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Daniel A. Herms

Don Cipollini
Wright State University - Main Campus, don.cipollini@wright.edu

K. S. Knight

J. L. Koch

T. M. Poland

See next page for additional authors

Authors
Daniel A. Herms, Don Cipollini, K. S. Knight, J. L. Koch, T. M. Poland, Chad Michael Rigsby, Justin G.A. Whitehill, and Pierluigi Bonello

THE QUEST FOR ASH RESISTANCE TO EMERALD ASH BORER: TOWARDS A MECHANISTIC UNDERSTANDING

D. A. Herms[1], D. Cipollini[2], K. S. Knight[3], J. L. Koch[3], T. M. Poland[4], C. M. Rigsby[2], J. G. A. Whitehill[5,6], and P. Bonello[5]

[1]Department of Entomology, The Ohio State University, 1680 Madison Ave., Wooster, OH 44691
[2]Department of Biological Sciences, Wright State University, 3640 Colonel Glenn Highway, Dayton, Ohio 45435
[3]USDA Forest Service, Northern Research Station,359 Main Rd., Delaware OH 43015
[4]USDA Forest Service, Northern Research Station, 1407 S. Harrison Rd., East Lansing, MI 48823
[5]Department of Plant Pathology, The Ohio State University, 2021 Coffey Rd., Columbus, OH 43210
[6]Current address: Michael Smith Laboratories, The University of British Columbia, 301-2185 East Mall, Vancouver, BC Canada, V6T 1Z4

ABSTRACT

Since emerald ash borer (EAB), *Agrilus planipennis*, was discovered in North America in 2002, it has killed many millions of ash trees in North America, and ash mortality now exceeds 99% near the epicenter of the invasion in southeast Michigan (Klooster et al. 2014). The development of EAB-resistant ash trees will be critical for restoration of ash in natural and urban forests. Goals of our collaboration are to identify, breed and screen ash germplasm for EAB resistance and silvicultural traits; and identify mechanisms of EAB resistance to facilitate breeding and screening.

In Asia, EAB does not devastate its endemic hosts, which suggests that Asian ashes are inherently resistant by virtue of their coevolutionary history with EAB. In a common garden study, patterns of ash decline and mortality were largely consistent with this hypothesis. Manchurian ash had the highest rate of survival and little canopy decline. The high EAB resistance of this Manchurian ash population of seedling origin is consistent with that observed previously for the clonal Manchurian ash cultivar 'Mancana' (Rebek et al. 2008), which suggests that EAB resistance is a species-level trait. *Fraxinus* x 'Northern Treasure' ash, which is a Manchurian (Asian) x black ash (North American) hybrid, had similarly high survival and low canopy decline, suggesting introgression of Manchurian ash resistance genes into the hybrid. However, this pattern contrasts sharply with that observed by Rebek et al. (2008), who found 'Northern Treasure' ash to be highly susceptible to EAB. One potential explanation for these divergent conclusions is that there may be taxonomic confusion surrounding this cultivar that needs to be resolved.

Most North American species and cultivars in the common gardens study experienced complete or nearly complete mortality, with green ash cultivars, black ash, and Oregon ash declining more rapidly than white ash cultivars. Blue ash has survived at a higher rate in the common garden than other North American species, but by 2014 had lower survival and greater canopy decline than Manchurian ash. Decline and mortality of blue ash has increased over time, suggesting that surviving trees in the plot may ultimately succumb to EAB. The European species and cultivars evaluated in the common garden also experienced high decline and mortality, including *F. ornus*, *F. excelsior* 'Aureafolia', and *F. angustifolia* subsp. *oxycarpa* 'Raywood,' which suggests that EAB has the potential to cause widespread economic and ecological impacts in Europe as it continues to spread in Russia and beyond.

Because the devastating impact of EAB on its host is due to larval feeding, we have focused much of our research on mechanisms that affect larval density, growth, and survival, including female oviposition preferences and phloem chemistry. In field studies, we evaluated the role of female oviposition preference as a determinant of interspecific variation in ash resistance to EAB (Rigsby et al. 2014). The "mother knows best" hypothesis predicts that ovipositing females should choose hosts on which their offspring will best perform, maximizing their own fitness. We found that susceptible green, white, and black ash consistently received more eggs than resistant Manchurian ash, and that preference for ash species was independent of tree size and vigor. These observations are consistent with the "mother knows best" hypothesis (Rigsby et al. 2014), and suggest that oviposition preferences may contribute to interspecific patterns of host resistance observed in previous studies (e.g. Rebek et al. 2008, Whitehill et al. 2012).

Eyles et al. (2007) identified constitutive phenolic compounds in phloem of the resistant Manchurian ash cultivar 'Mancana' that were not present the more susceptible green and white ash, including several hydroxycoumarins and two phenylethanoids, and suggested that they might represent potential EAB resistance mechanisms. However, in subsequent studies in which species comparisons were more phylogenetically controlled, these compounds were detected in highly susceptible black and European ash at concentrations comparable to or higher than in the closely related Manchurian ash (Whitehill et al. 2012). This strongly suggests that these hydroxycoumarins and phenylethanoids are, in fact, not responsible for the high resistance of Manchurian ash. The pinoresinol dihexoside and a tentatively identified coumarin derivative were the only phenolic compounds detected that were unique to Manchurian ash. Recent experiments have shown, however, the phenolic profile of Manchurian ash much more highly bioactive than that of black ash when consumed by EAB larvae, as they were oxidized to a greater degree by enzymes such as peroxidases that are expressed at higher levels in Manchurian ash. As a result, midgut tissues of larvae feeding on Manchurian ash experienced much higher levels of oxidation stress than did larvae feeding on black ash, even though the phloem phenolic profile of the two ash species is similar (Whitehill et al. 2012). In a comparative study of phloem proteomes of resistant and susceptible species, we found that other proteins implicated as defenses in other species were also constitutively over-expressed in Manchurian ash relative to green, white, and black ash, and thus might also contribute to high EAB resistance of Manchurian ash. These include a PR-10 protein, phenylcoumarin benzylic ether reductase, an aspartic protease, and ascorbate peroxidase (Whitehill et al. 2011).

As another approach towards identifying resistance mechanisms of Manchurian ash, experimental manipulations were used to alter performance of EAB larvae, and to relate larval performance to associated phytochemical changes in the phloem. Manchurian and black ash were exposed to variable water regimes and challenged with egg inoculations to elicit induced responses to larval feeding (Chakraborty et al. 2014). Growth of EAB larvae was lower on Manchurian ash, which provides evidence that antibiosis, as well as ovipositional non-preference, contributes to its high EAB resistance. EAB larval feeding induced higher concentrations of pinoresinol A in Manchurian than black ash, which may also contribute to higher resistance. Larvae grew faster on drought stressed Manchurian ash trees, which is consistent with the role of EAB as a secondary colonizer of stressed trees in its native habitat.

In another study, methyl jasmonate was applied to susceptible North American and resistant Asian ash species to determine if it can elicit induced responses in bark that enhance resistance to EAB (Whitehill et al. 2014). MeJA application decreased adult emergence in susceptible ash species to levels achieved by insecticide application. Concentration of the phenolic compound verbascoside sharply increased after MeJA application to green and white ash. When incorporated in an artificial diet, verbascoside decreased survival and growth of EAB neonates in a dose-dependent fashion. Lignin and trypsin inhibitors were also induced by MeJA, and analogs of both compounds reduced growth of EAB larvae in artificial diets (Whitehill et al. 2014). Application of MeJA prior to EAB attack may have potential for enhancing resistance of susceptible ash species by inducing increased concentrations of verbascoside and other defensive compounds (Whitehill et al. 2014).

We have initiated a breeding program based on hybridization of resistant Asian ash and susceptible North American ash (following the successful chestnut blight breeding program) (Koch et al. 2012), and have begun screening rare native genotypes that continue to survive ("lingering ash") where overall ash mortality is very high to see if they are truly resistant (Knight et al. 2012, 2013). We have made progress in overcoming barriers to hybridization of Asian and North American ash (Koch et al. 2012), and several extensive common garden plantations have been established in Delaware and Wooster, Ohio to evaluate EAB resistance and silvicultural characteristics of additional Asian species, Asian-North American hybrids, and lingering ash selections for use as parental lines to expand the breeding program. We have also developed bioassays to screen young plants for resistance, which will expedite breeding activities.

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