BACILLUS THURINGENIENSIS: POTENTIAL FOR MANAGEMENT OF EMERALD ASH BORER

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ABSTRACT

The active ingredients of microbial insecticides are live microorganisms pathogenic to certain insects. One such insect pathogen is *Bacillus thuringiensis* (Bt), a bacterium found naturally in soil, on leaves, in places were insects are abundant (such as grain silos and insectaries), and in infected insects. Bt-based microbial insecticides are formulated with spores and materials produced during fermentation, including crystalline insecticidal proteins—also known as Cry toxins. After ingestion, these Cry toxins are responsible for the majority of Bt's insecticidal activity and narrow host range. Cry toxicity results in damage to the insect midgut, followed by bacterial septicemia and death. As of 1997, ca. 190 Bt-based microbial insecticides were registered in the United States for control of certain lepidopteran, dipteran, and coleopteran pests. There are also Bt strains active against species of Homoptera, Hymenoptera, Orthoptera, Mallophaga, nematodes, mites, and protozoa. Microbial insecticides formulated with Bt are the primary management tools used to control forest insect pests due to their narrow host range, superior safety record, public acceptance, and compatibility with other management strategies such as biological control (Reardon et al. 1994).

In 2003, we initiated research to identify Bt strains active against EAB. Initially, we screened emerald ash borer (EAB) adults with four Bt-based microbial insecticides registered for control of lepidopteran and/or coleopteran pests. Although the products showed some activity against adult EAB, rates 4 to 12 times maximum labeled rates were required to achieve 66 to 98% mortality six days after treatment (Bauer et al. 2004).

To identify fractions responsible for EAB mortality in the Bt products, we bioassayed EAB adults with crystalline Cry3Aa from coleopteran-active *B. thuringiensis* subsp. *tenebrionis* (Btt); spore/crystal complex from lepidopteran-active *B. thuringiensis* subsp. *kurstaki* HD-1 (Btk); solubilized protein from Btk; soluble Cry1Aa, Cry1Ab, or Cry1Ac toxins cloned from Btk and grown in *E. coli*; spores of Bt 4Q22, an acrystalliferous strain; and toxin mixtures.

These were bioassayed by allowing EAB adults to imbibe droplets containing known quantities of toxin and/or spores; daily mortality was assessed for up to seven days. Neither Cry3Aa (30 µg toxin/dose) nor the Cry1A toxins (3 µg toxin/dose), dosed separately or as mixtures, were toxic to EAB adults. Bioassay of Btk crystal/spore complex (ca. 3 µg protein + $1.3x10^4$ spores/dose) in EAB adults resulted in ca. 78% mortality, whereas solubilized Btk proteins (3 µg protein/dose) caused 25% mortality. To ascertain the role of spores, we treated EAB adults with Bt 4Q22 ($7.8x10^2$ spores + 5 µg protein), a strain lacking Cry toxins; 63% of adults died within seven days. We then treated EAB adults with zwittermicin A (20 µg solubilized crude zwittermicin/dose), a spore-associated antibiotic produced by some Bt strains (including Btk HD-1); 100% of EAB adults died within six days. Although direct toxicity of zwittermicin in insects has not been reported, it is known to synergize Btk activity in gypsy moth (Broderick et al. 2000). Overall, these findings support the importance of Bt spores and/or other fermentation products in EAB mortality, as reported for several species including gypsy moth (Dubois and Dean 1998).

We are now focusing our research efforts on discovery of a Bt strain with high toxicity against EAB adults and a narrow host range. Initial bioassays will involve ca. 30 select strains, many with coleopteran toxicity, acquired from culture collections. We are also culturing Bt strains from EAB cadavers and ash leaves collected in southeastern Michigan in 2005. If we are successful, a new microbial insecticide will be developed and registered for management of EAB in forests and woodlots for ash tree preservation.

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