EXPLORING THE MOLECULAR AND BIOCHEMICAL BASIS OF ASH RESISTANCE TO EMERALD ASH BORER

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Larvae of the emerald ash borer (EAB) (*Agrilus planipennis*) feed on phloem of ash (*Fraxinus* spp.) trees. It is hypothesized that the resistance of Asian species of ash (e.g., Manchurian ash, *F. mandshurica*) to EAB is due to endogenous defenses present in phloem tissues in the form of defensive proteins and/or toxic secondary metabolites. We have taken a proteomic and metabolomic approach to understand the interactions between EAB larvae and ash phloem that are fundamental to elucidating mechanisms of resistance to EAB.

To identify proteins that may be responsible for resistance of Manchurian ash to EAB, we used Differential in Gel Electrophoresis (DIGE) to compare its constitutive phloem proteome with that of the closely related but highly susceptible North American black ash (*F. nigra*). Differentially expressed proteins were sequenced using nano-liquid chromatography-mass spectrometry (MS)/MS, and putative functions were assigned. Hierarchical clustering of 355 phloem proteins with concentrations that differed substantially (>twofold, $P < 0.05$) between black and Manchurian ash revealed protein expression patterns that were nearly diametrically opposed, despite the very close phylogenetic relationship between the two species. Proteins in Manchurian ash that may be associated with resistance include enzymes involved in the synthesis of lignans and flavonoids, oxidative enzymes, a PR-10 protein, and an aspartic protease. Future functional studies are needed to identify what roles these proteins may play, if any, in resistance of Manchurian ash to EAB.

Chemical characterization of phloem tissue of North American (green, white, black, blue), European (*F. excelsior*), and Manchurian ash revealed qualitative similarities in the constitutive phenolic profiles of black, European, and Manchurian ash, while individual compounds differed quantitatively. Phloem chemistry of green and white ash was similar qualitatively and quantitatively, as reported by Eyles et al. (2007). The phenolic profile of blue ash comprised mainly hydroxycoumarins and showed no similarity to the other species. The similarities and differences in phenolic profiles among species corresponded with their phylogenetic relationships (Wallander 2008).

The ability of methyl jasmonate (MeJA) (an endogenous plant stress hormone that regulates defense responses) to induce ash phloem chemistry was also investigated by applying 1 M MeJA to the outer bark of Manchurian and white ash. Treatment with MeJA increased concentrations of total phenolics in both ash species, suggesting that induced and constitutive phenolic chemistry may play a role in EAB resistance. These studies provide a foundation for ongoing investigations on the role phenolics may play in ash resistance to EAB. Discovery of resistance-related proteins and secondary metabolites that confer resistance to EAB would accelerate breeding and /or selection of resistant trees for restoration of ash to natural and urban forests.

LITERATURE CITED

Eyles, A.; Jones, W; Riedl, K; Herms, D.A.; Cipollini, D.; Schwartz, S.; Chan, K.; Bonello, P. 2007. **Comparative phloem chemistry of Manchurian (*Fraxinus mandshurica*) and two North American ash species (*F. americana* and *F. pennsylvanica*).** Journal of Chemical Ecology. 33: 1430-1448.

Wallander, E. 2008. **Systematic of *Fraxinus* (Oleaceae) and evolution of dioecy.** Plant Systematics and Evolution. 273: 25-49.