AILANTHUS ALTISSIMA INTERFERS WITH BENEFICIAL SYMBIONTS AND NEGATIVELY IMPACTS OAK REGENERATION1

Jenise M. Bauman2, Caitlin Byrne and Shiv Hiremath

Abstract: The invasion of Tree-of-Heaven (Ailanthus altissima) has been documented in disturbed landscapes leading to biodiversity loss and degradation of ecosystem function. Ailanthus interferes with the restoration of native species by its aggressive growth habit, alteration of nutrient cycles, and allelopathic chemical production. Recent studies suggest that allelopathy has a negative effect on the growth of red oak (Quercus rubra), possibly by interfering with the symbiosis of beneficial ectomycorrhizal fungi (ECM). This fungal symbiont is essential for healthy tree growth and the unavailability of these fungi may impede the success of seedling regeneration. This study investigated the effects of Ailanthus on biomass production and ectomycorrhizal fungal (ECM) colonization of red oak (Q. rubra) seedlings on a reclaimed coal mine site in eastern Ohio. Six plots were designated in an existing riparian buffer zone in a wetland at The Wilds Conservation Center in Muskingum County. Three of the plots were in an area where mature Ailanthus was present. The other three plots were located in the same riparian zone that was without Ailanthus. Naturally regenerating two-year-old red oak seedlings were selected for study (10 seedlings per plot, 60 seedlings total). The oak seedlings were sampled for biomass (g) and ECM root colonization. Two-year-old oak seedlings growing among mature Tree-of-Heaven produced significantly less biomass, specifically in root production, than the oaks growing without the invasive tree (P = 0.02). There was a decrease in ECM colonization (P = 0.001) and a shift in ECM community composition in plots where the Tree-of-Heaven was present (P = 0.0004). The increase in root biomass and ECM colonization may aid in the plant’s competitive ability for belowground resources, important for reestablishment. These data suggest that areas impacted by the invasion of Tree-of-Heaven may require restoration with plant species less reliant on ECM colonization when planting in soils immediately following invasive species removal.

Additional Key Words: native tree generation, riparian buffer restoration.

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**Introduction**

Riparian buffer areas form a transitional zone between upland habitat and streams, rivers, lakes, floodplains, and wetlands (Naiman et al., 2005). They are comprised of trees, woody shrubs, herbaceous perennials, annuals, grasses, and sedges that function as a protective barrier for aquatic ecosystems. The different rooting depths of the various plant groups control how water moves through the buffer zone and into the various aquatic habitats. The vegetation slows water flow and allows for sediments and other associated pollutants to be intercepted and filtered (Mitsch et al., 2001). Roots of the riparian vegetation also stabilize stream banks by preventing erosion, which also protects aquatic niches and related organisms from being destroyed by sedimentation (Bennett and Simon, 2004). Healthy riparian buffer zones are comprised of many plant species that contribute to ecosystem function, species biodiversity, healthy food webs, and breeding grounds for native wildlife.

There is a long history of wetland loss and riparian degradation in the U.S. that continues into the present day (Dahl, 2011). The state of Ohio is estimated to have lost the greatest percentage of its wetlands, 90% during the period of 1790s to 1980s (Dahl, 1990). Disturbances caused by draining, dredging, filling, and mining for coal disturb the groundwater and facilitate the conversion of wetland and riparian zones to upland soils (Mitsch and Gosselink, 2007). These disturbances invite the introduction of invasive plant species, particularly on mine lands where reclamation practices have established aggressive source populations. Further, diminished hydrology and loss of regular flooding creates homogenous non-native vegetation in place of the diverse plant community that once occupied the original riparian zone (Howe and Knopf, 1991). Non-native plant species have impacted thousands of hectares of Ohio’s natural areas causing changes to nutrient cycles, water tables, plant community, and wildlife habitat (Wedin and Tilman, 1996; Pritekel et al., 2006; Vaness and Wilson, 2007).

The invasion of *Ailanthus altissima* (Tree-of-Heaven) has been documented in the global landscape. Native to China, this tree was first introduced to the eastern U.S., from Europe, as an ornamental plant in the late 1700s (Feret, 1985). The invasion of this species causes biodiversity loss and degradation of ecosystem function, particularly in riparian buffer zones which require a diverse mix of species for functional water filtration. *Ailanthus* interferes with the regeneration of native species by its aggressive growth habit, alteration of nutrient cycles, and allelopathic
chemical production (Singh et al., 2003). Recent studies demonstrate that *Ailanthus* allelopathy has a negative effect on the growth of native red oak (*Quercus rubra*) (Gomez-Aparicio and Canham, 2008a). The authors speculated that components other than allelopathy, such as essential microbial symbionts that influence nutrient acquisition, may have played a role in oak growth suppression (Nilsson et al., 1993). *Ailanthus* effects are species-specific and do not impede the growth of certain non-mycorrhizal plants (Cipollini et al., 2008) or arbuscular mycorrhizal (AM) plant species such as red maple (*Acer rubrum*) (Gomez-Aparicio and Canham, 2008b). In addition, *Ailanthus* is an AM fungi host tree species (Huebner et al., 2007) and its allelopathic compounds may not be detrimental to the survival of certain AM fungi in the soil.

Ectomycorrhizal (ECM) fungi are the primary microbial symbionts important for the establishment of many forest tree taxa including Betulaceae, Fagaceae, Pinaceae, and Salicaceae (Smith and Read, 2008). These fungal symbionts are essential for healthy tree establishment and growth, particularly when using late successional species in restoration (Marx, 1991; Cordell et al., 1999). ECM fungi form characteristic root sheaths around the host plant root and produce radiating hyphae that allow for greater access to nutrients and water. Allelopathic compounds produced by certain invasive species can inhibit fungal colonization of tree roots and may impede the success of seedling regeneration and establishment (Wolfe et al., 2008). The primary objective of this study was to investigate the effects *Ailanthus* has on ECM fungal colonization of red oak (*Q. rubra*) seedlings in a reclaimed riparian forest patch located on a restored coal mine site. *Ailanthus* has been used experimentally as a restoration tree on mine sites, will quickly recruit in recently disturbed landscapes, and has been reported to invade older restored and remnant stands (described in Peugh et al., 2013). A better understanding of the mechanisms behind its invasion may aid in the restoration planning of plant and tree species whose establishment are dependent on a healthy microbial community.

**Methods**

**Plot Description**

This riparian forest patch is located at *The Wilds* Conservation Center in Cumberland, Ohio. *The Wilds* is a 3,700 ha center for conservation research located on reclaimed mined land in Muskingum County (Lat. 39 49.816576235, long -81 43.896718858). Mining for coal began in the 1940s by the Ohio Power Coal Company and continued until 2001. The primary coal seam
extracted was Meigs Creek No. 9 coal with an overburden that consisted of the Upper Sewickley Sandstone, Benwood Limestone, Fulton Green Shale, and Arnoldsburg Limestone Members. Portions of these strata contain 1.5% to 5% pyrite. Plots selected in this riparian buffer zone were mined from 1973 until 1976. The vegetation is primarily Pinus spp., planted upon reclamation. Other species that were either planted or naturally succeeded include native oaks (Quercus spp.), maples (Acer spp.), green ash (Fraxinus pennsylvanica), hickories (Carya spp.), and sycamore (Platanus occidentalis) (A. Campbell, unpublished data). This area is currently being invaded by non-native Ailanthus altissima (Fig. 1). In this riparian forest patch it was noted that oak regeneration was decreased in areas with mature Ailanthus when compared to areas where the invasive tree was not present (J.M. Bauman Per. Obs.).

Figure 1. This riparian buffer, which was once comprised of a mix of native trees and perennials, is currently being invaded by invasive Ailanthus. Healthy red oak regeneration was observed in areas where Ailanthus has not yet been established. In contrast, there was an obvious decrease in red oak seedlings in areas where mature Ailanthus is abundant.

The designated forest patch used in this study was 60 × 25 m. Within this forested riparian zone, six plots were established in areas where red oak were naturally regenerating, each plot measured 8 × 5 m. Of these, three of the plots were in an area where Ailanthus was not present; the other three plots were located among mature Ailanthus. In each 8 × 5 m plot, 10 red oak (Q. rubra) seedlings were selected for study (10 seedlings per plot, 60 seedlings total). Each plot was approximately 10 m apart and similar with regard to natural light. Soil samples were collected
from a depth of 0-18 cm using a soil probe, from each treatment plot. Three samples were collected (plot right, center, and plot left), mixed thoroughly, and allowed to air dry, and were sent (0.50 L) to Spectrum Analytic Inc., Washington Court House, Ohio for analysis. Average soil variables were pH (5.2), organic matter (6%), P (8.6 ppm), K (145.2 ppm), Mg (286.8 ppm), Ca (2,196.3 ppm), cation exchange capacity (20.9), S (39.5 ppm), B (0.4 ppm), Zn (4.41 ppm), Fe (319.7 ppm), Cu (1.83 ppm), and Mn (25 ppm). All macro and micronutrients were measured using the Mehlich 3 extraction method.

**ECM Field Sampling, ECM Identification, and Measurement of Red Oak Biomass**

The red oak (*Q. rubra*) seedlings were identified and tagged in September of 2011. In November of 2011, 60 seedlings were carefully removed from the soil and taken to the laboratory for seedling measurement and ECM quantification. Roots were washed with distilled water and transferred into a Petri dish. Two hundred and fifty root tips were randomly selected from each seedling and viewed under a dissecting microscope for the presence of a fungal sheath (60 samples, 15,000 root tips). A 3 mm section of root tip from each morphotype was transferred to a micro-centrifuge tube and used for isolating the fungal DNA.

The internal transcribed spacer (ITS) region was amplified using PCR primers ITS1 and ITS4 as described in Bauman et al., 2011. Sequencing was performed using capillary Sanger sequencing at the Plant-Microbe Geomonics Facility of The Ohio State University. ECM fungi were identified to genus by comparing the resulting fungal sequences to vouchered specimens in NCBI GenBank using BLAST (Altschul et al., 1997).

Seedling height was measured using a meter stick (cm) and basal diameter (mm) using a digital caliper. To measure plant biomass, roots and shoots were oven-dried for 24 hours at 100 °C and then weighed (g).

**Data Analysis**

Seedling biomass, root:shoot ratio, and percent ECM on roots were compared using a t-test. Stats were then verified and reported using a one-way analysis of variance followed by a Tukey's HSD post hoc test. Data were transformed using log+1 transformation to control for unequal variances. The differences were considered significant when p ≤ 0.05 according to the F test. All ANOVAs were performed using JMP 8.0 (SAS Institute, Cary, NC, USA). A non-metric multidimensional scaling (NMDS) ordination followed by a permutational multivariate analysis
of variance was used to determine differences in ECM species composition. Bray-Curtis dissimilarities were employed for the analysis with the maximum number of random starts in a search was set at 100 with $k = 2$ stress value. To improve the NMDS ordinations, data were standardized using Wisconsin double standardization. ECM fungal genera were standardized and fit onto the NMDS ordination by the envfit function. The correlation of ECM genera with NMDS axis was analyzed using Mantel tests. Significant differences among genera were detected by P-values ($< 0.05$) based on random permutations of the data. Both the dissimilarity matrices and Mantel tests were completed using the Vegan package of the R statistical program (R Development Core Team 2009, Oksanen et al., 2005).

**Results**

**Oak Biomass in Plots With and Without Ailanthus**

When seedling biomass was compared between oak seedlings growing in the absence of *Ailanthus* (2.72 g) and seedlings growing with mature *Ailanthus* (1.46 g) significant differences existed ($P = 0.02$). Oak seedlings produced more biomass when growing in the absence of the invasive tree (Fig. 2). When root:shoot ratio of the oak seedlings was compared, differences also existed. Oak seedlings growing within *Ailanthus* stands had a lower root to shoot average (0.32) as compared to seedlings in plots without *Ailanthus* (1.10; $P = 0.01$; Fig. 3).

**ECM Fungal Colonization and Community Composition on Red Oak**

When red oak root systems were observed for ECM colonization, there were significantly less ECM roots on seedlings in plots with abundant *Ailanthus* (29%) when compared to those growing without (41%; $P < 0.001$; Fig. 4). Of these, a total of 15 ECM fungal species were matched to the NCBI database (Table 1). Ten species were found in plots with *Ailanthus* and 11 were documented from plots without *Ailanthus* (Table 1). Although number of species sampled did not change significantly in the presence of the invasive tree, *Ailanthus* appeared to influence the occupancy of certain ECM fungi. *Russula* sp. 5, *Russula* sp. 6, and *Scleroderma* were more abundant on seedlings in plots without *Ailanthus* (Table 1). *Hymenogaster, Russula* sp. 1, *Tomentella*, and *Russula* sp. 4 were the most abundant on oaks sampled within *Ailanthus* plots.
Figure 2. Our results illustrate that *Ailanthus* (x-axis) had a negative effect on red oak (*Q. rubra*) biomass production (y-axis). Oak seedlings sampled from plots growing among mature *Ailanthus* were significantly smaller, averaging 1.46 g compared to the oak seedlings sampled from plots without *Ailanthus* (2.72 g). Bars represent the mean ± SE. Bars sharing common letters do not significantly differ at α = 0.05 determined by Tukey's HSD.

Figure 3. When red oak seedling root:shoot ratio was compared, seedlings growing within *Ailanthus* stands had a significantly lower root to shoot average (0.32) than oak seedlings in plots without *Ailanthus* (1.10; P = 0.01). Bars represent the mean ± SE. Bars sharing common letters do not significantly differ at α = 0.05 determined by Tukey's HSD.
Figure 4. When red oak root systems were observed for ECM colonization, there were significantly less ECM roots on oaks growing within Ailanthus plots (29%) compared to those growing without (41%). Values reported here represent the average percentage of root tips colonized. Bars represent the mean ± SE. Bars sharing common letters do not significantly differ at α = 0.05 determined by Tukey’s HSD.

Table 1. ECM fungal species sampled from red oak root tips ranked by the proportion of the genera sampled from counted root tips. The total number of ECM species present is listed on the last line of the table. Roots were collected from 60 oak seedlings (Total) from the six plots: three representing plots without Ailanthus (30 oak seedlings) and three plots where Ailanthus was present (30 oak seedlings). This table reports fungal colonization from sequences that were matched to vouchered ECM sequences available in GenBank.

<table>
<thead>
<tr>
<th>Species</th>
<th>Total</th>
<th>No Ailanthus</th>
<th>Ailanthus present</th>
</tr>
</thead>
<tbody>
<tr>
<td>Russula sp. 6</td>
<td>20.8</td>
<td>27.2</td>
<td>12.9</td>
</tr>
<tr>
<td>Russula sp. 5</td>
<td>14</td>
<td>25.5</td>
<td>0.0</td>
</tr>
<tr>
<td>Hymenogaster sp.</td>
<td>11.5</td>
<td>0.0</td>
<td>25.5</td>
</tr>
<tr>
<td>Scleroderma sp.</td>
<td>11.2</td>
<td>14.5</td>
<td>7.1</td>
</tr>
<tr>
<td>Tomentella sp.</td>
<td>10.1</td>
<td>0.0</td>
<td>22.4</td>
</tr>
<tr>
<td>Russula sp. 1</td>
<td>4.8</td>
<td>0.0</td>
<td>10.8</td>
</tr>
<tr>
<td>Russula sp. 4</td>
<td>4.3</td>
<td>0.0</td>
<td>9.5</td>
</tr>
<tr>
<td>Thelephora sp.</td>
<td>4.2</td>
<td>7.6</td>
<td>0.0</td>
</tr>
<tr>
<td>Lactarius sp.</td>
<td>4</td>
<td>4.2</td>
<td>3.8</td>
</tr>
<tr>
<td>Russula sp. 3</td>
<td>3.8</td>
<td>6.9</td>
<td>0.0</td>
</tr>
<tr>
<td>Inocybe sp.</td>
<td>2.6</td>
<td>&lt;1</td>
<td>5.7</td>
</tr>
<tr>
<td>Russula sp. 2</td>
<td>2.3</td>
<td>4.2</td>
<td>0.0</td>
</tr>
<tr>
<td>Boletus sp.</td>
<td>2.2</td>
<td>3.1</td>
<td>1.1</td>
</tr>
<tr>
<td>Sebacina sp.</td>
<td>1.5</td>
<td>2.7</td>
<td>0.0</td>
</tr>
<tr>
<td>Unknown ECM</td>
<td>&lt;1</td>
<td>3.0</td>
<td>1.0</td>
</tr>
<tr>
<td># of ECM spp.</td>
<td>15</td>
<td>11</td>
<td>10</td>
</tr>
</tbody>
</table>
A significant difference in ECM fungal species composition between plots with and without *Ailanthus* was detected ($F = 3.12, P = 0.0004$). NMDS ordination illustrated dissimilarity between these ECM communities in plots without *Ailanthus* when compared to plots where this tree was abundant (Fig. 5). Several ECM species showed a correlation with NMDS 1 and NMDS 2 (Fig. 5). *Boletus* sp. was correlated with NMDS1 ($R^2 = 0.29, P = 0.009$), which was associated with plots that did not contain *Ailanthus*. Fungal genera *Tometella*, *Thelephora*, and *Hymenogaster* were correlated with NMDS axis 2. Of these, *Thelephora* was found in association with oaks growing without *Ailanthus* ($R^2 = 0.20, P = 0.02$). Conversely, *Tometella* and *Hymenogaster* were found exclusively in plots that had *Ailanthus* (Table 1, Fig. 5) and significantly correlated to the invasive tree ($R^2 = 0.58, 0.18$ and $P = 0.001, 0.04$, respectively). *Scleroderma* sp. was marginally significant ($R^2 = 0.15, P = 0.08$) due to a decrease on oak roots when *Ailanthus* was present (Table 1).

Figure 5. NMDS ordination illustrating dissimilarity between ECM communities sampled from roots of red oak in plots without *Ailanthus* (○) when compared to plots where this tree was abundant (△). ECM species *Boletus* and *Thelephora* were associated with plots without *Ailanthus* and correlated to NMDS 1 and NMDS 2, respectively. *Tometella* and *Hymenogaster* showed a correlation with NMDS 2 and were associated with plots among established *Ailanthus*. Those species are annotated with vectors indicating direction of change based on presence of *Ailanthus*. 
Discussion

This study illustrated the negative effect *Ailanthus* had on seedling growth and ECM fungal root colonization of two-year-old red oaks (*Q. rubra*) in a riparian forest patch. Oak seedlings growing without *Ailanthus* were larger and had significantly more ECM roots. Specifically, there was a significant decrease in the root:shoot ratio of seedlings growing among established *Ailanthus*. Lower root to shoot ratios may suggest a stress response or inhibition of root development due to allelopathic effects of an invasive species (Alshahrani, 2008; Small et al., 2010). Castellano and Gorchov (2012) reported a similar finding on red oaks in forest stands invaded by garlic mustard (*Alliaria petiolata*); this herbaceous species is suspected to produce antifungal compounds toxic to mycorrhizal fungi (Wolfe et al., 2008). Along with other studies, our results suggest that the biochemical effects on mutualistic fungi contribute to the success of an invading plant species over its competitors in areas where that invader is not native (Stinson et al., 2006; Wolfe et al., 2008; Callaway et al., 2008).

The presence of *Ailanthus* may have selected for ECM fungi that tolerate allelopathic compounds in the soil. For example, *Tomentella* was one of the more abundant ECM species in *Ailanthus* plots and has also been reported in areas high in garlic mustard (Castellano and Gorchov, 2012). In addition, the decrease in root colonization may be in response to the plant’s decrease in carbon allocated to the root system (Saikkonen et al., 1999; Swaty et al., 2004; Bauman et al., 2012). This may have contributed to the shift in ECM composition from species that are better competitors when the carbon transfer from the host plant is high, to fungi that tolerate allelopathy and lower levels of carbon produced by its host plant. What is not known is whether fungal hyphae colonizing root tips are correlated to a more competitive fungus that can increase soil hyphal growth, thereby increasing plant nutrient and water acquisition (Kennedy and Bruns, 2005). Therefore, the tradeoff of allelopathic tolerance may not translate into fungal symbionts that are most beneficial to plant growth.

AM fungi are most abundant in riparian forest patches during early successional phases, proceeded by ECM fungi in later phases (Johnson et al., 1991; Piotrowski et al., 2008). Under natural conditions, the AM relationship may contribute to species coexistence by favoring inferior competitors (Grime et al., 1987). However, mutualistic associations that favor a superior competitor will contribute to the competitive exclusion of other species and have lasting negative impacts on the composition of the native forest community (Allen and Allen, 1990; Marler et al.,
Enhanced growth rates noted from red oak seedlings without *Ailanthus* had an overall improved carbohydrate status (indicated by increased root biomass and ECM colonization) that would help seedlings to be competitive following a canopy disturbance. It was not clear whether ECM activity was the driver of plant growth, or if plant fitness affected ECM colonization. However, regardless of the mechanism, both are strong indicators of plant vigor. Important to a plant that is competing for scarce resources, the increased vigor will aid in overcoming drought, pathogens, and other mortality causes. Therefore, the negative effect of *Ailanthus* on seedling growth may not only deter initial seedling regeneration, but also depress tree survival in the long-term (Jose et al., 2006).

Mycorrhizal association is known to increase the competitive ability of plants soon after disturbances (Bever, 2002). Documenting microbial interactions may have important restoration implications when planning re-plantings in areas where mature *Ailanthus* stands once deterred the growth of native species. It is not known how long the chemical effects of *Ailanthus* may last in forest soils. These data suggest that restoring riparian zones or other forest patches after *Ailanthus* treatment may not be successful using later successional tree species that rely heavily on an ECM fungal partner for establishment. Natural regeneration of oaks is unlikely after *Ailanthus* removal due to residual allelopathic soil compounds coupled with prolific seed banks and root sprouting typical of this non-native species. An active restoration plan should integrate chemical control (Peugh et al., in review), follow-up treatments, and a planting regime that incorporates native tree species with the ability to compete with *Ailanthus* re-sprouts (Moore and Lacey, 2009). It is not known how the presence of *Ailanthus* is altering nutrient availability at this site, or how that may influence ECM community. Follow-up studies are currently underway to better describe the changes in soil chemistry in stands heavily invaded by this non-native tree.

Using natural succession of riparian forest patches as a framework, a natural shift should occur within 10 to 15 years (Piotrowski et al., 2008), providing that the control of *Ailanthus* has been successful. Therefore restoration plantings using early successional species with AM fungal symbionts may benefit from high fertility patches, no dependence on ECM fungi, and tolerance to allele-chemicals. Although the presence of *Ailanthus* has been reported to stimulate the growth of an AM host, red maple (Gomez-Aparicio and Canham, 2008a), this species has been documented to significantly increase in abundance at the expense of species diversity. Therefore, a diverse mix of early successional trees or AM host species such as American sycamore (*Platanus occidentalis*),
black locust (*Robinia pseudoacacia*), hawthorns (*Crataegus* spp.), and sweetgum (*Liquidambar styraciflua*) may be the better tree selection to promote co-existence of a diverse community (Zeleznik and Skousen, 1996; Groninger et al., 2007; Moore and Lacey, 2009). The increased organic matter, soil moisture, and the accumulation of litter from early successional species may contribute to eventual succession of ECM fungi and host plants that will then promote the long-term restoration of a temperate riparian system (Read, 1991; Piotroski et al., 2008).

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