ASSESSING THE ECOLOGICAL IMPLICATIONS OF ARBUSCULAR MYCORRHIZAL FUNGAL
COLONIZATION OF THE INVASIVE SHRUB AMUR HONEYSUCKLE (*Lonicera maackii*)

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ASSESSING THE ECOLOGICAL IMPLICATIONS OF ARBUSCULAR MYCORRHIZAL FUNGAL

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ABSTRACT

ASSESSING THE ECOLOGICAL IMPLICATIONS OF ARBUSCULAR MYCORRHIZAL FUNGAL COLONIZATION OF THE INVASIVE SHRUB AMUR HONEYSUCKLE (*Lonicera maackii*)

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The introduction of non-native, invasive plants has significantly reduced the biodiversity of native plants and altered ecosystem processes and successional trajectories in novel environments. The invasive shrub Amur honeysuckle (*Lonicera maackii*) has negatively impacted the biodiversity and ecological balance of Eastern Deciduous Forests by reducing the growth and reproduction of native forbs, tree seedlings, and overstory trees. Previous research on the competitive success of *L. maackii* has focused on allelopathy and competition for light and below-ground resources. However, the disruption of native mycorrhizal networks by *L. maackii* is one potential mechanism that has been unexplored. We examined the vesicular arbuscular mycorrhizal colonization in *L. maackii* roots compared to a random sample of native control roots. Contrary to a reduction in mycorrhizae, the total mycorrhizal colonization levels in *L. maackii* roots were significantly higher than native roots in April and June. Additionally, arbuscular colonization, the site of nutrient exchange, was significantly higher in *L. maackii* across all months of the study; in April, arbuscular colonization was 4.1 times greater in *L.
maackii roots than control roots. Arbuscular colonization levels also peaked earlier in L. maackii roots than in native roots, which may be explained by L. maackii’s extended leaf phenology. These findings suggest that the high arbuscular colonization in L. maackii likely provides a significant benefit of increased nutrients to this invasive shrub and perhaps a competitive advantage over native plants. Additionally, these results complicate the potential role of allelopathy as a competitive mechanism – the allelochemicals must negatively affect the surrounding native plants, without disrupting the mycorrhizal network that these native plants and L. maackii depend on. The high arbuscular colonization of L. maackii is ecologically significant and likely an important mechanism enabling L. maackii to out-compete native plants.
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CHAPTER 1

LITERATURE REVIEW

Invasive Species

Invasive Species Introduction

The introduction of non-indigenous, native plants into novel environments has resulted in the reduction of native plant biodiversity and altered ecosystem processes and successional trajectories. For centuries, humans have transported plants beyond their native ranges into new environments through both accidental and intentional introductions (e.g. in cargo shipments and for agriculture/horticulture purposes, respectively). Although the majority of these introduced plants reside in natural communities with the native or resident plants, a few plants have become invasive, out-competing native plants and transforming natural communities. Invasive plants are non-native plants that are detrimental to the growth and fecundity of the native plants in the new environment. Over the past 100 years, approximately 2,000 – 3,000 invasive species have been introduced into the United States (Gurevitch et al. 2006). In Ohio, there are nearly 700 non-native plants, yet, only about 60 of these plants are considered invasive (Ohio Department of Natural Resources 2000). Invasive species pose the second greatest threat to biodiversity worldwide, with habitat loss as the greatest threat (Wilson 2002). Once an invasive species has invaded a habitat, the invasive out-competes and often displaces the native plants, forming a monoculture. The invasive species becomes the
community dominant and alters the way native plants coexist (Denslow and Hughes 2004). This change in community structure results in altered ecosystem processes of nutrient cycling, primary production, hydrology, food webs, succession, etc. (Bazzaz 1986; Denslow and Hughes 2004; Levine et al. 2003; Pimentel et al. 2000). Pimentel et al. (2000) suggests that more than $137 billion is spent annually on damages and controls of invasive species in the United States, with about $34 billion spent on plants alone.

Once an invasive plant is introduced, there are several stages of dispersal and growth before the invasive becomes dominant in the new habitat. First, an invasive plant must reproduce prolifically and disperse seeds. Then the seeds must successfully germinate in this new habitat, grow, and begin reproducing. Initial colonization events are often unsuccessful due to the small population size, unsuitable environmental factors, and selection pressures. Multiple introduction events at the same site are often required before populations become established. Once established, the population remains small for a length of time as the plants adapt to the habitat, grow, and reproduce. After this lag phase, the population begins growing rapidly and dispersing to new areas; many invasive species proliferate at exponential rates (Bazzaz 1986; Gurevitch et al. 2006). At this stage, the invasive species are no longer controlled by the native plants or environmental conditions, but are now driving and changing this invaded ecosystem (Huebner and Tobin 2006).

**Hypotheses for Invasive Species Success**

Several hypotheses have been developed to explain how non-native species have become invasive in new habitats. One hypothesis states that invasive species are released from
natural enemies in the new environment. The invasive species are no longer kept in balance by specialized herbivores and pathogens, releasing the population to expand exponentially. However, native plants are still kept in balance by coevolved natural enemies. Without predation, invasive plants may reallocate resources from defense mechanisms to increased growth and reproduction (Callaway and Ridenour 2004). The empty niche hypothesis states that the novel environment may have unused resources that the invasive species may utilize. For example, researchers have hypothesized that the invasive shrub *Lonicera maackii* dominates deciduous forests of the Midwest due to the absence of a predominant shrub layer in the forest understory (Collier et al. 2002). The third hypothesis, the evolution of invasiveness, suggests that invasive species evolve new genetic traits once introduced into the novel habitat. The biotic and abiotic factors of the new environment confer different selection pressures on the invasive species than its native range, resulting in rapid microevolution during the lag phase of establishment.

Another hypothesis for invasive success states that the invasive species brings novel traits or weapons to the new habitat. The resident species have not evolved defenses against these novel traits, where as plants in the original range are not harmed by these traits. These novel weapons disrupt coevolved relationships in the invaded range (Callaway and Aschehoug 2000). One such weapon is allelopathy, a biochemical inhibition of native plants by an invasive plant through the production and release of a chemical into the environment. Scientists speculate that allelopathic plants may have originally evolved the biochemicals for nutrient acquisition, anti-microbial properties (Callaway and Ridenour 2004), and disease and herbivore resistance (Cipollini, Stevenson, Enright, et al. 2008). However, in novel habitats, these properties provide a competitive advantage against the native flora. The success of these novel...
weapons in the new habitat may impose stronger selection pressures on these traits, perhaps increasing the allelopathic strength of these plants (Callaway and Ridenour 2004). The disturbance hypothesis states that exotic species have evolved to tolerate disturbance and can survive in highly disturbed sites, while native species cannot (Bazzaz 1986; Hierro et al. 2005). The final hypothesis states that propagule pressure, number of introduced offspring, greatly affects the success of the invasive species. High propagule pressure results in multiple reintroduction events, which may enable a successful invasive establishment in the new habitat (Hierro et al. 2005).

**Life History Traits and Habitat Characteristics Associated with Invasion**

Ecologists have identified several life history traits that are correlated with invasive success. Plant species that are invasive tend to have short juvenile periods, small intervals between seed production (Rejmanek and Richardson 1996), fast growth rates (Bazzaz 1986; Herron et al. 2007), vegetative reproduction, fast decomposition rates, a large native latitudinal range, long flowering period (Herron et al. 2007), and reliance on vertebrate seed dispersal (Rejmanek and Richardson 1996; Richardson et al. 2000). Invasive species also tend to have reduced seed weights, which can result in greater seed production, increased dispersal, germination, and growth rates of seedlings (Rejmanek and Richardson 1996). Colonizing plants generally have increased respiration, photosynthetic, and transpiration rates (Bazzaz 1986). Although these life history traits may apply to many invasives, some traits may be greater predictors of invasion in different types of plants. For example in woody plants, the greatest predictors for invasion are large native latitudinal ranges and rapid growth rates; however, evergreen plants are least likely to be invasive (Herron et al. 2007). When further sub-dividing
woody invasives into trees, shrubs, and vines, differences in reproductive strategy and shade tolerance become important indicators (Herron et al. 2007). Overall, successful invasive plants likely possess a combination of these invasive life history traits.

Ecologists have identified several characteristics of habitats that foster greater numbers of invasive species. One study found increased numbers of invasive species along roadsides, which may be due to greater light, nutrient rich soil, and frequent disturbance in these locations (Flory and Clay 2006). Additionally, edge and disturbed habitats have greater invasive species (Hunter and Mattice 2002), including former agricultural sites (Johnson et al. 2006). Also, early successional forests are more susceptible to invasion than late successional or mature forests (Flory and Clay 2006; Hunter and Mattice 2002; Hutchinson and Vankat 1997). Cipollini et al. (2009) found that forests without deer have decreased numbers of invasive species. Lastly, Huebner and Tobin (2006) determined that richness, diversity, number of native plants, as well as number of exotic plants, are factors important to invasion.

Many ecologists suggest that sites with greater light and close invasive propagules are more likely to be invaded than sites with low light and no adjacent invasives. Howard et al. (2004) challenges these hypotheses with results that suggest that light availability (size of tree and forest type) and propagule distance are unimportant in determining the likelihood of invasion. Howard et al. (2004) determined that hardwood forests had greater invasion than pine barren and pine-oak habitats; these sites greatly differed in canopy cover and soil properties, with pine habitats having less canopy cover and lower calcium and nitrogen levels. Soil properties positively correlated with invasion were calcium, nitrogen, magnesium, and phosphorus, while liter depth and foliar carbon to nitrogen ratios were negative correlated with
invasion (Howard et al. 2004). Howard et al. (2004) also found no correlation between pH, cation exchange capacity, and soil texture and invasion. However, other research has found that pH and finer textured soils influenced invasion (Johnson et al. 2006). Overall, sites that had greater soil nutrients were positively correlated with invasion (Howard et al. 2004).

Researchers have also determined that sites with greater biodiversity have increased invasive species. Early research on invasion ecology, which dated back to Elton in 1958, operated on the principle that habitats with low diversity and high disturbance were more likely invaded than habitats with high diversity (Howard et al. 2004). High diversity sites were thought to be protected from disturbance due to lack of niche space, less available resources, and high competitive exclusion (Hierro et al. 2005; Stohlgren et al. 2003). However, recent studies have found the opposite to be true; areas with high diversity actually have greater numbers of invasive species than less diverse sites (Howard et al. 2004; Stohlgren et al. 2003). These highly diverse areas may be positively correlated with habitat heterogeneity, which could result in areas with available resources for invasives to colonize (Davis et al. 2000; Howard et al. 2004; Stohlgren et al. 2003). Thus increased invasion in these habitats may be correlated to increased available resources, rather than greater biodiversity. Howard et al. (2004) determined that the degree of invasion varied greatly in highly diverse areas, further supporting the importance of other factors beyond plant biodiversity.

When grouping plants based on similar resource use, native plants prevent invasives with analogous resource needs from establishing. By grouping plants with parallel resource needs into functional guilds, the role of biodiversity in preventing or aiding assembly can be reexamined. Fargione et al. (2003) determined that native or resident functional guild plants
inhibit invasion of species of the same guild more than any other guild. This inhibition was due to competition for similar limited resources. Also, as the number of species increased in the native functional guild, the number, cover, and biomass of species in an invading functional guild decreased. Therefore, it appears that functional guild grouping supports the hypothesis that more diverse communities are less likely to be invaded.

As scientists continue to hypothesize about why some communities are more invaded than others, the theory of fluctuating resource availability may be a conclusion to the many hypotheses. The theory states that plant communities with increasing available resources are more likely to be invaded (Davis et al. 2000). Communities may have an increased resource supply due to an excess of resources beyond native plant use or a decreased resource use by native plants. Regardless, an increased resource supply results in decreased competition among both native and invasive species. Disturbance events also increase resource availability. These fluctuations, in addition to the natural fluctuations within any community, result in changing resource availability and perhaps, opportunities for invasion.

Amur Honeysuckle

Amur Honeysuckle Introduction and Life History Traits

*Lonicera maackii* or Amur honeysuckle is a non-native invasive shrub that has rapidly spread across the Eastern United States since its introduction in the late 1800’s. *L. maackii* originates from eastern Asia (China, Korea, and Japan) where it resides in floodplains and forests with open canopies (Luken and Thieret 1996). *Lonicera maackii* was first introduced to North America in 1896 and then to the United States two years later as an ornamental plant, cultivated
for its fruits and flowers (Luken and Thieret 1996). Between 1898 and 1927, the USDA encouraged the planting of *L. maackii* seeds and at least seven other introductions occurred (Luken and Thieret 1996). From 1960 to 1984, another branch of the USDA promoted planting of *L. maackii* cultivars that were genetically engineered to produce many flowers and seeds and vegetatively reproduce (Luken and Thieret 1996). The USDA encouraged *L. maackii* planting to improve habitat for wildlife, create windbreaks (Gadagkar *et al.* 2007), stabilize soil, and for ornamental decoration (Luken and Thieret 1996). In 1950, *L. maackii* growth and reproduction in the wild was first reported in the US (Luken and Thieret 1996), yet, no naturalizations have been reported in Europe. Braun reports the presence of *L. maackii* in Hamilton County, Ohio in 1961. Today, *L. maackii* resides in at least 26 states in the Eastern United States and in Ontario, Canada (Luken and Thieret 1996; United States Department of Agriculture 2010). The invasion of southwestern Ohio originates from an introduction in Oxford, Ohio in 1960 (Deering and Vankat 1999; Hutchinson and Vankat 1997). Unfortunately, nurseries continue to grow *L. maackii* and homeowners plant this shrub for its yellow and white aromatic flowers, red berries, and as a natural privacy fence.

*Lonicera maackii* population growth follows the invasion model with a lag period after colonization. The establishment of a population after a single introduction event requires large amounts of seed to remain viable in the seed bank over many years. However, *L. maackii* seeds lack dormancy mechanisms that enable survival for extended periods of time (Luken and Mattimiro 1991). Therefore, establishment of a population of *L. maackii* most likely occurs after a series of small colonization events (Deering and Vankat 1999). As seeds are dispersed into the same habitat over time, seedlings establish and grow, but the population remains small for a period of time. This lag in population growth is due to dispersal and the delayed reproduction
of *L. maackii*. *Lonicera maackii* do not become reproductive until 3 – 8 years of age (Luken and Thieret 1996) and by age 5, over 50% of the population is reproducing (Deering and Vankat 1999). Additionally, shrubs do not reproduce until a minimum height of 2.5m is reached (Deering and Vankat 1999). Young shrubs direct resources to height growth and stem production. However, when reproducing, shrubs redirect resources to height growth, basal growth and seed production (Deering and Vankat 1999). Deering and Vankat (1999) found height as a better indicator of reproductive status than age, due to a pronounced shift in resource allocation. After reaching a robust population size with many reproducing adults, seeds are dispersed and *L. maackii* begins establishing other populations away from the source population. Castellano and Boyce (2007) found that *L. maackii* have a clumped distribution, in which the older shrubs may be aiding in the growth of young *L. maackii* seedlings. Researchers estimate that *L. maackii* migrates at an annual rate of 0.5km (Deering and Vankat 1999; Hutchinson and Vankat 1997), spreading along roadways and other disturbance channels (Gadagkar et al. 2007).

*Lonicera maackii* has many growth characteristics that are advantageous to invasion. *Lonicera maackii* is a multi-stemmed, woody, deciduous shrub that can reach heights of 6m (Luken 1988; Luken and Thieret 1996). Additionally, *L. maackii* produces opposite, ovate leaves in early spring and senesce the leaves in late fall, retaining leaves longer than any other native plant (Gould and Gorchov 2000; Trisel and Gorchov 1994), including the native shrubs spicebush and pawpaw (McEwan, Birchfield, *et al.* 2009). Harrington *et al.* (1989) found that early leaf expansion accounts for 25-35% carbon gain/year (McEwan, Birchfield, *et al.* 2009), providing *L. maackii* with a photosynthetic advantage over native plants. In early spring, native trees and shrubs are negatively affected by frost, dropping frosted leaves and growing new ones, while *L.*
maackii is unaffected (McEwan, Birchfield, et al. 2009); perhaps L. maackii leaves are frost resistant because they are in a later stage of development due to early leafing. Lonicera maackii are reproductively plastic, alternating between vegetative and seed reproduction or using both at the same time (Luken and Mattimiro 1991). Given the short-lived seed bank, Luken and Mattimiro (1991) found that L. maackii populations are sustained through vegetative growth. Lastly, there are no known biological controls in the invaded habitats to limit L. maackii population growth.

Lonicera maackii produce an abundant source of red berries that provide food for many animals. Over 20 species of birds are known to disperse L. maackii fruit (Williams et al. 1992). The berries provide a food source for birds in late winter, when few native alternatives are available. Although the berries provide some carbohydrate-based nutrition, these berries are a poor food source compared to the lipid-rich native fruits (National Park Service and U.S. Fish and Wildlife Service 2010). Additionally, birds have begun nesting in L. maackii and in one study, robins preferred to use L. maackii for nesting over native trees (Schmidt and Whelan 1999). Unfortunately, robins and thrushes nesting in L. maackii had greater daily nest mortality rates than did nests in native trees (Schmidt and Whelan 1999). The lack of thorns in the branches, branch architecture, and lower nesting height may have played a significant role in the high nest predation of robins in L. maackii shrubs (Schmidt and Whelan 1999). Additionally, the early phenology of L. maackii may attract birds to nest in these shrubs (Schmidt and Whelan 1999). Williams et al. (1992) found that deer mice (Peromyscus maniculatus) also consumed L. maackii seeds and young stems; however, small mammals are unlikely to play an important role in seed dispersal. The invasive gypsy moth (Lymantria dispar) consumed little L. maackii leaf matter, which still resulted in moth mortality (McEwan, Rieske, et al. 2009). McEwan, Rieske, et al.
(2009) hypothesized that the chemicals in *L. maackii* leaves are unpalatable to generalist herbivores such as the gypsy moth. *Lonicera maackii* may benefit from forests invaded with gypsy moth because the moth would consume competing overstory trees, allowing more light to reach *L. maackii* (McEwan, Rieske, *et al.* 2009).

Although *L. maackii* colonizes a variety of habitats, the majority of research has focused on forest invasion. *Lonicera maackii* invades highly disturbed habitats ranging from forest, open fields, fence rows, urban areas, and along habitat edges (Dorning and Cipollini 2006; Luken and Thieret 1996; Miller and Gorchov 2004). Research on forest invasion has found that early successional forests are more susceptible to invasion than late successional or mature forests (Flory and Clay 2006; Hutchinson and Vankat 1997). Additionally, forests with canopy gaps are more prone to invasion than intact forests (Luken 1991). Researchers have hypothesized that the lack of a native shrub layer in the Midwest (empty niche hypothesis) may explain the abundance of *L. maackii* within forests (Collier *et al.* 2002). Luken (1991) determined that *L. maackii* grows better in non-forested habitats due to increased light availability.

**Effects of Light Availability on Lonicera maackii Growth and Invasion**

*Lonicera maackii* alters biomass allocation to growth depending on light availability. Forest grown *L. maackii* had greater stem death and new stem growth than open grown shrubs, which received greater light (Luken 1988). When repeatedly stressed, forest grown shrubs had greater mortality than open grown shrubs, signifying a drain in stored carbohydrates (Luken and Mattimiro 1991). Also, forest grown *L. maackii* had significantly lower amounts of net primary production (NPP) per stem and shrub than did open grown shrubs (Luken 1988). However,
regardless of habitat location, Luken (1988) determined that *L. maackii* is an important contributor to the primary production of invaded habitats. When comparing growth responses to light availability with a native shrub, *Lindera benzoin*, *L. maackii* had greater stem growth in partial and full sun than did *L. benzoin* (Luken *et al.* 1997). *Lonicera maackii* also had greater leaf plasticity than *L. benzoin*, producing more new leaves and thicker leaves than did *L. benzoin* when exposed to increased light (Luken *et al.* 1997). In shaded conditions, *L. benzoin* transported more nutrients below-ground, while *L. maackii* allocated more resources to shoot growth, perhaps enabling greater competition in the shade (Luken *et al.* 1997). Overall, *L. maackii* partitions resource use differently depending on light availability (Sanford *et al.* 2003).

Light availability plays an important role in the population growth of *L. maackii*. Initial limitations to *L. maackii* population growth are establishment and competition with native plants. As *L. maackii* shrubs reproduce and invade new areas, range expansion becomes limited by light availability (Luken and Thieret 1996). Luken and Mattimiro (1991) determined that forest grown *L. maackii* shrubs produce less seeds than open grown shrubs. Additionally, seed germination is lowered or inhibited in the dark compared to germination in the light (Luken and Goessling 1995; Luken and Mattimiro 1991). Hutchinson and Vankat (1997) found an inverse relationship between *L. maackii* presence and canopy cover and shade tolerance index, indicating that light plays an important role in *L. maackii* establishment.

**Negative Effects of *L. maackii* on Native Flora**

The negative effects of *L. maackii* on native tree seedlings and forest herbs have been well documented in the literature. *Lonicera maackii* has reduced the growth and fecundity of
three forest annuals (Gould and Gorchov 2000) and three perennial herbs (Miller and Gorchov 2004). Additionally, native species richness and abundance has decreased under *L. maackii* (Collier et al. 2002; Hutchinson and Vankat 1997). Also, tree seedling density and richness was negatively correlated to *L. maackii* cover (Hutchinson and Vankat 1997). Hartman and McCarthy (2007) found reduced growth and productivity of overstory trees in *L. maackii* invaded areas due to below-ground resource competition. In a study by Trammell et al. (2012), the biomass of overstory plants was reduced by 33% in forests invaded by *L. maackii*, while the leaf biomass of *L. maackii* increased (Arthur et al. 2012). Gould and Gorchov (2000) reported greater negative effects of *L. maackii* establishment in anthropogenic disturbed sites. Habitats long invaded by *L. maackii* had decreased herbaceous richness, density, and cover, decreased seedling and sapling richness (Collier et al. 2002; Hartman and McCarthy 2008), and less viable seed banks (Hartman and McCarthy 2008). Over time, reductions in native plant fecundity may lead to decreased population sizes (Miller and Gorchov 2004), isolated populations, and even local extinction of plants (Collier et al. 2002). These changes may result in different successional trajectories for forests invaded with *L. maackii* and those without (Hartman and McCarthy 2008).

*Altered Ecological Balance in Terrestrial and Aquatic Ecosystems Invaded by L. maackii*

*Lonicera maackii* invasion has altered nutrient cycling and food web dynamics in aquatic and terrestrial habitats. Several studies have found that *L. maackii* leaves decomposed significantly faster than native tree species (Arthur et al. 2012; McNeish et al. 2012; Trammell et al. 2012). Arthur et al. (2012) hypothesized that microbial colonization of *L. maackii* leaves prior to senescence may contribute to increased *L. maackii* decomposition rates. In aquatic systems, rapid *L. maackii* leaf decomposition has resulted in altered macroinvertebrate communities.
compared to native leaves (McNeish et al. 2012). McNeish et al. (2012) primarily found gather-collector macroinvertebrates in L. maackii leaf packs, signifying that macroinvertebrates use L. maackii leaves as habitat rather than food sources. With the reduction of native organic inputs into L. maackii invaded streams, significant changes will likely occur throughout all levels of these aquatic food webs (McNeish et al. 2012). In forests, L. maackii has significantly reduced the volume of rainwater reaching the forest floor and has changed the cation and NH₄⁺-N deposition under the shrubs (McEwan et al. 2012). Trammell et al. (2012) determined that L. maackii increased the rate of leaf decomposition of a native tree species, while Arthur et al. (2012) found that native leaf decay was slower under L. maackii.

Lonicera maackii Mechanisms for Competitive Success

Researchers have identified several mechanisms important for L. maackii competition with native plants. Lonicera maackii competes with native plants for both above-ground and below-ground resources. Below-ground, plants compete for soil nutrients, water, and root space. Hartman and McCarthy (2007) found that below-ground competition can have direct effects on overstory dynamics; Lonicera maackii negatively affects overstory trees through consumption of below-ground resources. Collier et al. (2002) suggests that competition does not occur deep below-ground, rather just below the surface of the soil where shallow L. maackii roots may reduce water and nutrients available for other native plants.

Over the past decade, researchers have identified allelopathy as a potentially important mechanism used by L. maackii to out-compete native plants. Allelopathy is a biochemical inhibition of native plants by an invasive plant through the production and release of a chemical
into the environment. Callaway and Ridneour (2004) hypothesize that allelopathy maybe a novel weapon used by *L. maackii* to compete against native plants in the introduced range. Allelopathic chemicals can directly affect surrounding plants by poisoning them or indirectly by harming the soil biota, such as mycorrhizae, that these plants depend on (Callaway and Ridneour 2004; Cipollini, McClain, *et al.* 2008). Plants release allelopathic chemicals into the soil through root exudates, leaf leachate in rain water, and from senesced leaves (Dorning and Cipollini 2006).

Several studies have found *L. maackii* extracts to be allelopathic and detrimental to native plants. Leaf and root extracts from *L. maackii* greatly reduced the germination of three understory herbs (*Impatiens capensis, Arabidopsis thaliana*, and *Alliaria petiolata*) (Dorning and Cipollini 2006). Additionally, as the extract concentration increased, herb germination decreased. However, when the same extracts were applied to *L. maackii*, no negative effects were found (Dorning and Cipollini 2006). *Lonicera maackii* may use allelopathy as a novel weapon to reduce competition of native plants, enabling resistant *L. maackii* offspring to colonize the newly vacated soil. Cipollini and Dorning (2008) also found that *A. thaliana* grown in *L. maackii* conditioned soils had delayed flowering, slower growth, and decreased survival. When nutrients were added to plants grown in *L. maackii* conditioned soil, growth and flowering increased, suggesting that *L. maackii* may affect soil nutrients. However, when *L. maackii* extracts were added to plants with additional nutrients, the benefits of nutrient addition were ameliorated (Cipollini and Dorning 2008). Cipollini, Stevenson, Enright, *et al.* (2008) have isolated 13 allelopathic compounds in *L. maackii* extracts, with flavones apigenin and luteolin and glucoside derivates being greatest. While the chemical composition of *L. maackii* leaves are not novel to invaded habitats, the quantity of chemicals present in the leaves/leaching into the
soil may be much greater than native plants have previously experienced (Cipollini, Stevenson, Enright, et al. 2008); the extended phenology and density of *L. maackii* may affect the concentration of chemical leachate. *Lonicera maackii* extracts also deterred the feeding of a generalist insect (Cipollini, Stevenson, Enright, et al. 2008) and resulted in invasive gypsy moth (*Lymantria dispar*) mortality (McEwan, Rieske, et al. 2009).

Allelopathy or the novel weapons hypothesis has been met with great skepticism because allelopathic and competitive effects are difficult to evaluate in the field. Additionally, experiments using activated carbon to remove allelopathic chemical from the soil have had mixed results (Cipollini, McClain, et al. 2008). Cipollini, McClain, et al. (2008) states that although allelopathy plays some role in native floral reduction, resource competition is most likely driving the reduction. Additionally, a study by Gould and Gorchov (2000) found that the fecundity of three forest annuals was not significantly different in sites where *L. maackii* had been removed vs. where it never existed. Therefore, research indicates that allelopathy (Miller and Gorchov 2004) and nutrient depletion do not play an important role in native floral reduction (Gould and Gorchov 2000).

Many ecologists attribute the invasive success of *L. maackii* to above-ground competition for light. Gorchov and Trisel (2003) state that above-ground competition for light is more important than below-ground competition. *Lonicera maackii* reduce light penetration to the forest floor with an extended leaf phenology (compared to native plants), long shoot growth, multiple stems, and an arching branch architecture (Gould and Gorchov 2000). Perhaps the answer to the competitive success of *L. maackii* is a combination of both above-ground and below-ground mechanisms, including the role of native mycorrhizal fungi.
Management and Restoration of Habitats Invaded by L. maackii

Managers must prioritize invasive species removal to achieve the greatest biodiversity in natural areas. The best management strategy is to prevent invasion and then remove small individuals as they invade. However, many natural areas are heavily invaded and require managers to prioritize resource use. Luken (1991) suggests that invasive species with greater resilience and high productivity (net primary production) should have high priority for removal. Additionally, recently disturbed areas, intact mature forests lacking gaps, locations with rare or endangered species, areas with high richness, and young shrubs should be given high priority. Luken (1991) has found that older populations of L. maackii have greater resilience and productivity, which decrease as trees establish and forests are formed. Managers will achieve best results and have less negative impacts if L. maackii shrubs are removed when young and in light-limited habitats (Luken 1991).

While several methods are effective for L. maackii removal, the cut and spray treatment yields the best results. Hartman and McCarthy (2004) found no significant difference in L. maackii mortality when using the cut and paint method versus stem injection; both methods resulted in about 99% L. maackii mortality. However, tree seedling survival was reduced with the injection method, where dead L. maackii biomass is left standing (Hartman and McCarthy 2004). Similarly, Cipollini et al. (2009) found reduced species richness and reduced light penetration with the injection method. Yet, greater numbers of garlic mustard and L. maackii seedlings were found after applying the cut and paint treatment (Cipollini et al. 2009). In areas with high deer densities, the injection method may be most beneficial to deterring deer and protecting native plants (Cipollini et al. 2009; Gorchov and Trisel 2003). When considering the cost and time involved in managing L. maackii, cut and spray is much more costly than injection.
(Hartman and McCarthy 2004) and both are more expensive than applying a foliar spray (a practice used by many managers with high densities of *L. maackii*). Lastly, some managers have begun shredding the *L. maackii* biomass with a high powered mulcher and then spray the resprouts the following season.

Regardless of removal method, native plant growth increases after *L. maackii* removal. Several studies report increases of native herb density (Gould and Gorchov 2000), tree seedling survival (Gorchov and Trisel 2003; Luken et al. 1997; Runkle et al. 2007), and percent cover and species richness after *L. maackii* removal (Runkle et al. 2007). In addition to removal, restoration is often needed to prevent reinvasion and accelerate natural succession, which may be retarded due to a decreased or absent seed bank (Hartman and McCarthy 2004). Luckily, allelopathic compounds from *L. maackii* should not affect restoration efforts, since these compounds do not remain in the soil for longer than a year after removal (Cipollini and Dorning 2008).

**Mycorrhizae**

*Mycorrhizae Introduction*

The ubiquitous mycorrhizal colonization of vascular plants reveals the significance of this symbiotic relationship for both fungus and plant species. Mycorrhizae are fungi that form a symbiotic relationship with plant roots, resulting in nutrient exchange of carbon to the fungus and organic soil nutrients to the host plant (Brundrett 2004; Garg et al. 2006). Mycorrhizae differ from pathogens in that mycorrhizae transfer nutrients to their host plant and typically do not cause decline in plant fitness, while pathogens generally reduce plant health without benefit
to the host (Allen 1991; Brundrett 2004). Mycorrhizae colonize about 95% of all vascular plants (Blee and Anderson 1998) and play an important role in nutrient cycling in nearly all types of ecosystems (Bonfante and Perotto 1995). Nonmycorrhizal plants are often found in aquatic habitats (Gerdemann 1968) or recently disturbed sites (Richardson et al. 2000); few nonmycorrhizal plants are found in intact, undisturbed communities (Richardson et al. 2000). The prevalence of mycorrhizae across the plantae kingdom indicates a significant benefit for plants, otherwise this unique relationship would not have evolved to the colonization level present today.

Early mycologists classified mycorrhizae into two groups, ectomycorrhizae and endomycorrhizae, based on the location of fungal structures within the plant cells. Ectomycorrhizae fungi densely encircle host roots with hyphae (called the mantle) and surround cortical cells within roots, but the hyphae does not penetrate plant cells. Nutrients transfer between the fungus and plant occurs through the Hartig Net, which is a network of interconnected hyphae located between cells in the root cortex (Brundrett 2004). Ectomycorrhizal associations are often formed with boreal and temperate deciduous trees (Richardson et al. 2000), particularly *Eucalyptus, Betula, Populus, Fagus, Shorea, and Pinus* genera, and provide an obligate mutualism, necessary for plant survival (Brundrett 2004). However, some seedling species are colonized by endomycorrhizae first and then become ectomycorrhizal as saplings (Renker et al. 2004); hosts that are colonized by both endo- and ectomycorrhizae are always colonized by endomycorrhizae first (Allen 1991). Ectomycorrhizae can greatly aid in seedling survival and growth in soils with low fertility by providing increased water and nutrient acquisition (Wardle et al. 2004). In coniferous ecosystems, ectomycorrhizae
produce a substantial portion of the total fine root matter. One study by Langley and Hungate (2003) found that 78% of fine root matter in one community was produced by ectomycorrhizae.

Most plants are colonized by endomycorrhizae, fungus that transfer nutrients within the root cells. Endomycorrhizae colonize four-fifths of vascular plants (Bonfante and Perotto 1995; Malloch et al. 1980) and are dependent on a host for survival, unlike ectomycorrhizae which can survive independently from a host plant (Langley and Hungate 2003). Endomycorrhizae are not host specific (Brundrett 2004; Garg et al. 2006; Renker et al. 2004; Richardson et al. 2000) and can vary anatomically, genetically and in the host plants they colonize (Brundrett 2004). However, none of the endomycorrhizae fungi form hyphal mantels around host roots like ectomycorrhizae (Allen 1991). The following sections will focus on one type of endomycorrhizae, vesicular arbuscular mycorrhizae (AM), the most ubiquitous mycorrhizae (Richardson et al. 2000).

**Arbuscular Mycorrhizae**

Vesicular arbuscular mycorrhizae (AM) form densely branched structures called arbuscules within the root cells to maximize nutrient exchange with the host plant. AM create lipid storage structures called vesicles and nutrient exchange sites called arbuscules within the cortical cells of the root (Brundrett 2004; Garg et al. 2006; Gerdemann 1968). Host plants are not harmed by AM colonization because the fungus does not penetrate the cell membrane (Garg et al. 2006). The dense branching structure of the arbuscules results in an increased surface area with the cell membrane, which increases nutrient exchange between plant and fungus. The arbuscules are formed in the cortex of the root tip (not in the rapidly growing
region of the root tip) near the endodermis, in an area where elongation no longer occurs and carbon receipt from the phloem is greatest (Blee and Anderson 1998). Arbuscules are generally short-lived, ranging from 2.5 – 4 days in some species (Bonfante and Perotto 1995) and up to 14 days in others (Blee and Anderson 1998). Arbuscular mycorrhizae do not colonize meristem and vascular tissue (Bonfante and Perotto 1995), nor roots deep within the soil (Allen 1991). Within the past decade, analyses of genetic and life history strategies of different AM species have resulted in the reclassification of AM fungi into the Phylum Glomeromycota.

The prevalence and importance of AM can be traced back to the early land plants. Fossil records indicate that mycorrhizae were present during the Early Devonian Period, over 400 million years ago (Remy et al. 1994). Scientists hypothesize that this ancient symbiosis either developed from a parasitic interaction that proved beneficial or a saprobic fungi that entered the roots (Remy et al. 1994). Regardless, these ancient AM may have played a pivotal role in the evolution of land plants (Bonfante and Perotto 1995; Remy et al. 1994; Renker et al. 2004) and movement of plants across land (Allen 1991).

Genetically compatible AM successfully colonize host plants without inducing a pathogenic response from the host. Under favorable conditions, a AM spore will germinate within the rhizosphere of a host plant. Thick-walled, large hyphae called runner hyphae emerge from the spore and expands along the length of the root (Allen 1991). From the runner hyphae, absorbing hyphae and penetrating hyphae are formed. The absorbing hyphae, which have fan-like dichotomous branches, transport absorbed nutrients to the host plant from the soil (Allen 1991). The penetrating hyphae contact and pierce the host root (Allen 1991). If the host plant and fungi are genetically compatible, an appresorium is formed at the site of fungal penetration
into the root (Bonfante and Perotto 1995). Additionally, the plant genome has control over different stages of AM development and will degrade mycorrhizae if specific plant genes are inoperable (Bonfante and Perotto 1995) or the plant is nonmycorrhizal. Similar to a pathogenic response, the host plant will induce apoptosis in sections of the root where the fungus has expanded or will release phenols to kill the fungus (Allen 1991).

To penetrate the cell wall, AM use both chemical and mechanical force. AM produce pectinase and cellulase, digestive enzymes that degrade the cell wall. However, these same enzymes cannot degrade suberin or lignin, which is found in the Casparian strip of the endodermis, thus preventing fungal penetration directly into the phloem (Bonfante and Perotto 1995). Due to the localization and low production of these cell wall degrading enzymes, scientists believe AM penetrate the cell wall by mechanical force, as well as, degradation (Bonfante and Perotto 1995). As the fungus continues to grow and expand, it causes changes in the infected plant cells – vacuoles split, nuclear size increases, amyloplasts disappear, and other organelles increase in quantity (Bonfante and Perotto 1995). Additionally, an interface membrane with an apoplast is formed at the infection site and is composed of both fungal and plant membranes (Bonfante and Perotto 1995). In an arbuscule, this new membrane, called the periarbuscular interface, is where nutrient exchange occurs (Bonfante and Perotto 1995; Garg et al. 2006).

The carbon sink in the arbuscules is a result of sugar flow from the phloem through the plasmodesmata of the roots. Photosynthates are likely transported from the phloem to cortical cells of the root through the apoplast and symplastically through the plasmodesmata (Blee and Anderson 1998). However, flow across the endodermis is regulated by the Casparian strip,
which would affect or prevent carbon uptake by arbuscules if photosynthates are only transferred through the apoplast. Therefore, Blee and Anderson (1998) hypothesize that arbuscules must form in cells with plasmodesmata, which results in a direct carbon transfer. Once carbon is transported to a cell containing an arbuscule, the carbon is then moved across the periarbuscular interface (Garg et al. 2006). Scientist are still debating about whether carbon exchange across the periarbuscular interface is due to passive transport and sink gradients or active transport with H⁺-hexose pumps (Garg et al. 2006). In the phloem, carbon is transported as hexose or sucrose and is then transformed into the fungal carbohydrates of trehalose and glycogen (Garg et al. 2006); since plants cannot use these new fungal molecules, reabsorption by the host plant is prevented (Allen 1991). The main form of stored carbon in AM is triacylglyceride (TAG) (Garg et al. 2006). Blee and Anderson (1998) believe that arbuscules may increase the carbon sink strength to the arbusculated cells of the root cortex by producing plant growth hormones.

Benefits of Mycorrhizae to Host Plant

Mycorrhizal colonization provides numerous advantages to host plants when compared to other nonmycorrhizal plants. One benefit to the host is increased root surface area with the addition of fungal hyphae. The hyphal extensions enable plants to acquire large quantities of water and nutrients, especially in areas outside the nutrient depletion zone around the roots; this increases the available niche space for the plant (Allen 1991). Additionally, hyphal diameter is much smaller than the roots, which allows hyphae to penetrate smaller soil pores that were previous unavailable to the plant (Allen 1991). This increased water absorption improves the hydraulic conductivity and leaf water potential in plants (Garg et al. 2006). Mycorrhizae are
essential for transporting inaccessible phosphorus to plants, which occurs due to the slow movement of phosphorus in the soil and the depletion zone around roots (Garg et al. 2006; Kaschuk et al. 2009). Plants require phosphorus for many enzymatic and signal transduction pathways (Garg et al. 2006). As phosphorus levels increase, plants may increase photosynthetic rates by raising the leaf area ratio and decreasing carbon allocation to roots (Bray et al. 2003). Mycorrhizae can also improve the uptake of other important soil nutrients such as K, Ca, Mn, and N, which are unavailable to nonmycorrhizal plants (Brundrett 2004; Garg et al. 2006; Quilambo 2003; Remy et al. 1994).

In addition to increased nutrient and water acquisition, mycorrhizal colonization provides other important advantages to host plants. Colonization results in increased soil stability, disease resistance in the host plant (Brundrett 2004), and improved stress tolerance of drought, soil acidity, salinity (Allen 1991; Garg et al. 2006; Rillig 2004), and heavy metal contamination (Quilambo 2003). In ectomycorrhizal plants, the fungus alters host root architecture, protecting the roots from decomposers (Langley and Hungate 2003). Additionally, plants with AM have greater dry biomass and increased height compared to nonmycorrhizal plants (Koch and Johnson 1984). In legumes, mycorrhizae can improve nitrogen fixation and photosynthesis (Garg et al. 2006; Kaschuk et al. 2009). Lastly, mycorrhizae can increase hormone production (Remy et al. 1994) and chlorophyll levels in leaves (Garg et al. 2006).

Cost of Mycorrhizae to Host Plant

Although many benefits are provided by colonization, mycorrhizae are heterotrophic and require a portion of the fixed carbon from the host. About 30-60% of the carbon fixed by
photosynthesis is transferred to the roots (Lynch and Whipps 1990). Approximately 40-90% of this carbon is then lost from the roots as respiration, rhizodeposition (carbon loss by exudates, secretions, lysates, and gases), and through micro-organisms (Lynch and Whipps 1990). Koch and Johnson (1984) estimate that mycorrhizae accounts for 0-12% of this carbon loss, while several other studies have measured about 4-16% of carbon loss by vesicular arbuscular mycorrhizae (Kaschuk et al. 2009; Lynch and Whipps 1990; Tinker et al. 1994), with estimates as high as 20% loss (Garg et al. 2006). The costs of colonization are highest in young plants where the mycorrhizae are much more efficient at obtaining water and nutrients than the immature roots, thus the relationship is essential (Kaschuk et al. 2009).

Carbon loss to mycorrhizae is proportional to the amount of nutrients acquired, which is often difficult to assess. Plant dependency on mycorrhizae decreases when nutrients are no longer limited in the surrounding soil (Kaschuk et al. 2009). When examining the costs of phosphorus acquisition, one study found that mycorrhizal plants required twice as much carbon as nonmycorrhizal plants (Kaschuk et al. 2009). However, another study argued that since mycorrhizal roots are smaller, more soil can be penetrated for the same carbon cost as nonmycorrhizal plant roots (Kaschuk et al. 2009). More accurate cost estimates can be obtained by calculating the amount of carbon required for production of the fungal structural components of nucleic acids, lipids, and proteins. Especially since mycorrhizal lipid production is energetically demanding (Kaschuk et al. 2009; Garg et al. 2006). Based on several different studies, Kaschuk et al. (2009) estimates that 400 to 1500 mg C g⁻¹ is taken from the plant for fungal growth and respiration. However, the prevalence of mycorrhizae across many plant taxa may suggest that the cost of mycorrhizal colonization is lowest compared to other nutrient and water acquisition methods (Tinker et al. 1994).
The benefits of increased photosynthesis by mycorrhizae outweigh the costs of reduced carbon to the host. Early research on mycorrhizae raised questions about whether the carbon drain by mycorrhizae negatively affected plant growth and reproduction. Several studies conducted over the past decade have revealed that mycorrhizae carbon costs are negated by increased photosynthesis of the host, which is a direct result of the mycorrhizal carbon sink (Kaschuk et al. 2009; Lynch and Whipps 1990; Tinker et al. 1994). The additional phosphorus supplied by the mycorrhizae flows into the chloroplast at the same rate that triose-phosphate is drawn out of the chloroplasts by mycorrhizae. As more carbon is drawn to the sink, greater amounts of carbon are produced, resulting in an increased rate of photosynthesis (Kaschuk et al. 2009; Taiz and Zeiger 2006). This positive feedback loop results in increased photosynthesis as carbon is used, however, increases in photosynthesis do level out over time. The phosphorus also plays a role in the regeneration of Rubisco through the phosphorylation of ADP, which can in turn increase photosynthesis as more phosphorus is supplied to the plant (Kaschuk et al. 2009; Taiz and Zeiger 2006). Researchers have also found that the mycorrhizal associations enhance plant nutrition, increasing leaf area size and the amount of carbon fixed per leaf (Kaschuk et al. 2009; Lynch and Whipps 1990). A combination of data from several studies shows that mycorrhizae increase rates of photosynthesis by 14% compared to nonmycorrhizal plants (Kaschuk et al. 2009).

Mycorrhizal colonization costs the host carbon, yet equal or greater amounts of carbon are made by increased photosynthesis than lost through the carbon sink. Several scientists estimate that approximately 4-16% of fixed carbon is lost from a plant by vesicular arbuscular mycorrhizae colonization (Garg et al. 2006; Kaschuk et al. 2009; Koch and Johnson 1984; Lynch and Whipps 1990; Tinker et al. 1994). However, Kaschuk et al. (2009) have found that
mycorrhizal colonization increases host photosynthesis rates by about 14%. Therefore, the costs of colonization are offset by the increase in photosynthesis alone (Kaschuk et al. 2009). When adding the increases in carbon fixation due to additional water, nutrients, and disease resistance, the benefits of colonization greatly outweigh carbon used by mycorrhizae. The symbiotic relationship between plants and fungus is evolutionarily conserved and abundant in numerous plant taxa, indicating that the costs of colonization must be much less than the benefits gained by this relationship.

**Mycorrhizae and the Effects on Community Composition**

Mycorrhizae may play a larger role in shaping community composition and ecosystem dynamics than previously realized. Classic ecological theory considers abiotic conditions, biotic interactions, and differential dispersal to be the principal drivers of plant community composition and diversity (Renker et al. 2004). However, recent studies have found competition and herbivory as key determinates to community patterns and organization (Renker et al. 2004). Unfortunately, relatively little attention has been given to the role of mycorrhizae in driving plant community structure (Renker et al. 2004; Richardson et al. 2000). Renker et al. (2004) hypothesizes that the distribution and abundance of plant communities in is fact dependant on the distribution of AM communities. Research has found an increased diversity and biomass of plant communities in areas with increased AM diversity (Renker et al. 2004; Wardle et al. 2004). As mycorrhizae impacts the nutrient acquisition and biomass of plants, the abundance of certain plant species changes, in turn, affecting community composition and other trophic interactions (Wardle et al. 2004). Additionally, mycorrhizae can
affect community composition by changing ecosystem processes of nutrient cycling,
decomposition, and soil structure (Langley and Hungate 2003).

Mycorrhizae can affect and influence several different plant communities at once through mycorrhizae networks. Common mycorrhizal networks (CMN) (Simard and Durall 2004) are interconnected networks of individual mycorrhizae that can transport carbon and nutrients between different plant communities (Friese and Allen 1991; Richardson et al. 2000). CMN can range in complexity, from connecting a few plants in one community, to quite complex networks of multiple fungal species interconnecting multiple plant species in several communities (Simard and Durall 2004). Only genetically compatible fungal species can fuse to the CMN; fusion sites are called anastomose (Allen 1991). Simard and Durall (2004) found that different types of mycorrhizae (ectomycorrhizae vs. AM) differentially transferred carbon between plants. Additionally, carbon transfer was bidirectional, with source and sink relations determining the direction and magnitude of carbon transfer (Simard and Durall 2004). CMN were found extensively in grassland and forests, with the greatest effects on seedling establishment (Simard and Durall 2004). These interconnected hyphal networks link dominant and subordinate canopy species, affecting nutrient transfer within the community (Quilambo 2003) and perhaps playing a role in determining the diversity and community structure (Simard and Durall 2004).

The interactions of aboveground and belowground processes affect different trophic levels and overall ecosystem functionality. In one study, aboveground trophic interactions, such as herbivory, reduced arbuscular mycorrhizal colonization in the roots; the wounded plant reallocated carbon for shoot growth, reducing carbon flow to the roots and to mycorrhizae (Wardle et al. 2004). Belowground nutrient cycling, comprised of decomposing roots/hyphae
and exudates, often exceeds that of aboveground litter and nutrient inputs (Langley and Hungate 2003). Plant community composition and even individual plant species impact the diversity and functioning of root organisms, such as soil pathogens, mycorrhizae, or root herbivores (Wardle et al. 2004). Conversely, soil organisms can impact the diversity and composition of plant community composition (Wardle et al. 2004).

Disturbance events greatly influence both mycorrhizal and plant community composition. Friese et al. (1997) states that disturbance may play the greatest role in determining the structure and functionality of mycorrhizal communities. Disturbance changes biotic and abiotic factors of the substrate, such as light, temperature, moisture, pH, mineral nutrients, water, and oxygen, which can affect mycorrhizal colonization and survival in the newly disturbed habitat (Friese et al. 1997). Large scale disturbances, such as volcanoes and fires, completely change these substrate factors, which affects the type of fungus that colonize the soil and the resulting vegetation that establishes. When examining disturbances caused by fire, the plant composition, which is influenced by fungal diversity, affects the severity and patchiness of the fire; then cyclically, the fire reduces plant biomass and ultimately, fungal colonization (Friese et al. 1997). Small scale disturbances are important as well, creating open niches for colonization and increased fungal diversity. Habitat fragmentation may play a role in the variation and distribution of AM, which can affect plant assemblages in specific communities, especially those plants requiring specific mycorrhizal symbionts (Renker et al. 2004).

Mycorrhizae can affect the successional trajectory of a disturbed site. Arbuscular mycorrhizal spores are dispersed by wind, water, and animals (Friese et al. 1997; Renker et al.
2004; Richardson et al. 2000). Smaller spores, which are carried by the wind, reach large scale disturbances first, while larger spores are later dispersed by rodents (Renker et al. 2004). The first plants to establish are mostly nonmycorrhizal, given the patchy distribution and low levels of mycorrhizae inoculum present in the soil. As mycorrhizal inoculum levels increase with additional spore dispersal (Friese et al. 1997), facultatively mycorrhizal plants, which are plants that benefit from mycorrhizal colonization in soils with low fertility, establish on the site. Arbuscular mycorrhizal diversity and distribution continually increases as mycorrhizae spread from root to root across the site and new spores are dispersed (Allen 1991). Finally, obligate mycorrhizal plants, which are fully dependant on mycorrhizal colonization for survival, become abundant on the site (Renker et al. 2004). Mycorrhizae colonization and distribution across the site can greatly affect which plant species establish and ultimately, the plant community composition in successional sites (Allen 1991). Therefore, restoration ecologists use mycorrhizae in habitat restoration by either retaining the original mycorrhizae or inoculate with a diverse mixture of local mycorrhizae (Renker et al. 2004).

Mycorrhizae and Invasive Plants

Mycorrhizae often form beneficial associations with invasive plants. A global study by Cronk and Fuller (1995) found that 82% of the 199 invasive plants surveyed had AM associations (Richardson et al. 2000). Given the prevalence of mycorrhizal colonization across plant taxa, the prolific distribution of mycorrhizae, and the low host specificity, this mutualistic relationship with numerous invasive plants is not surprising (Richardson et al. 2000). Mutualisms often aid in or are essential for successful invasion of non-native, invasive species into new habitats (Richardson et al. 2000), with animal pollination/seed dispersal and root organisms as the most
important mutualisms for invasion. Bray et al. (2003) found that mycorrhizal colonization increased invasive shrub invasion into grasslands. Other studies have found that the mycorrhizal associations have enabled invasive shrubs to invade previously closed, mature communities (Richardson et al. 2000). However, some facultatively mycorrhizal invasives may be favored over obligate mycorrhizal plants because they can thrive in habitats with little mycorrhizae (functioning as nonmycorrhizal species) and gain benefits from mycorrhizal colonization when competing with other plants (Richardson et al. 2000).

Invasive plants are only benefited by mycorrhizal colonization if the mutualistic relationship results in a greater competitive advantage for the invasive plant. Native mycorrhizae often colonize both native and invasive plants within the same habitat. Arbuscular mycorrhizal colonization of invasive plants should not provide a competitive advantage over native mycorrhizal plants, unless, the invasive species are able to use the mutualism in a new, unusual way (Richardson et al. 2000). For some invasive species, mycorrhizae have altered the competitive ability of the plant, which in turn, greatly changes the plant community composition in invaded habitats (Bray et al. 2003). Bray et al. (2003) found that mycorrhizae changed the morphology and physiology of the invasive shrub Ardisia crenata, enabling this plant to grow much faster than without mycorrhizae. Additionally, this shrub out-competes native plants in reduced light due to an increased leaf area ratio when inoculated with mycorrhizae (Bray et al. 2003). Another invasive plant, Asian knapweed (Centaurea maculosa), parasitizes multiple native prairie plants at once through the connected mycorrhizal network (Richardson et al. 2000).
Nonmycorrhizal invasive plants may invade new habitats by disrupting the mycorrhizal networks of native plants. Garlic mustard, *Alliaria petiolata*, is an invasive plant that produces an allelochemical, isothiocyanate (a glucosinolate derivative), which posses herbicide and antifungal properties (Roberts and Anderson 2001). The allelochemicals enter the soil through root exudates, leaf leachate, and/or decomposition of other plant tissues (Cipollini, Stevenson, and Cipollini 2008). In laboratory studies, Roberts and Anderson (2001) found that *A. petiolata* leaves inhibited germination of both mycorrhizae spores and native herbaceous plants. Several studies have confirmed that mycorrhizal colonization in native tree seedlings was significantly reduced in areas with *A. petiolata* (Barto et al. 2011; Stinson et al. 2006). Furthermore, these trees grew slower than other seedlings with intact mycorrhizae (Stinson et al. 2006). Barto et al. (2011) found that *A. petiolata* invasion did not affect overall AM richness, but resulted in a shift in AM communities toward less *A. petiolata* sensitive strains. These shifts in AM community composition occurred both locally and regionally (Barto et al. 2011). *Alliaria petiolata* has a greater negative effect on native plants with a high mycorrhizal dependency than on less dependent plants (Stinson et al. 2006). This disruption of mycorrhizal colonization may aid in *A. petiolata* invasion into mature forests (Stinson et al. 2006) and can greatly impact forest community composition by altering the growth rates and health of trees and herbaceous plants dependent on mycorrhizae (Stinson et al. 2006). Conversely, *A. petiolata* had no effect on the growth or reproduction of a nonmycorrhizal plant, *Arabidopsis thaliana* (Cipollini, Stevenson, and Cipollini 2008). Perhaps the negative allelopathic effects of *A. petiolata* are species specific, localized to contiguous plants, or are magnified by disruption of mycorrhizal networks (Cipollini, Stevenson, and Cipollini 2008). However, *Lonicera maackii*, another invasive plant, did negatively affect seed production in the nonmycorrhizal plant *A. thaliana*, indicating that the
negative effects of *L. maackii* are perhaps more extensive than simply disrupting mycorrhizal networks (Cipollini, Stevenson, and Cipollini 2008).
CHAPTER 2

ASSESSING THE ECOLOGICAL IMPLICATIONS OF ARBUCULAR MYCORRHIZAL FUNGAL COLONIZATION OF THE INVASIVE SHRUB AMUR HONEYSUCKLE (LONICERA MAACKII)

Introduction

Non-native, invasive plants can significantly alter the biodiversity and community structure of novel environments. Aside from habitat loss, invasive species pose the second greatest threat to global biodiversity (Wilson 2002). Once an invasive plant becomes dominant in a new habitat, interactions between native plant species are greatly changed, which results in altered community structure and ecosystem processes, such as of nutrient cycling, primary production, hydrology, food webs, succession, etc. (Bazzaz 1986; Denslow and Hughes 2004; Levine et al. 2003; Pimentel et al. 2000). At this stage, many invasive species are no longer kept in balance by the native plants, soil biota, or environmental conditions, but are now driving and changing terrestrial ecosystems (Huebner and Tobin 2006). These changes ultimately alter the successional trajectory of the invaded habitats.

*Lonicera maackii* is an invasive shrub that is radically transforming the diversity, structure, and functioning of Eastern Deciduous Forests. Since the late 1980’s, ecologists have documented the profound negative effects that *L. maackii* has on the growth and reproduction of native herbaceous plants (Gould and Gorchov 2000; Miller and Gorchov 2004), tree seedlings (Hutchinson and Vankat 1997), and native species richness under *L. maackii* (Collier et al. 2002).
Hartman and McCarthy (2007) determined that *L. maackii* also reduces the growth, fecundity, and foliar biomass (Trammell et al. 2012) of overstory trees in invaded areas. Additionally, the longer *L. maackii* has been established, the greater detriment to aquatic food webs (McNeish et al. 2012) and native plant communities (Collier et al. 2002; Hartman and McCarthy 2008). Over time, populations of native plants may become genetically isolated, leading to local extinctions (Collier et al. 2002; Miller and Gorchov 2004) and significant changes in the succession of forests with and without *L. maackii* invasion (Hartman and McCarthy 2008). Recent studies have found that rapid *L. maackii* leaf decomposition (Arthur et al. 2012; McNeish et al. 2012; Trammell et al. 2012), rainwater penetration, and nutrient deposition (McEwan et al. 2012) under *L. maackii* may significantly alter nutrient cycling and contribute to altered successional trajectories in habitats invaded by *L. maackii*.

Several life history traits of *L. maackii* have been correlated with invasive success. *Lonicera maackii* shrubs are reproductively plastic, alternating between vegetative and seed reproduction (Luken and Mattimiro 1991); which likely benefits *L. maackii* during times of rapid population growth. Additionally, *L. maackii* prolifically produces berries, which are dispersed by many native animals. *Lonicera maackii* differentially partitions resource use and biomass allocation for growth depending on the light availability (Luken 1988; Sanford et al. 2003). Perhaps the greatest advantage to *L. maackii* is the extended leaf phenology in comparison to native plant species (Gould and Gorchov 2000; McEwan, Birchfield, et al. 2009; Trisel and Gorchov 1994). Finally, there are no known biological controls for *L. maackii*, which enables this species to allocate resources for growth and reproduction, rather than defense.
For over a decade, researchers have debated about which mechanisms are most important for *L. maackii* competition with native plants. Hartman and McCarthy (2007) have determined that *L. maackii* competes with native species for below-ground resources, such as soil nutrients, water, and root space, which may direct affect overstory trees. Callaway and Ridneour (2004) hypothesized that allelopathy maybe a novel weapon used by *L. maackii* to out-compete native plants. This hypothesis is supported by several studies that have found *L. maackii* extracts to be allelopathic and detrimental to native plants (Cipollini and Dorning 2008; Dorning and Cipollini 2006) and lethal to the invasive gypsy moth (*Lymantria dispar*) (McEwan, Rieske, *et al.* 2009). However, allelopathy has been difficult to confirm in field experiments. Lastly, Gorchov and Trisel (2003) attribute *L. maackii*’s invasive success to above-ground competition for light, which is a greatly affected by *L. maackii*’s extended leaf phenology. However, one potential mechanism that has been previously unexplored is the disruption of native mycorrhizal networks by *L. maackii*.

The ubiquitous mycorrhizal colonization of vascular plants reveals the significance of this symbiotic relationship. Mycorrhizae colonize about 95% of all vascular plants (Blee and Anderson 1998), with arbuscular mycorrhizae (AM) accounting for the majority of endomycorrhizal colonization (Richardson *et al.* 2000). Arbuscular mycorrhizae exchange nutrients within host plant cells through structures called arbuscules. The densely branched arbuscules greatly increase the fungal surface area that comes into contact with the plant cell membrane, resulting in increased nutrient exchange between the plant and fungus. Mycorrhizal colonization provides numerous advantages to host plants when compared to other nonmycorrhizal plants. Several studies have determined that mycorrhizal plants have increased root surface area, which results in increased water and soil nutrients, such as phosphorus (Allen
1991; Garg et al. 2006; Kaschuk et al. 2009), K, Ca, Mn, and N, which are unavailable to nonmycorrhizal plants (Brundrett 2004; Garg et al. 2006; Quilambo 2003; Remy et al. 1994). Additionally, mycorrhizal plants have increased disease resistance (Brundrett 2004), and improved stress tolerance to drought, soil acidity, salinity (Allen 1991; Garg et al. 2006; Remy et al. 1994), and heavy metal contamination (Quilambo 2003). In return, the host plant provides fixed carbon to the heterotrophic mycorrhizae. Several studies have estimated that mycorrhizae remove 4-16% of carbon from the host (Garg et al. 2006; Kaschuk et al. 2009; Koch and Johnson 1984; Lynch and Whipps 1990; Tinker et al. 1994). However, mycorrhizal colonization results in a 14% increase in host plant photosynthesis (Kaschuk et al. 2009) and when combined with the benefits of additional water, nutrients, and disease resistance, the benefits of colonization normally greatly outweigh carbon use by mycorrhizae.

The impacts of mycorrhizal colonization extend beyond individual plant interactions and have been shown to play a large role in influencing community composition and ecosystem dynamics. The notion that plant community distribution may be directly related to AM community composition is supported by research that found an increased diversity and biomass of plant communities in areas with increased AM diversity (Renker et al. 2004; Wardle et al. 2004). As mycorrhizal colonization differentially affects certain plant species, plant community composition and trophic interactions change (Wardle et al. 2004). Interconnected networks of individual mycorrhizae (common mycorrhizal networks) are also having profound impacts on nutrient transfers within and between different plant communities (Friese and Allen 1991; Quilambo 2003; Richardson et al. 2000), which may affect diversity, competition, and plant community structure (Simard and Durall 2004). Mycorrhizae can also impact ecosystem
processes by altering nutrient cycling, decomposition, and soil structure (Langley and Hungate 2003).

Mycorrhizal fungi colonize a range of non-native invasive plants, however, it’s the potential competitive advantage gained by invasives that makes this mutualistic relationship ecologically significant. Richardson et al. (2000) determined that AM colonization of invasive plants should not provide a competitive advantage over native plant colonization unless the invasive used the symbiosis in a new, beneficial way. Several studies have found that mycorrhizae altered the competitive ability of some invasive plants, which in turn, greatly changed the plant community composition in invaded habitats (Bray et al. 2003). In the presence of mycorrhizae, Bray et al. (2003) found that the invasive shrub Ardisia crenata altered its morphology and physiology, allowing it to grow faster. Another invasive species, Asian knapweed (Centaurea maculosa), parasitized native prairie plants thorough use of a common mycorrhizal network (Richardson et al. 2000). Garlic mustard (Alliaria petiolata), an invasive allelopathic plant, reduced the growth of native herbaceous plants (Roberts and Anderson 2001) and tree seedlings by disturbing mycorrhizal networks (Barto et al. 2011; Stinson et al. 2006). Lonicera maackii is another invasive plant that has significantly reduced the growth and fecundity of native plants. However, the interaction between L. maackii and native mycorrhizae has been previously unexplored as a mechanism for competition with native flora.

The objective of this study was to quantify the mycorrhizal colonization of L. maackii roots as compared to native plant roots and asses the competitive advantages gained by L. maackii as a result of mycorrhizal interaction. We hypothesized that the total mycorrhizal colonization would be significantly lower in L. maackii roots than in native control roots.
Furthermore, we hypothesized that the percent total root length colonized by arbuscules would also be significantly reduced in *L. maackii* roots compared to native roots.

**Methods**

*Field Sampling/Data Collection*

This study was conducted in a secondary growth, beech-maple forest at Sugar Creek MetroPark, a Five Rivers MetroPark in Dayton, Ohio (USA) (Figure 1). Fifteen *Lonicera maackii* shrubs of similar height and canopy size were sampled (Figure 2). An additional fifteen control sites were established in contiguous areas of the forest lacking in *L. maackii* and with similar soil water holding capacity measurements (Figure 3). Control sites were positioned by a stratified random method along a transect running the length of the area without *L. maackii*. The native plant species in the control sites consisted of spring ephemerals, tree seedlings, forest herbs, and other common woodland flora. At each shrub or control site, three soil cores were randomly taken at a distance of 0.5m or 1.0m from the shrub base or control center along cardinal and intermediate directions. At each location, the cores were combined into one bag and all soil core holes were refilled. Samples were collected in April, May, June, and August of 2009. However, in April, only ten *L. maackii* shrubs and five control sites were sampled.

*Lab Processing*

The roots were prepared for arbuscular mycorrhizal (AM) assessment by removing the roots from the soil, clearing the roots of tannins with KOH, and then staining the roots with trypan blue (Phillips and Hayman 1970); any mycorrhizal structures within the roots were
stained by the trypan blue. The roots were scored for mycorrhizal colonization by a gridline-intersect method (Brundrett et al. 1994; Brundrett 2008; Giovannetti & Mosse 1980). Roots were randomly spread in a petri dish with intersecting grid lines spaced 1.0cm apart. With a dissecting microscope, the vertical and horizontal lines were scanned at 200x magnification for roots and the following information was recorded: the number of roots crossing a line, if the root was colonized or not, and the type of colonization (hyphae, arbuscule, vesicle, or spore). The total percent root length colonized was calculated by dividing the total number of colonized hits by the total number of root hits per sample. Similarly, total percent root length colonized with arbuscules was calculated by diving total arbuscule hits by the total root hits. Calculations were also made for vesicle and spore colonization. For all L. maackii samples, all roots analyzed were L. maackii roots and were easily distinguished from native herbaceous and woody roots based on color, size, and root architecture. For all control samples, the roots consisted of a random sample of native plant species and none of the native roots were identified or separately analyzed by species.

Statistical Analysis

A t-test (SigmaPlot 11.0) was used to compare differences in percent total root length colonized with arbuscules, vesicles, and spores between L. maackii and native or control roots for all months. Non-parametric data that could not be corrected with an Arcsin Square Root transformation were compared using a Mann-Whitney test (SigmaPlot 11.0). A Dunn-Sidak correction was used to adjust α. A one-way ANOVA was used to compare the seasonal variation in root colonization and arbuscular formation for both L. maackii and control roots. A Tukey post-hoc test was used to determine significant differences between comparisons. Differences
in vesicle and spore formation were not significant enough to compare seasonally with a one-way ANOVA. All bar graphs were created in SigmaPlot 11.0 with standard error bars.

Results

When comparing overall mycorrhizal colonization between *Lonicera maackii* roots and native control roots, *L. maackii* roots had a significantly higher percentage of total root length colonized in April and June than did native plant roots (*p* < 0.001), (Figure 4). However, there were no significant differences in colonization during May and August (*p* = 0.319 and *p* = 0.840, respectively). In control roots, colonization levels across all months were not significantly different (*p* = 0.602), (Figure 5). Colonization levels in *L. maackii* roots were significantly different in April compared to August (*p* = 0.005), with colonization levels the greatest in April (Figure 5). For all samples of *L. maackii* and control roots, hyphae accounted for the majority of the percent total root length colonized by mycorrhizae.

The percentage of total root length colonized by arbuscules was significantly higher in *L. maackii* roots across all months of the study (*p* < 0.001), (Figure 6). The greatest difference in arbuscule colonization occurred in April; *L. maackii* roots were colonized with an average of 4.1 times more arbuscules than native control roots. The next greatest differences in arbuscule colonization were June and August, with 3.8 and 3.7 times more arbuscules in *L. maackii* than controls (respectively). In May, *L. maackii* root colonization with arbuscules was 2.2 times greater than native control root colonization.

*Lonicera maackii* roots were densely packed with chains of arbuscules across all months of the study (Figure 7). When comparing arbuscule density in *L. maackii* and control roots,
greater masses of arbuscules were observed throughout *L. maackii* roots (Figure 8). Since all AM structures absorb the trypan blue stain, differences in arbuscule density were seen as different intensities of blue in the roots. In *L. maackii* roots, the dense chains of arbuscules appeared as one large, bright blue mass. However, in the native plant roots, less densely clustered arbuscules appeared as a light blue color, much lighter than the arbuscules in *L. maackii* roots.

Arbuscule colonization of *L. maackii* roots was highest in April, increased slightly in June and decreased again in August (Figure 9). However, only April and June arbuscule colonization were significantly higher than August (*p*=0.019 and *p*=0.032, respectively) with no other differences between months. In native control roots, arbuscule colonization was greatest in May and decreased by August (Figure 9). The only significant differences in colonization levels were May compared to August (*p*=0.033).

Overall, spore colonization was very low in both *L. maackii* and control roots. The percentage of root length colonized by spores was significantly higher in August *L. maackii* roots than controls (*p*=0.011, Dunn-Sidak corrected *p*=0.0127), (Figure 10). There were no significant differences in spore colonization across any other months (April *p*=0.827, May=0.247, and June *p*=0.323). For any single sample, the greatest numbers of spores were 26 in *L. maackii* roots and 11 spores in control roots (during June).

Few vesicles were found in *L. maackii* and control roots, with only 31% of all samples containing a vesicle. The percent of root length colonized with vesicles was significantly higher in *L. maackii* in August than in control roots (*p*=0.009, Dunn-Sidak corrected *p*=0.0127), (Figure 11). There were no significant differences in vesicle colonization during all other months (April
p=0.240, May p=0.339, and June p=0.666). Across all months, the greatest number of vesicles found in any *L. maackii* sample was 8 vesicles (June) and 2 vesicles in a control sample (found in April, May, and June).

**Discussion**

This study determined that *L. maackii* is readily colonized by arbuscular mycorrhizae, with colonization levels often greater than in native plants. When analyzing the functional aspect of the symbiosis, arbuscular colonization of *L. maackii* roots was significantly higher than native control roots during all months of the study. *Lonicera maackii* roots were densely packed with chains of arbuscules and contained 2.2 - 4.1 times more arbuscules than native plant roots. Additionally, arbuscular colonization levels peaked earlier in *L. maackii* roots than in native plants. While arbuscular colonization levels can be seasonally high, there are no records in the literature reporting the significantly high and sustained levels of arbuscular colonization as seen in the *L. maackii* roots in this study. The extended leaf phenology of *L. maackii* (Gould and Gorchov 2000; McEwan, Birchfield, *et al.* 2009; Trisel and Gorchov 1994) may explain *L. maackii*’s ability to sustain these earlier and higher levels of arbuscular colonization compared to native plants (Figure 12). If colonization levels were greater than what the host plant could support, the host would induce a pathogenic response, killing sections of the root to stop further fungal infection (Allen *et al.* 1989; Allen 1991). We did not observe any pathogenic responses in *L. maackii* roots, indicating that mycorrhizal colonization was within sustainable levels. Therefore, our hypotheses that total mycorrhizal and arbuscular colonization would be significantly reduced in *L. maackii* compared to control or native roots have been rejected.
The high level of arbuscular colonization in *L. maackii* roots indicates a significant benefit to this invasive shrub. As arbuscular colonization increases, the total surface area of the periarbuscular interface increases, resulting in greater nutrient exchange between the host plant and mycorrhizae. However, increased benefit to the host also means increased carbon demand by mycorrhizae. Several studies have shown that in mycorrhizal plants, the cost of mycorrhizal colonization was greatly offset by increased photosynthesis due to additional water, nutrients, and disease resistance provided by colonization (Kaschuk et al. 2009; Lynch and Whipps 1990; Tinker et al. 1994). With the significant level of arbuscular colonization in *L. maackii* roots, *L. maackii* must be able to fix greater amounts of carbon to sustain the arbuscular demand. Harrington et al. (1989) found that early leaf expansion accounts for 25-35% carbon gain/year (McEwan, Birchfield, et al. 2009), providing *L. maackii* with a photosynthetic advantage over native plants. The extended leaf phenology of *L. maackii* may facilitate the high level of arbuscular colonization compared to native plants, resulting in increased nutrients and, ultimately, benefit to the invasive shrub (Figure 12). Extended leaf phenology may also explain the significantly higher levels of spores and vesicles in *L. maackii* roots in August compared to native plants. Mycorrhizae may produce more spores and lipid storage units (vesicles) in *L. maackii* roots in response to increased carbon availability in the fall compared to native plants.

The benefit of increased nutrients from mycorrhizal colonization of *L. maackii* likely provides a competitive advantage for *L. maackii* over native plants. Native mycorrhizae often colonize both native and invasive plants within the same habitat. Arbuscular mycorrhizal colonization of invasive plants should not provide a competitive advantage over native mycorrhizal plants, unless, the invasive species are able to use the mutualism in a new, unusual way (Richardson et al. 2000). The increased nutrient acquisition, photosynthesis (Kaschuk et al.
2009; Lynch and Whipps 1990; Tinker et al. 1994), disease resistance, and stress tolerance (Allen 1991; Garg et al. 2006; Rillig 2004) provided by the high levels of arbuscular colonization would provide a large competitive advantage for L. maackii compared to native plants, enabling L. maackii to out-compete native plant species. Additionally, the increased root surface area likely aids in increased water acquisition for the shallow roots of L. maackii.

The significant mycorrhizal colonization of L. maackii roots complicates the possibility of allelopathy as a mechanism used by L. maackii to out-compete native plants. Several lab studies have determined that L. maackii extracts are allelopathic and detrimental to the growth and fecundity of native plants (Dorning and Cipollini 2006; Cipollini and Dorning 2008), yet do not affect the survival of other L. maackii plants. However, the allelopathic effects of L. maackii on native plants have been difficult to confirm in the field. Additionally, since L. maackii are significantly colonized by mycorrhizae, the allelopathic extracts produced by L. maackii must not negatively affect mycorrhizal networks. In another known allelopathic invasive plant, Garlic mustard (Alliaria petiolata), which is nonmycorrhizal, the allelopathic leaves inhibited the germination of mycorrhizal spores and native plants (Roberts and Anderson 2001) and reduced mycorrhizal colonization of native tree seedlings (Barto et al. 2011; Stinson et al. 2006). In a study comparing the effects of both L. maackii and A. petiolata on a nonmycorrhizal plant, Arabidopsis thaliana, L. maackii did negatively affect seed production in A. thaliana, while A. petiolata did not (Cipollini, Stevenson, and Cipollini 2008). Cipollini, Stevenson, and Cipollini (2008) speculate that the effects of L. maackii on native plants are more extensive than the disruption of mycorrhizal networks. If L. maackii uses allelopathy to reduce native competition, the allelochemicals must negatively affect the surrounding native plants, without disrupting the mycorrhizal networks that these native plants and L. maackii depend on.
The results of this study clearly indicate a significant symbiotic relationship between the native vesicular arbuscular mycorrhizae (AM) and the invasive shrub *Lonicera maackii*. The high level of arbuscular colonization in *L. maackii* is likely sustained by its extended leaf phenology and results in increased nutrients, photosynthesis, disease resistance, and stress tolerance and, ultimately provides a large competitive advantage for *L. maackii* to out-compete native plant species. Previous research has identified other mechanisms that may be important in the competitive success of *L. maackii*. Allelopathy and nutrient depletion may provide a competitive advantage for *L. maackii*, but likely play a minor role in native plant reduction (Gould and Gorchov 2000). Gorchov and Trisel (2003) hypothesize that light competition is more important than below-ground competition. However, light levels may also affect carbon production and allocation within *L. maackii*, which can directly affect arbuscular colonization levels. Therefore, the arbuscular colonization of *L. maackii* is a significant symbiosis and likely an important mechanism enabling *L. maackii* to out-compete native plants.
Figure 1: Map of the research site, Sugarcreek MetroPark, Five Rivers MetroParks, Dayton, Ohio. The red circle indicates the specific location where all the data was collected.
Figure 2: Photograph of an average *Lonicera maackii* shrub in April 2009.

Figure 3: Photograph of an average control site in April 2009.
Figure 4: Mean (SE) percent total root length colonized with mycorrhizae across all sampling dates. *Lonicera maackii* colonization was significantly higher than native control roots in April and June (p < 0.001).
Figure 5: Mean (SE) percent total root length colonized with mycorrhizae compared between months within L. maackii and control roots. Solid line depicts comparison among L. maackii samplings and dashed line indicates control comparisons. Lonicera maackii colonization levels were significantly different in April compared to August (p=0.005).
Figure 6: Mean (SE) percent total root length colonized with arbuscules across all sampling dates. *Lonicera maackii* arbuscular colonization was significantly higher than native control roots across all months of the study (p < 0.001).
Figure 7: *Lonicera maackii* roots densely packed with arbuscules during April (A), May (B), June (C), and August (D).
Figure 8: Photographs of *L. maackii* and control roots in June and August depict differences in arbuscule density. Arbuscule density in *Lonicera maackii* roots during June (A) and August (B) compared to native control roots during June (C) and August (D).
Figure 9: Mean (SE) percent total root length colonized with arbuscules compared between months within *L. maackii* and control roots. Solid line depicts comparison among *L. maackii* samplings and dashed line indicates control comparisons. *Lonicera maackii* arbuscular colonization levels were significantly higher in April and June compared to August (p=0.019 and p=0.032, respectively). Control arbuscular colonization levels were only significantly different in May compared to August (p=0.033).
Figure 10: Mean (SE) percent total root length colonized with spores across all sampling dates. *Lonicera maackii* spore colonization was significantly higher than native control roots in August (p=0.011, Dunn-Sidak corrected p=0.0127).
Figure 11: Mean (SE) percent total root length colonized with vesicles across all sampling dates. *Lonicera maackii* vesicle colonization was significantly higher than native control roots in August (p=0.009, Dunn-Sidak corrected p=0.0127).
**Figure 12:** A conceptual model illustrating the potential benefits, physiological changes, and competitive advantages gained by high arbuscular colonization of the invasive shrub *Lonicera maackii*. The arbuscular colonization of *L. maackii* is ecologically significant and likely an important mechanism enabling *L. maackii* to out-compete native plants.
CHAPTER 3
SYNTHESIS AND FUTURE DIRECTIONS

The significance of the ubiquitous mycorrhizal colonization of vascular plants is well understood, however, few studies have explored the importance of mycorrhizae in community or ecosystem processes. Additionally, the bulk of ecological research has focused on abiotic conditions, dispersal, and biotic interactions, such as competition and herbivory, as principal drivers of plant community composition and diversity (Renker et al. 2004), not mycorrhizal associations (Richardson et al. 2000). The many benefits of mycorrhizal colonization to host plants have been well documented in the literature (Allen 1991; Brundrett 2004; Garg et al. 2006; Kaschuk et al. 2009; Langley and Hungate 2003; Quilambo 2003; Remy et al. 1994; Rillig 2004), yet the majority of research continues to focus on species-specific interactions with mycorrhizae, rather than community or ecosystem dynamics/responses to mycorrhizae. The role of mycorrhizae in influencing community composition and ecosystem function is still poorly understood.

Several studies have begun exploring the effects of mycorrhizae on community dynamics. In communities with increased AM diversity, the diversity and biomass of plant communities were also increased (Renker et al. 2004; Wardle et al. 2004). Wardle et al. (2004) determined that the mycorrhizal impacts on nutrient acquisition and plant biomass differentially
affected plant species abundances, and in turn, community composition and trophic interactions. Additionally, Langley and Hungate (2003) found that mycorrhizae affected community composition through altering nutrient cycling, decomposition, and soil structure within the ecosystem. Other mycorrhizae and community dynamic research has studied the effects of common mycorrhizal networks on nutrient transfer and community structure (Simard and Durall 2004; Richardson et al. 2000), the interaction of above-ground and below-ground processes (Langley and Hungate 2003; Wardle et al. 2004), and the effects of disturbance on mycorrhizal diversity and the resulting plant community composition (Allen 1991; Friese et al. 1997; Renker et al. 2004). Within the past decade, several studies have begun exploring the importance of mycorrhizal associations on invasive species success.

For many invasive species, mycorrhizal interactions have provided a competitive advantage over native plants. Several invasive species, such as Centaurea maculosa and Alliaria petiolata, are non-mycorrhizal and negatively affect native plant species through parasitism (Richardson et al. 2000) and disruption (Barto et al. 2011; Stinson et al. 2006) of mycorrhizal networks. However, for other non-mycorrhizal invasive plants like Salsola kali, the mycorrhizae parasitized and eventually killed the invasive plant (Allen et al. 1989). Conversely, many invasive plants are benefited by mycorrhizal colonization, such as Ardisia crenata, which grew faster as a result of colonization (Bray et al. 2003). Lonicera maackii represents a new group of invasive plants in which heavy AM colonization provides a significant competitive advantage over native plants.

This study has determined that L. maackii is heavily colonized by arbuscular mycorrhizae and the symbiosis is likely an important mechanism in the competitive success of L. maackii over
native plants. However, many new questions arise with the conclusion of this study. Has the high level of arbuscular colonization and increased nutrient acquisition enabled *L. maackii* to extend its leaf phenology? Or, has the extended phenology with added carbon production allowed *L. maackii* to support higher arbuscular colonization than native plants with a reduced phenology (Figure 12)? Are arbuscular mycorrhizae equally attracted to *L. maackii* and native plant species or does *L. maackii* emit a signal through a pathway response to attract higher levels of arbuscular colonization? Given the significantly higher arbuscular colonization, is the mycorrhizal carbon drain in *L. maackii* significantly higher than the carbon drain in native plants? Also, is the mycorrhizal colonization of *L. maackii* really an equal mutualism or are the arbuscules draining more carbon than the amount of nutrients provided to *L. maackii*? Several studies have found that the phosphorus supplied by mycorrhizae flows into the chloroplast at the same rate that triose-phosphate is drawn out of the chloroplasts by mycorrhizae, resulting in proportional nutrient and carbon exchange (Kaschuk *et al.* 2009; Taiz and Zeiger 2006). Additionally, we did not observe pathogenic responses in *L. maackii* roots, indicating that the arbuscular colonization and resulting carbon drain was within sustainable levels. Lastly, how does light availability affect arbuscular colonization of *L. maackii*? Several studies have found that net primary production, seed production, and germination were greatly reduced in forest grown *L. maackii* shrubs than open grown shrubs (Luken 1988; Luken and Goessling 1995; Luken and Mattimiro 1991). Therefore, one would speculate that arbuscular colonization would be reduced in forest grown shrubs compared to open grown shrubs.

The high arbuscular colonization of *L. maackii* leads to additional questions about the importance of other competitive mechanisms used by *L. maackii*, such as nutrient acquisition and allelopathy. Does arbuscular colonization better enable *L. maackii* to out-compete native
plants for below-ground resources, such as nutrients or water? Additionally, how is allelopathy a mechanism for competition given the significant mycorrhizal colonization of *L. maackii* roots? If *L. maackii* uses allelopathy to reduce native competition, the allelochemicals must negatively affect the surrounding native plants without disrupting the mycorrhizal network that these native plants and *L. maackii* depend on. Future studies need to determine how *L. maackii*'s allelopathic compounds negatively affect native plants without harming the mycorrhizal networks. Additionally, how do the concentrations of allelochemicals differ in *L. maackii* roots compared to leaves and do these concentrations differentially affect soil surface and below-ground processes? Further research is needed to determine the extent to which mycorrhizal association with *L. maackii* provides a competitive advantage. Which mechanism is most significant for out-competing native plants – light, mycorrhizae, below-ground or above-ground competition for resources?

Additional research is needed to determine how mycorrhizae aids in *L. maackii* invasion into new habitats and the impact that *L. maackii* removal has on mycorrhizal inoculum potential of forest soils. Are the mycorrhizal inoculum levels in the soil different in areas where *L. maackii* has recently invaded vs. areas where *L. maackii* has established? How does the age of the *L. maackii* colony affect the mycorrhizal inoculum level? To preserve the biodiversity of plant communities, *L. maackii* must be removed and often restoration is needed to prevent other invasives from occupying the empty niche. In the literature, there are several effective methods to remove *L. maackii*; however, little to no research has been conducted on how different removal methods affect soil mycorrhizae levels. When herbicides are applied during the cut and spray, foliar spray or injection methods, are the chemicals transferred to the mycorrhizal networks and if so, what affects do the chemicals have on mycorrhizae? Which *L. maackii*
removal method retains the greatest amount of mycorrhizal inoculum in the soil? Additionally, how do different restoration practices affect mycorrhizal inoculum potential of forest soils? When comparing mycorrhizal inoculum levels, how are restoration practices different for habitats heavily invaded with *L. maackii* vs. Garlic mustard (*A. petiolata*)? Lastly, Luken (1988) determined that *L. maackii* is an important contributor to the primary production of invaded habitats. Future studies should examine how *L. maackii* affects carbon cycling in invaded forests.


