Fog as a Vehicle for Dispersal of a Microbial Insecticide in Shelterbelts

R. D. Frye, K. J. McMahon and R. A. Weinzierl

A cold fogger and a thermal fogger were effective in dispersing the entomogenous bacterium *Bacillus thuringiensis* Berliner (in Thuricide 16B) in shelterbelts.

Cankerworms are important as defoliators in shelterbelts in the northern Great Plains. Both the spring, *Paleacrita vernata* (Peck), and fall, *Alsophila pometaria* (Harris), cankerworms are involved. Severely defoliated shelterbelts do not protect adjacent crops from severe weather stresses and are aesthetically displeasing as well. Harper (1974) summarized work on the use of the entomogenous bacterium *Bacillus thuringiensis* (Berliner) for control of cankerworms, including results of efforts in North Dakota. The bacterium has demonstrated considerable effectiveness against both pests. However, improved formulations and application techniques would be desirable.

The investigation described herein was initiated in 1975 to evaluate ground fogging equipment for dispersal of *B. thuringiensis.* Falcon et. al. (1974) concluded that vertical coverage of cotton plants was better when *B. thuringiensis* was applied from the ground with a cold fogger than when the bacterium was applied from the air. The present study was conducted in North Dakota near Fargo in Cass county, and near Walhalla in Pembina county. It is part of a cooperative effort involving North Dakota State University and the USDA Forest Service, Rocky Mountain Forest and Range Experiment Station, Fort Collins, Colorado[1].

Methods and Materials[2]

A cold fogger (Microgen M52W-15), a thermal fogger (Dyna Fog 70B) and a conventional hydraulic sprayer (Spartan) were used to apply Thuricide 16B (*B. thuringiensis*) to Siberian elm, *Ulmus pumila* L., at a rate of 0.946 liter (1 quart) of product per acre (4.0 International Units). An International Unit (I.U.) is defined as 1,000 times the amount of a standard *B. thuringiensis* preparation required to kill 50 per cent of the test larvae (usually the cabbage looper, *Trichoplusia ni*) in a laboratory bioassay divided by the amount of the test material required to kill 50 per cent of a group of similar larvae (Burges 1966).

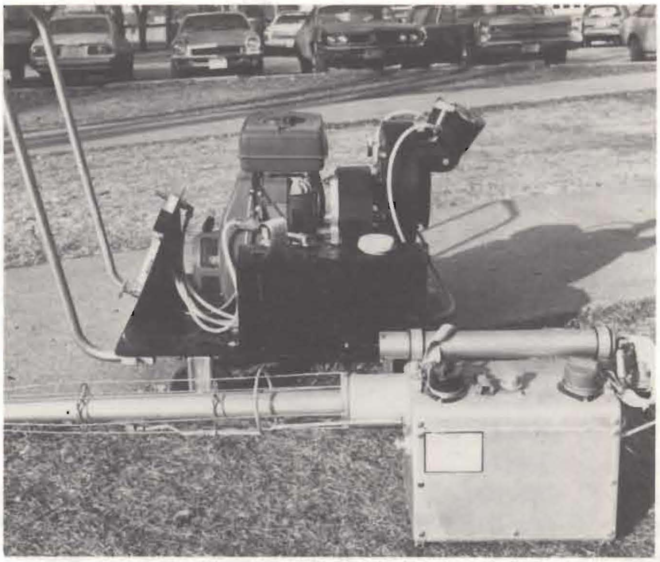

Figure 1. Cold fogger (rear) and thermal fogger (front) used to apply *Bacillus thuringiensis* to Siberian elm foliage.

The application rate for each type of equipment was calibrated and used to determine the time required to apply specific rates of insecticide to a known volume of foliage. The crown volume was determined by a method described by Stein and Doran (1975). The insecticide was applied from a pickup truck. Equivalents of approximately 18.2 (5 gallons) and 2.838 (3 quarts) liters of spray per acre were applied with hydraulic sprayer and foggers, respectively. The foggers are shown in Figure 1. Application methods used to apply the bacterium were utilized as treatments.

At Fargo, the treatments were applied August 5 to unreplicated single-tree plots. The trees were approximately 10.7 meters (35 feet) tall. At Walhalla, the treatments were applied to six single-row shelterbelts. The belts were approximately 0.805 kilometer (0.5 mile) long, and the trees in the belts averaged approximately 10.7 meters (35 feet) in height. Plots in the belts consisted of 10 trees; there was a minimum of 15.2 meters (50 feet) between plots. Treatments were applied June 3.

Dispersal and survival of the bacterium were evaluated on the basis of counts of viable spores on five 0.3167 square centimeter samples of foliage (0.25 square inch). Leaf samples were placed in 10 ml of sterile, distilled water and shaken in a

[1]*USDA Research Agreement 16-532-CA.*

[2]*The use of trade names is for brevity and specificity and does not imply endorsement by the USDA or North Dakota State University to the exclusion of other equivalent products.*

Dr. Frye is professor, Department of Entomology; Dr. McMahon is professor and chairman, Department of Bacteriology; and Weinzierl is graduate research assistant, Department of Entomology.

Acknowledgement: We thank Kevin Kallander for his assistance with the plate counts.

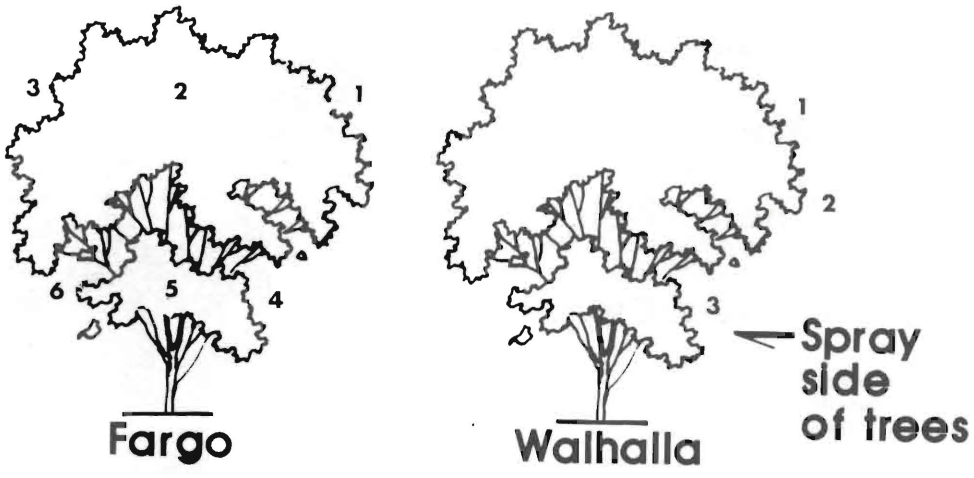

Figure 2. Location of sampling sites on the periphery and in the center of the crown of Siberian elm trees.

vortex mixer for 15 seconds. Serial dilutions were prepared in sterile, distilled water and spread-plated in replicate on five plates of plate count agar (Difco). Plates were incubated at room temperature for 24 hours and averages of counts of viable spores from the replicates were determined. At Fargo, samples were collected from six sites in each of the treated trees; two sites each on the periphery of the spray and lee sides and two sites in the middle of the crown, approximately 1.83 meters (6 feet) and 6.1 meters (20 feet) above ground. Commencing on August 5 (immediately after the treatments were applied), samples were collected daily through August 12.

At Walhalla, one sample was collected from each of the three sampling sites on the spray side of the crown of one tree in each plot in each of six belts (six replications per treatment) on June 4 (24 hours after application), 6, 8, 10 and 25; and on July 9. The sites were on the periphery of the crown 1.83 (6 feet), 3.66 (12 feet) and 6.1 (20 feet) meters above ground. The location of the sampling sites in trees at Fargo and Walhalla is shown in Figure 2. The samples were stored in coolers prior to processing.

Results and Discussion

Spore coverage on Siberian elm foliage at Fargo is presented in Table 1. The best overall coverage of the tree crown with *B. thuringiensis* spores was obtained with the hydraulic sprayer, followed by the cold fogger. Coverage with the thermal fogger was the lightest. When coverage in various portions of the crown is considered, the hydraulic sprayer and cold fogger were superior. In general, coverage with the hydraulic sprayer was better in portions of the crown including sampling sites 1, 4 and 5 (Figure 2). The most effective coverage in portions including sites 2, 3 and 6 (Figure 2) was obtained with the cold fogger.

At Walhalla (Table 2), there were no significant differences in overall crown coverage obtained with the hydraulic sprayer, cold fogger and thermal fogger. However, numbers of spores deposited on the foliage with the hydraulic sprayer and cold fogger were considerably higher than the number deposited with the thermal fogger. There were no differences in the number of spores deposited in various portions of the spray side of the crown. In general, the cold fogger and hydraulic sprayer deposited more spores in the upper portions (Figure 2). The hydraulic sprayer and thermal fogger deposited the most spores in the lower portion (Figure 2).

Results indicated that several differences should be noted. The numbers of spores deposited on elm foliage at Walhalla were considerably higher than at Fargo (Tables 1 and 2). At this time, an explanation might be that there was a decrease in the number of spores in the *B. thuringiensis* preparation from the time it was used at Walhalla (June 3) to the time it was used at Fargo (August 5). Weather conditions could have affected the results at Walhalla. Measurable and prolonged rainfall occurred within 24 hours after the bacterium was applied and periodically throughout the sampling period. Weather records for the period are missing. Weather conditions at Fargo were ideal at the time of application. Windspeed at both locations was 4.83 (3 miles) to 8.05 (5 miles) kilometers per hour. At Fargo, samples were collected immediately after application; at Walhalla they were collected 24 hours after application. At Walhalla, samples were collected from only the spray side of the trees (Figure 2), and an additional sample was collected from that side (Site 2).

Survival of *B. thuringiensis* spores on Siberian elm foliage at Fargo and Walhalla is summarized in Figures 3 and 4, respectively. Most spore in-

Table 1. Coverage of Siberian elm foliage with *Bacillus thuringiensis* spores immediately after application of the bacterium, Fargo, North Dakota, August 5, 1975.

Whole tree coverage		Coverage at sample sites in crown of tree		
Application equipment	Mean viable spore count	Application equipment	Site	Mean viable spore count
Hydraulic sprayer	605,000 a	Hydraulic sprayer	3	1,620,000 a
		Hydraulic sprayer	1	1,520,000 a
Cold fogger	153,503 b	Cold fogger	4	522,000 b
		Hydraulic sprayer	5	434,000 b c
Thermal fogger	9,867 c	Cold fogger	6	288,000 c
		Cold fogger	3	62,000 d
		Hydraulic sprayer	6	41,800 d
		Thermal fogger	4	41,600 d
		Cold fogger	5	40,800 d
		Hydraulic sprayer	4	9,400 d e
		Thermal fogger	6	8,200 d e
		Thermal fogger	5	5,800 d e
		Cold fogger	2	5,020 d e
		Hydraulic sprayer	2	4,800 d e
		Cold fogger	1	3,200 d e
Coefficient of variability = 39.6%		Thermal fogger	1	2,400 d e
		Thermal fogger	2	1,000 d e
		Thermal fogger	3	200 e

Viable counts based on five 0.3167 square centimeter (0.25 square inch) samples of elm foliage per treatment.

Means followed by the same letter do not differ significantly at the 5% level of error (Duncan, 1955).

Sites 1 (spray side) and 3 (lee side) on the periphery of the crown in each tree were approximately 6.1 meters (20 feet) above ground; sites 4 (spray side) and 6 (lee side) on the periphery of each tree were approximately 1.83 meters (6 feet) above ground; site 3 was in the center of the crown approximately 6.1 meters (20 feet) above ground; site 5 was in the center of the crown approximately 1.83 meters (6 feet) above ground.

Table 2. Coverage of Siberian elm foliage with *Bacillus thuringiensis* spores 24 hours after application of the bacterium, Walhalla, North Dakota, June 4, 1975.

Whole tree coverage		Coverage at sample sites in crown of tree		
Application equipment	Mean viable spore count	Application equipment	Site	Mean viable spore count
Hydraulic sprayer	1,170,689 a	Cold fogger	1	1,629,717 a
Cold fogger	1,160,589 a	Cold fogger	2	1,520,317 a
Thermal fogger	556,033 a	Hydraulic sprayer	1	1,460,333 a
		Hydraulic sprayer	2	1,212,900 a
		Hydraulic sprayer	3	838,833 a
		Thermal fogger	1	745,267 a
		Thermal fogger	3	701,333 a
Coefficient of variability = 75%		Cold fogger	3	331,733 a
		Thermal fogger	2	221,500 a

Viable counts based on five 0.3167 square centimeters (0.25 inch) samples of elm foliage from six replications per treatment.

Means followed by the same letter do not differ significantly at the 5% level of error (Duncan, 1955).

All sample sites were on the spray side of each sample tree, and on the periphery of the crown; site 1 was approximately 6.1 meters (20 feet) above ground; site 2 was approximately 3.66 meters (12 feet) above ground; site 3 was approximately 1.83 meters (6 feet) above ground.

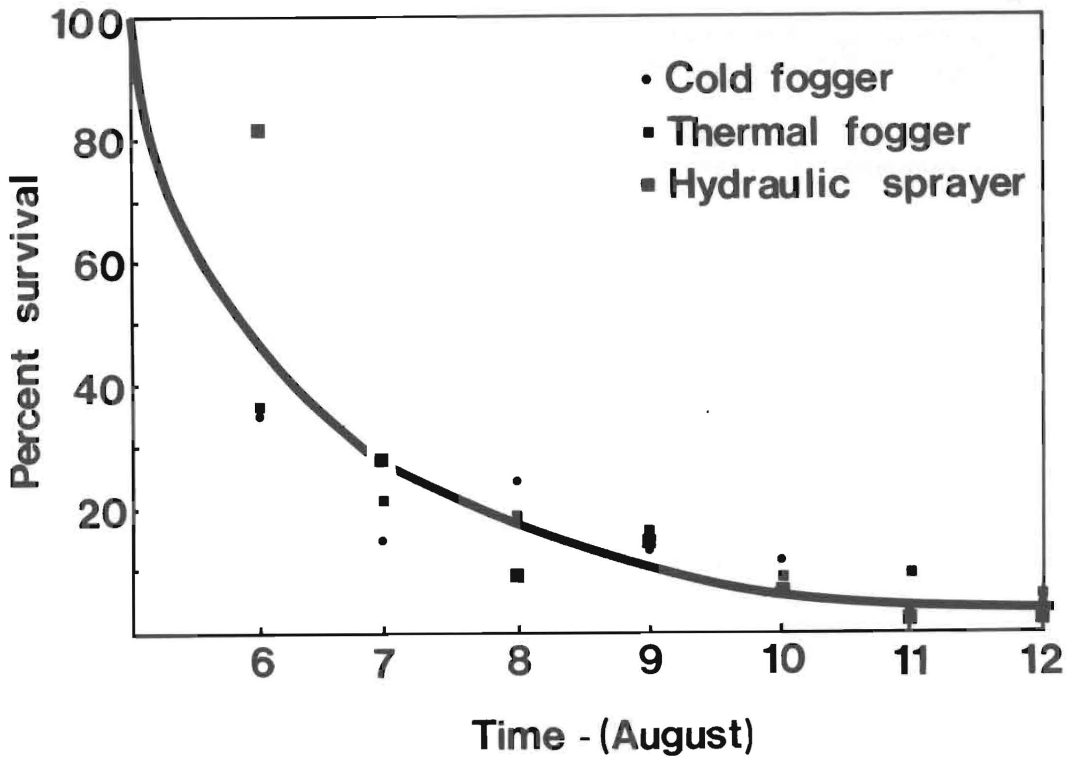

Figure 3. **Summary of survival of *Bacillus thuringiensis* spores on Siberian elm foliage, Fargo, North Dakota, 1975. The curve represents the average survival of spores applied with three types of equipment on August 5.**

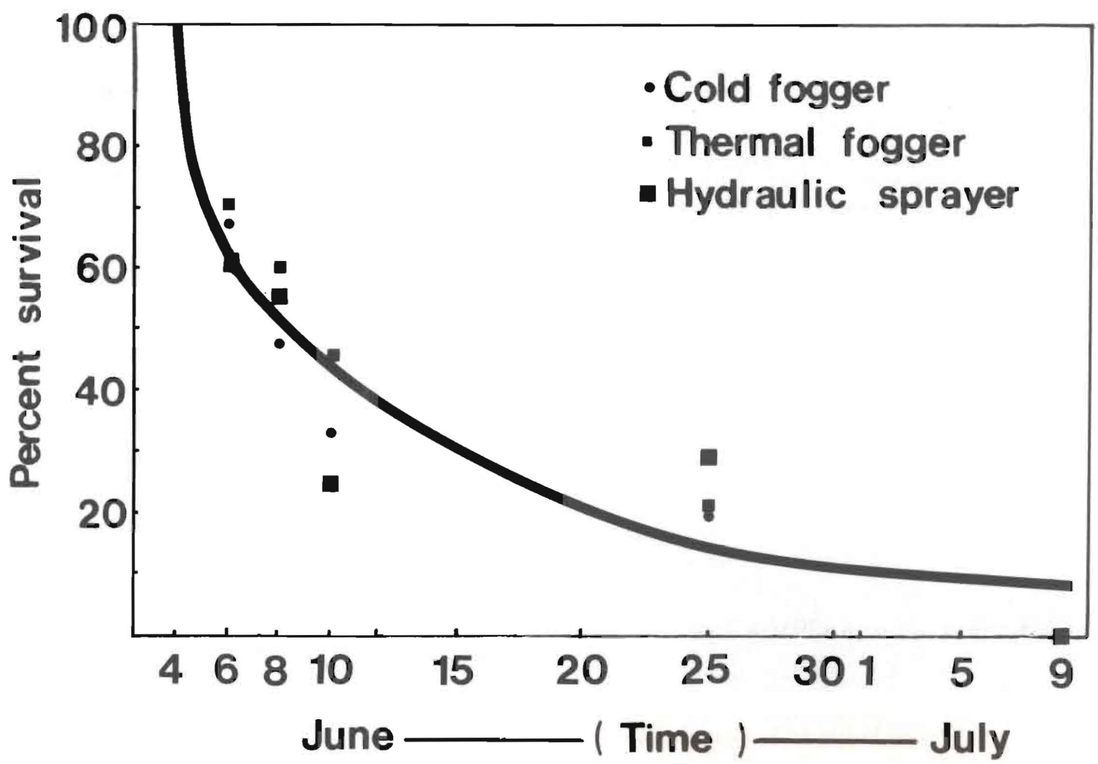

Figure 4. **Summary of survival of *Bacillus thuringiensis* spores on Siberian elm foliage, Walhalla, North Dakota, 1975. The curve represents the average survival of spores applied with three types of equipment on June 3.**

activation occurred during the first 24-48 hours of exposure to the weather at Fargo (Figure 3). Inactivation continued through August 10, followed by a stabilization of the decline in the number of viable spores during the remainder of the sampling period (1 week). At Walhalla, inactivation continued into early July, followed by a stabilization of the decline in the number of viable spores during the remainder of the extended sampling period (June 4 - July 9). The curve for the longer sampling period (Walhalla) was similar to the curve for the shorter sampling period (Fargo). In both cases, stabilization in the decline of viable spores occurred toward the end of the sampling period.

It is possible that more susceptible spores were killed during the first 24 to 48 hours, and the spores remaining on the foliage after that period were more resistant to the effect of weather, especially ultraviolet radiation. Davis et. al. (1968) described a logarithmic survival curve of microorganisms irradiated by ultraviolet light that tails off into a line whose slope corresponds to more resistant organisms. Sensitive organisms are inactivated first, and at the end only the more resistant survive.

At Walhalla, no significant differences in spore survival were associated with the three different types of equipment. At Fargo, overall survival (all sampling sites included) 24 and 48 hours after application was best when the bacterium was applied with the hydraulic sprayer. The highest survival rate after 72 hours was associated with the foggers, especially the cold fogger. After 96 hours, survival associated with the three types of equipment was similar. If we assume that the critical period for insect control with *B. thuringiensis* is from 1 to 3 days, application with the hydraulic sprayer or cold fogger would be most effective. The highest overall (all types of equipment included) spore survival throughout the sampling period (1 week) at Fargo was at sites 2 and 5 in the shaded center of the crown. Later in the sampling period, the percentage of survival was greatest at site 5. This is evidence of the effect of solar radiation on the spores (Buettner 1951).

Twenty-four hours after application, the greatest number of spores survived when applied with the hydraulic sprayer at most sites. The best spore survival after 72 hours of exposure to the weather was associated with the cold and thermal foggers, and this was true during the remainder of the sampling period (1 week, or 168 hours). Overall survival of spores was better at Walhalla. This could be related to the difference in the times of the season the tests were initiated (early June at Walhalla and early August at Fargo). There was considerable sky cover (clouds and haze) during early and mid-June at Walhalla. Buettner (1951) showed that the amount of ultraviolet radiation, which is lethal to bacterial spores, reaching the earth decreases as sky cover increases. Another factor could be the age of the *B. thuringiensis* preparation used in the tests. The preparation was used soon after it was formulated at Walhalla. Variability in the tests was high (Tables 1 and 2).

Summary and Conclusions

An investigation to determine the effectiveness of ground fogging equipment for dispersal of the entomogenous bacterium *Bacillus thuringiensis* (in Thuricide 16B) in shelterbelts was initiated in 1975. Tests were conducted at Fargo and Walhalla. A cold fogger and a thermal fogger were compared to a conventional hydraulic sprayer. Results indicate that fogging equipment should be considered promising for dispersal of this bacterium.

Although foggers did not improve on a conventional hydraulic sprayer regarding spore coverage on foliage and spore survival over extended periods in all cases, they have other advantages. Less water is required with foggers, and they were easy to handle when in use. The foggers required less directing of the spray than did the hydraulic sprayer. However, considerable difficulty was encountered in starting the thermal fogger. Heat generated by the thermal fogger burned substances in the insecticide preparation (corn oil, for example). The burned materials had to be cleaned from various openings frequently. The fog produced by the cold fogger was affected less by air currents than was the fog produced by the thermal fogger. Of the three types of equipment, the cold fogger was the easiest to calibrate.

References

Buettner, K. J. K. 1951. **Physical aspects of human climatology.** In Compendium of Meteorology, T. F. Malone (ed.). American Meteorological Soc., Boston, Mass., 1112-1125.

Burges, H. D. 1966. **The standardization of products based on** *Bacillus thuringiensis.* Proc. Intern. Colloq. Insect Pathology and Microbial Control, Wageningen, the Netherlands, P. H. van der Laan ed., North Holland Publ. Co., Amsterdam, 306-338.

Davis, B. D., R. Dulbecco, H. N. Eisen, H. S. Ginsberg and W. B. Wood, Jr., 1967. **Principles of Microbiology and Immunology.** Harper: New York.

Duncan, D. B. 1955. **Multiple range and multiple F. tests.** Biometrics, 11: 1-42.

Falcon, L. A., A. Sorensen and N. B. Akesson. 1974. **Aerosol application of insect pathogens.** Calif. Agr. 28: 11-13.

Harper, J. D. 1974. **Forest insect control with** *Bacillus thuringiensis.* Agr. Exp. Sta., Auburn Univ., Auburn, Alabama, 63p.

Stein, John D. and D. J. Doran. 1975. **A nondestructive method of whole tree sampling for spring cankerworm.** U.S. Dept. Agr. For. Serv. Res. Note RM-290, 4p., Rocky Mt. For. and Range Exp. Sta., Fort Collins, Colorado.