Evaluating the Treatment Efficacy of *Bacillus thuriengiensis* Var. Kurstaki: Reliability of Various Tools

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Abstract

The success of an operational or pilot insecticide spraying program against the spruce budworm (*Choristoneura fumiferana* Clemens) depends to a great extent on a variety of factors, and, in particular on: spraying efficacy and the meteorological conditions during and immediately following spraying. Among other things, spraying efficacy depends on: knowledge of the volume of insecticide necessary to kill this insect (droplet imbibing assays); adjustments to the spraying system in order to produce droplets containing a lethal dose (AT-802 calibration); and, finally, the capacity to achieve target objectives (optimization trials)—that is, the deposit of these droplets on foliage.

Droplet imbibing assays on the spruce budworm. Rousseau and Lemieux. 2001.

In Québec, the Société de protection des forêts contre les insectes et maladies (SOPFIM) is the organization mandated to efficaciously protect forests from certain forest pests. It performs quality control on products during all its spraying programs, including pilot programs. The quality control program devoted to formulations consists in: 1) determining insecticidal power; 2) searching for certain pathogenic microorganisms; and 3) identifying insecticidal species. All three aspects are the subject of laboratory analyses. Since 1997, SOPFIM has used a specific bioassay technique, developed by Kees van Frankenhuyzen and his team (van Frankenhuyzen et al. 1997.), in order to verify the insecticidal power of formulations. This technique, called an "imbibing assay," consists in measuring the mortality rate occurring after each insect has ingested a micro-droplet containing a known quantity of pesticide. In the present study, however, the results of imbibing assays are presented from the perspective of the efficacy of spraying and not that of the quality control program, whose purpose is to ensure that the insecticidal power of pesticide deliveries complies with the information printed on the label.

During 1999, the other members of the SOPFIM research team and I noted that the presence of an antibiotic in the diet used to raise insects appeared to have an impact on LD_{50}. The decision was thus made to verify in greater detail the effect of the antibiotic and its impact on calculations performed with a view to maximizing the efficacy of a treatment. In addition, droplet imbibing assays were also performed using Mimic™ (a tebufenozide-based insecticide) so as to compare its toxicity with that of Foray 76B™ (an insecticide having a base of *Bacillus thuringiensis,* var. kurstaki). The results of these assays were thus used to calculate the size of the droplets required in order to carry out an efficacious spraying program. Depending on the target efficacy level, most droplets should, at the least, contain a lethal dose for 50% of the individuals that ingested one of these droplets.

To begin with, spruce budworms were raised on a diet that either did or did not contain aureomycin, the antibiotic.Then, the assay method consisted in causing these budworms (instar V) to ingest a known quantity of the product. Generally speaking, groups of 40 insects were subjected to treatment involving each of the six serial dilutions (1:3) made using a diluted formulation. One group of insects that ingested only the dilution buffer served as a control during each assay. Individuals that either did not ingest the product or regurgitated it were eliminated; the others were put on a diet that either did or did not contain antibiotic. Then, larvae were placed in an environmental chamber: temperature was 25°C; relative humidity was approximately 45%; and photoperiod lasted 16 hours. Mortality was measured after 5 days. The ideal target mortality rate is 100% for the strongest dose and about nil for the weakest dose. Data were then processed using Polo™ software so as to establish the statistical validity of the lethal dose calculations. Assays were also performed using spruce budworms that had originated in a natural environment. This group of budworms were put on an aureomycin-free diet following ingestion of pesticide.

The antibotic has an effect on the results, the LD_{50} is 20 times lower when antibiotic is not used and the larvae continue to feed after ingesting a non mortal dose. In the case of a formulation of a Foray 76B™ type, a droplet as small as 29 µm in diameter at the time of release appears to have contained a lethal dose for 50% of individuals who absorbed it. These laboratory results

fit with those obtained with spruce budworms taken from a natural forest environment during the pilot program conducted in the Outaouais region. With respect to Mimic™, the toxicity of this product following ingestion is low compared to that of Foray 76B™. Thus, in order to kill 50% of a population (Instar V), droplets having a diameter of 72 µm at release are required (Sundaram and al. 1998.). Thus, a Mimic™ droplet this size represents a volume 15 times greater than that of a Foray 76B™ droplet (LD_{50}: 0.25 IU for instar V).

The toxicity data of this study should be used as inputs when mathematical calculations are performed in connection with treatment efficacy. The results of such calculation should be used prior to performing certain calibration tasks and wind tunnel tests so as to determine the best settings on a spraying system. In addition, this droplet imbibing assay technique could be used in order to discriminate between various *B.t.k.*-based formulations with respect to their insecticidal power and shelf life, thus constituting a valuable tool for spray program managers.

AT-802 calibration.
Rousseau and Lemieux. 2001.

In collaboration with Forest Protection Limited of New Brunswick, SOPFIM performed a characterization program centering on deposits of Foray 76B™ when this pesticide is sprayed from an AT-802 aircraft fitted out with 10 Micronair AU4000™ sprayers. This project was conducted during summer 2001 on the very location of the Longue-Pointe-de-Mingan (Québec) airport.

Before the plane arrived on the site of characterization work, FPL staff performed a series of adjustments on the spraying system. Wind tunnel test results were used to adjust flow rates and atomizer rotation speeds. As a result, settings on the spraying system were to produce droplets smaller than 100 µm in diameter for more than 90% of sprayed volumes (VMD=50 µm). These settings were established in relation to a swath of 70 meters. Testing was conducted into the wind and also under crosswind conditions for a swath of 70 meters. Testing was also performed into the wind for a swath of 100 meters. Prior to flying the plane, Kromekote™ papers and Petri dishes were placed on the ground every 2.5 meters for flights performed into the wind. The sampling system was set perpendicular to the wind, and aircraft generally flew over the centre of the sampling system, into the wind. During tests conducted under crosswind conditions, papers and dishes were set out every 5 meters. The sampling system was set parallel to the wind direction with the plane flying perpendicularly over the edge of the sampling system on the windward side. Ten minutes following flyover, Petri dishes and Kromekote™ papers were gathered. Papers were analyzed using the original Swath Kit™ equipment, using a modified version of the Swath Kit™, and by a hand count performed with a binocular microscope. These analyses were designed to determine the number of drops per area unit. Droplet diameter was measured with the microscope in order to identify the volume median diameter (VMD) and the number median diameter (NMD). Petri dishes were flushed and flushing liquid was analyzed using the Adam Kit™ so as to determine the active ingredients. Reference ranges were devised using a sample originating from the aircraft. The results of these analyses were expressed in BIU/ha.

The analysis of Kromekote™ papers using the Swath Kit™ proved to be rather unreliable. The system was thus modified, but once again, the results did not fit with the results produced by a "hand count" performed with a microscope fitted out with a filar micrometer. The difficulty, when using cameras, of differentiating dust from among the droplets made it necessary to read papers with a microscope. Depending on the equipment used, the results could vary by upwards of 100% and even 200%. The results of an analysis of the flushing liquid originating in the Petri dishes made it possible to accurately characterize the deposit in terms of the active ingredient. Using the Adam Kit™, it was possible to measure the presence of pesticide in droplets of less than 20 µm in diameter on Kromekote™ papers, the amount was sufficient to kill the larvae.

After having simulated several flyovers with planes equidistant to one another at 70 meters, the results obtained show an average deposit of 34 droplets/cm^2, for a coefficient of variation (COV) of 0.47. The VMD and DNM values were, respectively 95 and 33µm. In terms of active ingredient, 10.4 IU/hectare were detected, for a COV of 0.50.The results of trials done into the wind indicate a little overlap of the deposit occurs between spray lines, possibly denoting an overwide swath. With a narrower swath, the COV will decrease and the deposit will increase. For the crosswind trial, the results show that for wind speeds of approximately 8 km/h, practically no active ingredient or droplets were found on the papers or on the Petri dishes. It is difficult to target the product at the appropriate locations whenever very small droplets are released from a plane. Tests should be conducted over the forest in order to verify the deposit of pesticide on fir needles under normal spraying conditions, considering the low level of deposit recorded under crosswind conditions.

Optimization trials for insecticide spraying into small blocks. Mickle and Rousseau. 1998.

Deposit for dyed Foray 48B™ and Foray 76B™ to two 6-hectare (200m x 300m) collocated blocks was compared for two spray strategies. One block was sprayed using a normal application strategy of equally spaced swaths across the block. The other block was sprayed using an optimization technique developed from model (FSCBG, AgDRIFT™) predictions based on application parameters (Cessna 188, aircraft height, 4 Micronair AU4000™ rotary atomizers, drop size distribution) and local meteorology (winds, temperature and relative humidity). The two blocks were arranged as one 15-hectare (500m x 300m) block to allow for continuous spraying across both blocks on equivalent lines thereby reducing meteorological differences during the application. Five trials were realized.

Optimization strategies resulted in multiple passes (up to 6) on a single line displaced up to one half swath upwind of the block boundary. Swath displacement and multiple passes increased with increasing wind speed.

Measured deposit within the optimized block showed increases of 13% to 185% over deposit in the regular block (equal swath spacing). With increased deposit, average COV decreased from 0.64 to 0.43 reflecting the more uniform deposit across the block sprayed using the optimization strategy. Similar results were found for drop density with optimized to regular block ratios ranging from 1.0 to 2.87. Average COV declined from 0.75 in the regular block to 0.41 in the optimized block.

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