Genetically Engineering Resistance to the Emerald Ash Borer

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Abstract

An aggressive, exotic pest from Asia, the Emerald Ash Borer (EAB; *Agrilus planipennis* Fairmaire), first identified in Michigan in 2002, has now been detected in Ontario, Ohio, Maryland, Virginia, Windsor, and Indiana. All ashes (*Fraxinus* spp.) native to U.S. are susceptible (they have no innate resistant genes) and there are no known or chemical means for controlling the EAB (Fig. 1). In order to prevent its spread, large areas are under strict quarantine to prevent the transportation of ash products (*e.g.*, trees, branches, nursery stock, logs, and firewood).

We plan to genetically engineer white ash (*Fraxinus americana* L.) to be completely resistant to the EAB by using a gene from *Bacillus thuringiensis* (*Bt*). This bacterium produces a small protein that is toxic only to insects. There are hundreds of strains of *Bt*, each of which produces a unique toxin. Toxin produced by a given strain is generally only lethal to a narrow group of insects. Non-target effects are avoided because an insect must eat tissue from a plant into which the *Bt* gene has been inserted in order for the toxin encoded by the gene to have an effect.

Before producing transgenic trees, it is imperative to determine the *Bt* toxin to which the target insect is susceptible. To accomplish this *in vitro* screening, we developed a synthetic diet on which EAB adults and larva could be reared in a controlled environment. We then exposed the adults and larva to a variety of purified *Bt* toxins to determine which is effective. We will now begin assembling a genetic construct containing the corresponding *Bt* gene so that it can be transformed into white ash using an *Agrobacterium*-mediated protocol.

Hypothesis

We can genetically engineer white ash to be completely resistant to the Emerald Ash Borer (EAB) by using a gene from *Bacillus thuringiensis* (*Bt*).

Objectives

- Develop a suitable diet to rear the EAB in a controlled environment
- Expose the EAB to various *Bt* proteins to determine the toxin to which it is susceptible
- Assemble a binary vector for the appropriate *Bt* gene
- Develop a transformation and regeneration system for white ash
- Confirm *Bt* gene insertion and field-test for resistance of transgenics to the EAB

Figure 1. Ash tree infested with EAB.

Materials and Methods

Adult male and female insects were reared in a controlled environment, where they were fed fresh ash leaves and allowed to mate. Twice weekly eggs were transferred to a separate culture dish. The eggs were incubated in a temperature-controlled room until they hatched (after about two weeks).

Purified *Bt* toxins were supplied by Dow AgroSciences. To screen the *Bt* toxins, adults were fed one to two drops of various *Bt* toxin solutions. Newly hatched larvae were housed individually in 96-well plates along with the appropriate medium in which the toxin was incorporated.

The pART27 backbone will be used to assemble a binary vector containing the *Bt* gene encoding an effective toxin and the *GUS* reporter gene. It will also contain the neomycin phosphotransferase (*NPTII*)

selectable marker gene driven by the cauliflower mosaic virus (*CaMV*) 35S promoter.

We will transform the *Bt* gene into white ash using *Agrobacterium tumefaciens*. Transgenic plants will be regenerated *in vitro* via direct shoot organogenesis using the protocol of Bates *et al.* (1992). Explants from putative transgenic lines will be screened for GUS activity by histochemical assay. Stable transgene insertion will be verified using the polymerase chain reaction (PCR), and transgene copy number will be determined via Southern blotting. Finally, we will plant the *Bt* ash trees in areas where there is high EAB pressure to determine the level of resistance imparted by the transgene.

Results

- Developed a suitable diet for EAB larva (Figs. 2, 3, 4, and 5).

Figure 2. Diets #4, #5, and hylobius.

Figure 3. Diet #5 and hylobius.

Figure 4. EAB eggs on ash bark.

Figure 5. Neonate emerging from egg.

- Identified a *Bt* protein that was lethal to control EAB (Figs. 6, 7).

- Begun micropropagating white ash *in vitro* from mature-embryo explants (Fig. 8).

Figure 6. *Bt* bioassay. Three-day-old larvae in 96-well plate. Wells contain one part *Bt* powder in 10 parts diet.

Figure 7. *Bt* bioassay. Three-day-old larvae in 24-well plate. Wells contain one part *Bt* powder in 10 parts diet.

Figure 8. 4-week-old white ash embryos.

Literature Cited

Bates, S., J.E. Preece, N.E. Navarrete, J.W. Van Sambreek and G.R. Gaffney. 1992. Thidiazuron stimulates shoot organogenesis and somatic embryogenesis in white ash (*Fraxinus americana* L.). Plant Cell, Tissue and Organ Culture 31:21-29.

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