In-situ Genetic Conservation of White Ash (*Fraxinus americana*) at the Allegheny National Forest[1]

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Abstract

The emerald ash borer (EAB, *Agrilus planipennis*) is a non-native forest pest that has been sweeping across North America causing widespread mortality of trees in the genus *Fraxinus*, which includes the economically valuable white ash (*F. americana*). The rapid spread and lethality of EAB, paired with low levels of natural resistance in ash trees, has left forest managers with few management options to slow EAB or to conserve ash trees. Here we present the initial findings of a collaborative project to pursue regional genetic conservation of white ash trees across the Allegheny National Forest. The network of white ash conservation plots consists of 29, 3.24 ha (8 ac) plots distributed across the forest, each containing a subset of 20 ash trees that received insecticidal treatment with emamectin benzoate trunk injections. This design will allow us to test for associational protection of non-insecticide treated trees with treatment levels varying from 10 to 91 percent (i.e., proportion of protected ash trees in a stand). In conjunction with the ash conservation project, we monitored ash tree canopy health from 2010 (prior to the arrival of EAB) to 2015 across 193 permanent plots in the Allegheny National Forest. Following the arrival of EAB to the Allegheny National Forest in 2013, we conducted a follow up survey of ash canopy health in 2015 and discovered further canopy decline in both upper and lower slope positions, likely caused by EAB. Furthermore, canopy traps revealed that EAB, which was first discovered in the southern region of the forest in 2013, had now spread to the northern region.

Introduction

Native and non-native invasive forest pests represent considerable threats to host species and their associated forest ecosystems (Flower and Gonzalez-Meler 2015). Long-term persistence of affected species, as well as the maintenance of forest diversity, productivity and associated ecosystem services, is predicated on conserving susceptible individuals and populations of at risk species across the landscape. Furthermore, conservation practices that maximize the genetic diversity of the residual population and optimally mimic that of the initial population are preferred.

The emerald ash borer (EAB, *Agrilus planipennis*) is an invasive beetle which was inadvertently introduced into North America from Asia in the 1990s (Siegert et al. 2014). It feeds almost exclusively on ash trees (*Fraxinus* spp.) which are widely distributed across urban and forest environments of North America (MacFarlane and Meyer 2005). The widespread distribution of ash, coupled with EAB's rapid dispersal, has contributed to its swift invasion across the United States. Larval feeding of EAB creates serpentine galleries that girdle host trees, resulting in mortality in >99 percent of trees (Flower et al. 2013, Knight et al. 2014). Tree mortality and local ash population collapses occur in as few as 2 to 5 years. Because of the high degree of ash tree mortality, the future re-establishment of ash depends on post-EAB

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[1] A version of this paper was presented at the Gene Conservation of Tree Species – Banking on the Future Workshop, May 16-19, 2016, Chicago, IL

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seed germination and seedling recruitment. Furthermore, although EAB populations crash following host mortality, EAB populations subsist at low densities for years after canopy mortality, potentially threatening a recruited seedling and sapling cohort. Extirpation of the genus *Fraxinus* further threatens the diversity of temperate forests of the eastern United States, which are simultaneously threatened by a variety of other forest pests and pathogens. *In-situ* conservation approaches for maintaining ash genetic diversity across the landscape are essential for maintaining biodiversity and forests resilient to disturbances.

We are currently engaged in a collaborative project to examine the efficacy of insecticidal treatments of white ash (*F. americana*) trees as a conservation strategy to manage forests affected by the emerald ash borer. The goals of the project are to:

1. Provide *in-situ* conservation of ash genetic diversity on the Allegheny National Forest (ANF), Pennsylvania, through the treatment of a subset of 20 ash trees in each of 29, 3.24 ha (8 ac) plots across the forest (a total of 580 treated trees).
2. Test treatment efficacy across a range of conditions including initial tree health (ranging from healthy to some dieback), landscape positions (upper vs. lower slope), and across a range of ash densities (ranging from 21 to 201 ash trees per plot).
3. Test for associational protection of untreated ash trees in treatment plots across a range of ash densities. We hypothesize that, like herd immunity in vaccination, treating a high proportion of trees will provide some protection to untreated trees.
4. Monitor landscape-scale progression of EAB, ash mortality, and EAB population dynamics throughout the forest.

Methods

In 2010, prior to the arrival of EAB, we established a network of 193 ash health monitoring plots across the ANF. Plots were distributed across both upper and lower slope conditions allowing investigations into differential decline patterns associated with soil weathering and abiotic parameters. Using a 1 to 5 categorical scale modified for ash trees by Smith (2006), the canopy health of ash trees was assessed (post leaf expansion) to capture pre-EAB ash canopy health conditions. Canopy condition ratings were as follows: 1 represents a healthy tree with no defoliation; 2 represents a canopy with slight reduction in leaf density; 3 represents a canopy that is thinning and some of the top branches exposed to sunlight are defoliated (<50 percent dieback); 4 represents a canopy with >50 percent dieback; and 5 represents a dead tree with no leaves remaining in the trees canopy (see Flower et al. 2013 and Knight et al. 2014 for more details). The EAB was subsequently confirmed on the forest in June 2013. During the summer of 2015, the plots were re-measured to track the progression of canopy decline and its relationship with EAB.

In 2015, we began an insecticidal treatment study to conserve the genetic diversity of white ash on the ANF. We treated a subset of 20 ash trees per plot across 29 plots with emamectin benzoate stem injections, a systemic insecticide proven to provide multiple years of protection to healthy tress and those in moderate stages of canopy decline (Flower et al. 2015, Herms et al. 2014). All ash trees within each 100 m radius plot were measured, rated for canopy condition, and trees were randomly selected for insecticide treatment. Ash density in treatment plots ranged from 21 to 201 trees. Thus, treatment of 20 trees in each of these plots yielded a range of proportions of treated trees from 10 to 91 percent, allowing for a robust design to test for associational protection of untreated trees. We expect that untreated trees may benefit from the toxicity of their treated neighbors, and this design should determine what proportion of treated trees it may take to see these benefits. Finally, purple panel traps glued with tangle foot and baited with Manuka oil lures were deployed in a subset of insecticide treatment plots (n = 12 plots, two traps/plot) to track the distribution of EAB throughout the forest (fig. 1).

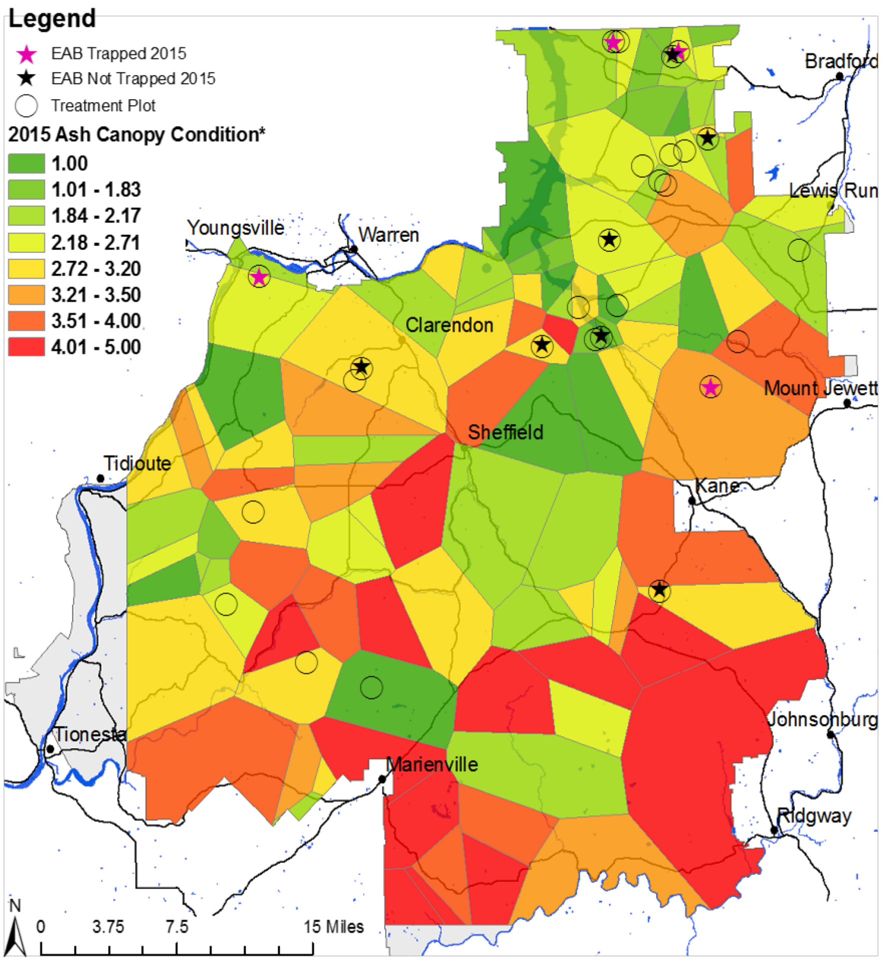

Figure 1—Map depicting the ash canopy health across the Allegheny National Forest, green to red gradient corresponds with ash canopy condition of 1 to 5 (healthy-dead). Open circles denote treatment plots and stars denote locations where emerald ash borer was trapped (pink) and not trapped (black).

In order to investigate ash canopy decline between 2010 and 2015, a repeated measures analysis of variance (RM ANOVA) was utilized with lower and upper slope positions as a main factor and ash canopy conditions in 2010 and 2015 as the repeated measure. This test was conducted using SYSTAT v. 12 statistical software (SYSTAT 2007).

Preliminary Results and Conclusions

Trapping efforts from 2015 revealed that, since its discovery in 2013 in the southern region of the forest, EAB is continuing to spread across the ANF. EAB trapping was confined to the central and northern portion of the ANF and detected EAB in four treatment plots (fig. 1). Despite the presence of EAB in these areas, ash canopies remain healthy, indicating EAB's recent arrival to the region.

Ash canopy deterioration is more severe and widespread in the southern ANF, which was expected based on the discovery of EAB prior to 2013 in counties adjacent to the southern extent of the forest (fig. 1). Additionally, it appears that canopy decline is more advanced in areas along roads and towns as predicted by the vehicle hitch-hiking spread mechanism proposed by Prasad et al. (2010). The 2010 ash survey revealed declining canopies on the upper slopes attributed to foliar nutrient deficiencies associated with base cation leaching from soils (Royo and Knight 2012; fig. 2). This difference between the canopy health of ash trees in the lower and upper canopies is consistent between time periods, with the lower slope canopies exhibiting healthier canopies relative to upper slope positions (RM ANOVA, $F = 20.527$, $P < 0.001$). The 2015 survey indicates continued canopy decline of ash across the ANF (RM ANOVA, $F = 121.272$, $P < 0.001$; fig. 2).

Efforts are underway to collect ash foliage from across the ANF to estimate population genetics parameters (using genus specific microsatellite markers) and to ascertain the proportion of ash genetic diversity that the insecticide treatments are conserving. Continued insecticide applications will be conducted to conserve the genetic diversity of ash. Additionally, EAB trapping across the ANF will continue in order to monitor EAB populations. Based on these and future findings, we will make recommendations to managers regarding the efficacy of emamectin benzoate injections on trees with varying initial canopy health. These results will provide insights into regional conservation efforts of tree species in decline from invasive forest pests.

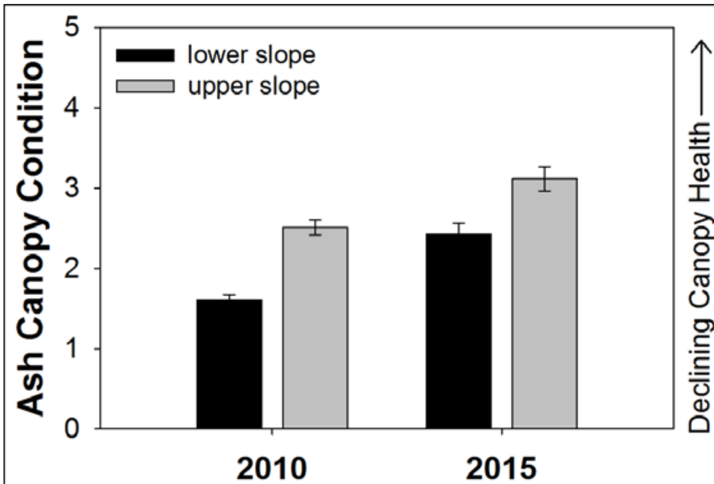

Figure 2: Ash canopy condition decline across control plots between 2010 and 2015 in both lower (black) and upper (grey) slope positions.

Acknowledgments

We thank Tim Fox and Matt Peters for their assistance with GIS, Kristen Giger of the National Wild Turkey Federation for her assistance with plot establishment, and Dan Herms and Erin O'Brien for their input on the initial experimental design. Finally, this research would not have been possible without funding and support from the U.S. Department of Agriculture, Forest Service, Northern Research Station, and U.S. Department of Agriculture State and Private Forestry.

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