EFFECTS OF *BACILLUS THURINGIENSIS* SDS-502 ON ADULT EMERALD ASH BORER

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ABSTRACT

Emerald ash borer (EAB), *Agrilus planipennis*, an intermittent pest of ash (*Fraxinus*) trees in northeastern Asia, was discovered in Michigan and Ontario in 2002. In North America, infestations of EAB are now known in 13 states and 2 provinces. This invasive buprestid attacks and kills ash trees and has caused the death of tens of millions of trees since its accidental introduction in the early 1990s. Tree mortality is caused by high population densities of EAB larvae feeding in the phloem. Although methods for controlling EAB in high value ash trees using systemic insecticides continue to be developed (Emeraldashborer.info 2010), management options are lacking for ash trees in forested and riparian ecosystems.

Bacillus thuringiensis. In environmentally sensitive habitats, the most widely used insecticides are made from strains of *Bacillus thuringiensis* (*Bt*), an insect-pathogenic bacterium. *Bt* is often used for control of invasive forest insects due to narrow host ranges, good safety records for human health and the environment, high public acceptance, and compatibility with other management strategies such as biological control. Generally, larvae are the target of *Bt* cover sprays; however, EAB larvae are inaccessible to topical sprays because they feed exclusively under tree bark. EAB adults, on the other hand, feed in the canopy on ash leaves and can be targeted by aerially applied foliar sprays. Our goal was to identify a *Bt* strain with sufficient virulence to control EAB adult populations below a tolerance threshold for ash survival (Bauer et al. 2006).

***Bt* toxicity in EAB adults.** In EAB adults, we screened 23 *Bt* strains with toxicity against species of Coleoptera using a droplet imbibement bioassay method. We found *Bt* SDS-502 was the most toxic of the strains tested (Bauer and Londoño 2009). *Bt* SDS-502, which expressed the Cry8Da protein toxin, has known activity in some scarabs, but not Lepidopotera (Asano et al. 2000). Phyllom LLC (Mountain View, CA) retains the exclusive license agreement for this patented strain from S.D.S. Biotech K.K. We confirmed toxicity of *Bt* SDS-502 resulted from native Cry8Da crystals (130 kDa protein protoxin) in EAB adults. After ingestion, Cry8Da is then solubilized by midgut pH and cleaved by midgut proteases to a 65 kDa activated toxin. We found the Cry8Da native crystals, the protoxin, and the activated toxin were equally toxic; denatured Cry8Da was not toxic. The median lethal dose (LD_{50}) of the native crystal-spore complex ranged from 0.16 to 0.35 µg of toxin (Bauer and Londoño 2009).

***Bt* intoxication in EAB adults.** During fermentation, *Bt* expresses one or more insecticidal crystal proteins or Cry toxins, which have varying levels of specificity to different insect groups. Upon ingestion by a susceptible insect, Cry toxins cause pores to form in the brush border membrane of midgut epithelial cells, resulting in cellular swelling and lysis; mortality by septicemia may result as bacteria invade the body cavity. To determine the mode of action of *Bt* SDS-502 in EAB, adult beetles were inoculated *per os* with 0.5 µL crystal-spore suspension containing 1µg Cry8Da toxin; control beetles were similarly dosed with water.

After 6 hours, the midguts from treated and control beetles were dissected, and the tissues fixed and embedded for transmission electron microscopy. We observed that Cry8Da caused similar ultrastructural damage in EAB adult midguts as observed for other *Bt* toxins in susceptible insect hosts, confirming a similar mode of action.

Lethal and sublethal effects of formulated Bt SDS-502 sprayed on leaves. Micron's ULVA+ hand-held, low volume sprayer was used to simulate aerial application. A 1-ml aliquot of *Bt* formulation or blank formulation was injected into the spray nozzle with a pipettor, which facilitates reproducible transfer of a known quantity spray into the nozzle. A spray room (1 m^2) was used to confine spray to the target area to allow comparisons between replicate spray assays and to compare formulations. Spray deposition on the excised ash leaves was measured using Teejet® water and oil sensitive spray paper (Spraying Systems Co, Wheaton, IL) placed at regular intervals between the leaves. The ash leaves were placed in a circle (8 to 10 leaves) along with four spray cards. The spray was applied 1 m above the leaves. After the leaves were sprayed, petioles were inserted into water vials, placed inside clear plastic boxes with three EAB adults per box, held in an incubator at 24ºC, and monitored daily for mortality for 5 to 7 days.

Bt SDS-502 paste was grown in a 200 L fermenter and spray-dried into technical powder; aliquots were formulated into flowable concentrates (FC) using paraffinic oils and other proprietary ingredients. Test formulations contained either 12 percent or 24 percent *Bt* SDS-502 technical powder. The mortality of EAB adults exposed to *Bt* SDS-502 occurred within 96 hours of feeding on sprayed leaves, and total mortality was similar for both *Bt* concentrations: 78 percent mortality for the 12 percent-*Bt* FC vs. 89 percent for the 24 percent-*Bt* FC. However, 39 percent of EAB adults died after 96 hours of exposure to the blank formulation, suggesting about half of the mortality resulted from the oil-based formulation alone. No significant sublethal effects of *Bt* SDS-502 on EAB adults surviving foliar sprays were found, although adult longevity and fecundity tended to be lower among the *Bt*-survivors than for controls.

This strain has much promise for controlling EAB using aerial applications; however, more research is needed on formulation and stabilization of a final product.

Literature Cited

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