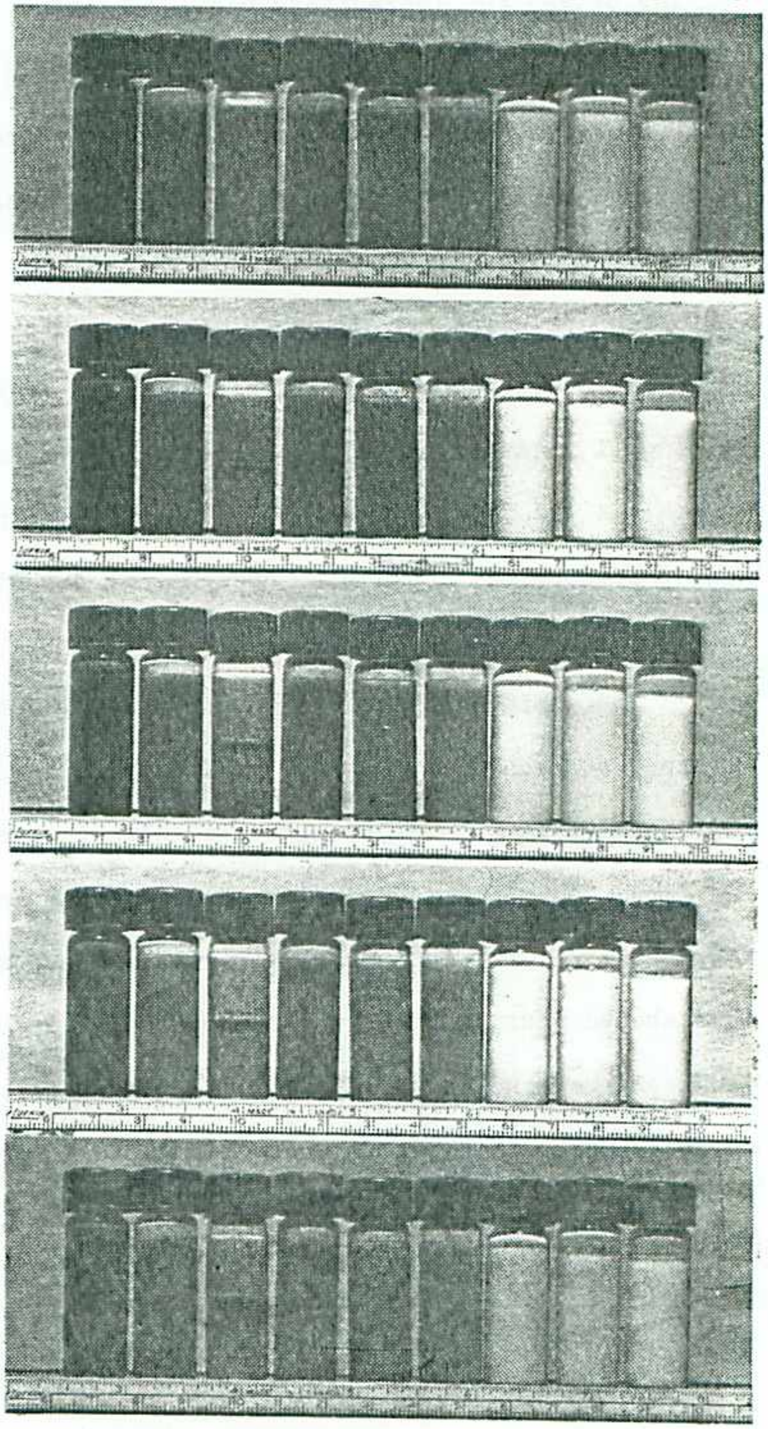

Fig. 5. Test tube technique for determining stability of *Bacillus thuringiensis* formulations; 0, 2, 4, 6 and 8 hours after mixing. A = 4.68 litres/hectare (64 US oz/ac), B = 5.85 ℓ/ha (80 oz/ac) and C = 9.35 ℓ/ha (1 USG/ac) D = 18.7 ℓ/ha. (2 USG/ac).

Time (days) Thuricide 16B (B C D) Thuricide 24Ba (A B C) Novabac 45 (A B C)

1 day

2 days

3 days

4 days

5 days

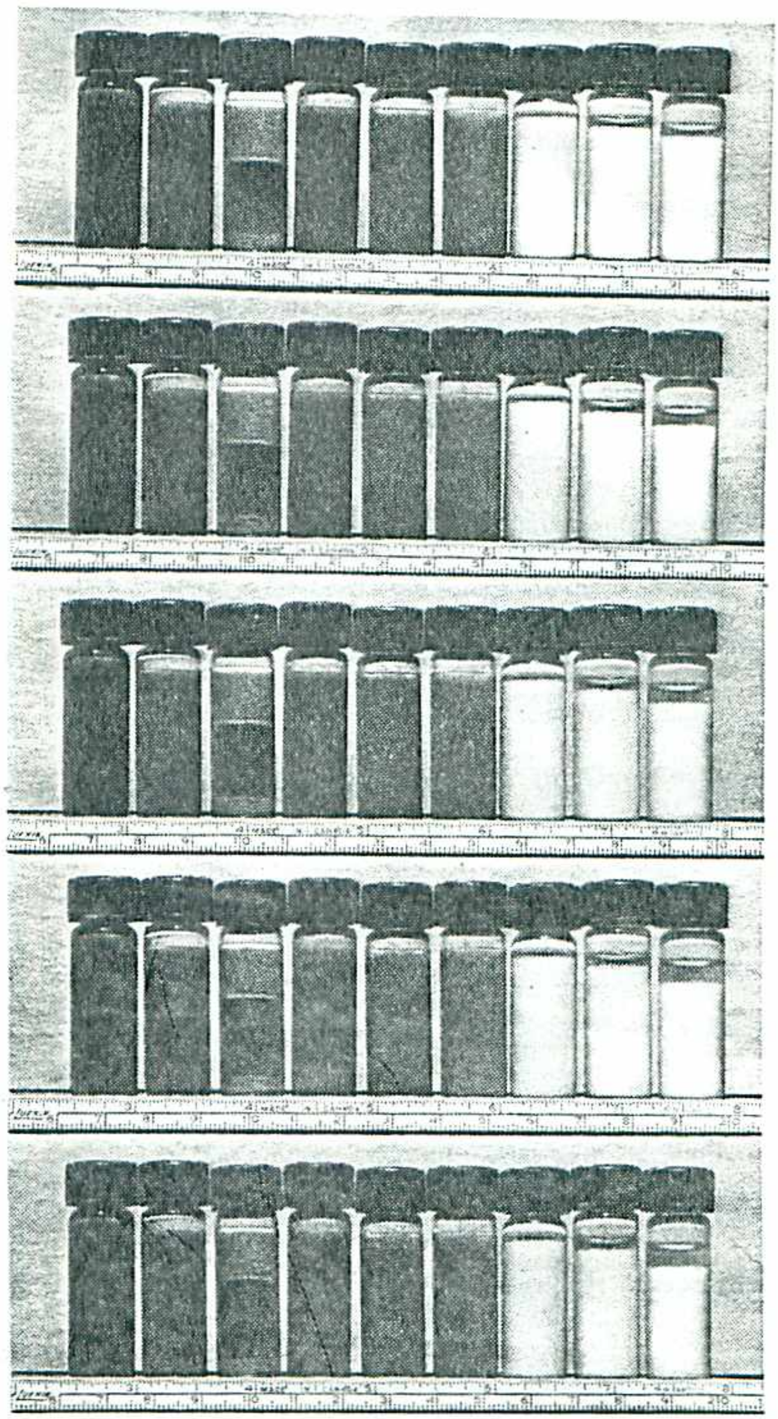

Fig. 6. Test tube technique for determining stability of *Bacillus thuringiensis* formulations; 1, 2, 3, 4 and 5 days after mixing. A = 4.68 ℓ/ha (64 US oz/ac), B = 5.85 ℓ/ha *80 oz/ac) and C = 9.35 ℓ/ha (1 USG/ac) D = 18.7 ℓ/ha (2USG/ac).

COLORIMETRIC DETERMINATION OF VOLUME DEPOSITS

The volume of spray deposited per unit area was assessed by measuring the absorbance of light by Erio Acid Red XB dye recovered from 51 x 75 mm glass slides exposed to the spray. These measurements were converted to litre per hectare data using a calibration curve produced for each tank sample.

Sample Preparation

Spray residue was washed off sample slides into centrifuge tubes using 4 ml. of water. These tubes were spun at 3,000 r.p.m. for 15 minutes to remove spores, crystals, and dust from the liquid. The absorbance due to Erio Acid Red XB in the supernatant of each tube was read using a spectrophotometer set at a wavelength of 562 nm. (the absorbance peak of the dye).

Calibration Curve

A calibration curve was produced for each tank mix. Ten microlitres of the suspension were applied to each of ten glass slides which were then placed in a vacuum oven set at 30°C and 15 inches vacuum. The following day, the slides were removed and washed using the same technique used for the field samples. Samples were diluted to make two replicates of each of the following concentrations:

CONCENTRATION (ml of tank mix per ml. of water)	EQUIVALENT DEPOSIT (L/ha)
9×10^{-4}	9.412
7×10^{-4}	7.320
5×10^{-4}	5.229
3×10^{-4}	3.137
1×10^{-4}	1.046

These calibration standards were centrigued and read in the same way as the field samples. The equation for a calibration curve was calculated by regressing absorbance readings on equivalent deposit values.

Notes

1) Ad hoc tests showed that distilled water was more efficient than toluene, benzene, and several concentrations of ethanol in water for removing *B.t.* deposits from glass slides.
2) It was found necessary to centrifuge or filter samples prior to spectrophotometry. Light scatter due to suspended dust and *B.t.* particles increased absorbance by as much as 100%. The effect of centrifuging both field samples and calibration standards was a net increase in deposit estimates of 78%. Table II contains both sets of data for comparison.
3) The spray formulations did not contain sufficient dye for precise estimates of deposit using the present method. Most of the samples gave absorbance readings of less than 0.100 A which means that most samples were read using only the bottom 5% of the sensitivity range of the spectrophotometer (range = 0-2A). Ideally, a tracer dye should be used at a concentration which produces samples with an absorbance of about one absorbance unit (1.0 A) at the target deposit rate. Using the present methodolcogy, the calculated ideal concentration of Erio Acid Red XB 400% for a planned 9.35 ℓ/ha (1 USG/ac) deposit is 2.8%.

An alternative to increasing the dye concentration is the use of a fluorescent tracer dye measured using a fluorometer, which is far more sensitive detector than a spectrophotometer. Choice of a visible/fluorescent dye such as Rhodamine B would still provide stains suitable for manual or automated counting for calibration purposes.

Spray Boom Collector Tube for rate of flow determinations of *B.t.* formulation

The collector tube was designed and built on location at the Gander Airport in order to conserve minimal quantities of experimental *B.t.* formulations and to reduce airport contamination. The tube was constructed from light weight 4" black plastic drain tile that was cut and angled to conform to the contour of the spray boom as shown in Fig. 8. The cut ends were taped or glued together to form a single continuous unit equal in length to each spray boom. The tube was then taped to the boom to locate the exact position of the spray nozzle relative to the collector tube. A series of one inch holes were cut into the top surface of the tube and the collector tube was adjusted to accommodate the appropriate spray nozzle as shown in Fig. 9. Both ends of the collector tube were sealed with a standard 4" plastic cap. A 2" hole was then cut into the bottom surface at the lowest end of the tube to provide an exit opening and collecting site for the spray fluid. A calibrated 15 gallon container served as the collecting tank for each boom.

Rate of flow calibration of the spray system can be undertaken with minimal quantities of material that can be collected and returned to the spray tank for future use. The system on the Cessna Ag-truck had a nominal flow rate of 22 USG/min., thus representing a considerable saving of experimental material that is normally sprayed out onto the ground as shown in Fig. 10. A close-up view of a typical-spray fan from a 8006 and 8004 Spraying System Teejet nozzle is shown in Fig. 11.

Standard procedure for rate of flow calibration required the system to be calibrated using water as the test liquid to ensure proper operation of the system and to establish a standard for comparison. The spray tank and booms were then drained and the experimental *B.t.* material added to the spray tank. The system was primed to ensure that all nozzles were operative and then the collector tubes were installed on the booms. The system was then primed to show 40 psi on the pressure guage within the cockpit and the spray booms were opened for a 60 second spray run. The spray material collected in the 15 gallons tanks was weighed and measured for volume displacement as compared to the water standard. Care was taken to ensure that the *B.t.* solution had sufficient time to "de-bubble" in order to obtain the correct air free volume of formulation (approximately 12 hrs.)

The system can provide data on rate of flow and approximate specific gravity values using water as standard. A typical "ad hoc" field set up is shown in Fig. 12.

Fig. 8. Construction of the spray collector unit for rate of flow calibration of *B.t.* forumulations.

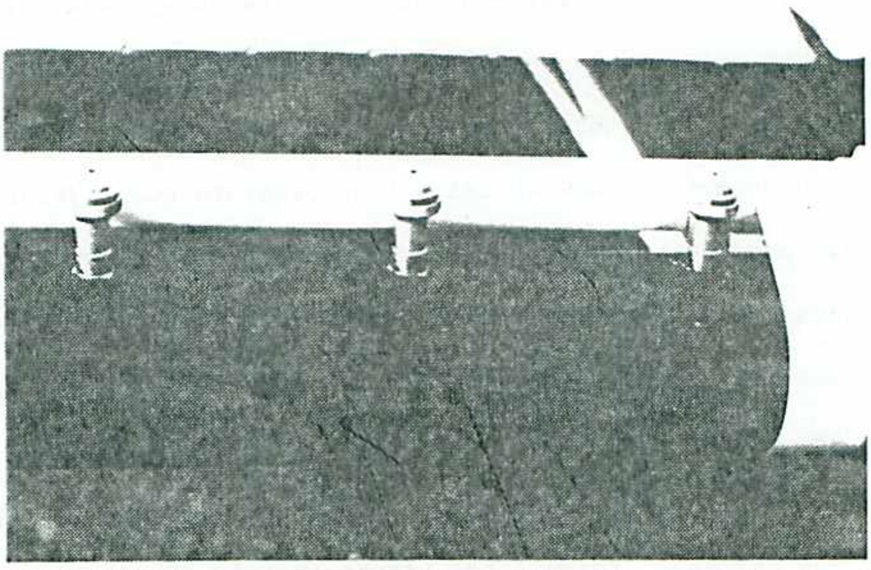

Fig. 9. Close up view of collector tube showing arrangement of tube openings and nozzles.

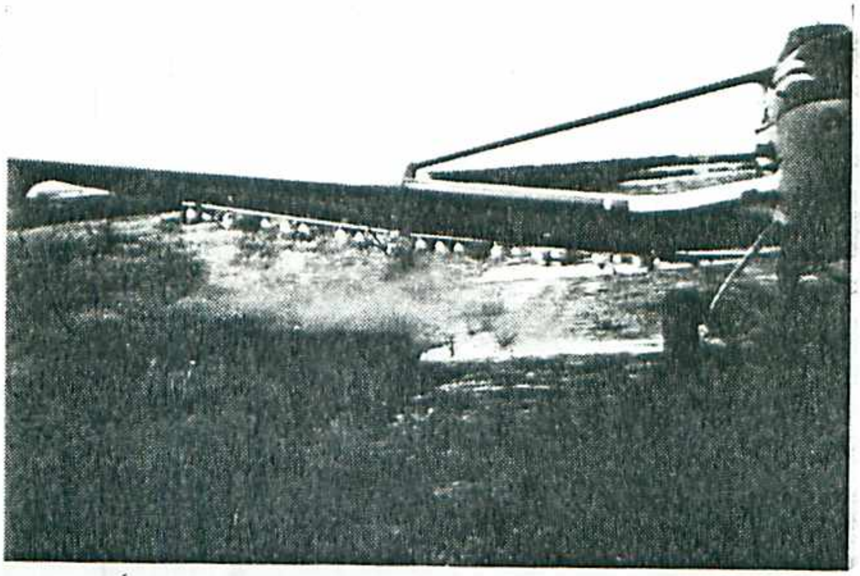

Fig. 10. Rate of flow calibration of Thuricide 16B formulation through Spraying Systems 8006 and 8004 Teejet nozzles at 40 psi.

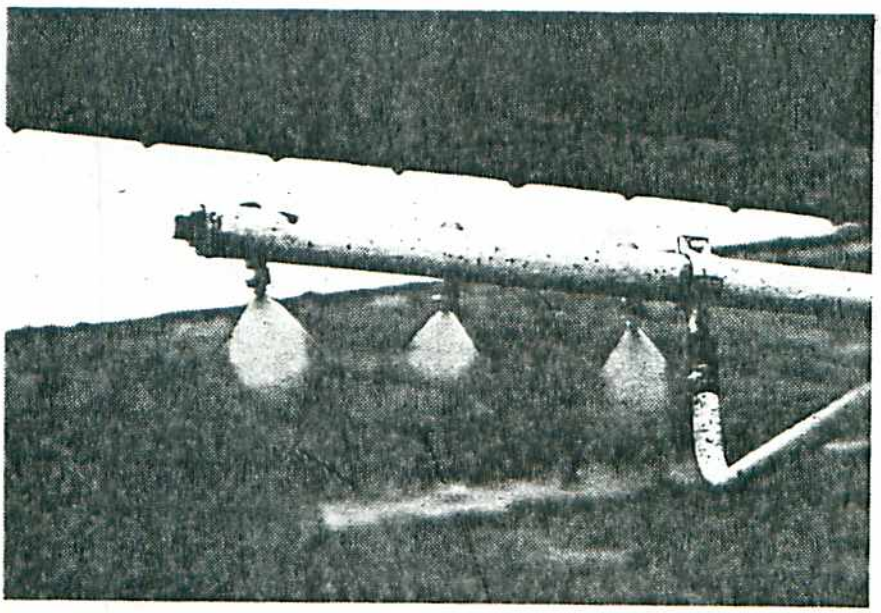

Fig. 11. Close up of terminal end of boom showing spray fans from 8006 and 8004 nozzles.

Fig. 12. "Ad hoc" field arrangement for weight volume measurement of *B.t.* formulations during rate of flow calibration trials.