PROCEEDINGS OF THE

2010 Ohio Invasive Plants Research Conference:

"Connecting Research and Land Management"

February 18, 2010
Indoor Adventure Center
Frankin Park, Columbus, Ohio

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*Authors submitted an abstract of their presentation only, without a full research summary.

Conference Information

The Ohio Invasive Plants Council (OIPC) is a non-profit coalition of organizations and individuals throughout the state of Ohio who have a mutual interest in Ohio's natural ecosystems and the effects of invasive plants and other organisms on their biological diversity. As its primary mission, OIPC participates in statewide efforts to address the threats of invasive species to Ohio's ecosystems and economy by providing and promoting stewardship, education, research, and information exchange. OIPC has been hosting research conferences every three years since 2004.

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OIPC also received a generous grant from The Columbus Foundation in 2009, part of which was used for this conference.

OIPC's Research Work Group designed and hosted the 2010 conference and consists of:

Don Cipollini, Chair
Nicole Cavender
Theresa Culley
Kathleen Knight
Brian McCarthy
Joanne Rebbeck
Jennifer Windus

The following individuals contributed to conference planning and logistics:

Karen Adair
Kendra Cipollini, Editor of Proceedings
Mark Gilson
Nora Hiland
Carrie Morrow

The Ohio Invasive Plants Council is guided by a 12-member Board of Directors, elected to 3-year terms by the members of the Council. The Board of Directors at the time of this conference is composed of:

Jennifer Windus, President , representing state government (ODNR Division of Wildlife)
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Sandy Woodthorpe, representing interested public

Conference Agenda

8:00 Registration, Indoor Adventure Center

8:45 Welcome (Don Cipollini, Wright State University)

9:00 Karen Goodell, Ohio State University-Newark, *Invasions and plant-pollinator interactions: perspective and context determine outcomes*

9:30 Amy Campbell, Ohio State University, *Sexual Reproduction in non-native common reed,* Phragmites australis

10:00 Break, poster session, and refreshments

11:00 **Keynote Address:** Richard Mack, Washington State University, *Eradication or Control? Combating invasive plants through a lump sum payment or on the installment plan*

12:00 Lunch

1:00 Kathleen Knight, USDA Forest Service Northern Research Station, *Effects of emerald ash borer on forest ecosystems: facilitation of invasive plants*

1:30 Theresa Culley, University of Cincinnati, *Defining invasive plants in Ohio: A case study of the ornamental Callery pear (*Pyrus calleryana*)*"

2:00 Break, poster session, and refreshments

2:45 Don Cipollini, Wright State University, *Microbial pathogens of garlic mustard: hope for control, or risk of parasite spillback?*

3:15 Land managers' panel

4:20 Closing remarks (Jennifer Windus, Ohio Department of Natural Resources, Division of Wildlife; OIPC President)

4:30 Tours of Franklin Park Conservatory

5:30 Cocktails at the Conservatory

Invasions and plant-pollinator interactions: perspectives and context determine outcomes

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We investigate the interactions between invasive plants and pollinators. These mutualistic interactions comprise a key element in the successful spread and establishment of invasive plants that require pollinators, but they could also impact the populations of pollinators that use them. Changes in the foraging patterns or the population abundances of pollinator populations could in turn affect the reproduction of native plants that also rely on the same pollinators. We use *Lonicera maackii* as a model system to examine how the interactions between an invasive plant and pollinators affect both the reproduction of the invasive plant and reproduction of native plants. We find that the nature of these interactions is highly contextual, depending on local variation in both abiotic and biotic conditions. These findings complicate the classification of this invasive species as "harmful" or "benign", but provide insights into its ecological role that could guide control and management strategies.

Sexual reproduction in non-native common reed, *Phragmites australis*

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Non-native *Phragmites australis* (common reed) invades fresh and saltwater wetlands in North America. This aggressive species can rapidly form monotypic populations, decreasing native species diversity. In the US, past research suggests *P. australis* spreads primarily through vegetative propagation. We investigated seed viability, dormancy and germinability of *P. australis* populations growing in four freshwater wetlands adjacent to Lake Erie, Ohio. In June of 2006, we located 24 sampling sites in three hydrologic zones: upland (never inundated), shoreline (moist soil), and inundated (submerged throughout the growing season) and collected seeds from current-year individuals. The majority (96%) of seeds tested had embryos that stained normally using a tetrazolium test, indicating seeds are mostly viable. Germination of scarified seeds increased significantly from 70% in water, to 95% in gibberellic acid, which suggests *P. australis* seeds exhibit some dormancy. Nonscarified seeds had low germination percentages (6-25%) when exposed to a constant temperature of 25 ºC, but germination improved significantly (90-100%) when the seeds were exposed to a diurnal temperature fluctuation of 10/30ºC. Results were consistent across hydrologic zones, suggesting water level to which a parent plant is exposed does not play a significant role in seed performance. Seeds may play a larger role in the spread of non-native *P. australis* than previously thought.

Phragmites australis is a cosmopolitan species that occurs in fresh and saltwater wetlands around the world. In North America, a non-native European haplotype has invaded many wetlands along the eastern seaboard and the Great Lakes region. This aggressive species can rapidly become mono-dominant, decreasing native plant and animal species diversity. Past research suggests *P. australis* populations spread primarily through vegetative propagation in North America (Harris & Marshall 1960, Gervais et al. 1993). Sexual reproduction has been thought to be a minor component of the species' reproductive strategy. But if this is true, how has this non-native species spread so rapidly over such long distances? Vegetative reproduction cannot account for this. Seed production, viability and dispersal must be more successful in common reed than previously thought.

To explore this possibility, we looked at seed viability and germination rates of seeds collected in stands of non-native *P. australis* in four wetland systems located on the southern shore of Lake Erie: Mentor Marsh, East Harbor Wetland, MHD Marsh, and Ottawa National Wildlife Refuge. Because water depth affects the performance of *P. australis* (Mauchamp et al. 2001), study sites in each wetland were selected where populations occurred in three hydrologic regimes: inundated, shoreline, and upland water levels. The objectives of this study were to: (1) determine the effect of parent plant hydrology on seed viability and germinability, and (2) ascertain whether *P. australis* seeds exhibit any dormancy.

Mature inflorescences were randomly selected from different parent plants at each site. Seeds used in viability and dormancy tests were scarified by cutting through the endosperm on the side opposite the embryo. Seeds used in germination tests remained whole.

To investigate seed viability, a tetrazolium test was performed on a total of 200 seeds (50 seeds x 4 replicates) from each of 12 sites. Imbibed seeds were covered with 1% tetrazolium chloride solution for 18 hours, washed and covered with 85% lactic acid solution for 2 hours. Seeds were examined under a dissecting microscope, and normally stained embryos were counted to obtain percent seed viability. Seed dormancy was tested using a total of 400 seeds (50 seeds x 4 replicates x 2 treatments) from each of 12 sites. Seeds were germinated in either distilled water or 10^{-3} M gibberellic acid solution (GA), a common plant growth hormone, at 25 °C in constant light for 7 days. To test seed germinability, 400 seeds (50 seeds x 4 replicates x 2 treatments) from each of the 24 sites were germinated in distilled water at either constant temperature (25°C) or diurnally fluctuating temperatures (10°C for 16 hours and 30°C for 8 hours) in constant light for 12 days. All test results were analyzed using analysis of variance (ANOVA).

Nearly all (96%) of the tested seeds stained normally when exposed to tetrazolium chloride solution, indicating seeds were mostly viable. Germination increased significantly when seeds were exposed to gibberellic acid (70% in distilled water, 95% in GA; $F = 99$, $p < 0.0001$), indicating there is some level of seed dormancy present (Figure 1). Germination increased significantly when seeds were exposed to diurnally fluctuating temperatures (18% in constant T, 96% in fluctuating T; $F = 1993$, $p < 0.0001$) (Figure 2). In all tests, there were significant differences among wetland systems, which suggest the local environment affects *P. australis* seed characteristics.

Figure 1.

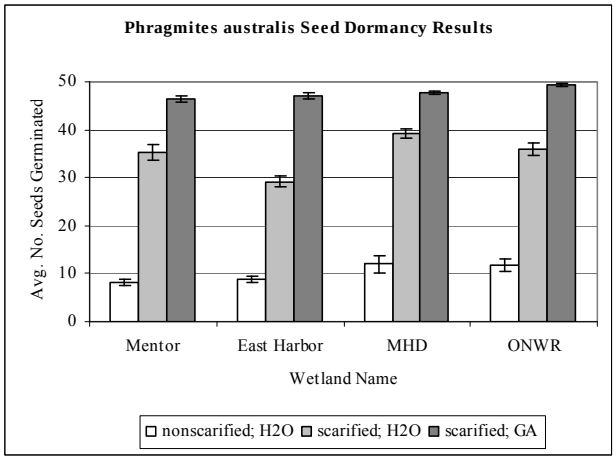
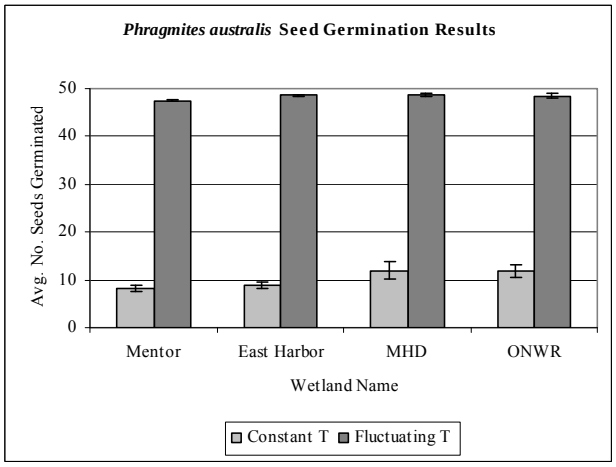


Figure 2.



The rapid and almost complete germination of the seeds from these sites is in sharp contrast to previous *P. australis* germination studies in the US that found that few seeds will germinate (Wijte & Gallagher 1996, Pellegrin & Hauber 1999). Studies in Europe and Japan have also produced varying accounts of *P. australis* seed characteristics (Ekstam & Forseby 1999, Nishihiro et al. 2004). These different results could be due to site-specific differences between locally adapted *P. australis* populations. It is likely that local adaptations around Lake Erie allow seeds to remain dormant until an environmental trigger, such as a suitable range of fluctuating temperatures in early spring, signals optimal growing conditions.

Phragmites australis populations are actively managed in many areas, making information regarding the species' potential for spread very useful. An investigation of seed production, seed longevity, and susceptibility to predation would provide further insight into the relative significance of *P. australis* seeds. Another potential area of research would be to determine whether *P. australis* seeds produce gibberellic acid endogenously. If GA is the hormone responsible for stimulating germination in *P. australis* seeds, this could open a new avenue of control, as commercial anti-gibberellins are currently used in the agricultural industry to control weed seed germination.

The importance of sexual reproduction to Lake Erie reed populations is still unclear, as seedling survival and establishment should be taken into account. It seems probable, though, that *P. australis* spreads aggressively and rapidly because of a two-pronged approach: seedling establishment in mudflat and disturbed areas, followed by vegetative propagation. In this way, a few seedlings' survival would be sufficient to start a new population.

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Eradication or control? Combating invasive plants through a lump sum payment or on the installment plan

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The advantages of eradicating, compared with simply controlling, potentially invasive plants seem clear: the pest species is eliminated and additional resources (other than to detect the species' re-entry and destroy the immigrants) will not be needed. The counter argument is that eradication is either prohibitively expensive or logistically impossible, or both. Control is however often a poor bargain, as it requires the commitment of public funds for an indefinite period. If the control effort is reduced or abandoned for whatever reason, all the accumulative effort may be rapidly undone once the invasive species resumes its spread. If a well-developed eradication protocol is followed scrupulously, it could be a practical option in combating potentially invasive plants in many more cases.

Effects of emerald ash borer on forest ecosystems: facilitation of invasive plants

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Emerald ash borer (EAB) (*Agrilus planipennis*), an introduced insect pest, has killed millions of ash trees in the Midwest and is spreading rapidly. The effects of EAB on forest ecosystems are being studied through a collaborative research program between the US Forest Service and the Ohio State University. We are monitoring the decline and mortality of >4500 ash trees and saplings, as well as changes in understory light availability, the responses of both native and invasive plant species, changes in species composition and forest structure, and effects on other organisms and ecosystem processes in over 250 monitoring plots in forests in Ohio and Michigan, representing a gradient of EAB infestation duration. Yearly monitoring began in 2004 and is continuing. The plots are located in forest stands representing different ages and habitat types to include all five ash tree species native to the region: pumpkin ash (*Fraxinus profunda*) and black ash (*Fraxinus nigra*) are found in swamp ecosystems, green ash (*Fraxinus pennsylvanica*) is typical of floodplains, white ash (*Fraxinus americana*) inhabits upland forests, and blue ash (*Fraxinus quadrangulata*) has an affinity for calcium-rich areas. Forest canopy gaps, formed by dying ash trees, allow increased light to the understory in ecosystems without a well-developed midstory. The increased light affects both native and invasive plants in these ecosystems. We have identified 14 species of invasive plants in the monitoring plots, with at least one invasive plant species present in most plots. Initial cover of invasive species was low in most plots, which may indicate an opportunity to control plants in these ecosystems before they respond to gaps. Individual invasive species distributions were associated with habitat, geography, and land use history.

Defining invasive plants in Ohio: a case study of the ornamental Callery pear (*Pyrus calleryana*)

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Exactly how to define and identify invasive plants is a difficult topic that relies on available data on plant traits, behavior, and impacts on the ecosystem. An invasive plant is a species or cultivar, which once introduced into a new location, spreads to different natural areas and persists in populations which negatively impact the ecosystem. Why and how some plant species become invasive is not always known but is of great interest to scientists, land managers, government agencies, and nursery professionals alike. In Ohio, a collaborative effort involving these groups is underway to update the list of top invasive plants created over a decade ago. This Ohio Invasive Plants Council (OIPC)-sponsored endeavor is centered on using objective, scientifically-based research to assess invasiveness of plants. To highlight the challenges and opportunities of this effort, a case study is presented of the Callery pear (*Pyrus calleryana*). This Chinese species is one of the most popular ornamental trees today but is also increasingly found in natural areas in Ohio. Research now indicates that wild populations form from cross-pollination between different cultivars and rootstock. Preliminary evidence also suggests that wild pear can negatively impact the above-ground and below-ground biotic communities, and thus should be of concern statewide.

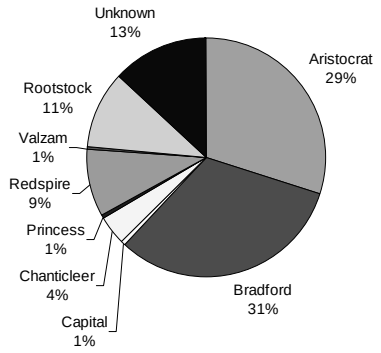
Despite the dramatic economic costs that invasive plants impose in terms of control efforts and damage to the ecosystem (Pimental et al. 2005), identifying such plants can be challenging, especially if they are in the early stages of spread. Most invasive species are only noticed once they have reached substantial numbers although theoretical models are now being developed to predict invasiveness (e.g., Herron et al. 2007). More recently, scientific interest has focused on understanding the mechanisms of invasion – that is, how a species or cultivar may become invasive over time. For example, are certain taxa predisposed to invasiveness because of traits brought from the native range? Or is invasiveness a condition that evolves over time, reflecting genetic rearrangements and adaptation to new environments? In ornamental plants, can invasiveness be unintentionally promoted by selection for desired horticultural traits that also happen to promote spread (i.e. abundant flowering, environmental tolerance, rapid growth)? These and other questions underlie the difficulty in defining and identifying invasive plant species, and specifically in creating a list of invasive species in Ohio. Despite these challenges, such information is of great interest to scientists studying evolutionary adaptation in plants, and is critical for land managers and governmental agencies if they are to effectively manage invasive species. Given that many woody invasives have a past or current horticultural use (Reichard and White 2001), the nursery industry is also an important contributor to this dialogue.

In Ohio, a collaborative effort is now underway involving these groups to update the list of top invasive plants, created primarily from input from land managers over a decade ago. This new Ohio Invasive Plants Council (OIPC)-sponsored endeavor follows that of other states and is based on objective, scientifically-based research to assess invasiveness of individual species or cultivars (cultivated varieties). Although still ongoing, this project involves both the creation of an assessment protocol as well as its application to introduced species throughout the state.

The Callery pear (*Pyrus calleryana*) is a species currently being examined in the assessment procedure and is an example of the challenges and opportunities facing this endeavor. This ornamental tree is one of the most popular landscaping trees in the US, sold commercially as different cultivars including 'Bradford', 'Cleveland Select', 'Chanticleer', 'Aristocrat' and 'Autumn Blaze' (Culley and Hardiman 2007). Collectively known as the Callery pear cultivars, these trees are prized for their abundant production of white flowers in early spring, fast growth rate, and general hardiness. Originally imported into the US from China in the early 1900's to breed fireblight resistance into the edible *P. communis*, this species has more recently begun to appear along roadsides, along the edges of

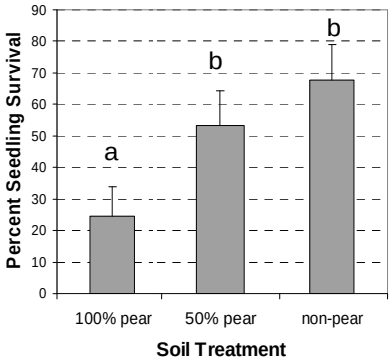
parks, and even occasionally within forests in Ohio. In the southern US, Callery pear is already perceived as a problematic species and is on several invasive or watch lists because it spreads so readily and can form dense thorny thickets that outcompete other species. Why these wild populations form in the first place is now known. As a Rosaceous species that exhibits gametophytic self-incompatibility, individuals of *P. calleryana* are generally unable to self-pollinate, although this appears to be a leaky system in which selfing can occur on rare occasion (Culley and Hardiman, unpubl. data). As revealed by microsatellite markers, wild populations across the US are now known to originate largely from cross-pollination between genetically different cultivars planted in surrounding commercial or residential areas (Culley and Hardiman 2009). In these cases, wild individuals are F_1, BC, or advanced generation hybrids. Within Ohio, wild populations are generally younger and primarily composed of F_1 hybrids. More recently, it has become evident that rootstock of grafted commercial trees can also contribute to the formation of wild populations if rootstock is allowed to sprout and flower (Culley et al., in review). Figure 1 displays the percent contribution of cultivars, rootstock, and unknown individuals in a parentage analysis of 100 wild individuals from Benedict Nature Preserve in Cincinnati, OH. Thus this species differs from many other invasive taxa in that a single Callery pear cultivar or rootstock is not necessarily invasive, but rather it is the presence of more than one cultivar or rootstock in a given area that can trigger fruit formation, resulting in wild populations.

Figure 1.



Invasiveness of a species also depends upon the existence of detrimental impacts on the surrounding ecosystem, a topic just beginning to be quantified in the Callery pear. In addition to forming large thickets that displace neighboring species, preliminary evidence suggests that *P. calleryana* may also be allelopathic. For example, the species is resistant to fireblight disease because it produces benzoquinone, a phytotoxic compound which also inhibits germination and growth of various plant species. Although it is still unknown whether benzoquinone itself can leach from the rootzone or from fallen leaves of *P. calleryana* to detrimentally impact neighboring plants, preliminary soil tests indicate that radish seedling survival can be reduced in soil collected from under pear trees (Figure 2). Additional tests are now ongoing to determine the strength and repeatability of this effect, and to identify any additional allelopathic compounds that may be released by wild *P. calleryana* individuals. Investigations are also continuing to determine the effect, if any, of these compounds on the soil community, including fungi and bacteria. As such, more information is needed on the ecosystem effects of the Callery pear before a comprehensive assessment of its level of invasiveness can be completed.

Figure 2.



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Microbial pathogens of garlic mustard: hope for control or risk of parasite spillback?

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In the last decade, garlic mustard in southwestern Ohio has increasingly shown evidence of attack by several pathogenic fungi and bacteria. Thorough study is required to know whether such pathogens are potential natural biocontrols whose distribution should be promoted. A powdery mildew fungus has been identified through morphological and molecular analyses as a strain of *Erysiphe cruciferarum*, the causal agent of powdery mildew disease in crucifers. Diseased populations of garlic mustard are distributed throughout southwestern Ohio, with an apparent epicenter in Montgomery and Greene Counties, but disease incidence can vary greatly. In the greenhouse and field, powdery mildew disease reduced growth and fitness of garlic mustard and nullified its competitive ability with a native plant. In limited host specificity tests under greenhouse conditions, only one cruciferous crop cultivar was moderately susceptible to the local strain, but three wild cruciferous plants were moderately susceptible. In the field, none of the wild species showed evidence of disease in areas known to have held diseased garlic mustard, which may be due partly to phenological escape. Susceptibility of different garlic mustard populations from its native and invasive range appeared to vary in a qualitative fashion, suggesting major gene resistance, and most of the susceptible populations were localized to southwestern Ohio. Most of the European populations of garlic mustard examined were resistant to the local strain of powdery mildew. Recently, black rot disease caused by the bacterium *Xanthomonas campestris* pv. *campestris* was identified on garlic mustard through symptomology and biochemical analyses. This disease causes substantial visible mortality of garlic mustard in the field, but as a potential pathogen of crops and wild crucifers, it requires further study.

Physiological responses of invasive *Alliaria petiolata* to elevated atmospheric CO_2 and warm temperatures

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Global environmental changes such as increasing atmospheric CO_2 and temperature may influence invasion dynamics in complex ways. Garlic mustard (*Alliaria petiolata*) is a widespread, invasive biennial whose responses to future climate scenarios are not well understood. In a growth chamber study, *A. petiolata* and the co-occurring native *Geum vernum* were grown separately from seed in a factorial design under ambient and elevated (+ 3 °C) spring temperatures, and ambient and elevated atmospheric CO_2 conditions (380 vs. 550 ppm). Measurements included total biomass, leaf expansion rates, gas exchange, and leaf concentrations of allelopathic compounds for *A. petiolata*. Both species grew significantly faster at high temperatures than at ambient temperatures. The elevated CO_2 treatment had only slight effects on growth, but increased leaf thickness significantly in both species, and *A. petiolata* showed photosynthetic down-regulation at high CO_2. Concentrations of flavonoids and cyanide decreased significantly in *A. petiolata* leaves at elevated CO_2, possibly due to dilution by starch accumulation. Glucosinolate concentrations did not differ significantly among treatments. These data suggest that warm temperatures will be more important stimulants of growth for *A. petiolata* and *G. vernum* than elevated CO_2 and that there may be reduced allelopathy in *A. petiolata* in future climate scenarios.

Species invasions are now so pervasive and significant in their effects on the world's ecosystems that many ecologists consider invasive species to be an important global change, comparable to human-induced environmental changes such as rising atmospheric CO_2 and associated increases in temperature (Mack et al. 2000). It is critical to understand how invasions interact with these environmental global changes, as there is some evidence that these may facilitate invasive species. For example, elevated CO_2 is known to enhance the growth and photosynthetic efficiency of many C_3 species, and invasive plants have been observed to benefit to a greater extent than native plants in some situations (e.g., Smith et al. 2001). In addition, many invasive plants are allelopathic and so produce more secondary compounds compared to native plants. Elevated CO_2 has been shown to increase the production of toxins in *Toxicodendron radicans* (poison ivy, Mohan et al. 2006), suggesting that allelopathy could intensify in a high CO_2 environment. Finally, high temperatures may change growth rates and therefore the population dynamics and competitive interactions between native and invasive species (e.g., Williams et al. 2007).

Garlic mustard (*Alliaria petiolata*), a biennial herb native to Europe, is an important invasive plant in the United States with known allelopathic effects (e.g., Prati & Bossdorf 2004, Stinson et al. 2006). To our knowledge, its responses to predicted future increases in CO_2 and temperature have never been explored in the published literature. In this study we grew *A. petiolata* and *Geum vernum*, a co-occurring native perennial, in a factorial design under ambient and elevated (+ 3 °C) early spring temperatures, and ambient and elevated atmospheric CO_2 conditions (380 vs. 550 ppm). Our goal was to describe and compare the physiological and growth responses of these species to future climate scenarios.

Each species was grown separately from seed in 15 cm deep pots filled with a mixture of field soil, commercial potting mix, and sand. Pots (30 per treatment per species) were placed in one of four growth chambers in the Duke Phytotron Facility, each representing a different temperature-CO_2 treatment combination to give a full factorial design. Temperatures simulated average conditions in central Ohio for April and May, and were gradually increased during the study. Plants were grown for nine weeks and pots receiving a particular treatment were rotated to a different chamber every two weeks to minimize chamber effects on plant growth. Pot positions were rotated within chambers three times per week. Plants were watered with ¼ strength

Hoagland's solution three times per week, increasing to daily treatments as plants increased in size. RO water was applied to maintain moist soil conditions as needed. Plants received light levels of 800 microeinsteins within a 12 hour day/night schedule.

Leaf length was measured regularly in all plants. When the blade of 5th leaf was between 5 and 6 cm in length, 12 *Alliaria petiolata* were randomly selected in each of the four treatment combinations and the 5th leaf was sampled, weighed, frozen in liquid N and placed on dry ice for measurements of allelopathic compounds. Overall growth was assessed by collecting all plants at the end of the study for shoot biomass, and a subset of plants was sampled for root biomass. Photosynthetic rates, light response curves, and A-C_i curves were measured on a randomly chosen subset of 8-week old plants with a portable LI-6400 infrared gas exchange system.

Both *A. petiolata* and *G. vernum* grew significantly faster at high temperatures than at ambient temperatures and this was particularly pronounced in the first four weeks of the study when temperatures were coolest. The elevated CO_2 treatment had only slight effects on growth, but increased leaf thickness significantly in both species, a common response in many plants grown at high CO_2. There was a tendency for *A. petiolata* to invest more in root tissue in the high temperature treatments compared to plants grown at ambient temperature. *A. petiolata* showed photosynthetic down-regulation (suppression of photosynthesis) at high CO_2, particularly in the low temperature treatment. In contrast, *G. vernum* photosynthesis was not affected by high CO_2 and was increased by temperature. Concentrations of flavonoids and cyanide decreased significantly in *A. petiolata* leaves at elevated CO_2, possibly due to dilution by starch accumulation. Glucosinolate concentrations did not differ significantly among treatments.

These data suggest that warmer temperatures will be more important than high CO_2 in determining early spring growth rates for *A. petiolata* and *G. vernum* under predicted future climate conditions. This makes sense since both species remain green overwinter, are photosynthetically active at temperatures slightly above freezing (L. J. Anderson, unpublished data), and probably are adapted to maximize growth during warm days in the variable weather conditions of early spring. The sensitivity of gas exchange to temperature in *G. vernum* can also be explained in this context.

Photosynthetic down-regulation has been observed in other species in response to high CO_2 and can be interpreted as an efficiency response to reduce carbon capture when the photosynthetic system becomes CO_2-saturated. However, this response can also be an artifact of root system constraints in pot studies. Our plants were grown from seed over nine weeks and did not appear excessively pot bound at the end of the study, but *G. vernum* did not show photosynthetic down-regulation and also had a smaller root system than *A. petiolata*. Therefore, it will be important to explore *A. petiolata* gas exchange responses to high CO_2 in a field setting without root constraints.

Contrary to the work of Mohan et al. (2006) on *T. radicans*, plant defense compounds in *A. petiolata* did not increase at elevated CO_2 and even showed evidence of dilution in the thickened leaf tissue. This response should also be explored more extensively in a field environment, but suggests that the allelopathic effects of *A. petiolata* may be reduced a warmer, CO_2-rich world. This may have important implications for competitive interactions among species and exploration of these physiological changes in a community context is a critical next step.

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Assessing the effects of Amur honeysuckle control on native forest plant communities four years after honeysuckle removal

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Amur honeysuckle (*Lonicera maackii*), an important invasive shrub, has a phenology and dense canopy that can exclude native herbs and interfere with regeneration of woody plants in forests. I established modified Whittaker plots in four stands in a Woodland Mound Park, Hamilton County Park District, in southwest Ohio, with a gradient of honeysuckle infestation intensities in 2005. Honeysuckle canopies were removed by herbicides in fall 2005. Plant cover was monitored every year from 2005 to 2009. Total species number has increased at all sites but the one with lowest initial honeysuckle cover, and it appears to have stabilized by 2009 at most of the sites. Herb cover has also increased at all sites, with the sharpest increase in 2009. Much of this increase in 2009 is due to a rapid cover increase by the invasive herb garlic mustard (*Alliaria petiolata*). Surprisingly, *A. petiolata* increased the most at the site with lowest initial honeysuckle cover. The Shannon diversity index *H´* also increased sharply in 2009 at all sites. Invasive species presence has increased at all sites, with the possible exception of the site with lowest initial honeysuckle cover; the presence of flowering by invasive species has followed similar trends.

The spread of invasive plant species is now recognized as a major problem for native plant communities (Pimentel et al. 2005). Woody invasive plants can have particularly large impacts as they can affect multiple strata of forest communities (Webster et al. 2006). Control efforts can be effective in temporary control of many of these woody species (see Boyce 2010). Invasive plants can change ecosystem function in a variety of ways, and removal of the invasive species may not necessarily result in complete restoration of the plant community.

One of the most important invasive plants in the Ohio River Valley and adjacent areas of the US is *Lonicera maackii* (Rupr.) Maxim (Amur honeysuckle). It was introduced from eastern Asia to the United States as an ornamental shrub in the mid-19th century (Luken and Thieret 1996). It has been shown to reduce species richness and the survival and growth of both herbaceous and woody species, including even the growth of mature trees. This has been attributed to its extended-deciduous leaf habit and dense canopy, competition for soil resources, and allelopathic effects on herbaceous species (Dorning and Cipollini 2006, Cipollini et al. 2008a, b).

A recent study (Runkle et al. 2007) has shown that recovery of the understory can take at least a year after *L. maackii* removal, and also that invasive *Alliaria petiolata* (M. Bieb.) Cavara & Grande (garlic mustard) increased its coverage after *L. maackii* removal. Because native species may have difficulty recolonizing a stand from which they have been excluded, removal of *L. maackii* may result in increases in *A. petiolata*. Thus, the objective of this study was to document the changes in the plant community that occurred after *L. maackii* stands of various ages and coverages were removed.

This project was carried out at Woodland Mound Park, Hamilton County Park District, in southwest Ohio. Elevations in the park range from roughly 150 to 240 m. Much of the park consists of second-growth forest, with a significant cover of *L. maackii*. Four sites were chosen to represent a "chronosequence" of *L. maackii* stand ages. *Lonicera maackii* stand ages were estimated on the basis of its cover; stands were designated Sites 1-4, with Site 1 having the least cover and Site 4 the most. Stand age was later estimated by aging the largest stump found in each stand. A modified Whittaker plot, consisting of a large 20x50 m (1000-m^2) plot, with subplots of various sizes, was placed at each site. The *L. maackii* canopy was removed in late fall 2005. Foliar herbicide (glyphosate) was sprayed at all sites except Site 1, where *L. maackii* shrubs were cut and glyphosate was painted on the stumps.

Every site was visited frequently enough from late March into fall in 2005, 2006, 2007 and 2009 to estimate coverage and flowering of all species. Herb coverage (%) for each species was estimated on the 10 1-m^2 subplots. Shrub coverage (%) was measured on the 2 10-m^2 subplots in 2005 only, before *L. maackii*

removal. Tree basal areas (m^2 ha^{-1}) were estimated by measuring diameter at breast height (dbh) in 2005 and 2007 on the 100-m^2 subplots. Species presence, as well as evidence of flowering or fruiting, was noted on subplots and plots of all sizes (1-m^2 to 1000-m^2).

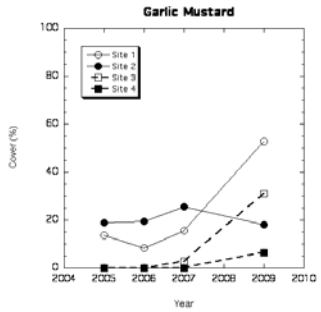
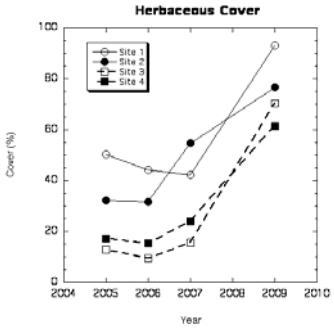


Figure 1.
Figure 2.



The number of species at Site 1 has remained at 50-55, while it appears to be converging on 80-85 species at the other sites. Herb coverage also increased dramatically at all sites (Figure 2), including Site 1. Unfortunately, *A. petiolata* is one of the species that has increased its coverage, and is now one of the dominant species at all sites.

Why this is true is unclear. If weather were responsible, or if an invasion "front" was passing through Woodland Mound Park, there should be increases at all sites, but Site 2 has stayed fairly constant. If site history were the case, we would expect the smallest change in garlic mustard at Site 1, which had the smallest initial honeysuckle coverage and in many ways has changed little. *Alliaria petiolata* appears to be affecting diversity parameters; the Shannon diversity index *H´* for the herb stratum has increased 2-3X at all sites due to the increase in number of species, but the increase in *A. petiolata* has kept the Shannon evenness index *J´* from also increasing, as evenness is decreased by a species with high coverage. Other changes are starting to appear. Species turnover rates per year were calculated. If Site 2 is excluded, then mean species turnover rate/year increases as one goes from Site 1 (0.02) to Site 3 (0.03) to Site 4 (0.18), indicating that herbs have a greater response when a more honeysuckle canopy was present. Site 2, however has a greater rate of turnover (0.11) than expected. Honeysuckle on this site was treated differently; shrubs were cut and herbicide applied to stumps. It is possible that the cut shrub tops provided refuges for herbs from deer browsing, allowing more herbs to establish and thereby accelerate the rate of species turnover. It is also possible that the increased native herb abundance has kept *A. petiolata* from increasing at this site (Figure 2).

When 1-m^2 plots are examined, there has been an increase in the mean number of invasive species over time (~1.5X at all sites). The largest change has been at Site 4 (more than 2X). Very similar trends were seen when the total number of invasive species on the 1000-m^2 plots, although Site 1 does not appear to increase. Flowering among invasive plants had trends somewhat like those for species presence, but there were also differences. Site 1 has been fairly stable across the period for both the 1-m^2 plots and the 1000-m^2 plots. While Site 2 showed increases from 2005 to 2007, there was a sharp decrease in 2009 back to levels similar to those seen in 2005. Sites 3-4 have shown increases.

The largest changes have been 1) large increases in herb coverages at all sites, and 2) increases in *A. petiolata* coverage, which are associated with changes in diversity. The total number of species may be leveling off at most sites. Two results are puzzling. 1) Why is garlic mustard now so prevalent at Site 1? 2) Why is Site 2 responding so differently from other sites?

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Does *Alliaria petiolata* or its extracts affect invertebrate abundance or behavior?

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The allelopathic effects of *Alliaria petiolata*, garlic mustard, on other plants and mycorrhizae have been well-documented, but we know less about the direct effects of allelopathic chemicals on the fauna that live in invaded communities. To investigate these effects, we conducted invertebrate surveys in garlic mustard-invaded and control plots and also tested the direct effects of garlic mustard extracts on invertebrate behavior in the lab. We ran surveys across four sites in Ohio to extract invertebrates from leaf litter in invaded and uninvaded sites. More invertebrates were found in leaf litter from invaded habitats, although this depended on the site under scrutiny. Garlic mustard extract deterred terrestrial isopods but attracted *Spodoptera exigua* larvae. These results suggest that allelochemicals from garlic mustard may negatively affect certain invertebrates but may also attract other taxa. One possible mechanism for the attraction of invertebrates is garlic mustard's ability to increase nutrient availability in hardwood forests.

Invasive plant species often have major effects on ecosystems, and can affect not only other plants but higher trophic levels (Stout & Morales 2009). One such plant is *Alliaria petiolata*, or garlic mustard (GM), a plant native to Europe and introduced to North America over a century ago. Researchers have determined that GM's success may be through allelopathic effects on mycorrhizal fungi (Wolfe et al. 2008) or other species' growth (Barto & Cipollini 2009). We know little, however, about the negative effects of GM on other organisms. The only study looking at ground invertebrates in areas with and without GM could not find a significant difference in numbers of ground beetles between the two areas (Davalos & Blossey 2004). Other researchers, however, have found that removal of GM increased both arthropod and salamander abundances in a central Ohio forest (G. Smith, unpublished manuscript). There have been few studies to determine whether any effects are due to direct allelopathic effects of GM. Our study thus seeks to answer two questions: 1) do the abundances of invertebrates differ between GM-invaded and uninvaded plots across Ohio and 2) does GM extract affect the behavior of invertebrates?

We performed Berlese funnel sampling of invertebrates in leaf litter at four different populations within Ohio across a N/S transect: Two populations near the Denison University Campus (Taylor-Ochs (T) and Norpell Woods (N)), the Kenyon College biological Reserve (K), and The Bath Nature Preserve near Akron (A). At each population we chose a site currently or recently invaded by GM and an adjacent site less than 100m away where no GM was growing. We marked ten, $1m^2$ adjacent quadrats in each site and 20g of leaf litter was collected from the middle of each quadrat. Invertebrates were extracted using Berlese funnels within a week of collection. Invertebrates included ants, larvae, terrestrial isopods, Annelids, beetles, Molluscs, spiders, springtails, centipedes, and Hemipterans. Because there were too many zeros if we separated the organisms into categories, we analyzed the total number of organisms per funnel using a Generalized Linear Model (GLM) with an exponential link function with site and treatment as crossed effects.

GM extracts were made by soaking 50g of whole plant material in 1000 mL of distilled deionized water for 24h. The liquid fraction was then pulled through a funnel with filter paper using a vacuum to remove coarse debris. This was repeated and then the fraction was sterilized by pulling the liquid through a 0.2 micron filter. Control treatments were produced by sterilizing distilled deionized water through the 0.2 micron filter only. Control and GM extract (1.5 ml of each) were squirted onto two half circles of filter paper and each semi-circle was placed into a single 15-cm diameter petri dish. A single organism was then placed in the

middle of the petri dishes and allowed to choose one side or the other. The position of the organism was noted every 15 minutes up to an hour after initial placement and the proportion of organisms on the control or GM extract side was analyzed using a Chi-square test. If the organism was found in the middle of the dish or on the lid it was not counted. The following organisms were tested using this method: *Spodoptera exigua* (beet armyworm, n = 31) larvae, *Galleria mellonella* (waxworm, n = 34) larvae, and terrestrial isopods (*Porcelio* spp., n=38). All organisms were purchased from commercial sources and so presumably did not have an ecological history with GM.

We found that there was a significant site by treatment interaction in the GLM ($X^2 = 9.12$, 3 df, $p = 0.027$). Looking at the simple effects within each site, GM-invaded sites had more organisms per Berlese trap at Kenyon and Norpell Woods but not at Akron or Taylor Ochs (Figure 1). We did not find evidence that GM extract significantly affected the behavior of *G. mellonella* ($X^2 = 0.11$, $p = 0.732$), but that GM extract did affect *S. exigua* ($X^2 = 3.9$, $p = 0.048$) and the isopods ($X^2 = 8.52$, $p = 0.004$). Seventy-four percent of the isopods were found on the control side, while 32% of *S. exigua* larvae were found on the water-only side (Figure 2).

Figure 1.

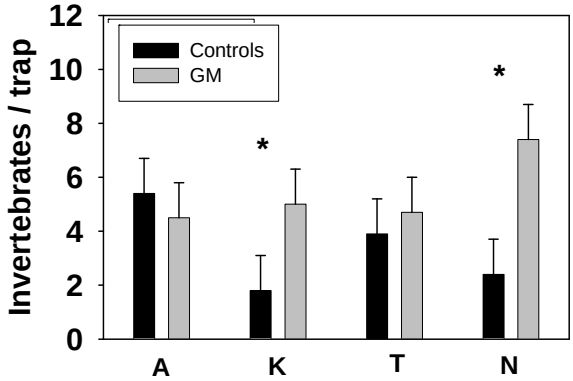
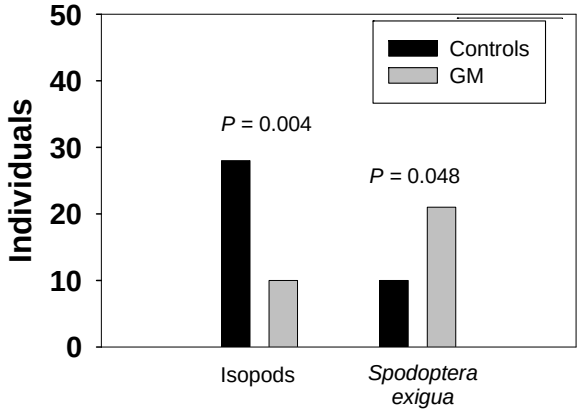


Figure 2.



These results suggest that invertebrates are attracted to or perform better in GM-invaded leaf litter. Other researchers have shown that GM can increase rates of decomposition and nutrient availability in hardwood forest litter (Rodgers et al. 2008). This mechanism might explain the higher numbers of organisms where GM had invaded, but does not explain the behavioral experiment results. Another possibility is that sites where GM had invaded were more also more shaded than areas that were typically more barren, with more shaded areas being more attractive to invertebrates (Lindhe et al. 2005).

Although our results are tantalizing and suggest that GM exudates may attract or benefit invertebrates, more research is needed to confirm our findings. It would be helpful to know if organisms prefer GM extract over other plant exudates because the invertebrates could be simply cueing into any plant volatiles in hopes of a meal rather than being attracted to GM itself. Finally, more experiments should remove GM and observe whether the fauna changes in composition or abundance over time. These types of experiments would probably prove more powerful than observational studies alone.

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Comparison of mechanical, foliar and dormant stem control methods on mortality of autumn olive (*Elaeagnus umbellata*); a study on reclaimed surface mine land

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The Wilds is a conservation organization located on 3,700 hectares of reclaimed surface mine land in southeastern Ohio. Improvement of wildlife habitat and invasive species management are central to *The Wilds*' Restoration Ecology program. The shrub *Elaeagnus umbellata* is of particular concern. Historically, it was planted to improve soil stability and nitrogen content during the reclamation process. Since reclamation, *E. umbellata* has spread prolifically, forming dense stands and reducing the quality of grassland habitat. Control of *E. umbellata* is difficult due to prolific seed production and aggressive re-sprouting. The primary goal of this study was to assess the effectiveness of three control methods (mechanical removal, foliar herbicide and dormant stem herbicide applications) on the mortality of *E. umbellata*. In 2007-2008, nine 200m^2 study plots with three replications of each treatment were established. Effectiveness of each treatment was evaluated in 2009 with a total of 225 individual shrubs tracked. The foliar herbicide treatment resulted in 98% mortality of *E. umbellata*; dormant stem herbicide caused 71% mortality, and mechanical control yielded only 15% mortality. Statistical comparisons indicated the foliar herbicide application was more effective ($p < 0.025$) in causing total mortality than either the dormant stem herbicide treatment or mechanical removal.

The Wilds is a large scale wildlife research center dedicated to advancing conservation through science, education and personal experience. Functioning as a living laboratory, the center is situated on nearly 4,000 ha of reclaimed surface mine land and is visited by over 75,000 people annually. *The Wilds*' Restoration Ecology staff seeks to demonstrate effective land management techniques that improve wildlife habitats and increase biodiversity on a landscape scale through eradication of invasive species such as autumn olive (*Elaeagnus umbellata*). Native to Eastern Asia, *E. umbellata* is an aggressive deciduous shrub up to 6 m in height with alternate shimmery leaves and red fruits speckled with pale scales (Gleason & Cronquist 1991). Due to its tolerance for poor soils and nitrogen-fixing properties, *E. umbellata* was one of several non-native species utilized in re-vegetation plantings during surface mine reclamation in the early 1980's (ODNR 1983). This shrub continues to reproduce prolifically, encroaching into the open pastures and reducing quality of grassland habitat. This is of particular concern for imperiled species, such as Henslow's sparrow and other obligate grassland nesting birds, which have been shown to utilize reclaimed mine land (Bajema et al. 2001, Ingold et al. 2009). Removal of *E. umbellata* is particularly challenging due to variable reproduction strategies that include abundant seed germination, vegetative root suckers and vigorous stump sprouting following mechanical treatments (Kohiri et al. 2002).

The primary goal of this study was to assess the effectiveness of three distinct control methods on the mortality of *E. umbellata*. Control methods included mechanical removal, foliar and dormant stem herbicide applications. Activities began with the establishment of twelve 200 m^2 (4 ha) study plots. Among the plots, three replications of each treatment and a control group were designated. Prior to treatment, GPS location data and metal marker tags were recorded for 25 random individual shrubs per plot for a total of 225 individuals tracked. During the 2007-2008 seasons, *E. umbellata* was controlled using the three treatment methods. The foliar herbicide applications were conducted in August 2007 using the following mix of product and concentration: 26.7% imazapyr isopropylamine salt (Arsenal Powerline™) at 16 oz/100 gal, 60% metsulfuron methyl (Escort XP™) at 2 oz/100 gal flowable product, surfactant (Surf Plus 584 MSO™) at 16 oz/100 gal and drift retardant (Mist Trol 336™) at 4 oz/100 gal. The mechanical removal was conducted in November 2007 using a John Deere 3110D backhoe to extract the aboveground plant material and the main root ball. Following removal, the plant materials were placed in a central pile within the plot. The dormant stem treatments were completed in February 2008 using the following mix of product

and concentration: 61.6% triclopyr: 3,5,6-trichloro-2-pyridinyloxyacetic acid, butoxy ethyl ester (Garlon 4™) at 1.5 gal/100 gal, 27.6% imazypyr isopropylamine salt (Stalker™) at 16 oz/100 gal, 90% alkyl aryl polyoxyethylene ether, free fatty acids & isopropanol (Invade 90™) at 1 gal/100 gal, surfactant (Axit Oil™) and drift retardant (Mist Trol 336) at 4 oz/100 gal. Both herbicide applications were completed utilizing a two-person crew and a 300-gal trailer sprayer unit with handgun attachment.

During the 2009 season, all recorded shrubs were evaluated according to the effectiveness of each treatment. Treatments were measured based on whether or not the method resulted in total mortality. Data were statistically compared using an independent two-sample Student's *t*-test. Significance was set at $p < 0.05$ (see Table 1).

Table 1.

Comparison of mechanical, foliar and dormant stem herbicide control methods on % mortality of autumn olive (*Elaeagnus umbellata*). Values with different letters are significant ($p \leq 0.05$) using Student's *t*-test.

Treatment	% Mortality
Foliar herbicide: Arsenal Powerline™ Escort XP™	98 (a)
Dormant stem herbicide: Garlon 4™ Stalker™ Invade 90™	71 (b)
Mechanical removal: John Deer 3110 D backhoe	15 (c)

Note: Percentages are based on total number of shrubs effectively killed.

The results showed that the foliar herbicide application was the most effective in causing total mortality than either the dormant herbicide application or the mechanical removal ($p < 0.025$). Mechanical treatments resulted in the greatest occurrence of stump sprouting following removal. No mortality was observed in the control plots. Both the foliar and dormant stem herbicide treatments were subcontracted at a cost of $750/ha. Mechanical treatments were conducted by *the Wilds* staff at a cost of $160/ha.

We conclude that the foliar herbicide blend utilized in this study may provide an effective tool for the removal of *E. umbellata.* The cost of utilizing this treatment, however, may be prohibitive for many land managers and private landowners. Therefore, future management techniques may consider utilizing a mechanical-foliar combination method that reduces the quantity and cost of herbicide.

Additional observations from this study showed a secondary invasion of non-native species into the newly disturbed areas, primarily Canada thistle (*Cirsium arvense*) and teasel (*Dipsacus fullonum*). Given this, future studies and management at *the Wilds* will include a native plant restoration component in order to reduce further invasive species establishment. *The Wilds* plans to continue demonstrating invasive plant management and habitat improvement techniques throughout the property as a means to increase biodiversity, benefit native wildlife and create greater public awareness of these issues.

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Using ^{15}N stable isotope to label *Lonicera maackii* (Amur honeysuckle) seeds: Linking seed to source

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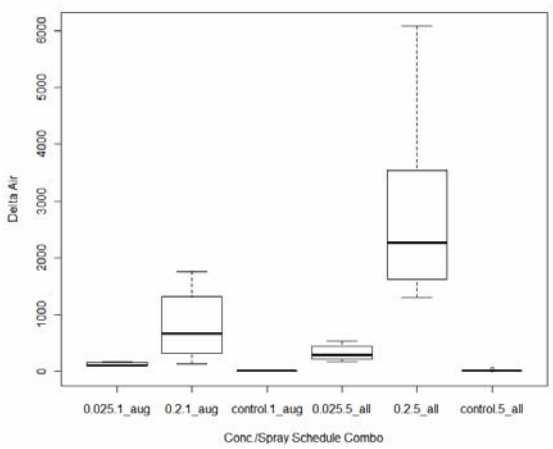
Seed dispersal of invasive plants has a profound influence on spatial patterns of new populations and is of critical interest for understanding invasion dynamics. However, the inability to match recruits to their source makes it difficult to study dispersal. Here we report preliminary results demonstrating a novel method to label seeds of the invasive shrub *Lonicera maackii* (Amur Honeysuckle), with a stable nitrogen isotope (^{15}N) by treating reproductive adult shrubs with a foliar application of ^{15}N-urea. Two temporal treatments (5 applications, 1 August application) and three ^{15}N-urea concentrations (0.0 g/l (control), 0.025 g/l, and 0.20 g/l) were compared on 11 adult shrubs to determine effective concentrations and spray regime. Concentration treatments of ^{15}N-urea produced significant differences from control (ANOVA $p = 0.016$); all treated shrubs showed ^{15}N values above controls. The five spray treatment showed pronounced differences in ^{15}N across concentration groups. These results demonstrate that ^{15}N can be used to label offspring of this woody invasive shrub, thus linking seed to its source, and may prove to be a useful tool in dispersal and recruitment studies and provide valuable information of invasion dynamics in general.

Dispersal is an integral part of species invasion (Allendorf & Lundquist 2003) and largely influences the pattern of invasive spread. While diffuse dispersal is important at the local scale, rare, long-distance dispersal events are important for establishing new foci from which new populations can spread (Moody & Mack 1988). Effective management of invasive species depends on an understanding of how new sites become colonized (Moody & Mack 1988, Edwards & Leung 2009); therefore an understanding of dispersal dynamics and recruitment patterns is critical.

In plant invasions, seed dispersal has a profound impact on the spatial patterns of resulting populations, but it is difficult to link population patterns to dispersal patterns due to the difficulty in determining the source of the new recruits; this is especially true of long-distance and secondary dispersal events (Nathan & Muller-Landau 2000, Wang & Smith 2002, Nathan 2006). Several techniques have been employed to try and link plant recruits to their parent source but an emerging method has great potential: stable isotopes (Wang & Smith 2002, Carlo et al. 2009). While stable isotopes have been used for numerous ecological and environmental applications (Michener & Lajtha 2007), their application in seed dispersal studies is just being realized (Carlo et al. 2009). Carlo et al. (2009) found that the offspring of labeled adults reliably have ^{15}N levels above that of controls. They also show that the ^{15}N signal is retained in the growing seedling for a period of time following germination and that offspring from adults treated with different dosages can be differentiated and traced back to their source following dispersal. This method, while never applied to invasive species, could provide a cost effective means of understanding dispersal and recruitment patterns of these species and therefore could provide valuable information for management plans.

Here we present preliminary results demonstrating the effectiveness of isotope labeling of seeds of *Lonicera maackii* (Amur Honeysuckle), a rapidly spreading invasive shrub in the eastern US. Using a hand sprayer we treated reproductive shrubs during fruit development, with foliar applications of approximately 750 ml of ^{15}N-urea and wetting agent, in a 3 x 3 design: 3 temporal treatments (5 sprayings throughout summer, 1 Aug. spraying, 1 Sept. spraying) crossed with 3 concentrations (0.025, 0.20, 0 g/l). Preliminary data presented here were collected from seeds of 11 shrubs from the first two temporal treatments, with shrubs from all concentration groups. Seeds were removed from ripe fruit and air dried. Eight seeds from each shrub were analyzed at the University of California Davis Stable Isotope Facility (Davis, CA) for ^{15}N and results compared using a linear mixed-effect model with variance weighted by concentration.

Figure 1-Distribution of values for $\delta^{15}N$ (Air) (^{15}N of seed compared to that of air) for seeds of 11 *Lonicera* shrubs treated with ^{15}N-urea; three concentrations of ^{15}N-urea [0.025, 0.2, control (0.0) g/l] were crossed with two application treatments (one application in Aug. ('1_aug') and five applications throughout the summer ('5_all'). Each box plot represents data from 8 seeds each of two or three shrubs.



Seeds from all treatment/concentration combinations showed $\delta^{15}N$ values greater than untreated controls (Figure 1), with significant differences in concentration ($p = 0.016$). For each of the two temporal treatments, seeds from shrubs sprayed with the lower concentration of ^{15}N-urea had $\delta^{15}N$ values intermediate between those of controls and those sprayed with the higher concentration. Shrubs sprayed five times over the growing season had higher $\delta^{15}N$ values than those sprayed only once, although this difference was not significant. We believe this is a result of the small number of shrubs analyzed and we predict that as more seeds are analyzed, the difference between a single spraying and five sprayings will be significant.

These results provide evidence that ^{15}N- urea applied to the foliage of reproductive *L. maackii* shrubs is an effective way to label its seed. While all concentrations and spray regimes were effective at producing a ^{15}N "signal", different concentration treatments were better distinguished where shrubs were sprayed five times.

For this method to be of use for field studies, further investigation is required. We want to be able to detect the ^{15}N "signal" in seedlings that have established under natural conditions, in order to link new recruits to a ^{15}N-urea treated maternal source individual or population. Therefore, we are investigating the ability to detect the ^{15}N in seedlings grown from seeds from these sprayed shrubs, as well as the rate of signal decline over time as ^{15}N becomes more dilute with ^{14}N acquired from the environment. Isotopic labeling in this manner may provide a very useful tool in dispersal and recruitment studies.

We plan to apply this method to our investigation of the invasion dynamics of *L. maackii* in a fragmented landscape. Specifically, we hope labeling potential source plants will enable us to assess the relative importance of local spread (diffuse) vs. long distance dispersal. An understanding of these dispersal and recruitment dynamics can provide valuable information for management programs designed to prevent, control, or eliminate this invasive species.

We suggest that isotopic labeling of offspring with ^{15}N can be applied to other invasive plants, furthering our understanding of invasive species dispersal and invasion dynamics in general.

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A comparison of allelopathic effects of three Midwestern invasive plants

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Bush honeysuckle (*Lonicera maackii*), garlic mustard (*Alliaria petiolata*) and lesser celandine (*Ranunculus ficaria*) are three species which invade Midwestern forests. Allelopathy was investigated by making leaf extracts of each of these three species. In a fully factorial experiment, the effect of extract type and extract concentration on germination, growth and reproduction of *Arabidopsis thaliana* in potting soil and in field soil was investigated. A control of distilled water was also used. A subsequent experiment investigated the effect of these treatments solutions on germination over 7 days of three agricultural species (*Lactuca sativa*, *Brassica oleracea*, and *Ocimum basilicum*). Germination of *A. thaliana* in potting soil was significantly delayed by the two highest extract concentrations of *L. maackii* compared to the control in potting soil. There were significantly fewer siliques when plants were treated with extracts of *L. maackii* in potting soil. There were significantly fewer siliques when plants in field soil were treated with extracts of *L. maackii* and *R. ficaria* compared to *A. petiolata*. Across all test species, *R. ficaria* and *L. maackii* extracts affected germination the least, while *A. petiolata* extracts affected germination the most. However, the extracts impacted species germination differentially; *L. sativa* and *O. basilicum* were more sensitive to *A. petiolata* and *R. ficaria* extracts and *B. oleracea* was more sensitive to *L. maackii* extracts. These results provide evidence of differential allelopathic effects of three important Midwestern invasive species.

Invasive species pose a threat all over the world, (Pimentel et al. 2005). One trait that may enhance invasive success is allelopathy, which is the release of a substance from the plant that is harmful to surrounding plants. Plants that exude these chemicals are more likely to take over a new environment their native neighbors have evolved tolerance to their chemicals (Callaway & Ridenour 2004).

In this research we focus on three invasive species to the Midwest: *Lonicera maackii*, *Alliaria petiolata*, and *Ranunculus ficaria*. *Lonicera maackii* (Amur honeysuckle) is native to Asia and extracts have been shown to inhibit germination (Dorning & Cipollini 2006). *Alliaria petiolata* (garlic mustard) is native to Europe, and has been shown to exhibit allelopathic effects on *Geum* sp. (Prati & Bossdorf 2004). The allelopathic effect may vary by target species, as there was no effect of *A. petiolata* on *Arabidopsis thaliana* (Cipollini et al. 2008). *Ranunculus ficaria* is native to Europe and is an emerging invasive species in riparian zones. To date, there has not been any published research on its effects as an invasive, nor on its allelopathic potential.

Extracts were made from *Lonicera maackii*, *Alliaria petiolata*, and *Ranunculus ficaria* by soaking fresh leaves for 48 hours in distilled water followed by filtration. The extracts were then diluted to three different concentrations: 0.3, 0.2, and .01g fresh leaf tissue/mL distilled water. Ten mL of these solutions were added every other week to *Arabidopsis thaliana* (Brassicaceae) planted in 100 mL pots with potting soil. Control plants were given only distilled water. Plants were monitored for percent germination, flowering, and rosette diameter. After 12 weeks, the number of siliques per plant was counted. A subsequent experiment used the same design, yet utilized field soils. The allelopathic potential on germination was explored by applying similar extracts and extract concentrations to three agricultural species in three separate families: *Lactuca sativa* 'Grand Rapids, Tipburn Resistant'(Asteraceae), *Ocimum basilicum* (Laminaceae), and *Brassica oleracea* 'Copenhagen Early Market' (Brassicaceae). Ten seeds of each were placed on paper towels and watered with 10 ml of extract solution. Germination (measured as emergence of the radicle) was followed for 14 days. There were four replicates of each treatment combination for each experiment. Data were analyzed using appropriate (M)ANOVAs.

Figure 1.

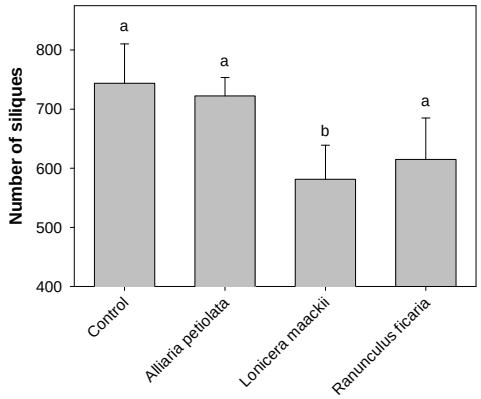


Figure 2.

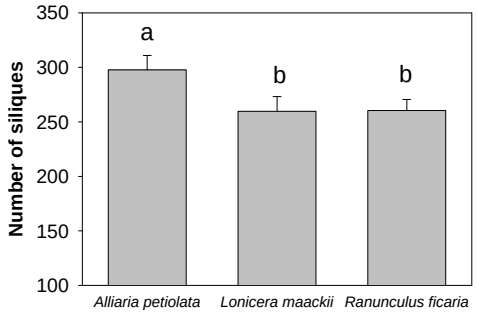
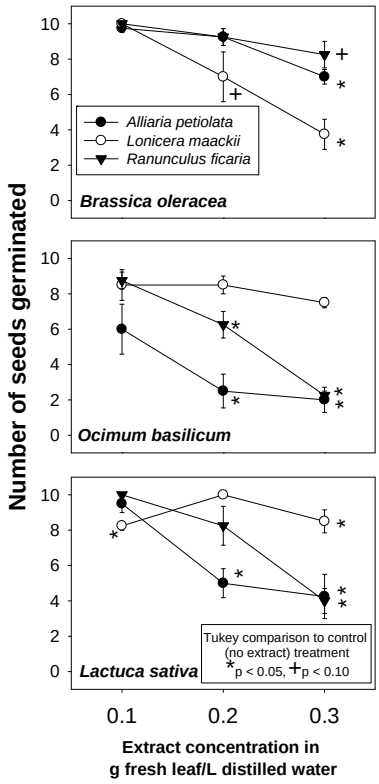


Figure 3.



For *A. thaliana*, There was significant delay in germination for the first two days ($p = 0.002$ and $p < 0.001$) in the two highest extracts of *L. maackii*. Reproduction, as measured by number of siliques, was suppressed by extracts of *L. maackii* in potting soil ($p = 0.049$, Figure 1). Extracts of *R. ficaria* showed a trend ($p < 0.071$) in reducing reproduction and delay flowering in *A. thaliana*. In field soil, there were significantly fewer siliques of *A. thaliana* when treated with *R. ficaria* and *L. maackii* extracts compared to *A. petiolata* extracts (Figure 2).

There was a significant ($p < 0.001$) interaction between extract type, extract concentration and test species on seed germination. Germination of *B. oleracea* was most affected by extracts of *L. maackii*, while germination of *O. basilicum* and *L. sativa* were most affected by extracts of *A. petiolata* and *R. ficaria* (Figure 3). Germination of *B. oleracea* decreased with increasing concentration of *R. ficaria* extract, though not as steeply as in *L. sativa* and *O. basilicum*.

In our experiments, we found evidence of allelopathic effects of three invasive Midwestern species: *R. ficaria*, *L. maackii*, and *A. petiolata*. Effects of extracts of *L. maackii* were greatest on species from the Brassicaceae, while extracts of *A. petiolata* and *R. ficaria* had the highest inhibitory effect on species in other families. Extracts of *A. petiolata* did not strongly affect the two species in the Brassicaceae, as in previous work (Cipollini et al. 2008). This is most likely caused by the similar chemical composition of plants in the same family, which makes *A. thaliana* and *B. oleracea* more resistant to the effects of these chemicals. Effects of extracts of *R. ficaria* were generally weaker though still inhibited germination, particularly at the highest concentration.

Our study illustrates the importance of using multiple test species and experimental conditions to incorporate consideration of differing sensitivities to allelopathic effects.

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Southeast Ohio Non-Native Invasive Species Interest Group: Building collaboration for landscape level impacts

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In 2007 a group of interested stakeholders, including private landowners, land managers, researchers and non-profit organizations began meeting to explore opportunities to share knowledge and experience and coordinate efforts across the landscape. This group identified three watersheds in Southeastern Ohio where the presence of public lands and private non-profit lands intermixed with private holdings could create the right conditions for successful Cooperative Weed Management Areas (CWMAs). Groups and individuals within these three areas began working together to remove the most vigorous of invasive species, to prevent new infestations through early detection and rapid response and to educate and engage landowners, citizens and youth about non-native invasive species (NNIS) in order to have positive landscape-level impacts. Future direction for the group includes participation in the Central Hardwoods Invasive Plant Network (CHIP-N), which will inventory, map and control NNIS along the Ohio River and its tributaries.

It has long been known that non-native invasive species (NNIS) do not recognize man-made boundaries. Furthermore, the pattern of landownership in the eastern US is such that large tracts of public land are not contiguous and contain many private in-holdings, with a pattern of roads and utilities further dissecting ecosystem blocks. This "checkerboard" of ownership and rights-of-way creates conditions ripe for NNIS invasion. Therefore, it has become necessary to develop partnerships, to share information and to coordinate projects that cross boundaries and prioritize areas of treatment for landscape-level impacts.

The Cooperative Weed Management Area (CWMA) model has been successfully used extensively in the western US to allow land managers to combine resources and work across boundaries to control weeds. A CWMA is a partnership comprised of landowners within a determined boundary that agree to work together to combat NNIS populations. Typical partners include federal, state and local agencies, private landowners and concerned citizens. Adaptation of this model to the eastern US has been more slow-going, with issues arising because of the greater number of landowners (public and private) and the smaller acreage of most tracts of land.

In 2007, a group of partners who were concerned about NNIS came together to share knowledge and experience and coordinate efforts across the landscape. The group named itself the Southeast Ohio NNIS Interest Group and created a map of three geographic areas that have the potential to become future CWMAs. Each area contains public lands or areas where private grassroots organizations are engaged in non-native invasive species control. These areas loosely mimic the watershed boundaries of the Hocking River Valley, Lower Muskingum River and Little Muskingum and Ohio Rivers respectively (Figure 1). These three watershed areas have been working to control the NNIS of concern in their localities and in the areas identified as the most important ecologically or the most viewable and educational to the public.

In the **Hocking River Valley** a group of concerned citizens and students have been working with Dr. Phil Cantino (Ohio University and the Athens Conservancy) primarily on the control of *Alliaria petiolata* (garlic mustard). In 2009, 76 volunteers clocked 356 person-hours in NNIS mapping and removal. Of this total, 48 person-hours were spent mapping the distribution of *A. petiolata* in the Athens Conservancy's conservation easement on the Baker property. The remaining 308 hours were spent removing *A. petiolata* from Strouds Run State Park, Ohio University Ridges Land Lab, Hockhocking-Adena Bikeway, the Athens Conservancy's Bluebell and Blair preserves and the Baker conservation easement. These pulls have been an on-going volunteer effort and comprise every weekend during the *A. petiolata* pulling season. Partners include the Athens Conservancy, Rural Action, Ohio University and Hocking College.

The **Lower Muskingum River** group has been working with Marilyn Ortt (retired ODNR botanist) in eradication efforts on lands held by the Friends of the Lower Muskingum River (FLMR) Land Trust

and watershed organization. Over 3,000 *Ailanthus altissima* (tree-of-heaven) stems ranging from seedlings to mature trees were treated on a 30 acre parcel, and additional NNIS have been treated on other FLMR properties. Local students and other volunteers have participated in *A. petiolata* pulls in KrisMar Woods, a public greenspace in Marietta.

Materials aimed at public awareness have been developed by the Lower Muskingum group and distributed throughout the watershed. These materials include an interpretive panel installed near Marietta Harbor, signs for lock chambers on the Muskingum River and launching ramps along the Lower Muskingum, a tabletop display for events, Plantwise brochures for garden centers and brochures detailing species of concern locally.

The group conducted two NNIS workshops (September 2008, September 2009) for local land managers and private landowners. The day-long workshops included topics on biological control of *Persicaria perfoliata* (mile-a-minute), managing *Pueraria lobata* (kudzu), treating *A. altissima*, government programs to help fund NNIS control on private lands, *Elaeagnus umbellata* (autumn olive) removal techniques and using native plants in backyard settings for wildlife habitat. Partners include FLMR, Buckeye Hill RC&D Council, The Wilds, NRCS, Washington County Master Gardeners and OIPC. In-kind match was provided by The Wilds, FLMR, Duke Energy and Washington State Community College.

Efforts in **Little Muskingum and Ohio Rivers** watersheds have focused around the Leith Run Recreational Area, which is part of the Wayne National Forest. Help came from two volunteer groups to control NNIS at the Leith Run Recreation Area along the Ohio River. The Social Involvement Through Education and Service (SITES) volunteers pulled 28 garbage bags full of *A. petiolata* from around the campground. Youth Conservation Corps volunteers assisted in the removal of *Fallopia japonica* (Japanese knotweed) from 2.50 acres of the Ohio River bank. Partners include the Wayne National Forest, Youth Conservation Corps and SITES volunteers.

Resultant to the efforts ongoing in these three regions numerous partners have been engaged, funding has been leveraged, citizens have been involved (including many youth) and countless acres have been treated.

Figure 1.

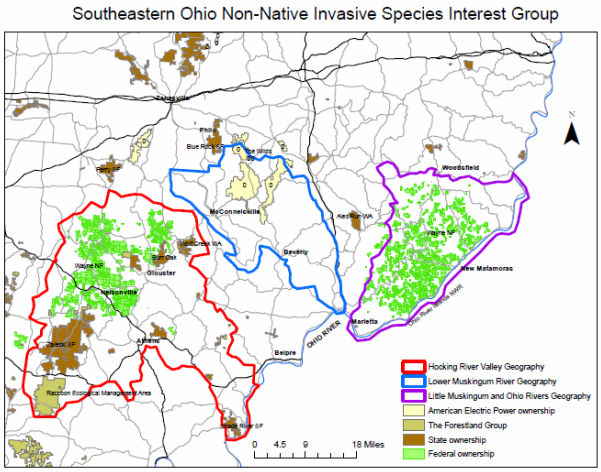
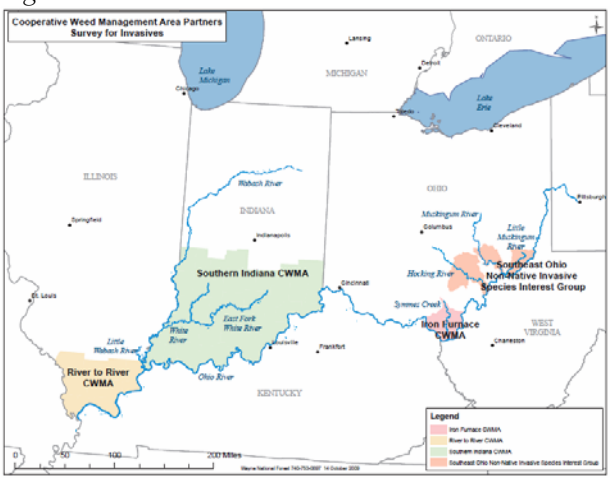


Figure 2



As a whole, the SE Ohio NNIS Interest Group identified the need to share information on where and how NNIS are treated within southeast Ohio. A GIS dataset and map were created to be shared with partners, landowners, funders, non-profits, cities and townships in order to leverage support in managing NNIS on additional acreages. Once treatment areas are mapped the group will be able to more strategically decide where to focus efforts for the most ecological benefit. In addition, the GIS dataset includes specific treatment information and allows for photograph attachment to show changes over time.

In addition, the coordination of this Interest Group with three other CWMAs lead to the creation of the Central Hardwoods Invasive Plant Network (CHIP-N), which will identify, inventory and map infestations along the riparian corridor of the Ohio River and several tributaries (Figure 2). This partnership will be implemented in 2010 and will lead to the development of an inter-state strategic plan for the prioritization and treatment of invasives.

Replacing invasive sweet-flag, *Acorus calamus* with native *Acorus americanus*

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This study was undertaken to identify and control stands of invasive sweet-flag in Ohio wetlands and replace them with diverse communities containing native sweet-flag. A contribution in Flora of North America cleared the longstanding confusion over sweet-flag species identity which impeded control of the invasive species. The native, fertile diploid *Acorus americanus* (Rafinesque) Rafinesque, with its smooth leaf surface covering the major veins is clearly distinguished from the invasive, sterile triploid sweet-flag *Acorus calamus* Linnaeus with its conspicuously raised leaf midrib. Propagation and characteristics of native sweet-flag were observed first-hand in plants raised from seed obtained from a quality wetland in Holmes County, Ohio. Unlike native sweet-flag with its scattered distribution, *A. calamus* forms dense monocultures with seasonally-persistent rhizomes, making eradication difficult. Small populations were controlled by removing the rhizomes, while light exclusion methods were tested for control of large populations. After leaves were removed plants were covered by a layer of opaque plastic, or wood chips overlaying newspapers or clear plastic. To enhance diversity in the invaded fen community, native wetland plants, including *A. americanus*, were planted in the plant community adjacent to the invasive sweet-flag management area.

An accurate description of how to recognize the genetically-distinct invasive species/varieties of purple loosestrife, reed canary grass, reed grass, cat-tail and sweet-flag plants is essential for successful control of invasive populations of these plants. This study was undertaken to learn how to identify the invasive sweet-flag in Ohio wetlands, control populations and replace them with diverse communities that include native sweet-flag. Les and Mehrhoff (1999) described the confusion in taxonomic literature concerning species identity in the genus *Acorus* in North America dating back to the 1600s. Such confusion resulted in inconsistent control of invasive sweet-flag and in mistaken plantings of the invasive species in wetland restorations.

The authoritative characterization of *Acorus* species in North America published in the Flora of North America is the first to treat the two North American species formally and provide a key to distinguish them (Thompson 2000). The native sweet-flag *Acorus americanus* (Rafinesque), Rafinesque, a fertile diploid, is clearly distinguished by its main leaf veins and midvein that are more or less equally raised to form a smooth leaf surface. By contrast, the introduced sweet-flag *Acorus calamus* Linnaeus, a sterile triploid, has leaves with a midvein prominently raised above the leaf surface (see figure below). Clear separation of the two North American species is based on morphology, essential oil biochemistry, cytology, isozymes and ethnobotany (Packer & Ringius 1984, Thompson 1995).

Acorus americanus *Acorus calamus*

First-hand familiarity with the taxonomic traits that distinguish the two sweet-flag species is important for locating and identifying populations in the field. Plants of the non-native *A. calamus* are common in the Dayton area. On the other hand, native sweet-flag has not been reported for this part of the state. To become familiar with traits needed for accurate identification and successful culture of

the species, plants of *A. americanus* were raised from seed obtained from Holmes County.

The value of *Acorus* as a medicine and a food likely had a marked influence on the distribution of both species in Ohio, thus providing leads that can help track the distribution of sweet-flag populations. Today, native sweet-flag is found in 17 counties, mainly in northern Ohio (Rick Gardner, pers. comm.).

A coefficient of conservatism of 6 on a scale of 1 to 10 indicates that the species is characteristically not found growing in low quality wetlands. The native species is often found near Native American village sites or camping areas (Gilmore 1931). In Holmes County, the location of former Native American trails and hunting grounds, *A. americanus* is found scattered throughout botanically-significant sedge meadow communities that include *Platanthera leucophea*, prairie fringed orchid.

Mabry (1977) reported that the non-native *A. calamus* first arrived in North America from Europe in 1567, likely introduced for its medicinal value. The presence of monocultures of *A. calamus* in a number of Greene and Montgomery County wetlands appears to reflect the presence of the former Watervliet Shaker community, with its herb gardens and herbal trade.

Knowledge of the morphology, life cycle, physiology and growth form of an invasive species can be useful in designing effective control measures. For example, systemic herbicides like glyphosate target the metabolically active portions of plants and are not as effective in controlling the less metabolically active region of the rhizome behind the growing tip.

Physical removal of the network of rhizomes can be used to control small clusters of *A. calamus*. Cutting roots with a planting knife along the length of a rhizome frees it so it can be lifted out carefully with a spading fork. Pieces that break off are difficult to retrieve and can resprout eventually. Plants that sprout after an area is cleared can be controlled by wiping the leaves with a 6% solution of glyphosate-based herbicide approved for wetland use. Three to five separate applications may be needed to eliminate the invasive plant.

To avoid the labor-intensive removal of rhizome networks, experimental control methods based on covering leaves and starving the plants are being conducted on a site in which invasive sweet-flag has displaced approximately 10,000 square feet of wet meadow. Leaves were cut off close to the soil and the plants were covered by layer of opaque plastic, or 3 inches of wood chips overlaying newspapers or clear plastic (see figure below).

Placing newspaper and covering newspaper and clear plastic with wood chips

It is important to know whether the rhizomes of asexually-reproducing *A. calamus* are adapted to support shoot growth for a long period in the dark. To hasten the demise of the subsurface rhizome network, segments with emerging shoots are severed and removed. These rhizome pieces, with attached shoot sprouts, are being kept dark in moist soil at room temperature and examined periodically to determine how long they remain alive.

An important element of this study is to increase diversity of the wetland plant community being threatened by the invading sweet-flag. Along with the measures to control invasive *A. calamus*, native sweet-flag and other wetland plants with medium to high coefficients of conservatism will be propagated and planted to enhance the diversity of the plant community in the impacted wetland.

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The effect of an allelopathic invader, *Alliaria petiolata* (Brassicaceae), on native plant-mycorrhizae mutualisms

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Garlic mustard (*Alliaria petiolata*; GM) is a widespread invader of forests. GM produces a suite of allelochemicals that are toxic to arbuscular mycorrhizal fungi (AMF). However, many native forest herbs rely on the AMF mutualism for survival and reproduction. We hypothesized that exposure to GM allelochemicals diminishes growth of AMF external hyphae, resulting in minimal nutrient uptake and decreased physiological rates in native plants. We conducted a field experiment in which one *Maianthemum racemosum* plant from each of nine pairs received a treatment of fresh GM litter, with the paired plant as a control. Prior to treatment, leaf gas exchange rates did not differ within the pairs. Two weeks after treatment, GM treated plants showed a significant decline in leaf gas exchange (MANOVA on all 3 response variables, $p < 0.05$). Stomatal conductance decreased by 36% in treated plants compared to controls with similar decreases in photosynthetic and transpiration rates (ANCOVA $p = 0.003$; $p = 0.05$; $p = 0.01$, respectively). To determine GM's anti-fungal effects, we used phospholipid fatty acid analysis and found no difference in AMF external hyphal biomass between the two treatments. Overall, our results suggest that GM impacts native plant physiology. Future experiments will determine whether these effects are direct or mediated through AMF death.

Mutualisms are cooperative relationships between two organisms. However, conflict can occur when one partner experiences an increase in costs or a decrease in benefits. Over time, conflict causes the relationship to erode into parasitism (Johnson et al. 1997). Invasive plant species can create conflict between native plants and their mutualists. For example, the disruption of native plant pollination mutualisms by invaders is well-documented (Morales & Traveset 2009).

For plants, one of the most important mutualisms occurs with arbuscular mycorrhizal fungi (AMF). In this relationship, AMF colonize both plant root tissue (internal hyphae) and the surrounding soil (external hyphae). AMF external hyphae mine the soil for mineral nutrients and water and transport them back to the internal hyphae inside the plant root. The nutrients and water can then move through arbuscules and into the plant host. In return, plants supply the AMF with carbon (Smith & Read 2009).

In an eastern deciduous forest, it is estimated that approximately ~70% of all plants rely on nutrients from AMF for survival ("obligately mycorrhizal"; Brundrett & Kendrick 1988). However, the recent invasion of forests by the exotic biennial herb, garlic mustard (*Alliaria petiolata*; Brassicaceae; hereafter, GM) could be altering the conditions favoring the plant-AMF mutualism. GM allelochemicals are lethal to AMF external hyphae in soil, while the arbuscules and vesicles inside the roots of native plants can remain intact and are likely protected by the root tissue of the host plant (Barto 2008). Thus, when the native plant-AMF mutualism is exposed to GM allelochemicals, the plant should experience no change (or even an increase) in the carbon cost of supporting the AMF, yet the physiological benefits likely decline dramatically as the AMF external hyphae die. As a result, the invasion of GM may establish the conditions that destabilize the native plant-AMF mutualism and shift it towards a parasitism.

This study was conducted at Trillium Trail, a mixed mesophytic forest that is owned and managed by the Fox Chapel Borough, Pennsylvania. This forest was invaded by GM in 1992. We selected 18 *Maianthemum racemosum* plants (False Solomon's Seal; Liliaceae). Like many other forest understory herbs, *M. racemosum* often co-occurs with GM and has been suggested to be obligately mycorrhizal (Brundrett & Kendrick 1988). These plants were grouped into 9 pairs that were matched for size and microhabitat. Prior to imposing the treatments, we took baseline physiological measures on all plants as follows: one leaf was placed in the Li-Cor 6400 infrared gas analyzer leaf chamber and allowed to acclimate to

the light level (600 $\mu mol \cdot m^{-2} \cdot s^{-1}$) for 5 minutes. Photosynthesis, transpiration, and stomatal conductance were recorded every 15 seconds for 1 minute, giving a total of 5 measures for each physiological variable. These 5 measurements were averaged to give an estimate of each trait.

To create a GM and control treatment, mesh screen bags were made. Half of these bags were stuffed with fresh leaves, stems, and roots from 5-6 large GM plants collected at Trillium Trail. As the leaf tissue in the mesh bags senesced, all chemical compounds leached into the soil. The other bags were left empty. Within each native plant pairing, one plant was selected to receive a control bag, while the other received a GM bag. Treatments were left in place for 2 weeks.

After two weeks, we measured three aspects of the plant-AMF mutualism: 1. *Physiology*: All physiological traits described above were measured again. 2. *Root colonization*: After physiological traits were measured, a subset of plants from the control and GM treatments were excavated. Ten roots were removed at random, stained with trypan blue and analyzed for % root length colonized by AMF. 3. *Biomass of AMF in soil*: Four weeks after the treatments had been imposed, soil cores were collected around control and GM-treated *M. racemosum*. Phospholipid fatty acid analysis (PFLA) was used to assess the biomass and abundance of live AMF hyphae in the soil. PLFA is a method that is used to assess diversity of microbial taxonomic groups (Hill et al. 2000). All microbes produce phospholipid fatty acids as a critical component of cell membranes; however, their chemical composition differs among taxonomic groups, yielding a unique PLFA for each group. AMF were identified by PLFA 16:1 w5c. Results are expressed as nmol PLFA g^{-1} soil C.

Prior to treatment, there was no significant difference in physiology within pairs of *M. racemosum* (MANOVA, $p = 0.72$). However, after two weeks of treatment, GM-treated plants displayed significantly lower rates of photosynthesis, transpiration, and stomatal conductance when compared to control plants (MANOVA, $p = 0.05$; ANCOVAs, $p < 0.05$; $p < 0.01$; $p < 0.003$, respectively). These data strongly suggest that GM treatment negatively impacts native plant physiology. We found that both GM-treated and control *M. racemosum* retained internal AMF structures in equal abundance, indicating that GM treatment did not affect AMF within the roots. We did not detect a significant difference in AMF external hyphal biomass between soils from GM treated and control plants. There are two reasons why AMF external hyphae did not decline as predicted. First, AMF external hyphae have a turnover rate in the soil of just 5-6 days (Staddon et al. 2003). Because soil cores for PLFA were taken 4 weeks after GM treatment, it is likely that the hyphae had sufficient time to regenerate. Second, certain AMF taxa are more sensitive to GM than others (Burke 2008). PLFA analysis cannot detect species-specific differences in AMF abundance (Hill et al. 2000).

The results of this study are in part consistent with the prediction of a GM-mediated decline in native plant physiology via disruption of AMF external hyphae. However, these results could also be explained by direct effects of GM allelochemicals on *M. racemosum* physiology rather than indirect effects via AMF. Future work will tease apart direct vs. indirect effects. Overall, this experiment is an important step forward for invasion biology as it highlights the fine-scale physiological changes that can occur in native species as a result of invasion.

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Disturbance facilitates a secondary spread of invasive plant species: management concerns for emerald ash borer (EAB)-infested forests

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When the emerald ash borer was first identified in 2002, eradication efforts were implemented to control the beetles' spread. Preliminary research identified the environmental impacts of the eradication program which included accelerated canopy gap formation and thus greater light reaching the forest floor. In addition, tracked-vehicles used during the eradication efforts caused significant soil compaction. The result of this habitat alteration was a measurable shift in community composition, predominately attributed to the establishment of invasive plants. Removal of EAB-infested ash trees remains a necessary practice to minimize safety hazards in active recreation areas. The current project seeks to understand establishment patterns of invasive plants across a gradient of disturbance intensities in order to determine the best management practice (BMP) for EAB-infested forests. In 2007, an intermediate disturbance treatment, designated as cut without compaction, had all ash removed but without using vehicles. This treatment was compared to forested areas that received the EAB eradication protocol and to uncut control areas with ash trees remaining. We determined that the use of tracked-vehicles during the eradication protocol facilitated a greater establishment of invasive plant species. Furthermore, there was no change in the native plant community composition in the intermediate disturbance treatment.

Eradication efforts of the emerald ash borer (EAB) were implemented soon after the beetles' discovery in an attempt to eliminate or contain the beetles' spread. The eradication protocol included removal of every ash tree within a half-mile radius around an infested tree. Impacts of the eradication protocol to the native forest were first identified in 2005 in Pearson Metropark Lucas County, Ohio. Hausman et al. (2010) compared areas that received the eradication treatment (Cut: all ash trees removed), to areas that were left as uncut control sites, (Uncut: ash trees still standing) and found the eradication protocol accelerated the formation and size of gaps within the forest and thus increased the duration and intensity of light penetrating through to the forest floor. In addition, the vehicles used during the eradication efforts caused significant soil compaction. This change to the abiotic environment caused a dramatic shift in the understory plant community. Nearly 18% of the plant species surveyed were non-native. A total of 13 invasive species were identified and all but one species were present in cut areas including 10 species that were only found within the eradication areas (Hausman et al. 2010). While EAB eradication is no longer an active program, removal of dead/dying ash trees continues to be a necessary management practice. The spread of EAB continues to cause mortality and those unstable trees are major safety hazards. Therefore, land managers will need to consider methods for proper tree removal to minimize any compounding effects that may adversely affect the native plant community. Our objectives are: 1.) to create various disturbance intensities with corresponding habitat changes 2.) to determine invasive plant establishment patterns across our disturbance gradient and 3.) to propose land management practices to minimize the likelihood of invasive plant establishment.

This project compares areas that received the eradication treatment (Cut plots: all ash trees removed using trucks), to areas that had all ash trees removed by hand (Cut w/out: all ash trees removed without use of trucks), and to areas that were left uncut (Uncut plots, ash trees still standing). The cut without compaction treatment represents an intermediate level of disturbance and was added to isolate the impact of an increased light environment without the effects of soil compaction. This treatment was added in 2007; therefore, all comparisons between treatments are based on a "Response time" or the time from when the disturbance treatment was applied. Eighteen 20x25m-plots (6 plots per treatment) were

established within Pearson Metropark, Lucas County, Ohio. The light environment was assessed using hemispherical photos; soil compaction was measured using a soil penetrometer with readings taken at 5 depths through the soil profile. The herbaceous understory was determined using % cover of all species present from seven $1m^2$-subplots within each plot. All measurements were collected annually for 3 years.

Figure 1.

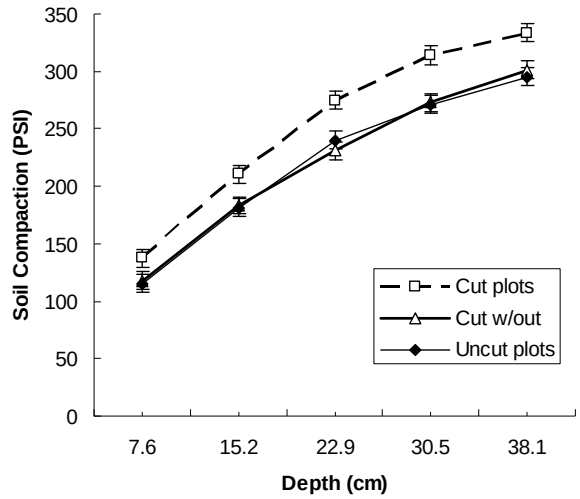
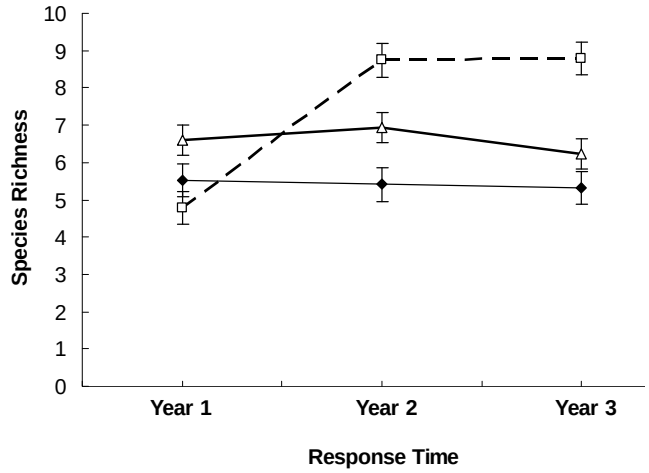


Figure 2.



Immediately following the application of a disturbance treatment, the light environment in the Cut w/out treatment was comparable to the light environment of the Cut treatment in the eradication zone. However, there was a significant difference in soil compaction between the Cut treatment compared to the Uncut control and Cut w/out treatment ($p < 0.001$) with no difference detected between the Uncut control and Cut w/out treatment (Tukey's HSD) (Figure 1). The understory plant community remained similar over the three year study in both the Uncut control plots and the Cut w/out compaction plots. There was no difference in species richness or diversity within either treatment over time ($p = 0.27$) even though between the treatments there was greater species richness in the Cut w/out compaction treatment (6.5 species) over the Uncut (5.3 species) (Figure 2). The Cut treatment however, nearly doubled the number of species that colonized after the eradication disturbance (4.7 species in Year 1 to 8.8 species in Year 2) ($p < 0.001$). All of those newly established species were non-native and persisted 3 years after the disturbance was first created.

Treatments in this study represent three possible scenarios to manage the spread of EAB and can be used to identify how the forest community responds to each treatment scenario. The Cut plots in the eradication zone reflect an intense level of anthropogenic disturbance, characterized by a high light environment and compacted soils, resulting in the greatest sensitivity to invasive plant species establishment. The Cut w/out compaction plots reflect an intermediate level of anthropogenic disturbance also characterized by a higher light environment but with out the effects of soil compaction. The understory plant community however, was not compromised at this level of disturbance due to a lack of detectable change found to the native understory. By the termination of this experiment, the Uncut plots were exhibiting signs of infestation with some ash tree die back from the continued spread of EAB, and therefore reflect a natural level of disturbance with more gradual changes to the environment. Here as well, there was no change through time in the understory plant community.

From a land management perspective therefore, prioritization of ash tree removal should consider the technique necessary to be effective, the impact that technique will have on the future native plant understory and the proximity of the area to potential invasive seed sources. If tree removal is necessary, cutting by hand with chainsaws has the most minimal disturbance impact on the native plant community. Caution should be taken when cutting trees close to trails and edges as these are likely source locations for invasive plant species.

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Occurrence of *Schoenoplectus mucronatus* at the U.S. Department of Energy Fernald Preserve

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Bog bulrush (*Schoenoplectus mucronatus*) is a perennial wetland species native to Africa, Asia, and Europe. Its documented occurrence in the United States includes California, Hawaii, Iowa, Kentucky, Missouri, New Jersey, New York, Pennsylvania, Washington, and Tennessee. This species spreads through seed, rhizomes, and stolons, and has shown resistance to certain herbicides. Online plant databases do not show the distribution of the species reaching into Ohio or Indiana; however, local experts have indicated that specimens are in both states. Various reports indicate that *S. mucronatus* is locally abundant but not yet widespread regionally. In recent years, *S. mucronatus* has become increasingly abundant at the Fernald Preserve, a U.S. Department of Energy site in northwest Hamilton County, Ohio. The 425-hectare (1,050-acre) site has undergone extensive remediation and subsequent ecological restoration, and various wetlands have been constructed across the site. *Schoenoplectus mucronatus* was first observed at the Fernald Preserve in 2008, in one constructed basin. During monitoring in 2009, *S. mucronatus* was seen in 8 of 23 basins surveyed. This increase has raised concern that the species may become a regional problem. This research serves to alert botanists and ecological restoration personnel of the species' regional occurrence, and prompt discussion of its potential impacts and how it can be controlled.

Bog bulrush (*Schoenoplectus mucronatus*), also known as rice field bulrush, is a perennial obligate wetland species native to Africa, tropical and temperate Asia, and Europe (USDA/GRIN 2005). Because of its invasive nature in rice fields, *S. mucronatus* is considered one of the world's worst weed species. This species affects the rice fields of Bangladesh, France, India, Malaysia, the Philippines, Portugal, Spain, and the United States (Bryson & Carter 2008). While the species' economic risk to agriculture proves its significance as a weed, this project investigates whether the species threatens regional ecosystems by destroying wildlife habitat, limiting biodiversity, or changing the natural composition of the native area. Reports of *S. mucronatus* claim local abundance; however, it does not appear to be widespread in the region.

The Fernald Preserve is situated on a 425-hectare (1,050-acre) tract of land, approximately 29 kilometers (18 miles) northwest of Cincinnati, Ohio. The site is near the unincorporated communities of Ross, Fernald, Shandon, and New Haven in Hamilton County. It is a former uranium-processing facility that was shut down in 1991. Since then, the site has undergone extensive remediation pursuant to the Comprehensive Environmental Response, Compensation, and Liability Act (CERLA). Remedial activities and subsequent ecological restoration have converted the site from an industrial production facility to an undeveloped park, encompassing wetlands, prairies, and forest. When the large-scale soil remediation and waste disposition were completed in the fall of 2006, the site was successfully transferred from the U.S. Department of Energy (DOE) Office of Environmental Management to the DOE Office of Legacy Management. The Fernald Closure Project was then renamed the Fernald Preserve.

Wetland creation is a central component of ecological restoration at the Fernald Preserve. Approximately 57 hectares (140 acres) of wetland and open-water communities have been established at the site. The control of invasive species has been and will continue to be a part of the long-term ecological restoration and maintenance plan. *Schoenoplectus mucronatus* recently emerged in several of the created wetlands. It first appeared in 2008 in one wetland, but by summer 2009, the species was seen in 8 of the 23 wetlands monitored, as well as in two additional locations on site.

This increase in observed patches of *S. mucronatus* raised concern about its potentially invasive nature. In response, we conducted both a literature review of the history, impacts, and control

of *S. mucronatus*, and a local review of *S. mucronatus* distribution.

This sedge fruits during the summer to fall months and at maturity reaches 2 to 3 feet. This plant is characterized by sharply triangular culms—its leaves consist of 1 or 2 bladeless sheaths per culm. *Schoenoplectus mucronatus* reproduces through seeds, rhizomes, and stolons. The stolons end with result in round, dark tubers, which will grow into new plants when constantly submerged. However, the tubers will become dormant in unfavorable conditions with potential to sprout when conditions improve. (Washington State NWCB 2007). Plants typically sprout 60 to 70 days after a flood event (UC IPM Online 2008). Studies have shown this weed to be resistant to some herbicides, primarily ALS-inhibitors such as bensulfuron (e.g., Londax) and bispyribac (e.g., Regiment) (Busi et al. 2006, Fischer & Hill 2006). However, manually removing this weed has been reported as an effective means of eradication (Washington State NWCB 2007).

Schoenoplectus mucronatus was first sighted in the United States in New England in the early 1900s but did not persist. The species has been seen in California rice fields since 1942. Regional presence in the Midwest began in 1971 (FNA 2003). Currently online databases show distribution in California, Hawaii, Iowa, Illinois, Kentucky, Missouri, New Jersey, New York, Pennsylvania, and Tennessee (FNA 2003, USDA/NRCS 2009). Although *S. mucronatus* is not yet listed in online databases for the state of Washington, Washington State Noxious Weed Control Board has listed it as a class A noxious weed due to its presence on the Ridgefield National Wildlife Refuge in Clark County (Washington State NWCB 2007).

To learn more about the regional distribution of *S. mucronatus*, we asked 13 local herbariums about any *S. mucronatus* vouchers. The herbariums, contact persons, and positive responses are listed in Table 1. There is only one vouchered location in Indiana, one in Kentucky, and four in Ohio. Counties include Butler, Fairfield, Pickaway, and Hamilton, in Ohio; Perry in Indiana; and Pulaski in Kentucky. The Pulaski County voucher notes that this species may have been introduced into the area by contaminated ornamental water lilies planted in a local pond.

Wetland monitoring activities at the Fernald Preserve will continue to document the frequency and distribution of *S. mucronatus*. In addition, control alternatives will be evaluated and implemented as part of ongoing maintenance activities. The apparent spread of this species across created wetlands raises concern. Botanists and land managers should be on the lookout for *S. mucronatus* in created and preserved wetland communities across Ohio.

Table 1.

Contact	Affiliated University	Response and Voucher Locations
Dr. Michael Vincent	Miami University, Oxford	Butler, Pickaway, Fairfield, and Hamilton counties, Ohio
Dr. Maggie Whitson	Northern Kentucky University	Pulaski County, Kentucky
Dr. Ron Jones	Eastern Kentucky University	Pulaski County, Kentucky
Alice Lawrence and Robert Neidlinger	Western Kentucky University	No known vouchers
Dr. Rebecca W. Dolan	Butler University	Perry County, Indiana; Portugal
Dr. Nick Harby	Purdue University	Taiwan
Dr. David Winship Taylor	Indiana University Southeast	No known vouchers
Eric Knox/Sarah Rosenberg (assistant)	Indiana University	No known vouchers

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Flora of North America (FNA). 2003. *Schoenoplectus mucronatus*. FNA Volume 23. http://www.efloras.org/florataxon.aspx?flora_id=1&taxon_id=242101173 (accessed December 8, 2009).

University of California (UC), IPM Online. 2008. Weed gallery: ricefield bulrush. http://www.ipm.ucdavis.edu/PMG/WEEDS/ricefield_bulrush.html (accessed December 8, 2009).

USDA, Germplasm Resources Information Network (GRIN). 2005. *Schoenoplectus mucronatus*. http://www.ars-grin.gov/cgi-bin/npgs/html/taxon.pl?409608 (accessed December 8, 2009).

USDA, NRCS. 2009. PLANTS profile for *Schoenoplectus mucronatus*. http://www.plants.usda.gov/java/nameSearch?keywordquery=schoenoplectus+mucronatus&mode=sciname (accessed December 8, 2009).

Washington State Noxious Weed Control Board (NWCB). 2007. Class A noxious weed ricefield bulrush *Schoenoplectus mucronatus* and Written findings of the Washington State Noxious Weed Control Board. http://www.nwcb.wa.gov/weed_info/Schoenoplectus_mucronatus.html (accessed December 8, 2009).

Native and invasive plants in the understory of forests impacted by Emerald Ash Borer

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Plant biodiversity is an important component for the sustainability of native forest ecosystems. Non-native invasive plants may alter the native biodiversity and affect the integrity and function of native forest communities. Disturbed areas, such as canopy gaps, are highly susceptible to colonization by invasive plants. The exotic insect pest, emerald ash borer (EAB; *Agrilus planipennis*), has killed nearly all ash (*Fraxinus* spp.) trees in southeastern Michigan (the point of introduction of EAB), and is expected to continue to spread throughout eastern forests. The resulting canopy gaps will potentially facilitate the spread of many woody, herbaceous, and vining invasive species that are already established in the surrounding landscape. Currently, there is limited information available to help predict how invasive species will respond to EAB-induced forest gaps or if they will be affected differently than native species. We established plots in seven state or metropolitan parks in southeast Michigan that have extensive public forested lands (Highland, Hudson Mills, Indian Springs, Island Lake, Kensington, Pontiac Lake, and Proud Lake). In 2008, we located, tagged, and measured the volume index (length × width × height) of ten different woody invasive species (*Berberis thunbergii*, *Celastrus orbiculatus*, *Elaeagnus umbellata*, *Euonymus alatus*, *Lonicera* spp., *Rhamnus cathartica*, *Rhamnus frangula*, and *Rosa multiflora*) within each plot. We also located, tagged, and measured the volume index of a native woody plant within 1 m of each invasive plant. In 2009, we re-located each native-invasive pair and measured the volume indices; growth rate was then calculated and compared between native and invasive species. Within each plot, we also established four 4-m^2 quadrats centered at 8 m in each cardinal direction and counted the number of all invasive woody plants as well as native plants greater than 12 inches tall. After one season, we have yet to see any clear trends in relative growth rate of native relative to paired invasive plant species. The most frequent exotics encountered were *R. cathartica*, *R. frangula*, and *C. orbiculatus*. The most frequent native species in the understory were *Fraxinus* spp. followed distantly by *Carpinus caroliniana*. Volume measurement and understory woody plant diversity surveys will be repeated for two more seasons.

A preliminary assessment of herbivore damage and the potential for enemy release as an explanation for the success of the woody invasive *Lonicera maackii*

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The 'enemy release hypothesis' argues that when an exotic species is introduced to a novel habitat, it experiences a release from regulation by herbivores resulting in increased distribution, abundance, and vigor. This study aims to assess the amount and incidence of herbivory occurring on *L. maackii* in edge and interior habitats. In October of 2008, leaves were taken from shrubs located in interior and edge habitat from 8 natural areas in Ohio. A follow up study was conducted in 2009, which included multiple sampling dates and a separation long and short shoots. Leaves were assessed for the amount and type of damage. For the 2008 season, approximately 70% of leaves had no herbivory and the damage present on the remaining leaves was between 1-5%. Of those experiencing herbivory, 85% were damaged by chewing. Edge habitat sustained more damage than the interior. Damage rates from this study revealed that levels of herbivory experienced by *L. maackii* are likely too low to impact the fitness of these shrubs. These findings indicate 'enemy release' may contribute to the success of *L. maackii*. Future studies will be conducted to further clarify the role of ERH in the invasion biology of *L. maackii* in Ohio.

The ecological consequences associated with the invasion of non-native species are a threat to ecosystems worldwide (Mack & D'Antonio 1998, Pimentel et al. 2005). One prominent theory for the success of invasive plants in their new habitats is the 'enemy release hypothesis' (ERH) which argues that when an exotic plant species is introduced to a novel habitat, it can experience a release from regulation by specialist herbivores often resulting in a rapid increase in distribution, abundance, and vigor (Keane & Crawley 2002).

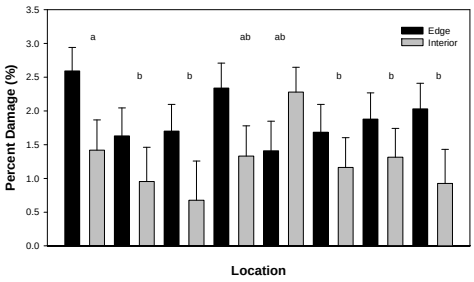
Lonicera maackii (Rupr.) Maxim (Amur honeysuckle), a prominent Ohio invasive shrub was the focus of this study. *Lonicera maackii* is an upright, deciduous woody shrub native to Asia (Luken & Thieret 1996). There are no studies indicating whether ERH contributes to the successful invasion of *L. maackii* in the Midwest. Evidence to support ERH often involves accounts of larger plants, differences of herbivore and pathogen loads, and herbivory assessments in the invaded range as compared to the native range (Carpenter & Cappuccino 2005, Maron & Vila 2001). Observations of *L. maackii* in its native versus introduced habitat indicate that the shrub grows more vigorously in introduced range than its native range, an indication that ERH might be a factor in invasion (Luken & Thieret 1996).

Leaf samples were collected from eight sites throughout Ohio in October 2008 from forest edge and the interior habitats. Leaves were scored for damage on a percentage basis using a standardized scale for leaf area lost or infected. Based on previous assessment studies of leaf herbivory, categories for leaf damage were established as follows: 1) chewed or skeletonized, 2) scraped, 3) mined, 4) infected with pathogen (i.e. fungal infection), and 5) mixed (Adams et al. 2008). The incidence of herbivory was also calculated from these data. In 2009, a season long assessment was conducted at 3 sites to determine the timing of leaf area removal. Because of the difference in phenology, an additional distinction was made between long and short shoots.

Results of our 2008 survey indicate approximately 69.3% of all leaves surveyed had no herbivory, 24% of all leaves experienced 1-5% herbivory, and the mean amount of herbivory was 1.8%. Nested ANOVA revealed significant differences in herbivory between locations within the sites (Figure 1). When pooling all leaves that were positive for herbivory, 85% were chewed while scraping, mining, rolling, and mixed herbivory contributed an insignificant amount of herbivory. Results of the 2009 survey were also dominated by leaf chewing. A low level of infection was observed on *L. maackii* sampled at all sites (2008) with only 1.93% of all leaves positive for the presence of pathogen damage. The main symptom of infection was simple leaf necrosis and the majority of these leaves were located in the edge habitat at Hueston Woods State Park, Oxford, Ohio. Preliminary results

of 2009 indicate a difference in mean percentage removed through the season for pooled leaves from Taylorsville Metropark site (1.10% June, 2.69% August, and 2.14% October). Also, the differences between long and short shoots were more dramatic in the interior habitat as illustrated by the August sampling where the mean damage for edge long was 3.58% and interior long was 1.28%. A nested ANOVA will be performed to illustrate the differences between sites, locations, and shoot types.

Figure 1.



Damage rates from this study revealed that levels of herbivory currently experienced by *L. maackii* are likely too low to impact the fitness of these shrubs. Edge habitats are a more dynamic system with higher biodiversity and increased exposure, which would help explain increased herbivory in this habitat location. While it is true that the shrub experiences higher herbivory in edge habitat, the amount was very low, and the amount light available to the plant on the edge might facilitate compensatory growth, which would counteract any effect the herbivores might have on the performance of *L. maackii.* The dominance of leaf chewing indicates that the damage that is occurring to *L. maackii* can be attributed to generalist herbivores.

Results support the view that 'enemy release' may contribute to the success of *L. maackii*. Previous research has shown *L. maackii* produces defensive compounds which have inhibited herbivory in laboratory feeding trials (Cipollini et al., 2008). The absence of specialist herbivore feeders, coupled with evidence of both resistance and tolerance strategies within this genus further suggest enemy escape has occurred. To clarify the role of ERH on the invasive success of *L. maackii*, future research will include a common garden experiment with native, non-native/non-invasive, and invasive congeners, simulated herbivory experiments with native and invasive genotypes, and leaf level chemical analyses of defensive compounds for all experiments. If ERH plays a major role in the success of *L. maackii* we can predict that this shrub will not experience significant amounts of herbivory without the introduction of a specialist herbivore or pathogen as is the case with classical biological control program.

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Invading from the garden? A comparison of leaf herbivory for exotic and native plants in natural and ornamental settings

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Invasive plants are a significant threat to biodiversity and ecosystem function. The enemy release hypothesis proposes that exotic species become invasive due to higher performance allowed by low levels of damage by pathogens and predators. We tested this hypothesis in ornamental and natural settings to determine if ornamental settings confer a release from herbivory. We compared levels of leaf herbivory among native and exotic tree and shrub species in ornamental and natural settings in the Cincinnati region. Overall we found lower levels of leaf damage for exotic species than for native species, supporting the enemy release hypothesis. We found no differences in the amount of leaf damage suffered in ornamental or natural settings or between invasive or non-invasive exotic species. Our results indicate that ornamental settings do not provide any added release from herbivory for exotic species.

The enemy release hypothesis proposes that exotic species are subject to lower levels of herbivory than native species (Elton 1958). Many studies have shown that herbivore damage can be context specific, varying in both space and time. Agrawal et al. (2005) argued that times and areas where damage to an introduced species are low may allow windows of opportunity when they can invade native communities. Ornamental settings may provide such a context by providing areas rich in exotic species potentially experiencing low levels of herbivory.

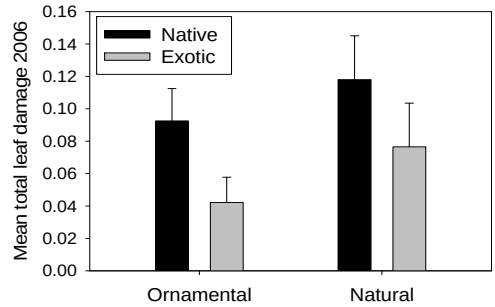
We conducted two separate experiments, one in 2006 and a second in 2007, to evaluate levels of leaf herbivory. All observations were conducted in and around the Cincinnati metropolitan area. In 2006 we selected common tree and shrub species that were either native to the region or were not. In 2007 we focused on a wider array of exotic species with varying degrees of invasiveness. In both years we examined leaf herbivory in natural and ornamental settings. Our "natural" settings were areas subject to minimal management, e.g. natural areas and wooded parks; our ornamental settings were areas subject to considerable management. We defined an ornamental location as one with a managed understory.

In 2006 five leaves were randomly sampled per tree. In 2007 we increased sampling to 10 leaves per tree. All sampling was done in September and October, prior to leaf fall. We visually assessed the proportion of leaf area damaged by herbivory.

For 2006 data, analyses were conducted using general linear models. Each tree or shrub was considered a subject and leaf damage averaged across the five leaves within a tree. Native/exotic status and location (natural or ornamental) were fixed effects, each with two levels. A full factorial design including all interactions was analyzed. The proportion of damage was angularly transformed prior to analysis to better meet the assumptions. In 2007, we analyzed data in a similar manner, averaging across the 10 leaves per tree. Here, all species were exotic and we compared species based on their invasiveness. Because some species were not found in natural settings, we conducted separate tests between natural and ornamental settings and between invasive and non-invasive exotic species.

Slightly less than 9% of leaf area was damaged in 2006 (Figure 1). Native species showed significantly greater damage than exotic species ($F_{1,80} = 7.60$, $p = 0.007$), but there was no difference between ornamental or natural settings ($F_{1,80} = 1.96$, $p = 0.17$) or any interaction between setting and status ($F_{1,80} = 0.03$, $p = 0.87$).

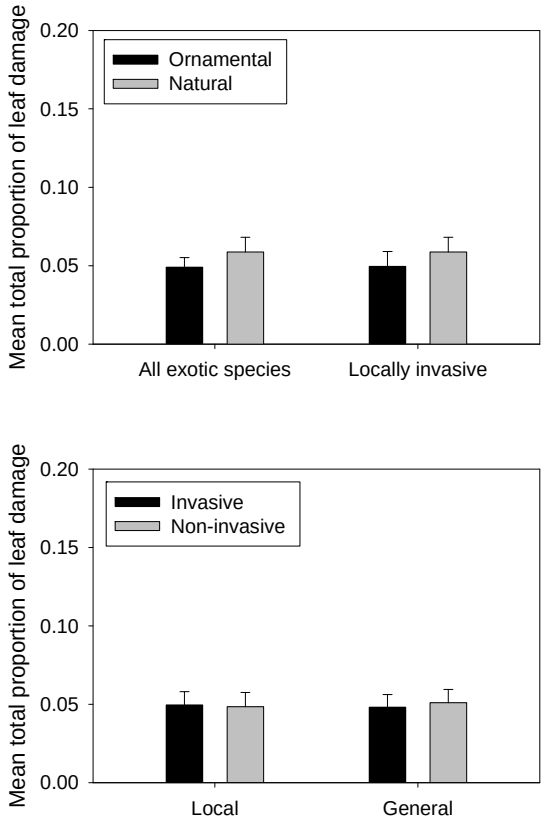
Figure 1



Several non-native species invasive elsewhere were not found in natural settings around Cincinnati. We classified exotic species as "locally invasive" if we found them in natural settings in our searches. These species as well as others listed as being invasive either by USDA or APWG were classified as "generally invasive." Exotic species that we did not find in natural settings and that were not listed by either USDA or the APWG were considered non-invasive.

In 2007 the total amount of leaf damage for non-native species was 5.23% (Figure 2). There were no differences in leaf damage between natural and ornamental settings when considering all species ($F_{1,112} = 0.28$, $p = 0.28$) or when considering only locally invasive species ($F_{1,76} = 0.00$, $p = 0.99$). Within ornamental settings there were no differences in damage suffered by non-invasive species and either locally invasive species ($F_{1,73} = 0.95$, $p = 0.33$) or generally invasive species ($F_{1,73} = 2.30$, $p = 0.14$).

Figure 2



Only a portion of exotic species become invasive, and many studies have sought to characterize plant characteristics or conditions that promote invasiveness. Broadly, our results support the enemy release hypothesis in that exotic species suffered lower levels of herbivory than did native species. Our results across an array of species indicate that ornamental settings provide limited if any added advantage through an additional release from herbivory. We note that this does not mean the invasion of native habitats does not occur from ornamental settings, there are countless examples. What we show is that *in general* there is no *added* release from herbivory due to being in an ornamental setting. Depending on the conditions and amount of care, ornamental settings may offer many other advantages potentially facilitating invasion.

The results of our study largely mirror Nuckols and Connor (1995) who found no difference in the level of leaf herbivory on native trees between urban and natural settings. Our study extends this result to show that native and non-native species show little difference in leaf herbivory suffered in ornamental or natural settings. Our study contrasts with the results of Cappuccino and Carpenter (2005) who found that nine invasive, exotic species in northeastern North America suffered less herbivory than did nine non-invasive, exotic species. We found no difference in the total amount of herbivory suffered by locally invasive exotic species versus locally non-invasive exotic species. Similarly there was no difference if we compared generally invasive exotic species and non-invasive exotics. By necessity these comparisons were limited to ornamental settings. Overall it does not appear that amounts of herbivory vary with invasiveness in ornamental settings for the species studied.

Overall the results of our study suggest that if release from herbivory is important for invasion, ornamental settings provide little inherent additional benefit. Non-native species suffer less leaf herbivory than do native species, but the context of an ornamental versus natural setting does not affect this relationship across an array of species.

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Bringing science and management together: Effective strategies for dealing with invasive plants in the Iron Furnace Cooperative Weed Management Area

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Non-native invasive plant species have expanded into almost every ecosystem in North America and are presently altering most natural system processes. The alteration of these biological systems is forcing creating a high cost to human society. Managers are faced with a variety of difficult decisions with respect to prioritizing their activities and are usually restricted to certain agency level boundaries. One solution to this problem has been the formation of cooperative weed management areas (CWMAs) which rely on the cooperation of adjacent landowners (private, state, and federal) to document and manage invasive plant species. We will discuss the formation of Ohio's only CWMA through the integration of many partnerships and activities. The Iron Furnace Cooperative Weed Management Area (IFCWMA) is 241,000 ha in size where 20% is federal land (national forest), 4% is state land (state forest), and 76% is private land. Here we focus on an early science-management initiative of the IFCWMA: to develop an easily deployed methodology to map the distribution and abundance of invasive species, using handheld devices equipped with GPS technology, in specific watersheds so that distribution and abundance can be documented prior to forming a management strategy and assessing future research needs among partners. We hope that the formation of IFCWMA and the example of management-scientist collaboration can serve as an example for the formation of other CWMAs elsewhere in Ohio and throughout the central and southern Appalachian region.

Cooperative Weed Management Areas (CWMAs) are collaborations between federal, state, and private entities to combat the establishment and spread of non-native invasive plant species (NNIS) throughout the landscape. Complicating matters is the checkerboard pattern of land ownership that exists in most localities, where state, federal, and privately owned lands are interspersed, thus confounding management strategies. NNIS, once established do not conform to these political boundaries.

CWMAs have gained popularity in the last decade as a means to combat the spread of NNIS. Cooperation between state, federal, and private agencies and landowners can minimize NNIS by effectively utilizing a common management strategy across ownership boundaries. The benefits of CWMAs include the sharing of information on the NNIS infestations and control techniques, access to funding sources not otherwise available to individual organizations, and creating avenues for public outreach.

The Iron Furnace Cooperative Weed Management Area (IFCWMA) was formed in September 2006. The IFCWMA's boundary includes Lawrence Country and parts of Scioto, Jackson and Gallia counties in southern Ohio (Figure 1). The perimeter was determined based on 5th order watershed boundaries and thus includes significant watersheds for Symmes Creek, Pine Creek and the Ohio River.

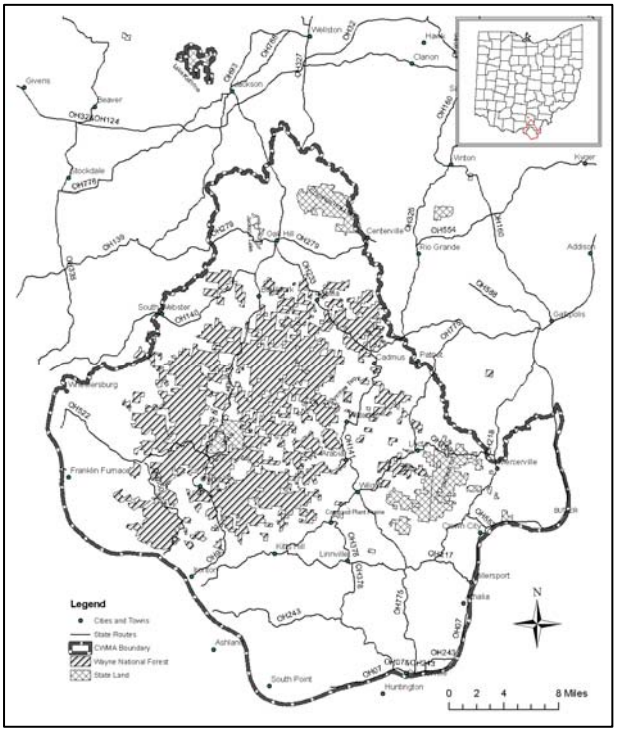
The IFCWMA derives its name from southern Ohio's historical significance in the iron industry. Timber harvesting to fuel the furnaces and to clear land was extensive in the 19th century; indeed, land management including logging and mineral extraction continue to play a vital role in the region's economy. These intensive management activities led to ample opportunities for NNIS invasion and proliferation.

The goals of the IFCWMA are

- To increase public awareness through education about invasive, non-native plants by hosting weed workshops, field tours, and public meetings and by providing informational materials on NNIS.
- To seek grants and other funding sources to support the goals of the IFCWMA.

- To provide coordination and leadership for a local volunteer monitoring program to collect information on the locations and spread of invasive, non-native plants and for early detection of new invasive, non-native plant infestations. This information would aid in prioritizing sites for invasive plant control.
- To develop and implement a strategic plan for the IFCWMA which will determine the long-term direction and priorities of the CWMA.
- To systematically control, over time, NNIS in the IFCWMA.

Figure 1.



The IFCWMA is managed by 17 partner organizations, including representatives from federal, state and private organizations. We have a coordinator, secretary and two working group committee leaders who serve one-year term positions.

To date, we have organized six workshops and conferences for the general public and land managers. We've received two grants which were used to map invasive species on state, private and public lands. The IFCWMA and its partners helped start the Ohio Kudzu Eradication Initiative and worked with the U.S. Fish and Wildlife Service, the U.S. Forest Service, and a private land owner to control invasive plants to protect a federally listed plant species.

In 2010, we are continuing our mapping efforts by partnering with Ohio University, the National Forest Foundation and the Central Hardwoods Invasive Plant Network to map invasive species on state, private and federal lands in the IFCWMA. We also plan on organizing at least one workshop to train community members in the identification and mapping of invasive species.

The poster we present here is the results of an inventory performed with a grant from the U.S. Forest Service State and Private branch. The grant was received and administered by the Ohio Invasive Plants Council (OIPC) and the mapping work was carried out by Ohio University through a contract with OIPC.

We first met with land managers from organizations within the IFCWMA to determine which areas were most important for them to be inventoried. These areas were surveyed for the presence of all NNIS. Surveying equipment consisted of a HP iPAQ 210 Enterprise® PDA with an integrated Trine GPS receiver. ArcPad® software was downloaded into the PDA and used for data acquisition. Invasive plant populations were identified and mapped as polygon shape-file layers over a topographic base-layer, thus providing accurate location and distribution data for NNIS occurring on the property. Each polygon included four data fields: 1) NRCS code name of the NNIS, 2) Height estimate of NNIS in feet when applicable, 3) Percent coverage of the NNIS species within the shape-file, and 4) Notes on the mapped infestation. All field data was subsequently edited using ArcMap® 9.2 software to produce high-quality map layers for each species of NNIS occurring the different properties. At the end of the project each land manager or land owner received a report which included a map the property with the NNIS infestations, treatment recommendations and GIS shapefiles.

For more information on the IFCWMA and this project and future project please visit www.ironfurnacecwma.org. The IFCWMA meets quarterly at the Jackson Library. If you would like to attend a meeting or join the IFCWMA please contact Chad Kirschbaum.

Exploring the potential influence of *Lonicera maackii* on nutrient fluxes and pools: leaf fall, rainwater throughfall and soil

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Invasive species usurp habitat space in the ranges they invade, in some instances creating a virtual monoculture. The impacts of this phenomenon on community level traits (e.g., species diversity) are relatively well-understood— less is known about the impacts of invasion on ecosystem-level processes. We are approaching this knowledge gap using *Lonicera maackii* invasion in the Ohio Valley as a model system. In a series of studies, we compared ecosystem-level processes in areas dominated by *L. maackii* to those free of the invasive. In a litter basket study we measured leaf litter input under and away from honeysuckle shrubs. We found that, surprisingly, leaf-fall phenology of *L. maackii* mirrored that of the native species canopy, contributing leaf material to the forest floor from 5-Oct to 28-Nov, but differed from the forest canopy in also exhibiting a late-season pulse of leaves 12-Dec. While leaf input from native species was similar under and away from shrubs, leaf mass of *L. maackii* was much higher under *L. maackii* shrubs than away. In a separate study, we installed funnels under and away from *L. maackii* shrubs and compared the chemistry of throughfall that passed through a native tree canopy (without the influence *L. maackii*) with that which passed through the *L. maackii* shrub canopy. We found that the amount of water was lower under shrubs than away, and also that throughfall chemistry that passed through *L. maackii* had higher cation content than that passing through a native species canopy. In a third study, we sampled and analyzed soil and used *in situ* incubations to assess nitrogen mineralization under and away from *L. maackii* shrubs. Overall, we found that the invasion of *L. maackii* has important implications for a series of ecosystem processes. Future work will seek understanding of the mechanisms driving shifts in nutrient cycling associated with *L. maackii*.

Inferring pattern of colonization of the invasive shrub Amur Honeysuckle (*Lonicera maackii*) in southwestern Ohio from the genetic structure of their populations

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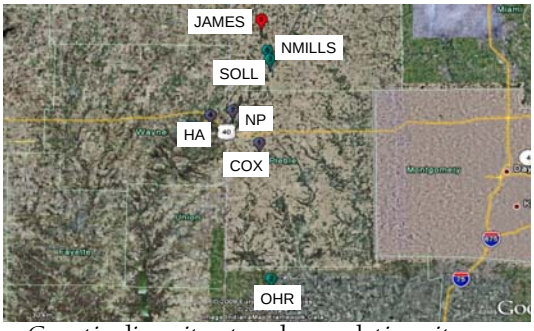
Genetic diversity and levels of differentiation between seven well-established populations of Amur honeysuckle were examined using five polymorphic microsatellite loci. These samples were examined to determine allelic variation, percent polymorphic loci, expected heterozygosity, and relatedness between populations. In 158 samples, 58 alleles were identified across all loci. The average number of alleles found at each locus was 11.6. The mean number of alleles found within each population ranged between 5.2 and 9.0, with the average being 7.0. Mean numbers of effective allele within populations were lower, ranging from 3.5 to 5.1 with an overall average of 4.1. Analysis of molecular variation (AMOVA) revealed that only 8% of the observed variation arose from differences between populations. The majority of variation (92%) was found within populations. Overall, the observed heterozygosity among loci was found to be slightly less than the expected heterozygosity (H_O=0.7066, H_E=0.7415). The degree relatedness between populations indicates that geographical distance is correlated with the genetic distance separating them. Two different approaches were employed to examine how the populations were related to each other and to determine whether this species spreads along an advancing front or through long-distance dispersal.

A fundamental question about invasive plants is whether they proceed along expanding fronts or through long-distance dispersal events followed by local expansion (Auld & Coote 1980). Understanding how invasive plant species spread will have important implications for management and control. *Lonicera maackii* (Rupr.) Maxim. (Caprifoliaceae), Amur Honeysuckle, is a large, self-incompatible (Deering & Vankat 1999) shrub native to Asia that was introduced into the United States in 1898 (Luken & Thieret 1995). Additional introductions were made by the USDA at least 14 times over the following 86 years (Luken & Thieret 1995). Naturalized populations now occur in nearly every state east of the Mississippi (Trisel & Gorchov 1994). This study outlines a first attempt to describe the genetic structure of *L. maackii*. In particular, the aim is to find interpopulation genetic differences to support the three proposed invasion paths, one from Dayton, OH, via Miami County to the southeast, one from Cincinnati, OH, via Preble County to the south (Hutchinson & Vankat 1997), and one from Richmond, IN, via Randolph County to the southwest.

This study was conducted in three Ohio counties (Darke, Preble, and Miami) and Wayne County, IN. Each study plot was established in a landscape of privately owned woodlots in an agricultural matrix. Leaf tissue from seven well-established populations was collected for a total of 158 samples. Distances between study populations ranged from 2.00 km to 57.07 km (Figure 1). Genetic variation was determined using five microsatellite marker loci.

Figure 1



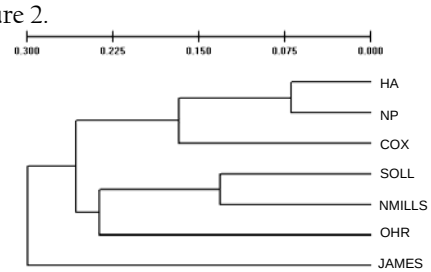
Genetic diversity at each population site was quantified by the mean number of alleles per locus (Na), the effective number of alleles per locus (Ne), observed heterozygosity (H_O), and expected heterozygosity (H_E), for each locus and averaged across all loci. Relatedness between populations was determined using Nei's genetic distance and used to produce a UPGMA tree. Relatedness between populations was also examined using Structure (Hubisz et al. 2009).

A total of 58 different alleles were found in the five loci, with an overall average of 11.6 alleles per locus. Mean Na in each *L. maackii* population ranged from 5.2 in the NMILLS population to 9.0 in OHR with an average of 7.0. Ne was lower, ranging between 3.5 and 5.1 per population (NMILLS and OHR, respectively) with an overall average of 4.1. Average H_O in each population ranged between 0.6476 in the SOLL populations and 0.7571 in NMILLS, with an overall average of 0.7066. The average H_E for each population varied between 0.7047 and 0.8041 (NP and OHR, respectively) with an average of 0.7415. High H_E and Na found in these populations suggest high levels of genetic diversity and a low level of inbreeding, which are further supported by the F-statistics calculated for each locus. The mean fixation index (F_{IS}), the inbreeding coefficient, was found to be 0.0183 when calculated across all loci. The analysis of molecular variance (AMOVA) showed that 92% of the observed variation arises from within population differences.

A Mantel test conducted to determine the relationship between the genetic and geographic distances between populations showed a positive correlation ($r = 0.3566$, $p < 0.001$). Figure 2 more clearly depicts the relatedness between the seven studied populations. The dendrogram separates the seven populations into three distinct groups. COX, HA, and NP are all closely related and in close proximity to each other. This is expected due to the correlation of geographic and genetic distances as described by the Mantel test. The second cluster, comprised of SOLL, OHR, and NMILLS, shows close relatedness even though OHR is geographically separated from the other two.

The third group is most noteworthy, showing that the JAMES population is genetically distinct from any of the other populations examined. This occurs despite close geographic proximity to the NMILLS and SOLL populations.

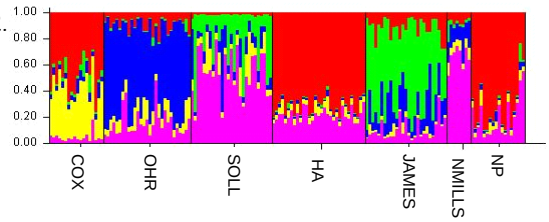
Figure 2.



Further analysis using Structure separates these seven populations into five different clusters. HA and NP are still grouped together in red and SOLL and NMILLS both fall within the same cluster in pink. JAMES, OHR, and COX are each distinct clusters shown in green, blue, and yellow respectively.

While the POPGENE and Structure provide two different explanations for the genetic structure of *L. maackii* in this region, each may be valid. The dendrogram does show three groupings; however, the OHR, SOLL and NMILLS sites need further attention. All three are related to each other, but SOLL and NMILLS are more closely related to each other than to OHR.

Figure 2.



Comparing this to the populations' locations (Figure 1), it is clear that SOLL and NMILLS are very close in proximity while OHR is some distance to the south. Something similar occurs with the HA, NP, COX populations. Here, the three are all more closely related than to any of the other studied populations, but COX separates, both genetically and geographically, from the other two. COX is not as genetically distinct as OHR was in its group, but it still remains different. Looking at the corresponding Structure plots, the populations seem to split nicely into three groups. However, when assuming additional populations, OHR and COX become their own distinct clusters. The results from both POPGENE and Structure support the correlation between geographical and genetic distances. These findings suggest that *L. maackii* spreads through long-distance dispersal followed by outward expansion.

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The impact of invasive hydrophytes on tadpole growth, survival and development

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Invasive species are second to habitat loss in the list of threats to native biodiversity. At the same time, amphibians are facing declines globally. Understanding the mechanisms by which invasive species may affect native amphibians is critical to conserving amphibian biodiversity. This study followed enclosed *Rana clamitans* (green frog) and *Lithobates catesbeianus* (American bullfrog) tadpoles in non-invaded wetlands, and wetlands invaded by either *Typha angustifolia* or *Phragmites australis* over the course of the summer of 2008. The tadpoles consisted of three clutches or "frog groups" of early-laid *R. clamitans*, late-laid *R. clamitans* and *L. catesbeianus*. All individuals came from a non-invaded pond. Tadpoles and abiotic measures were monitored weekly. Abiotic measures included nitrate, ammonium, dissolved oxygen, pH, and temperature, and biotic measures included survival and total length. Wetlands varied in dissolved oxygen, nitrate, and ammonium based on wetland type, and temperature and pH varied among and within wetland types. Temperature, wetland type, and frog group all influenced growth and survival. There were also numerous interaction effects across the study. The host of interactions and confounding variables makes interpretation difficult, but effects are there, even if the mechanisms are not clear at this point. This work is part of an ongoing dissertation research project.

Potentially of great concern for amphibians, which are already experiencing declines, are invasive plant species (e.g. Maerz et al. 2005). Invasive plants are not only novel organisms, with chemical arsenals to which many amphibians are naïve, but often change the structure of the entire habitat by rapidly establishing a monoculture or dominance of a single species, thus reducing habitat complexity (Gerber et al. 2008). Between the disproportionate number of invasive plants that are hydrophytes (Zedler 2004), and amphibians' need for wet or aquatic breeding areas and juvenile habitat, invasive plants have a potential to affect amphibians and other partially to fully aquatic organisms, more so than the threat invasive plants pose to non-aquatic organisms. Amphibians' susceptibility to environmental factors such as temperature (Brattstrom 1962), UV radiation (Zimskind et al. 1955), pH (Grant et al. 1993), and some chemicals (Raimondo et al. 1998) - all factors that may by influenced by invasive plants (Gordon 1998) - serves to compound the problem. Novel chemicals associated with, decreased diversity due to, and restructuring of habitats by invasive plants may lead to detrimental effects on native amphibians through a variety of pathways.

This study monitored growth and survival of enclosed *Rana clamitans* and *Lithobates catesbeianus* tadpoles in non-invaded wetlands and invaded wetlands. Invaded wetlands had well established populations of either *Typha angustifolia* or *Phragmites australis*.

This study utilized five wetland complexes. Each complex includes three independent ponds of comparable sizes- one dominated by *T. angustifolia*, one dominated by *P. australis*, and one with predominantly native plants. These were wetlands used in the summer of 2007 to ascertain the chemical profile differences among the three types of wetlands. These wetlands consist of mixed land uses, from protected state preserves to storm water retention swales.

In late May, three *R. clamitans* clutches were caught from an independent, non-invaded wetland shortly after amplexion. Egg masses were divided into groups of approximately 10-20 eggs each. Masses were placed in enclosures, and after hatching clutches were standardized to ten larvae per enclosure. This procedure was repeated in late June with clutches of *R. clamitans* and *L. catesbeianus*. Tadpoles were monitored weekly. Biotic measures included survival and total length.

In order to account for differences in body size over the study duration, tadpole growth relative to size was also examined. This growth metric, Relative Growth Rate (RGR), indicates weekly increments in body length relative to tadpole size at the beginning of the week. In native ponds, there

was no significant difference in RGR among the frog groups. In *T. angustifolia*-invaded wetlands, early *R. clamitans* had a higher RGR early, and then around week 4 approached the RGR for late *R. clamitans* and *L. catesbeianus*. In *P. australis*- invaded wetlands, again early *R. clamitans* had higher RGR than the other two, and in week six had a lower RGR than late *R. clamitans* and *L. catesbeianus.* Overall, early *R. clamitans* showed a faster initial growth relative to *R. clamitans* and *L. catesbeianus* later in the season.

Analysis revealed no significant differences in size across the three frog groups in the native wetlands. Analysis of variance showed that in the *T. angustifolia* wetlands, early *R. clamitans* had a higher growth rate ($p < 0.001$), but experienced early die offs in week 6. In *P. australis* wetlands, early *R. clamitans* again experienced higher initial growth rates, and then their growth slowed noticeably around week 5. Late *R. clamitans* and *L. catesbeianus* showed similar responses throughout the growth period.

Weekly measurements of the physical and chemical characteristics of all experimental ponds revealed significant differences among pond types. There were higher dissolved oxygen ($p < 0.001$) and nitrate ($p < 0.001$) in *T. angustifolia*-invaded ponds than in the other two pond types. There were also differences in water temperature where non-invaded ponds were warmer than either of the invaded ponds ($p < 0.001$). Finally, ammonium content was higher in *P. australis*-invaded ponds ($p < 0.001$).

Seasonal affects within *R. clamitans* and interspecific differences were seen in tadpole development. The potential seasonal effects could be attributed to any chemical compounds produced by the plants (i.e. secondary metabolites), or to environmental stressors early in the season, such as high competition, unstable temperatures, etc. The identity of the invading species seems to be of less importance than the presence or absence of the invader. In this case, tall, foliose, densely growing monocultures may create a functionally different environment than what is provided by non-invaded wetland plant communities. This may indicate that the problem for amphibians is due to the potential abiotic changes that the altered plant community structure provides- e.g., temperature profiles, light availability, and periphyton community structure. It is apparent that there is a faster rate of growth in *R. clamitans* for both of the invaded communities. It is possible that the invasive species are providing some stress that makes this growth rate unsustainable for long term green frog development.

If wetlands become dominated by invasive hydrophytes, then the functional result may be similar to amphibian habitat loss, or a population sink for amphibians. While many individual frogs are thought to have site fidelity to their natal pond, they do often move to nearby ponds. If individuals are continually moving into an invaded pond, where the offspring may suffer decreased survival and growth, the existence of these ponds may contribute to population declines for otherwise stable metapopulations.

Even in the face of no-net-loss wetland policies, this transfer of habitat to functional non-habitat via plant invasion may lead to similar population level effects as habitat loss, which is currently a leading cause for many population declines. This would lead to the illusion of conservation when in fact critical breeding habitat is being lost, but that loss is not recognized officially. The conservation of amphibians in particular is a critical concern. Amphibians have a unique role within the vertebrate community due their lifestyle, which creates important aquatic-terrestrial links not seen in many taxa. They also function as indicators of biotic integrity for many agencies that assess environmental quality because of their life history traits.

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The response of European buckthorn, *Rhamnus cathartica* to soil amendments in restoration

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European buckthorn, *Rhamnus cathartica*, is a non-native, woody, shrub in the Midwest which has been shown to leave "legacy effects" in the soil even after removal. Studies show that *R. cathartica* increases gravimetric water and nitrogen contents in invaded soils. Field studies indicate that amending soil with mulch after invasion and removal reduces reinvasion of this species. This study investigated the impacts of soil amendments with mulch on non-native *R. cathartica* sapling growth and seed germination in a controlled environment compared to field results. Our experiment utilized the soil amendments of commercial and *R. cathartica* mulch to simulate treatments used in the field. We examined the effects of these treatments on native *R. alnifolia* seed germination. We examined the alterations of nitrogen and moisture levels that occur in each treatment. In the field, we observed that mulch amendments reduced *R. cathartica* reinvasion. In the greenhouse, mulch amendments reduced *R. cathartica* sapling growth and available nitrogen. After 4 weeks, no native *R. alnifolia* seeds germinated but several non-native *R. cathartica* seeds germinated in all treatments, though less germinated in *R. cathartica* mulch-amended soil. Our results suggest that addition of mulch may be a useful tool in reducing elevated soil nitrogen levels and preventing reinvasion and growth of *R. cathartica*.

Restoration of native ecosystems after invasion by European buckthorn has become an important issue in the Midwest. European buckthorn, *Rhamnus cathartica*, is a non-native, woody shrub invading North American woodlands. Over time, this species forms a dense thicket modifying a number of ecosystem properties in these woodlands including soils, moisture and available nitrogen (N) levels (Heneghan et al. 2005).

A field project which focused on "soil amelioration" after removal of *Rhamnus cathartica* suggests that application of mulch to previously invaded soil reduces re-invasion of that species (Heneghan, unpublished). The addition of high carbon (C) mulch may lead to microbial immobilization of plant available N, thereby potentially restoring the soil properties to their pre-invaded conditions. This experiment observes the impacts of soil amendments on *R. cathartica* growth both in the field and in a controlled environment, and compares the seed germination of *R. cathartica* to the native shrub in the same genus, *R. alnifolia*, in these soil treatments.

In the field experiment, *R. cathartica* saplings and seedlings were counted in three $0.25m^2$ quadrats in 5 replicate restoration plots that had *R. cathartica* mulch addition, commercial mulch addition and no soil amendment at Whippoorwill Farm in Mettawa, IL.

In the greenhouse experiment, 288 *R. cathartica* saplings were collected from Mary Mix McDonald Woods in Glencoe, IL then rinsed, dried, weighed and planted randomly in each of three soil types and labeled. Saplings were watered daily in the greenhouse at Chicago Botanic Garden (CBG), Glencoe, IL. Height and leaf number of each sapling were taken twice at weeks 0, 3, 5 and 10. A sample of each type of soil was analyzed before and after planting for gravimetric moisture content and NO_3 and NH_4 concentrations using a 2M KCl extraction and colorimetric method with a HACH DR 5000 Spectrophotometer and reagents (Robertson et al. 1999).

In the germination experiment, 128 seeds each of the non-native *R. cathartica* collected from Lake County, IL and the native *R. alnifolia* collected from Chippewa National Forest, MN were planted in three soil treatments and left to germinate in flats in a greenhouse at CBG. Seedling emergence was observed and counted every three days.

Figure 1

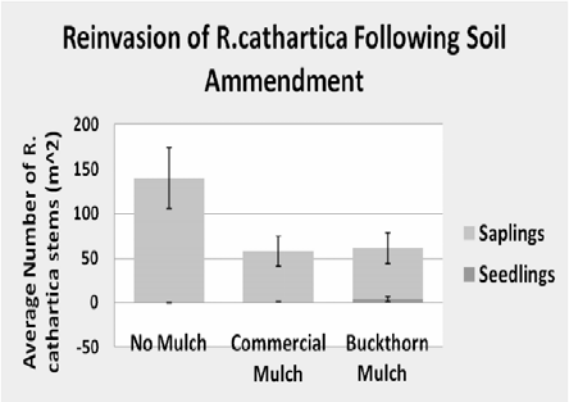


Figure 2

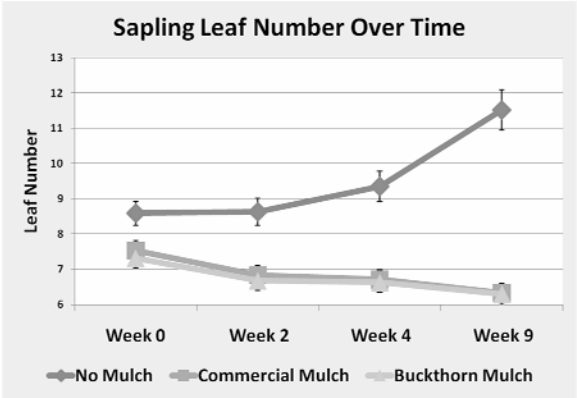
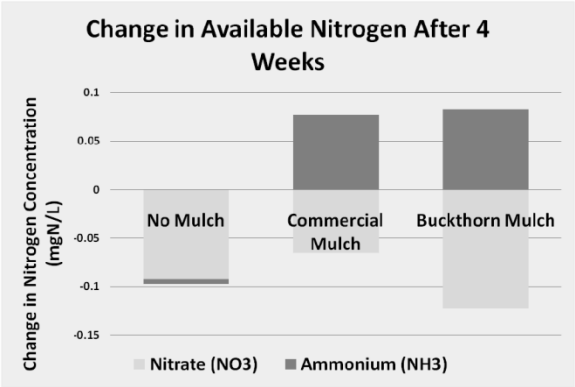


Figure 3



In the field experiment, reinvasion by *R. cathartica* saplings and seedlings was significantly higher in the No Mulch treatment and lowest in both mulch treatments. There is no significant difference in reinvasion between the two mulch plots suggesting
that in the field the type of mulch added to the soil has no significant impact on reinvasion (Figure 1).

In the greenhouse experiment, *R. cathartica* height increased in all soil treatments but was reduced in both mulch treatments. Sapling leaf number increased throughout the experiment in the no mulch treatment but began to decrease in both mulch treatments (Figure 2). As with the field experiment, while *R. cathartica* grew taller and had more leaves without soil amendment, there was no difference between the two mulch additions.

Soil nitrate (NO_3) and ammonium (NH_4) decreased in the no mulch treatments during the first 4 weeks of plant growth (Figure 3). This decrease in the no mulch soil could be due to the greater plant growth observed in this treatment. Soil NH_4 increased while NO_3 decreased in both mulch treatments (Figure 3). The mechanism of this is unclear and will be subject to investigation throughout the remainder of this experiment.

In the germination experiment, *R. cathartica* seeds have germinated in all soil treatments but no *R. alnifolia* seeds have germinated to date. Germination was highest in no mulch soils and lowest in *R. cathartica* mulch soils. These initial results suggest that the rapid germination of *R. cathartica* relative to the native *R. alnifolia* may be one of several mechanisms contributing to the success of *R. cathartica* in the Midwest. The lack of germination of *R. alnifolia* also suggests that perhaps an additional treatment is necessary for the seeds to break dormancy and germinate.

Our results indicate the addition of mulch to invaded soil reduces overall growth of the *R. cathartica* sapling and seedling germination both in the field and in the greenhouse. *Rhamnus cathartica* mulch may cause a reduction of soil N levels in soil greater than the commercial mulch. These factors may result in the reduction of the plants overall growth rate and ability to germinate. We therefore conclude and suggest further investigation of high C soil amendments to reduce the reinvasion of *R. cathartica* in restoration.

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Use of aerial surveys to map and treat *Ailanthus altissima* trees on large forested landscapes in Ohio

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Ailanthus altissima is a highly invasive non-native tree that is present in many forested landscapes in Ohio. Managers often observe an expansion in *Ailanthus* populations following forest disturbances such as harvesting and prescribed burning. A single female *Ailanthus* tree can produce 350,000 seeds, which are commonly wind-disseminated distances exceeding 100 to 200 meters. A cooperative research project was initiated to study the distribution and abundance of *Ailanthus* within Tar Hollow State Forest located in southeastern Ohio. We employed geo-referenced digital aerial sketch mapping technology in a low-flying helicopter to identify female trees (seed-producers) and patches (non-seeders) of *Ailanthus* in winter 2008, when persistent seeds were highly visible. This winter survey method was effective in detecting seed-producing *Ailanthus* across a landscape. During a two-hour flight, 98 seed-bearing females and 42 patches, ranging in size from 0.18 to 13.4 ha were identified within a 3885 ha (9600 acre) area. Seventy percent of the aerially-identified females were ground-truthed using hand-held GPS units; 4.3% were either misidentified or not located. In summer 2009, sampling of individual trees as well as a systematic grid was initiated to quantify *Ailanthus* abundance and demography in relation to management practices such as harvesting and prescribed fires and landscape/stand attributes, to better understand and model the key factors related to the presence, abundance, and spread of *Ailanthus*. Research plots were also installed to study the direct effects of prescribed fire and herbicide treatments on *Ailanthus* demography and spread. Herbicide-stem injections (hack-n-squirt with imazapyr) of these geo-referenced trees began in the autumn of 2009. Treatment effectiveness and subsequent woody plant regeneration will be monitored over time.

A study of *Elaeagans umbellata* dispersal based on the ages and relative locations of individuals in a stand

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This study examines the spread of the invasive species *Elaeagans umbellata* (Autumn Olive) based on the ages and relative locations of 76 individuals in a 2.95 hectare stand. All individuals of *E. umbellata* in the stand were mapped using GPS in a 20 meter grid with locations subsequently mapped using ESRI ArcGIS. The locations of younger individuals were compared to locations of older individuals to examine dispersal routes and test the null hypotheses that age was homogenous across the stand. The age and diameter of each individual was recorded to allow investigation of the age-to-diameter ratio. Analysis of the ages and diameters showed no consistent age: diameter ratio. Analysis of the relative ages of nearest neighbors indicated a non-random age-class structure ($\chi_{df=9} = 56.38$, $p < 0.0001$). Individuals aged 1-4 years most often had 5-9 year old neighbors. Individuals aged 5-9 most often had neighbors of the same age. Individuals aged 10-14 most often had neighbors aged 15+, and individuals aged 15+ most often had neighbors aged 9-14. A map presenting the ages and relative locations of individuals showed a cluster of older individuals which appear to be the founder plants during colonization of the stand.

This study examines the spread of invasive exotic species in natural ecosystems, which is an increasing problem in natural areas management (Andersen et al. 2004). *Elaeagnus umbellata* Thunb. (Elaeagnaceae, Autumn Olive) is a perennial exotic shrub introduced from Asia into North America in the 1830's as an ornamental and for erosion control on disturbed ground. Seeds of *E. umbellata* are spread by birds and other wildlife, which eat the berries and distribute them in their feces (LaFleur et al. 2007).

The questions addressed in this study investigate the colonization habits of *E. umbellata*. Is there a pattern of dispersion in *E. umbellata*? Is there a consistent age to size ratio in *E. umbellata*? It is hypothesized that the stand will have an uneven and spatially heterogeneous age structure: older individuals will be more likely to have older neighbors, and younger individuals will be more likely to have younger neighbors.

The study site is located within Crab Orchard National Wildlife refuge in Marion, IL. It is an abandoned agricultural field which was not managed and became overgrown by *E. umbellata*. This is an ideal site for invasive species invasion because the area was disturbed and abandoned; invasive species are known to colonize an area after a disturbance (Lonsdale 1999). Dispersal of *E. umbellata* to this particular site was likely facilitated by planting of *E. umbellata* by the forest service in other areas of Crab Orchard National Wildlife Refuge during the 1930's (USFWS 2009). The study area is densely colonized by *E. umbellata.* Areas of dense, older growth gradate into areas of high grass and dense Box elder saplings with little or no *E. umbellata.*

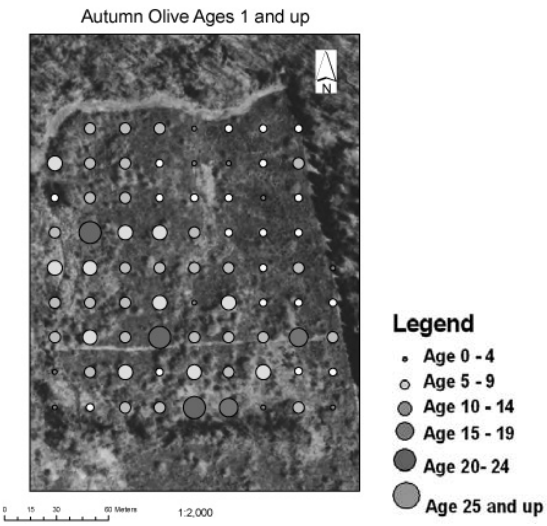
The study site is a 2.95 ha abandoned agricultural field. A grid of GPS points 20 m apart was laid over a map of the site on ESRI ArcGIS software, and the map with the points were loaded onto GPS device. The GPS device was used as a guide to each point, where an *E. umbellata* age sample was taken.

The age and diameter were both determined by sampling the base of the trunk, since *E. umbellata* often branches several times before reaching breast height. The samples of *E. umbellata* were cut at the base with large gear loppers to allow age determination. Larger individuals were sampled by taking a core of the trunk. The rings were revealed with sandpaper, counted, and the age was noted. The age of an individual was recorded, as well as the diameter of the trunk. The diameter of the trunk was determined using a logger's tape measure. If no individual is found at the exact GPS sample point, a

sample was taken from the closest individual to the sample point.

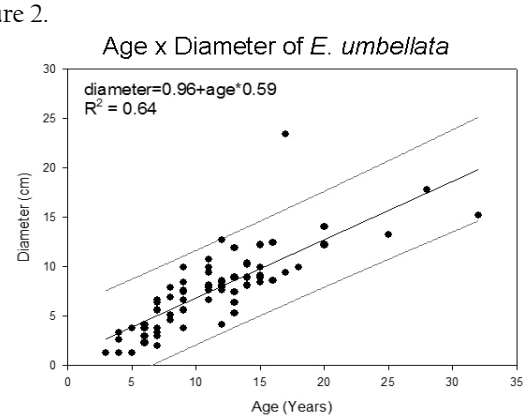
Analysis of the relative ages of nearest neighbors indicated a non-random age-class structure ($\chi_{df=9} = 56.38$, $p < 0.0001$). Individuals aged 1-4 years most often had 5-9 year old neighbors. Individuals aged 5-9 most often had neighbors of the same age. Individuals aged 10-14 most often had neighbors aged 15+, and individuals aged 15+ most often had neighbors aged 9-14. The three oldest individuals in the southeast region of the site appear to be founding individuals during colonization. (Figure 1).

Figure 1.



There is a relationship between age and diameter (Figure 2). Older individuals have a larger diameter than younger individuals. The age of an individual can therefore be estimated using diameter.

Figure 2.



The relationship between age and diameter allows diameter to function as a surrogate for age during data collection. There is more scatter among the diameters of older individuals. This may be due to environmental factors influencing growth. It could also be attributed to the small sample sizes of the older individuals. The older individuals have fewer individuals to average (only 1 or 2 for ages older than 15) so the number produced is more erratic.

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Competition and allelopathic effects of native and invasive populations of *Lonicera maackii*: a comparative analysis

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It is unknown if the novel weapons or evolution of increased competitive ability hypotheses explain the invasiveness of *Lonicera maackii* in eastern United States woodlands. We tested if the allelopathic properties of *L. maackii* have a significant impact on the fitness of native *Pilea pumila* in addition to below ground competition. In addition, we tested if *L. maackii* populations vary in allelopathic and/or competitive ability within the invasive range and between native and invasive ranges. To our knowledge, this is the first study to separate the effects of belowground competition and allelopathy of *L. maackii* on a native competitor as well as compare native and invasive populations of *L. maackii*. Addition of activated carbon to potting soil increased the ability of *L. maackii* to inhibit the fitness of *P. pumila* in addition to competition. *Lonicera maackii* from Ohio had a greater effect on its competitors and responded less to competition than *L. maackii* from a population in China. Results indicate that *L. maackii* can alter soil chemistry resulting in inhibition of its neighbors and *L. maackii* from Ohio is a better competitor both inter- and intra-specifically.

Microsatellite markers for an invasive aquatic plant, *Myriophyllum spicatum*

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Myriophyllum spicatum is an aquatic, invasive weed responsible for displacing native plant communities, obstructing water flow, altering water chemistry, and providing breeding grounds for mosquitoes. When removing this plant, it is necessary to confirm that the method of control is being applied to *Myriophyllum spicatum* and not a native, American watermilfoil, *Myriophyllum sibricum*. Currently, physical observations are the only way to distinguish between the two plants. This study attempts to develop a more accurate means of identification by using microsatellites as molecular genetic markers. Microsatellites from *Myriophyllum spicatum* were isolated and cloned into *E. coli*. A total of 46 positive colonies were sequenced. For sequences containing microsatellites, primers were designed for the flanking regions. Four sets of primers were developed and utilized in polymerase chain reactions using *Myriophyllum spicatum* DNA. The reactions showed an amplification of DNA when visualized on polyacrylamide gel. Reactions with *Myriophyllum sibiricum* DNA, however, showed no amplification. This suggests that the primers can be used to effectively differentiate between the two species and could be applied in tandem with physical observations in order to confirm the identity of a possible *Myriophyllum spicatum* growth before treatment.

Eurasian watermilfoil is an aquatic, invasive weed that was introduced to the United States sometime between 1880 and 1940 and has since spread rapidly throughout the continental states (Reed 1977, Couch & Nelson 1985). It grows into dense, tangled canopies that effectively displace the majority of native plant communities obstruct water flow, alter water chemistry, and provide breeding grounds for mosquitoes (Donaldson & Johnson 1998, Madsen 2005, Jacobs & Mangold 2009).

Eurasian watermilfoil bears a striking resemblance to American watermilfoil (*Myriophyllum sibiricum*), a native, benign species, such that it is exceedingly difficult to tell the two apart by physical characteristics alone (Eiswerth et al. 2000). Accurate identification, however, becomes a necessity when attempting to control a growth of the invasive plant with mechanical, chemical, or biological means. A case of mistaken identity could result in the destruction of a growth of the native watermilfoil species, clearing the environment for the spread of its invasive cousin.

Instead of relying on physical observations as a means of identification, this study utilizes molecular genetic markers, which are recognizable sequences within the genome of an organism that can be used to determine relatedness or variation. Specifically, this study utilizes simple sequence repeats (SSR), also known as microsatellites. Microsatellites are regions of DNA composed of several copies of tandemly repeated nucleotide sequences. While microsatellites themselves are extremely susceptible to mutation, the areas flanking microsatellites are greatly conserved within a species (Tautz 1989). The invariable nature of these regions makes the sequences they encompass prime candidates for amplification by a polymerase chain reaction (PCR). While there are other genetic markers that can be used for identification, most of them rely on length polymorphisms. Unfortunately, because different species can produce similarly sized DNA fragments, such methods can yield inconclusive results (Rongwen et al. 1995).

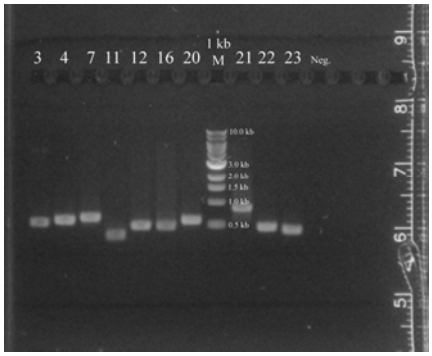
The purpose of this study is to identify microsatellites unique to Eurasian watermilfoil and develop primers that would be able to amplify these sequences in a PCR. If successful, such primers could aid in the control and management of this invasive species by providing a quick, cost-effective way to distinguish Eurasian watermilfoil from American watermilfoil.

In order to develop these primers, samples of Eurasian watermilfoil were collected from Cabinet Gorge, Noxan Rapid Reservoir, and Pond Oreille Lake and River in Montana. DNA was extracted using a DNeasy® Plant Mini Kit (Qiagen, Ca), and

digested with a combination of Pvu II and Xmn I restriction enzymes. Double stranded SuperSNX linkers (IDT) were then attached to the ends of the digested DNA. Enrichment of the DNA fragments containing microsatellites was accomplished using biotinylated oligos, BioAG13, BioAC13, BioATT10, and BioCAG9 (IDT), and magnetic Dynabeads. The probes, which were attached to the dynabeads, were designed to bind to complementary microsatellite sequences. Capturing the beads magnetically allowed for the isolation of DNA fragments with microsatellites from those without.

The enriched DNA was amplified in a polymerase chain reaction (PCR), using SNXF as a primer (IDT), and cloned using an InvitrogenTM TOPO TA Cloning® Kit (Invitrogen Corporation, CA). Cells from all positive colonies and one negative colony were added directly to PCR mixtures containing M13 forward and reverse primers. After the PCR, samples were run on a 0.4% agarose gel and visualized with ethidium bromide (Figure 1). Colonies that yielded bands of 500 bp or greater were chosen for sequencing. Forty-six samples were selected, purified using a QIAquick® PCR Purification Kit (Qiagen, CA) followed by sequencing at Geneway Research, CA.

Figure 1.



The sequenced DNA samples were visualized and scanned for microsatellites. Thirty-eight of the samples contained microsatellites. Of this number, four samples were chosen to have primers designed for the regions immediately flanking their tandemly repeated DNA. The resulting four sets of primers were used in PCRs with DNA samples from Eurasian watermilfoil and Northern American watermilfoil. PCR amplicons were run on 6% polyacrylamide gels and visualized with SYBR Green. PCR amplicons containing Eurasian watermilfoil DNA yielded bands corresponding to fragments 50 bp in length as expected, while the American watermilfoil failed to amplify any DNA. These results suggest that the primers can differentiate between the two species and could be applied in tandem with physical observations in order to confirm the identity of a possible *Myriophyllum spicatum* growth before treatment.

Future endeavors will involve the creation and testing of additional primers using the remaining thirty-four Eurasian watermilfoil sequences. The more primers that are available for testing, the more accurate this means of identification becomes.

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Negative impacts and allelopathic potential of the invasive ephemeral *Ranunculus ficaria*

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Ranunculus ficaria has been identified as invasive in the United States, despite a lack of published evidence of its negative impacts. We examined if *R. ficaria* negatively affected the growth and reproduction of the native *Impatiens capensis* and, if so, whether it is by allelopathy, nutrient competition or some combination thereof. We performed a fully-factorial field experiment, manipulating the presence of *R. ficaria*, nutrients, and allelopathy (with the use of activated carbon). The presence of *R. ficaria* tended to negatively affect survival of *I. capensis* ($P = 0.058$). Nutrients showed a significant effect on the number of seeds produced, stem diameter and height. The interaction between the factors of *R. ficaria* and carbon on seed production was significant. In the absence of carbon, *R. ficaria* significantly decreased seed production, illustrating the negative impact of *R. ficaria*. In the presence of carbon, there was no effect of *R. ficaria*, suggesting that carbon ameliorated the negative allelopathic effect of *R. ficaria*. This is the first study to demonstrate the negative impact of *R. ficaria* and the possible role of allelopathy in this species. Further, we show that the negative impacts of this species persist well beyond its early growing season. More research is needed to more fully understand the scope and mechanism of negative impacts of this emerging invasive species.

Ranunculus ficaria, or lesser celandine, is an invasive groundcover that appears to be affecting native plants in forested floodplains in many states (PCA 2009). *Ranunculus ficaria* was brought to the United States from Europe by the 19th century for horticultural purposes (Bailey 1935). *Ranunculus ficaria* is currently documented in at least 22 US states and 4 Canadian provinces (USDA 2009). It has been identified as invasive in at least 9 states (PCA 2005). Unpublished and preliminary data indicate that the presence of *R. ficaria* is associated with reduced abundance of native species (Homan 2005). However, there is no published research on the negative impacts of *R. ficaria*. Allelopathic compounds may be responsible for the success of a number of important invaders of North America (Hierro & Callaway 2003, Orr et al. 2005, Cipollini et al. 2008).

For our study, we examined if *R. ficaria* negatively affects the growth and reproduction of the native annual *Impatiens capensis* and, if so, whether it is by allelopathy or nutrient competition or some combination thereof. We expected that the presence of *R. ficaria* would have an overall negative impact, that nutrients would have an overall positive impact and that addition of carbon would have no overall effect on the performance of *I. capensis*. If allelopathy were important, we expected to see a significant carbon by *R. ficaria* interaction and if nutrients were important, we expected to see a nutrient by *R. ficaria* interaction.

In the field we performed a fully-factorial experiment with the main factors of: presence/absence of *R. ficaria*, presence/absence of activated carbon and additional/no additional nutrients, replicated 8 times (2 *R. ficaria* levels x 2 carbon levels x 2 nutrient levels x 8 replicates = 64 experimental units). The treatment combinations were haphazardly assigned to each plot and each plot was located approximately 25 cm apart.

In the *R. ficaria*-present treatments, we removed *R. ficaria* and replanted them with *I. capensis* seedlings. In *R. ficaria*-absent treatments, we removed the *R. ficaria* completely before transplanting *I. capensis*. In activated carbon-present treatments, we worked 10 ml of activated carbon into the top 8 cm of soil of each plot. In nutrient-added treatments, we added the manufacturer-recommended amount of 1.5 teaspoons of Scotts Osmocote slow-release fertilizer in the top 8 cm of soil in each plot. We disturbed the soil in each subplot regardless of treatment combination to control for soil disturbance effects. We also removed other surrounding vegetation within 25 cm^2 of the transplants and watered each plot thoroughly.

The number of fruits, number of seeds and survival (days to death) of the seedlings were recorded once each week. Height and stem diameter (measured directly beneath bottom node with a digital caliper) were measured. The *R. ficaria* had lost all of its foliage by June 2. The leaf litter decomposed by June 12, exposing the bulblets. Measurements began on May 5 and ended on August 21, with the final seed count taken on August 28.

Figure 1

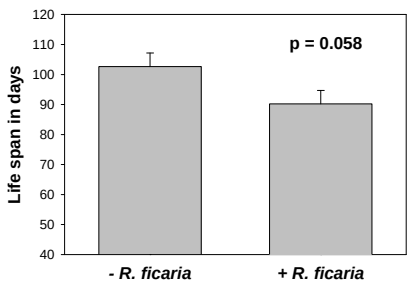


Figure 2

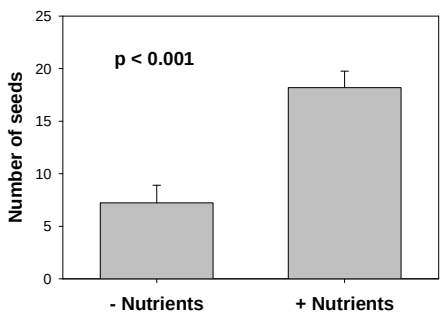
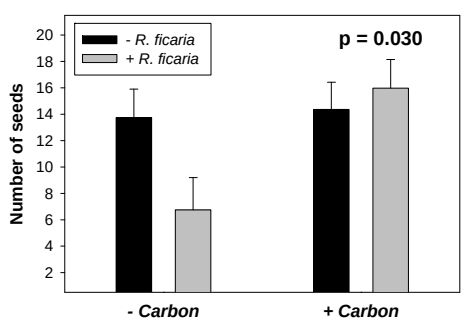


Figure 3



For survival, there was a near-significant effect of *R. ficaria* ($F_{1,55} = 3.75$, $p = 0.058$), with *I. capensis* tending to die sooner when *R. ficaria* was present (Figure 1). For total number of seeds produced, there was a significant effect of nutrients ($F_{1,47} = 20.53$, $p < 0.001$) and a significant interactive effect between *R. ficaria*-presence and carbon ($F_{1,47} = 5.03$, $p = 0.030$). The presence of nutrients nearly tripled seed production (Figure 2). When carbon was absent, *R. ficaria* reduced seed production. When carbon was present, seed production was similar whether *R. ficaria* was present or absent (Figure 3). Nutrients increased both height ($F_{1,23} = 7.28$, $p = 0.013$; 32.1 ± 1.8 cm without nutrients vs. 39.9 ± 2.1 cm with nutrients) and stem diameter ($F_{1,31} = 30.34$, $p < 0.001$; 0.482 ± 0.02 cm without nutrients and 0.689 ± 0.03 with nutrients).

Our results show that *R. ficaria* does indeed negatively impact *I. capensis*, providing confirmatory evidence of invasive status of this species. The presence of *R. ficaria* showed a tendency to have a negative overall impact on the survival of *I. capensis*, and in the absence of carbon, *R. ficaria* significantly decreased seed production. However, in the presence of carbon, there was no effect of *R. ficaria*, suggesting that carbon ameliorated the negative allelopathic effect of *R. ficaria*. Unpublished data indicate that presence of *R. ficaria* exhibits allelopathy (Cipollini, unpublished data) and is associated with reduced abundance of native species (Hohman 2005). As expected, nutrients showed a significant effect on the number of growth (in terms of height and stem diameter) and seed production. There was no significant interaction between nutrient level and presence of *R. ficaria*, suggesting that nutrient competition is not the primary mechanism of impact of *R. ficaria*.

Interestingly, we found that *R. ficaria* had lasting effects beyond its brief growing season. Even though *R. ficaria* had completely senesced by June 2, it still negatively impacted *I. capensis*, which lived without the presence of *R. ficaria* for about half of its life span.

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The recent expansion of hybrid cattails in a created Ohio wetland

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Spontaneous crosses between native, broad-leaved cattail (*Typha latifolia*) and introduced, narrow-leaved cattail (*Typha angustifolia*) have resulted in populations of hybrid cattail known as *Typha x glauca*. Although *T. angustifolia* is considered to be invasive, *T. x glauca* is considered more vigorous and invasive than either parent species. Hence, we hypothesize that *T. x glauca* could displace populations of *T. angustifolia* and *T. latifolia*. The objective of our research was to determine whether F_1 hybrids, advanced generation (AG), and backcross (BC) progeny have displaced parental species in a naturally colonized wetland at the Olentangy River Wetland Research Park (ORWRP) in Columbus, OH. We used species-specific SSR and RAPD markers to identify the two species and hybrid progeny. In 2000, only *T. angustifolia* and *T. latifolia* were identified in the wetland. Surveys conducted along permanent transects in 2008 and 2009 indicate that the wetland now includes F_1 hybrids, AG, and BC progeny. By 2009, 54% of the sampled shoots (n = 50) were of hybrid origin. Hybrids appear to be displacing their parental species while also invading uncolonized areas of the wetland.

Native, broad-leaved, cattail (*Typha latifolia* L.) and narrow-leaved cattail (*Typha angustifolia* L.), a putative introduction from Europe, spontaneously hybridize to form *Typha x glauca* Godron (Smith 1967). Although *T. angustifolia* is considered invasive, *T. x glauca* is reportedly even more invasive (e.g., Galatowitsch 1999). Hybridization of *T. angustifolia* and *T. latifolia* has been implicated in invasions of cattails in the Western Great Lakes Region (Travis et al. 2010). The increased vigor of *T. x glauca* compared to its parents could lead to the displacement of native species, including *T. latifolia*, in wetlands. The objective of our research is to document changes in frequencies of *T. latifolia*, *T. angustifolia*, and *T. x glauca* F_1 hybrids and advanced-generation/backcross progeny (collectively referred to as *T. x glauca*) in a naturally colonized wetland at ORWRP (see Wetland 2 in Mitsch et al. 1998; this 1-hectare wetland was created in 1994).

Morphologically, *T. x glauca* is intermediate between its two parents, and most characteristics that distinguish the parental species overlap. This makes positive identification of hybrids and their progeny difficult, especially when plants are immature. Historically, *T. x glauca* has been considered primarily sterile (Smith 1967). However, AG or BC progeny of *T. x glauca* may resemble either parental species and, until recently, those progeny likely remained unidentified. Kuehn et al. (1999) developed species-specific RAPD markers for identifying *T. angustifolia*, *T. latifolia*, and their progeny. We recently verified a subset of microsatellite (SSR) markers developed by Tsyusko-Omeltchenko et al. (2003) as species-specific for cattails in the Great Lakes Region (Snow et al. 2009). These molecular markers allowed us to accurately identify cattail species and hybrids in the ORWRP wetland.

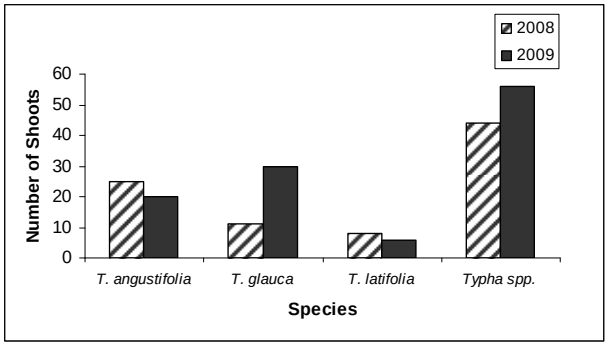
When Selbo and Snow (2004) used morphological characteristics and RAPD markers to identify cattails in a 2000 collection at the wetland, they found no evidence of hybridization between the two species. At that time, *T. angustifolia* dominated 88% of the area colonized by cattails, while *T. latifolia* occurred on 12%. In 2006, using RAPD markers, we discovered an F_1 hybrid in a small sample of plants from the same wetland. Many more hybrids and hybrid progeny were identified in 2007.

To document the expansion of hybrids in 2008 and 2009, we collected leaf tissue at two meter intervals along permanent transects (boardwalks) and at junctions of transects and edges of cattail stands in the wetland. Selection of cattail ramets was random except that reproductive shoots were preferred. After extracting DNA from leaves, we used three RAPD and six SSR primers to amplify the DNA. RAPD amplification products were separated on agarose gels, visualized under uv light, and documented using Kodak® 1D 3.6 gel imaging system. SSR amplification products were analyzed using ABI Prism® 3100 genetic analyzer and GeneMapper 3.7 software.

Based on the results of these molecular markers, 57% of the samples collected in 2008 were identified as *T. angustifolia* and 25% were *T. x glauca* (Figure 1). Preliminary results based on a subset of markers for

2009 reveal that 54% of the samples were of hybrid origin, representing a large increase over a single year.

Figure 1. Frequencies of *Typha* taxa sampled along permanent transects in a created wetland at the ORWRP, Columbus, OH (n = 44 in 2008 and n = 56 in 2009).



The area along the permanent transects colonized by cattails increased from 2008 to 2009 (Figure 1, *Typha* spp.). Our results to date suggest that *T. x glauca* was the most prevalent taxon in newly colonized areas in 2009. Overall, the prevalence of the two parental species decreased by about 20% from 2008-2009. Although it is tempting to speculate that these changes were due to the more invasive nature of *T. x glauca*, these results reflect the changes of a single year in a dynamic system. The predominant *Typha* species may fluctuate with changing biotic and abiotic conditions. The water depth at this wetland was intentionally subjected to major pulses during the growing season of 2004 and then held steady throughout 2005 (Mahlik & Mitsch 2008). In Wisconsin, Boers and Zedler (2008) reported that stable water levels appear to favor the growth of *T. x glauca* more than fluctuating levels.

The motivation for this research was our discovery of *T. x glauca* at the ORWRP for the first time in 2006. Selbo and Snow (2004) documented limited overlap in flowering between the two species in 2000, suggesting that hybridization between the species was possible during a short time period. Although the overlap was brief, flowering times could vary from site to site and year to year. In addition, conditions favoring hybrid seedling establishment and growth could allow colonization by *T. x glauca* in some years, but not others. Comparisons of the cattail stands in 2008 and 2009 with 2000, when no hybrids were identified, suggest that once conditions favor hybridization and establishment, *T. x glauca* may quickly displace its parents and invade uncolonized areas.

We identified numerous advanced generation and backcross progeny of *T. x glauca* in the wetlands. Although this is contrary to evidence reported by Smith (1967) and Kuehn et al. (1999), it agrees with recent studies of cattail species at three national parks in the Western Great Lakes Region (Travis et al. 2010). The existence of advanced generation and backcross progeny suggests that *T. x glauca* is fertile and that its progeny should be considered when evaluating the invasiveness of the *Typha* spp. complex.

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