LABORATORY BIOASSAY OF EMERALD ASH BORER ADULTS WITH A *BACILLUS THURINGIENSIS* FORMULATION SPRAYED ON ASH LEAVES

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ABSTRACT

The emerald ash borer (EAB) (*Agrilus planipennis*), a buprestid native to Asia that feeds on ash trees (*Fraxinus* spp.), was discovered in southeast Michigan and nearby Ontario in 2002. It apparently arrived in the 1990's via infested solid-wood packing materials from China. As of 2011, areas considered generally infested with EAB in North America include Michigan, Ohio, Indiana, and Ontario, Canada. Additionally, infestations are known in Illinois, Iowa, Kentucky, Maryland, Minnesota, Missouri, New York, Pennsylvania, Tennessee, Virginia, West Virginia, Wisconsin, and Quebec, Canada (www.emeraldashborer.info). Since 2002, we have been studying biological and microbial controls for management of EAB in forested ecosystems of North America.

Select strains of *Bacillus thuringiensis* (Bt), a group of insect-pathogenic bacteria, are active ingredients of host-specific bioinsecticides, several of which are sprayed aerially to control certain forest defoliators. Bt-based insecticides have been used for over 50 years and have an excellent safety record with respect to human health and the environment, a high public acceptance, and good compatibility with other management strategies such as classical biological control. Thousands of Bt strains, each containing different crystalline protein toxins and host ranges, have been isolated from diseased insects, soil, and leaves.

Background of Bt toxicity in EAB adults. We are evaluating the potential of Bt SDS-502 for use as a microbial insecticide to suppress EAB adult populations by aerial application. Bt SDS-502 expresses the Cry8Da insecticidal crystalline protein, which is toxic to some scarab species but not Lepidopotera (Asano et al. 2003). Previously, we reported on results of our research including 1) the toxicity in EAB adults to the native crystal-spore complex, purified Cry8Da protoxin (130 kDa), and the activated Cry8Da toxin (65 kDa); 2) Bt mode of action as observed in electron micrographs of midgut epithelial cells taken from adults during intoxication; 3) lack of toxicity in adult hymenopteran parasitoids important in EAB biocontrol (*Oobius agrili*, *Tetrastichus planipennisi*, *Spathius agrili*, *Atanycolus* spp.); and, 4) EAB adult toxicity following exposure to ash leaves sprayed with an oil-based formulation (Bauer et al. 2009; Bauer and Londoño 2011). Below, we summarized the methods and results of a recent spray trial of a newly developed water-based formulation.

Mortality of EAB adults fed ash leaves sprayed with formulated Bt suspensions. A batch of Bt SDS-502 was grown in a 40,000 L fermentor, concentrated, spray-dried into a technical

powder (TP), and formulated into water-dispersible granules (Bt-WDG). A sample of the Bt-WDG containing 50% TP (Lot #PHY-3-11) was provided by John Libs of Phyllom LLC, the licensor of the Bt SDS-502 (Patent US6962977) for a laboratory-spray trial against EAB adults. The Bt-WDG was suspended in a 10% sucrose solution, vortexed, and a 1-mL aliquot of suspension was pipetted into a sprayer reservoir; the sprayer was a rotary atomizer (Micronair ULVA+), which was used to simulate aerial spray deposits (Dimond 1989). The sucrose functioned as a feeding stimulant. The spray was applied 1-m above 6 to 8 ash leaves (greenhouse-grown *F. uhdei* leaves trimmed to 3 to 4 leaflets/leaf), which were laid horizontally over one-square meter. To keep the leaves fresh, the petiole of each leaf was inserted in a hole punched into the lid of a water-filled 1-ml plastic vial. Water- and oil-sensitive spray paper (Teejet®, Spraying Systems Co., Wheaton, IL) was placed at regular intervals among the leaves to assess spray deposition. After spray, each leaf was placed inside a clear plastic box with three EAB adults (3- to 4-d-old) and held at 24ºC for a 4-d-exposure to the sprayed leaves, at which time the beetles were given fresh, untreated leaves. We used 30 EAB adults for each of the following treatments: three concentrations of Bt-WDG (25, 50 or 100 mg Bt-WDG/ml sucrose solution) and two controls (10% sucrose and WDG-blank formulation). Daily mortality of the EAB adults was monitored for a total of 7 d.

The mortality of EAB adults after 7 d was: 20% at 25-mg Bt-WDG/ml spray; 43% at 50-mg Bt-WDG/ml spray; 50% at 100-mg Bt-WDG/ml spray. Control mortality was 3% for the WDG-blank formulation and 0% for the 10% sucrose. Time to death averaged 4.3 d, and was similar at the three Bt-WDG concentrations. Droplet density and diameter were determined from the spray paper using a dissecting microscope with a micrometer. The droplet density was positively correlated with WDG concentration and highly variable between leaves; e.g. a range of 12 to 265 droplets/cm^2 was determined at the highest Bt-WDG concentration. We categorized the droplet diameters as small (<100 μm, medium (101-200 μm), or large (>201μm). We found the medium-sized droplets were by far the most abundant, averaging 32.1 droplets/cm^2. Droplet density for the small- and large-sized droplets was similar, averaging 3.5 and 6.4 droplets/cm^2, respectively.

Conclusion. After almost a decade of research on EAB and ash in North America, we are getting closer to developing an IPM program to suppress populations of this invasive beetle in forested ecosystems. An essential part of such a program is an environmentally friendly, narrow-spectrum bioinsecticide that can be applied aerially. Previously, we reported on the toxicity of Bt SDS-502 in EAB adults, which feed in the forest canopy on the leaves of ash trees. Laboratory studies have confirmed that Bt SDS-502 is compatible with exotic and native EAB parasitoids, Lepidoptera, and select nontarget insects. EPA registration for Bt SDS-502 is in progress. We are continuing to optimize the efficacy of Bt-test formulations in the laboratory and anticipate the first field trials in 2012.

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