2007 Ohio Invasive Plants Research Conference

Continuing Partnerships for Invasive Plant Management

Proceedings

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Preface

The Ohio Invasive Plants Council (OIPC) is a coalition of organizations and individuals throughout Ohio who have a mutual interest in Ohio's natural ecosystems and the effects of invasive plants and other organisms on their biological diversity. The group has had a 501c(3) nonprofit status since 2005. As their primary mission, the OIPC participates in statewide efforts to address the threats of invasive species to Ohio's ecosystems and economy by providing leadership and promoting stewardship, education, research, and information exchange.

The OIPC has several active working groups including Research, Education, Communication & Public Outreach, Restoration, Control, and Mapping. The Research Working Group hosted the first Ohio Invasive Plant Research Conference in 2003. The proceedings are available to download at the OIPC website: http://www.oipc.info/ohioresources.html . The 2007 Ohio Invasive Plants Research Conference took place on January 18th at Ohio Wesleyan University in Delaware, Ohio under the theme, *Continuing Partnerships for Invasive Plant Management*. Over 250 people were in attendance. The conference included speakers addressing many topics relating to invasive plant species as well as case study speakers. The keynote speaker was Valerie Vartanian from the Nature Conservancy and The Missouri Botanical Garden. Twenty one posters covering a wide range of topics were also presented as part of the poster session. These proceedings include short papers from some of the speakers and poster presenters or summaries/abstracts of their presentation. These proceedings have been made possible from a grant given by the **US Forest Service Northeastern Area State and Private Forestry**. The highly successful conference was made possible through the efforts and financial contribution of many people and organizations. The Ohio Invasive Plant Council would like to acknowledge the following contributors:

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Integrated vegetation management for invasive plant control and native grass establishment; applications for natural resource managers.

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Abstract. The Department of Forestry and Natural Resources at Purdue University has an ongoing research project that seeks to develop 1) control options for a variety of invasive plants and 2) management techniques for native grassland restoration. The primary objective of the project is the research and development of integrated vegetation management options for controlling invasive species, establishing desirable vegetation, and the restoration of native landscapes. Using an integrated vegetation management approach for invasive species ensures that control methods are adaptable to multiple habitats and management objectives. The primary control methods focus on the combination of multiple vegetation management techniques, application methods, and herbicide combinations. Methods are evaluated either individually or in combination and include mechanical cutting, herbicide use, controlled burning, and natural re-vegetation. Invasive plants of interest include Amur honeysuckle, Amur maple, autumn olive, garlic mustard, and multiflora rose. Herbicide treatment scenarios include basal stem, cut stump, dormant stem, and foliar. Herbicides of major focus include glyphosate, imazapic, imazapyr, fosamine, metsulfuron, and triclopyr. Mechanical treatments include traditional flail mowing and the Brown Brush Monitor in concert with herbicides and stage of regrowth. Establishing native warm season grasses is the major focus of re-vegetation efforts and focuses on a variety of seed mixes and cultural techniques. This is often aided by the use of control burning as a reclamation, maintenance, and establishment tool when it is feasible.

Introduction

Understanding Invasive Ecology

The terms invasive species and exotic species are defined differently within many conservation and environmental circles. For this summary, we offer the following definitions. An *invasive plant* is any plant that is unwanted and grows or spreads aggressively. Typically invasive organisms are also categorized as species that do harm because they create either economic or environmental damage. An *exotic species* is an organism that occurs outside of the eco-geographical area where it evolved.

To understand invasive plant control, we must evaluate the problem plant's biology and ecology. Once the life processes of an invasive plant are understood, we can determine when that plant will be most susceptible to a specific control measure. For example, many perennial thistle species are best controlled with herbicide in the fall of the year. After the thistle produces seed, the plant will focus on replenishing energy to the roots until it goes

dormant in the fall. As a result, herbicide applied after seed production is readily translocated to the roots as the plant stores resources, effectively providing superior control.

Invasive exotic plants are often able to usurp resources such as light, water, nutrients from native plants, thus, prospering at their expense. When Invasive plants become established within a natural system they cause a loss of biodiversity or ecosystem function.

Once an invasive plant is established, it is difficult to completely eliminate it. Control efforts typically focus on protecting high-value natural areas and preventing the additional spread of the invasive species. Control efforts are the most efficient in the early stages of invasive plant population establishment.

Successful invasive plant control stems from the use of an Integrated Vegetation Management (IVM) approach. IVM is the process of combining multiple control techniques to produce a desired vegetative condition. A hypothetical example of IVM for removing Canada thistle from a prairie is as follows:

1) Patches of thistle within the prairie are delineated and mapped.
2) The prairie is burned in the early spring / late winter.
3) Depending on emergence, 1 to 2 weeks after burning the thistles are treated with a broadcast foliar application of a broadleaf selective residual herbicide.
4) In late summer, the areas are checked again for any remaining thistle and spot treated accordingly with the same broadleaf selective herbicide.
5) The following spring the area is burned again, checked for thistle seedlings, and spot treated as needed.
6) The treated areas can then be seeded with a native grass and forb seed mix to prevent any further establishment of invasive vegetation.

Purdue University Research Case Studies

We conducted a number of herbicide and planting trials that varied in IVM technique, seasonality, and site condition. This study involves a large representation of forestry and right-of-way labeled herbicides and application methods, with particular emphasis on application methods that limit non-target damage. An abbreviated description of selected herbicide trials and native grass establishment research follows. Regional adjustments to herbicide rates and application methods may be needed if you want to duplicate these methods in your region of the United States.

I. Invasive Woody Plant Control

Application method: Basal and cut-surface

Description: Basal application involved application of herbicide to the bottom 18 inches of the woody stem to the point of wetting only. Cut-surface application required mechanical removal of the woody stem and then application of a ring of herbicide to the cambium layer of the remaining stump. CAUTION!: Do not spray to the point of run off or pooling at the base of the stem with either method. Application was made in the dormant (winter/spring) season but will work year round as a technique for invasive plant control. Rate: 15% Garlon 4A + 3% Stalker + 82% Ax-it basal oil. Mix by volume. Species tested: Amur honeysuckle, autumn olive, black cherry, and multiflora rose. Results: 100 % mortality was achieved with both methods on all species.

Application method: Dormant stem low-volume spot treatment

Description: The spray solution was applied to individual stems. This application requires that the majority of the bark surface area is covered to the point of wetting, not run off. This includes the main stem, branches, and twigs. A narrow cone nozzle operated at 40 PSI provided an adequate spray pattern. Application was made in the dormant (winter/

spring) season to reduce the risk to non target species.

Rate: 6 qt. Garlon 4 + 16 oz. Stalker + 4 gal. Ax-it basal oil + 1 qt. 90% nonionic surfactant. Water was added to these herbicide amounts to equal 100 gallons of spray solution. CAUTION!: Keep solution agitated when spraying. Do not spray stems over vegetation that is not dormant or non-target plant injury can occur. Species tested: Amur honeysuckle, autumn olive, and multiflora rose. Results: 100 % mortality was achieved with proper coverage.

Application method: Broadcast low-volume foliar application

Description: The spray solution was broadcast over unwanted woody vegetation and also used to spot-treat individual plants. Application was made at the end of the growing season (fall) prior to heavy frost or the senescence of leaves on target plants. It was applied as a foliar broadcast at a rate of 45 gal/acre using 2 Boom Buster nozzles at 35 PSI.

Rate:

1) 8 oz/A Arsenal + 2 gal/A Accord + Nonionic surfactant
2) 12 oz/A Arsenal + 2 gal/A Accord + Nonionic surfactant
3) 12 oz/A Arsenal +1.5 gal/A Krenite + 1.5 oz/A Escort + Nonionic surfactant

CAUTION!: This is a nonselective herbicide mix that has some residual control. It effectively controls many grasses, broadleaves, and woody plants. Species tested: Amur honeysuckle, autumn olive, multiflora rose, viburnum, grapevine, sassafras, and black cherry.

Results: Treatments 1-3 all resulted in excellent control (95-100%) of woody stems that had been cut the previous winter and allowed to re-grow for 1 growing season. Previous field trials suggest that the results will not change in the second growing season. However, these results only represent data from 1 year after treatment. Caution must be used when applying this information to a specific spray program. These areas were planted into native grasses the next growing season and showed good grass establishment 4 months after planting (see savannah restoration).

II. Non-woody Herbaceous Plant Control

Application method: Dormant season broadcast foliar for garlic mustard control.

Description: A broadcast application was made in the late winter an estimated 6 weeks before bud break. The application was made to the forest floor and basal leaves of the garlic mustard seedlings. Application was made with a backpack sprayer at a rate of 15 gallons per acre. Rate: 3 rates of Plateau were used. 2, 4, and 6 oz./A. + nonionic surfactant. Species tested: Garlic mustard. Results: 90 - 100 % mortality was achieved with all herbicide rates. CAUTION!: The impact to native species for this treatment has not been adequately researched at this time. This method is effective but should not be used in areas that have a high risk of non-target damage. (Journey herbicide could be substituted for Plateau at the appropriate conversion rate.)

III. Native grass establishment

Thirty-six species of native grasses and forbs were used to create 12 different seed mixes. The seed mixes were used in establishment trials and planted on prior row crop agricultural ground. Truax Company Flex II native grass drill was used to plant the establishment trials. Each seed mix establishment trial included a control, low, medium, and high rate of Plateau/Journey (imazapic) herbicide. Plateau / Journey rates were tested on each seed mix as follows: control (no imazapic), low (2 / 5.2 oz/A), medium (4 / 10.4 oz/A), and high (6 / 15.6 oz/A).

Regardless of seed mix, NWSG establishment trials consistently showed that the use of Plateau herbicide significantly increases the success of plantings. Notably, NWSG stands were often established in the first year allowing many of the native species to produce flowers in their first growing season.

Increases in the per acre imazapic herbicide rate resulted in improved residual weed control and promoted the establishment of NWSG. However, many forbs did not survive the high herbicide rate. Low to medium rates of imazapic are recommended, on prior agricultural ground, if forbs are included in the native grass seed mix. Higher rates are appropriate for sites with high weed pressure, fallow ground, or seed mixes with only grasses.

IV. Savannah restoration: combining invasive plant control and NWSG establishment.

We attempted to restore a savannah ecosystem (grass and forb dominated understory with scatter trees) by combining our invasive plant control and NWSG establishment research and applying these techniques to a savannah test area that was dominated by invasive woody plants. Research efforts for the savannah concurrently looked at invasive plant control and techniques for native warm season grass (NWSG) establishment. Our objective was to efficiently remove the invasive woody plants, preserve the oak overstory, and establish native grasses within 2 growing seasons (18 months). We were successful in meeting our objectives. The function of the established savannah and long-term species diversity of the resulting plant community is currently unknown. However, we have successfully established the foundation and structure needed for a functional savannah ecosystem.

The steps used to create this foundation are:

1) A mechanical flail mower was used during the winter months to reduce the majority of the woody invasive understory. (For this project we used a Hydro-Ax mower). The cut woody stems were mowed and mulched by the machine leaving 3-6 inch high stumps and a significant layer of woody debris. The undesirable (non-oak) overstory trees that remained after mowing were cut and removed from the site.
2) The cut stems prolifically re-sprouted the following spring. In October, the area was treated with a nonselective low volume foliar herbicide application. This application (see above) was applied in the fall prior to heavy frost or the senescence of leaves on target plants.
3) The treated area over-wintered and was prepared for planting the next spring by spraying an herbicide solution of 2 qt. of glyphosate and 10.4 oz. of Journey. Spraying was done after bud break and the first flush of pioneering annuals to help control competing vegetation.
4) Planting was done using a Truax Rough Rider Range Land Drill. It is a native grass drill specifically designed to plant into rugged uneven site conditions with large debris. It is capable of precisely planting native seeds into and over debris, 18 inches and smaller, without stopping.

The seed mix included the following species (all rates are in ounces of pure live seed per acre): big bluestem (*Andropogon gerardii*) - 48oz.; Eastern gama grass (*Tripsacum dactyloides*) - 56oz.; Indian grass (*Sorghastrum nutans*) - 24oz.; little bluestem (*Schizachyrium scoparius*) - 16oz.; side-oats grama (*Bouteloua curtipendula)* - 48oz.), black-eyed susan (*Rudbeckia hirta*) - 2oz.; false sunflower (*Heliopsis helianthoides*) - 2oz.; partridge pea (*Chamaecrista nictitans*) - 8oz.; prairie coreopsis (*Coreopsis palmata*) - 2oz.; purple coneflower (*Echinacea purpurea*) - 4oz.; and, showy tick-trefoil (*Desmodium canadense*) - 4oz. The final seeding rate, of 13.4 lbs. PLS / A , was used to plant the site. The severity of the site required the heavy seeding rate since

some of the seed was inevitably planted on top of the debris.

By using the above technique we were able to establish successfully native grasses into the formerly invasive understory without losing the oak overstory. Grasses established well the first year with little competition from weeds and invasive plants. It will take several more growing seasons to fully assess the results of the planting and herbicide efforts. Based on our prior research and field trials we are confident that this method will continue to produce favorable results.

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Are roadsides a red carpet for invasive species?

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Abstract. Non-native species are common along roadsides, and it is widely assumed that roadsides function as conduits for invasion, facilitating dispersal of plant species along their length. We test this idea by examining spatial distribution of three common nonnative species occurring in forest roadsides of southeast Ohio: *Rosa multiflora*, *Tussilago farfara*, and *Microstegium vimineum*. Our results suggest that roadside is superior habitat for all three species. *Rosa* and *Tussilago* appear to move along roads slowly at rates determined by their natural dispersal mechanisms: vegetative propagation and seed dispersal, respectively. Both species appear to be limited to roadside primarily by their intolerance of shade. There is no evidence of facilitated dispersal. By contrast to *Rosa* and *Tussilago*, *Microstegium* spreads to new sites very rapidly despite having no obvious dispersal mechanism. Qualitative observations suggest it is carried along the roadside as dormant seeds in gravel moved by a road grader – a form of road-dependent facilitated dispersal. *Microstegium* can also leave the road and enter adjacent forest as gravel is carried off the road by runoff water. We recommend that road construction be avoided near natural areas, and that construction and maintenance of necessary roads be conducted in such a way as to minimize disturbance which offers habitat to non-native species.

Introduction

Deciduous forests of southeastern Ohio are famous for their lush and diverse displays of native wildflowers. Of course there are also non-native plant species in the region, but non-natives have been of only minor concern to forest managers because they are largely restricted to disturbed habitats and open-canopy sites. A long-established forest generally has few such species, and those present rarely attain great size or density. Invasion has become more common however, as a number of shade-tolerant invaders have appeared. Most worrisome are garlic mustard (*Alliaria petiolata*) and Nepalese stilt grass (*Microstegium vimineum*) which can establish populations in long-established deciduous forest. Both species potentially compete with our native flora, and pose a threat to biodiversity of the region.

How can we manage shade-tolerant invasive species to avoid colonization of forests? As the presentations in this meeting show, eradication of established non-native populations is generally time consuming, labor intensive, expensive, and often unsuccessful. In most cases, it is probably more efficient to prevent an invasion in the first place. From this perspective, we must ask how non-native species get into long-established forest areas. A commonly heard explanation is that invasions proceed along roadsides, and move off the road into adjacent forested areas. On the surface this idea is reasonable. Non-native species are certainly abundant along roadsides ("wayside species" is a common descriptor for weedy plants). Roadsides are homogeneous strips of habitat extending over long distances, which could easily support incremental advance of a plant population. Anecdotal and qualitative accounts

of range extension along a roadside are common in the published literature.

Progress along a roadside is not necessarily the mode of invasion, however. It is possible that roadsides merely serve as congenial habitat, and that propagules disperse between roadsides independently of their linear structure (Christen and Matlack 2006). This situation would occur if dispersal range of a species greatly exceeded the interval between roads. It is also possible that non-native species are pervasive and that roadside occurrence is a mistaken impression based on the position of the observer on the road. The assumptions of road habitat and road corridor function must be tested.

To help understand the interaction of roadsides and nonnative species it is helpful to envision three models of road function (Christen and Matlack 2006). In the first model, dispersal may be independent of road presence. Alternatively, if dispersal could not reach other roadsides, a species might leap-frog along a single roadside, extending daughter populations outward from the original mother population (model 2). Extension along the road would be channeled by unfavorable habitat on either side. In the third model, dispersal may actually be facilitated along the road axis by some assisting agent such as wind, animals or cars.

To test the role of roads in facilitating invasions, my students and I have described distributions of three common non-native species in our region. We ask three questions. First, do roadsides really promote the growth of these species, or is this merely a mistaken impression? Second, how often do propagules arrive on roadsides? Third, do populations preferentially expand along the road axis, and if so, how?

Methods

We examined distributions of three non-native species chosen for varied life histories at sites in the Wayne National Forest and Vinton Furnace Experimental Forest. Multiflora rose (*Rosa multiflora*) is a large perennial shrub which spreads by clonal propagation and by dispersal of fleshy fruits by birds. The species has been in southern Ohio since at least the 1930's. Nepalese stilt grass (*Microstegium vimineum*) is an annual grass with no apparent seed dispersal, and very limited ability to propagate vegetatively. Seeds do not have any obvious morphological features such as hooks, fleshy tissues, or wind-catching spines which would suggest a dispersal mechanism. Seed is, however, capable of remaining dormant in the soil for 2-4 years. Stilt grass has only been in the region since around 1995. The third species, coltsfoot (*Tussilago farfara*), is a low-growing perennial with very limited vegetative propagation. Like other members of the Compositae, it disperses seed by wind. All three species occur along roadsides in southeast Ohio; the first two form dense populations in some woodland areas away from roads.

We surveyed 100m transects along woodland roadsides, noting the number of stems of each species in 1m x 1m plots. Similar transects were surveyed in forest > 50m away from the road. Roadsides were examined on ridge tops and in valley bottoms.

To test habitat quality, seeds of *Microstegium* were experimentally sown into plots next to roads and away from roads, under open and closed crown canopies in each location.

Plants of *Tussilago* were counted in 50cm x 50cm plots along 10m transects perpendicular and parallel to roads. The distribution parallel to a road would show the effects of roads in promoting dispersal, if such was indeed occurring, whereas the distribution perpendicular to the road which reflect dispersal from the same source, but without the road influence. By comparing perpendicular and parallel distributions we hoped to infer the effect of road proximity.

To examine dispersal in *Microstegium*, plots near (5m from) large reproducing populations were examined for seedlings. To test for limitation of seedling recruitment by availability of seeds, seeds were experimentally sown into adjacent plots. To test for limitation of recruitment by habitat quality, we removed leaf litter from another plot (leaf litter has been shown to control *Microstegium* germination)

Results

Roadside as habitat

All three species had road-oriented distributions, consistent with high habitat quality in roadsides. *Tussilago* was never encountered anywhere except roadsides. *Rosa* occurred much less frequently in undisturbed forest than along roadsides, and strongly preferred valley road transects to ridge road transects, indicating a road x landscape position interaction. Bias towards valleys probably reflects a need for soil moisture, indicating that this non-native species' distribution is responding to several environmental gradients at once.

Microstegium also clearly prefers roadsides. Experimentally sown *Microstegium* seeds germinated significantly more often in roadside plots than in plots away from roads, and seedlings grew more vigorously beside roads. There appeared to be an interaction with canopy openness: germination and growth were always greater under an open canopy regardless of road proximity.

Population extension

The distribution of stems along the roadside offers clues as to the mechanism of invasion. *Rosa*, for example, colonizes roadsides by vegetative propagation from stout rhizomes – we have rarely observed seedlings. *Rosa* shoots are significantly clustered (positive spatial autocorrelation) on roadsides at scales of 0 – 3m. This distance corresponds to the length of rhizome extension. It appears that *Rosa* is slowly expanding its population along roadsides by extension of rhizomes, but is unable to enter adjacent forest due to the unsuitability of forest habitat.

Tussilago is clustered in patches 10-15m long. Unlike *Rosa*, it has only very limited capacity for vegetative spread. We conclude that *Tussilago* is extending its population by wind dispersal of its plumed seeds. Complete absence away from the road suggests that its extension is channeled by suitability of roadside habitat. Comparison of stem distribution parallel and perpendicular to roads shows no difference. *Tussilago* does not seem to be expanding more rapidly along the road axis, so there is no evidence of facilitation of movement along the road.

Microstegium completely saturates the roadsides in which it occurs. Evidently it is able to move easily within sites although the mechanism is not evident. In our dispersal experiment, seedlings were absent from the undisturbed forest near the reproducing population indicating that the species is not spreading in sites away from roadsides. A few seedlings appeared when litter was removed, suggesting limitation of invasion by habitat quality. Many more seedlings were apparent in the experimentally sown plots, indicating dispersal limitation. Evidently *Microstegium* does not disperse far in populations away from roads, suggesting that that dispersal is controlled by a process peculiar to roadsides.

We noticed that *Microstegium* distributions in roadsides often closely correspond with mounds of gravel created by road maintenance. Distributions extend from roadsides down water diversion channels, and from the water diversions into natural stream beds. It appears that *Microstegium* is being moved into intact forest by movement of runoff water.

Long range colonization

Arrival in a roadside site may occur by a mechanism distinct from movement within sites. It is difficult to observe rare colonization events, but colonization frequency can be estimated from the frequency of distinct patches in that same way that frequency of bacteria is estimated by counting the number of colonies on a petri dish. *Rosa* typically shows 4 – 6 distinct patches in a 100m stretch of roadside. Knowing that *Rosa* spreads very slowly within sites and has been in our region for ca. 70 years, we conclude that *Rosa* arrives in roadside sites at a rate of ca. 1 established plant per 100m every fourteen years.

Microstegium also shows distinct clusters in roadside populations, and much larger numbers of individual plants. In our road-parallel transects, however, abundance of *Microstegium* was significantly correlated with canopy openness. Clusters of stems indicated good places to grow rather than colonization events. It appears that the roadsides we examined are so completely saturated with *Microstegium* that stem density has become an indicator of local environmental quality. *Microstegium* is absent from most forested roadsides in southeast Ohio, indicating a degree of dispersal limitation at a coarser scale. Although we are unable to estimate the frequency of colonization events, it seems safe to say that colonization from sources within sites is much more common that colonization from remote sources.

Discussion

The three species we considered all have distributions concentrated in roadsides. The impression of roadside occupation is true, and not merely an artifact of human observation. Both *Rosa* and *Multiflora* appear to exhibit stronger recruitment in roadsides, although the reason is unclear. It is evident, however, that roadside position interacts with other environmental gradients (e.g. landscape position, canopy openness) influencing distribution of the species. *Tussilago* is even more narrowly restricted in its distribution, never appearing away from roadsides. These observations are consistent with many recent reports of non-native species distributions biased to roadsides (Tikka et al. 2001; Gelbard and Belnap 2003, Pauchard and Alaback 2004, Lu and Ma 2006, Flory and Clay 2006) although few have asked what process causes this distribution. We suggest that bias to roadsides is caused by environmental gradients related to the road construction and maintenance.

All three species appeared to be moving progressively along the road axis. Spread away from the road appeared to be much more limited in each case. In *Rosa* and *Tussilago* spread appeared to progress slowly, at a rate determined by the growth form and dispersal mode of each species. Our results offer no evidence of preferential movement along the road in these species. Movement along the road corridor is a default condition caused by poor habitat quality away from the road. *Rosa* does not appear to have completely colonized the stretches of road we examined in several decades despite presence in the sites. Although *Rosa* is a serious concern for land managers, we conclude that movement along road corridors is not a threat. *Tussilago* is also not a threat to nearby communities despite movement along the road axis, owing to its extreme limitation to roadside habitats.

Microstegium progresses along the road axis much more rapidly. The close correspondence of its distribution with microtopography created by road maintenance suggests passive movement as gravel is shifted by road graders. We speculate that *Microstegium* is able to move by virtue of its dormancy in the soil. Dispersal by road maintenance is ironic. Although *Microstegium* has no obvious

dispersal mechanism, it spreads through local sites much more rapidly than the wind-dispersed *Tussilago* or the animal-dispersed *Rosa*. Further evidence for movement of dormant seed is provided by the appearance of *Microstegium* in water-moved sediment in the water diversion channel.

Finally, invasion appears to be a hierarchical process in at least two of these species, involving multiple dispersal mechanisms. Both *Rosa* and *Microstegium* appear to arrive in sites much less frequently than they propagate within sites, leading to distinctly patchy distributions. *Microstegium* invasion includes another step – movement by water – which has the potential to spread more rapidly than roadside colonization once seeds reach a natural stream.

These findings probably do not apply to all non-native species, nor to all sites in which non-native species occur, but they give an indication of the variety of roadside responses which are possible and the hierarchical complexity of the invasion process. Clearly the degree of threat offered by a species is determined by the interaction of its life history with the circumstances of roadside microhabitat. *Tussilago*, for example, seems to be little threat to adjacent forest despite its ability to reach remote roadside sites because it is intolerant of forest habitat. *Microstegium* is a greater threat because it can tolerate a degree of shade, and its seed dormancy allows it to be moved by humans. Ironically the greatest agent of invasion appears to be us – land managers seeking to protect the forest through conscientious road maintenance.

Management recommendations

These observations suggest several steps to prevent invasion along roadsides. Roadsides are a totally artificial habitat created by human land management. If humans are the agents of invasion, we can also alter our management practices to limit such invasion. We suggest the following management practices.

1. Education. All road workers should be able to recognize the prominent invasive species in their work area, and understand the negative consequences of moving gravel and soil.

2. Soil/gravel movement. All soil or gravel should be inspected before transport for invasive species. Infested piles should be treated with herbicide before transport or not used.

3. Planning. Roads should not be constructed near sensitive natural areas, as such roads are sure to provide habitat for invasive species which could enter the surviving habitat from the roadside.

4. Construction. If road construction is unavoidable, the project should minimize disturbance to the area next to the road surface. Road margins should be as narrow as possible to minimize the amount of invasion-vulnerable habitat.

5. Canopy. In a forest, preserve the crown canopy above the road. Because most invasive plant species are to some degree shade intolerant, a degree of shade will discourage colonization.

6. Water diversion channels. Place sediment traps in road drainage channels, and clean them out regularly. Avoid constructing water diversion channels leading directly into natural streams.

7. Maintenance in short sections. Apply roadside maintenance in short sections. For example, raise the blade of the road grader every 100m. Brush loose material off the sickle bar of the mower every 100m. Complete one short section before moving to the next.

8. Break the homogeneous habitat. Roadsides are easily followed by non-native species because they are homogeneous over long distances. Roadside movement can be slowed by introducing different management regimes at short intervals, and thereby interrupting the continuity of habitat. For example, intersperse mown roadside sections with unmown sections.

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Invasive species: Focusing on the pathway and not the person (and other strategies for successful invasive species control)

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The Nature Conservancy

Summary of Presentation

The Nature Conservancy's Global Invasive Species Initiative (GISI) was established to provide a better understanding of the incipient, overwhelming threat to biodiversity from introduced organisms that cause harm. We work with our operating units, partners, and other land managing agencies and organizations to share information about invasive species. TNC has managed natural areas for many years and can show the benefits and costs to invasive species control. Many of our sites have successfully controlled local invaders, from bullfrogs to knapweed. But that isn't the only approach we take in trying to get ahead of this biological pollution. The GISI has been very successful in working with the public and private sectors in strategies to avoid, detect, and control invasives.

So what has worked so far? In many regions, invasive plant councils are being established that include a good cross-section of local stakeholders in this issue. In many cases, they become a safe support group in which to hold frank discussions on the impacts to the environment and to the local economy. Most importantly, due to the diversity of stakeholders and the geographic range in which they represent, their collective experience and knowledge help to craft meaningful, effective detection and control strategies.

There have been some efforts that have not worked as well in either their ability to effectively control the target species, or create a long lasting commitment to a program. In some cases strategies have been attempted in too small a geographic area without the support from neighboring regions with the same problems. Though the work needs to be done at the local level, sharing success and failure stories at a larger scale have been greatly helpful.

Where do we go from here? Attempting to regulate each individual invasive species may be cumbersome and ultimately not effective. We could accomplish more if we focus on the pathways in which invasives arrive and develop plans to address those introductions as quickly as possible. Importing and exporting crops, ornamental plants, and pets have are directly responsible for the introduction of potentially invasive species. Other forms of trade can indirectly bring unwanted invasives through ballast water or wood shipping materials.

For example, once an organism has made its way here, it can spread if there isn't a sound detection and response system in place. In many regions around the country, municipalities use planting palettes that have not been checked for listings of locally invasive species. This demand for an invasive street tree for example, forces certain nurseries to maintain these plants in stock. Gardeners (myself included), often trade plants with friends or take our favorite plant with us when we move. If that plant was not invasive to the original location, it may be invasive to the new location.

There are still many issues that need to be resolved if we are to develop sound strategies for protecting our environment from harmful invasive species. A positive step in that direction has been more participation in groups by a wider diversity of stakeholders to which difficult issues can be discussed and resolved. It may not be an easy process, but it is a process that has had it share of success stories.

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Controlling Amur Honeysuckle (*Lonicera maackii)* in the Hamilton County Park District

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Hamilton County Park District

Abstract. The Hamilton County Park District, located near Cincinnati Ohio, has been working towards the control and management of the invasive shrub, Amur Honeysuckle (*Lonicera maackii*), which has invaded thousands of acres of the park district's green space. Management is prioritized based on road access, accessibility by the public, quality of habitat, availability of resources, and feasibility. Methods of control include a combination of hand removal techniques, mechanical treatments, foliar herbicide treatments and basal bark treatments. Costs vary considerably between control methods, the least expensive and efficient method being foliar treatment. The Hamilton County Park District supports research and monitoring of invasive species and believes that the results of increased native species diversity after honeysuckle removal have been well worth the effort.

Introduction

The Hamilton County Park District, (HCPD), manages more than 15,600 acres (6313 ha) in the southwest corner of Ohio. Most of the district's 20 parks and preserves are covered by second growth or successional hardwood forest. About 5% of park natural area is comprised of tall grass prairie or cool season grassland. 6% of the total acreage has wetland or aquatic ecosystems.

In her book *The Woody Plants of Ohio: Trees, Shrubs and Woody Climbers, Native, Naturalized and Escaped,* (1961 Ohio State University Press), E.L. Braun stated that Amur Honeysuckle was "Reported only from Hamilton County, where it is becoming abundant in pastures and woodlands". Forty-five years later, much of that invaded land is park property. About 4,000 acres (1619 ha) of HCPD has at least 20% cover of non-native bush honeysuckle. Control of Amur Honeysuckle has therefore become a priority for HCPD.

Criteria used to prioritize invasive plant control locations

- *First priority:*
  - Easy access. Edges of roads and fields allow for the use of larger spray equipment to reach target plants. This provides control over the most acreage and in areas with plants that produce most of the seed. There is also immediate savings of time that otherwise would be spent trimming these plants. Native plants that replace the exotics are usually not as aggressive, so require much less maintenance.
  - High use areas. This provides the public with the benefits of more diverse plant life and a more visually appealing view.
  - High quality natural areas and rare species. Removing invasive species protects and promotes plants and animals of special interest and their environment.

— Availability of resources. When volunteers are available or other opportunities arise in a specific area, take advantage of it.

— Completion of whole parks or sections within a park. These may be sites where there is a small area with invasive species that can be treated easily.

— Previously treated sites. Once an area is sprayed, occasional maintenance is required to keep invasive species from returning.

— Stop the spread of new threats. If a species has been proven to be very invasive elsewhere, eliminate it before it becomes the "next honeysuckle".

- *Second priority:*
  — Other lower quality natural areas. Remote, less significant natural areas can be worked on if time allows. Some areas will never be reached.

Methods for the Control of Amur Honeysuckle

- **Pull or dig**.
  When a sparse population of small honeysuckle occurs in a wooded area, and it is not a good time to apply herbicide, we pull the honeysuckle and hang the plants to dry with roots pointing upward.

  If volunteer help is available, pulling or digging is an option.

  This method creates the most soil disturbance, and is the most labor intensive. We remove about ½ acre (.2 ha) of honeysuckle per year this way.

- **Basal bark treatment**.
  Apply an herbicide with a penetrating oil carrier to the base of each shrub. This can be done any time the bark is dry. Even when applied in winter there is some residual effect on non-target species. We use Pathfinder II, a ready to use formulation with Garlon 4 as an active ingredient in non-petroleum oil penetrant.

  We control about 2 acres (.81 ha) per year by basal bark treatment.

- **Cut and treat stumps.**
  Cut honeysuckle off close to the ground, then spray or paint a 33% solution of glyphosate to cut surface. We cut about 15 acres (6.1 ha) of honeysuckle each year. This is a good project for volunteer groups.

- **Cut or mow, then spray leaves the following fall**.
  If large mowing equipment is used to cut down honeysuckle, instead of trying to find stumps under all the mowed debris, we wait until the plants resprout, then use a foliar application of 1¼% glyphosate in the fall.

Average Costs of Amur Honeysuckle Control Methods

Treatment	Cost per acre
Contracted foliar treatment	$250
Contracted cut and chip brush	$3,200
Contracted brush mowing	$900
In house foliar treatment	$150 plus equipment expenses
In house cut and treat stumps	$1,200

- **Fall foliar application of glyphosate.**
  Spray honeysuckle with 1¼% glyphosate after desirable species are no longer green and when the honeysuckle is continuing to photosynthesize. We controlled over 300 acres (121.2 ha) using this method in 2006.

Results and Discussion

The Hamilton County Park District began controlling honeysuckle more than 20 years ago. At that time, the method of choice was to dig it up with a pick and shovel. It was very labor intensive and caused a lot of soil disturbance. We also would just cut it off at the ground. The results were that each cut stem would sprout into three or more stems. Much has been learned since then about controlling Amur Honeysuckle and other invasive plants. By far the most efficient way to control honeysuckle is fall foliar treatment. The question some people have is, is it worth all the effort? We think it is. The park district supports research and monitoring of invasive species, their control and their effect on the ecosystem.

Our research and observations have shown that native species diversity and density increase after honeysuckle management.

Acknowledgements

We would like to thank Donald R. Geiger for introducing us to the fall foliar application of glyphosate to control Amur Honeysuckle.

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Strategic management of five deciduous forest invaders using *Microstegium vimineum* as a model species

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Abstract. This paper links key plant invasive traits with key landscape traits to define strategic management for five common forest invaders, using empirical data of *Microstegium vimineum* dispersal into forests as a preliminary model. *Microstegium vimineum* exhibits an Allee effect that may allow management to focus on treating its source corridor populations at local scales. Forest interior populations without a constant seed source from the corridor populations could potentially go extinct on their own. In contrast, species with long-distance dispersal are likely best managed at a landscape scale, focusing on new establishment sites rather than on removal of source corridor populations.

Introduction

Effective management of invasive species in forests requires the ability to predict their establishment and spread. While there are several studies evaluating the characteristics of both invasive species (Rejmanek and Richardson 1996, Reichard and Hamilton 1997, Kolar and Lodge 2001) and landscapes vulnerable to invasion (Burke and Grime 1996, Higgins et al. 1999, Huebner and Tobin 2006), literature evaluating the effects of landscape spatial patterns on the invasion process is relatively depauperate (Higgens and Richardson 1996, With 2002). Linking spatial patterns of disturbance, resource availability, and vegetation types with demographic and biological processes of establishment and spread should result in a more strategic approach to management (Figure 1).

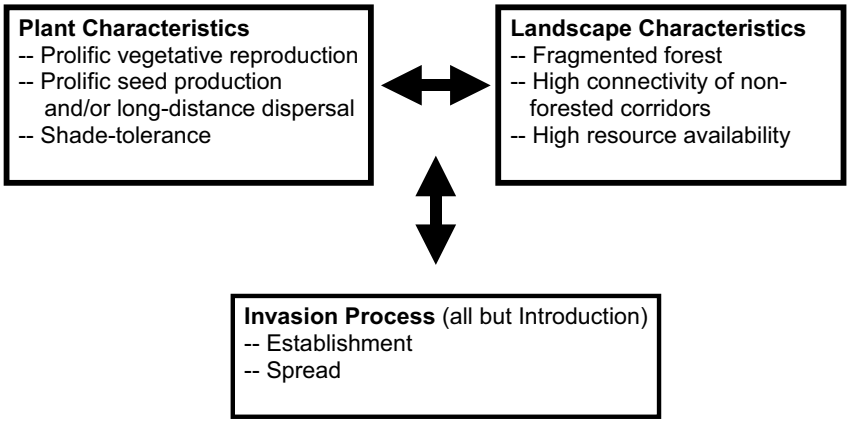


Figure 1. Linking key plant invasive characteristics with key landscape characteristics to predict spread of an invasive plant.

The ability to reproduce prolifically over long distances by seed (Cain et al. 2000, Wang and Smith 2002, Nathan 2006) and vegetatively (Kolar and Lodge 2001) under both high and low light conditions have been documented as traits shared by many known invasive plants. Likewise, several studies attribute relatively high resource availability (which may change with disturbance; Huenneke et al. 1990, Bergelson et al. 1993), fragmentation, and increased connectivity of non-forest corridors (Parendes and Jones 2000) as landscape attributes that promote invasion.

The objectives of this paper are to (1) predict establishment and spread of *Microstegium vimineum* (Japanese stiltgrass) using empirical data linking plant demography and biology with landscape characteristics, and (2) hypothesize how *Alliaria petiolata* (garlic mustard), *Ailanthus altissima* (tree of heaven), *Rhamnus cathartica* (common buckthorn), and *Celastrus orbiculatus* (oriental bittersweet) would differ from *M. vimineum* spread.

Methods and Materials

The Species. Table 1 summarizes the five species based on three key invasive traits – shade-tolerance, long-distance seed dispersal, and clonal growth. *Microstegium vimineum* is shade-tolerant (Winter et al. 1982) and reproduces by seed (Gleason and Cronquist 1993). It may spread vegetatively by rooting at the nodes (Hoshikawa 1969) but is not clonal. Both cleistogamous (closed) and chasmogamous (open) flowers are produced (Tanaka 1975), though the chasmogamous flowers tend to be associated with populations located in high light (Barden 1987). The lack of chasmogamous flowers in shaded plants may place such populations at a disadvantage in terms of lower flower and seed production (due to smaller plants), and seed dispersal over much shorter distances (seeds remain on the plant until plant senescence). Seeds of *M. vimineum* are thought to be dispersed by water and animals (Mehrhoff 2000). Wind dispersal is possible but has not been confirmed. Some research suggests that short-lived grasses, such as *M. vimineum* would favor short distance dispersal because the risk of finding a non-safe site for germination is too great (Collins and Uno 1985). *Alliaria petiolata* is similar to *M. vimineum* in that it is shade-tolerant (Byers and Quinn 1998, Welk et al. 2002) and relatively short-lived (it is a biennial; Gleason and Cronquist 1993). It may be dependent on short distance dispersal unless water or animals serve as secondary vectors (Cavers et al. 1979). *Ailanthus altissima* is an invasive tree with long-distance seed dispersal, primarily by wind. Seed have been documented to travel as far 200 m (Kota 2005) or further; other wind dispersed plants have seed dispersal distances as far as 300 m (Robinson and Handel 1993). This tree is dioecious and clonal, spreading via root suckers (Gleason and Cronquist 1993) and is often associated with disturbed habitats in high light (Hu 1979, Hamerlynck 2001). *Rhamnus*

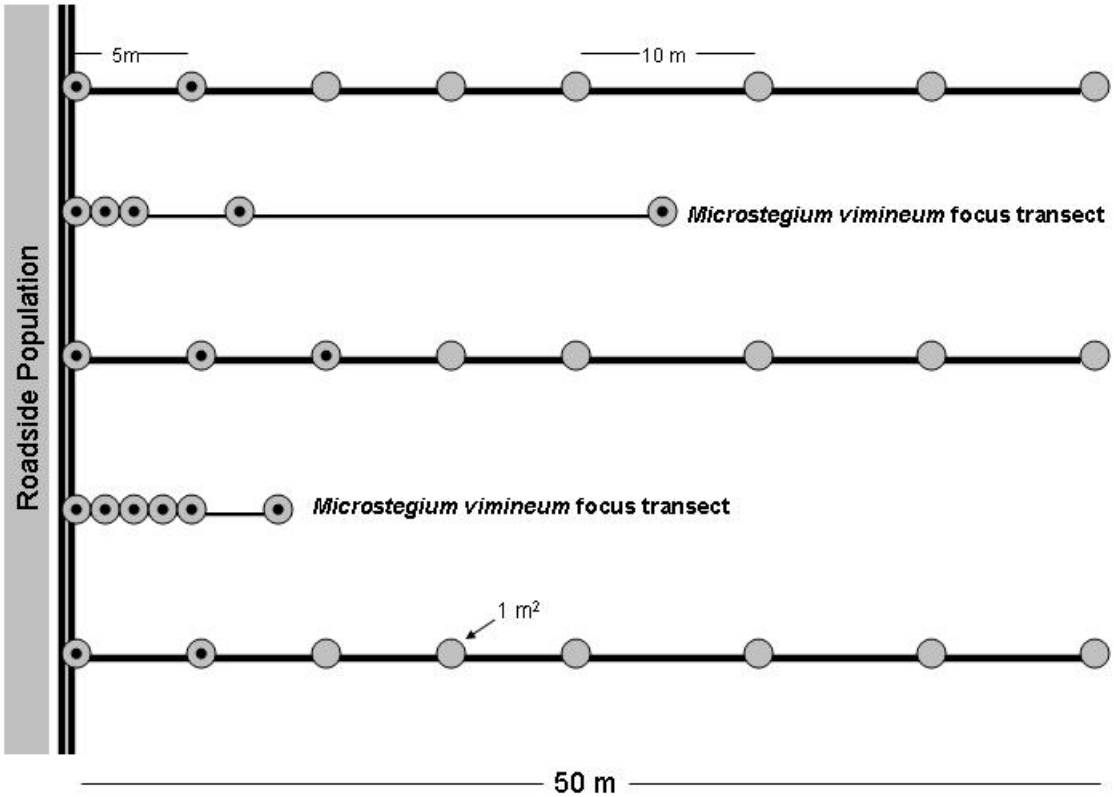
Table 1. Comparison of the five invasive species in terms of three key invasive plant traits. + indicates that the trait is well-documented in the literature and – indicates that the trait is not documented.

Plant Trait	Shade-tolerance	Prolific long-distance seed dispersal	Clonal growth
Microstegium vimineum	+	—	—
Alliaria petiolata	+	—	—
Ailanthus altissima	—	+	+
Rhamnus cathartica	—	+	—
Celastrus orbiculatus	—	+	+

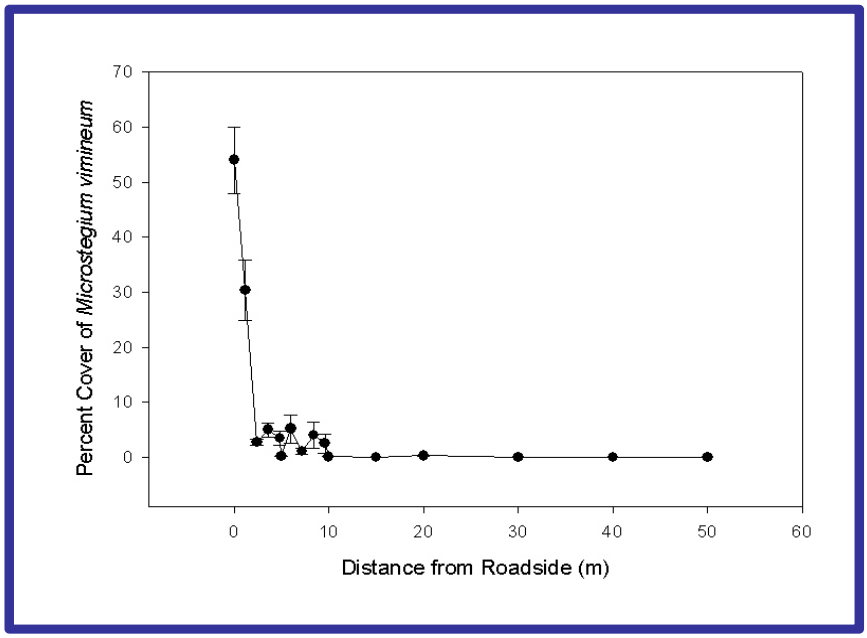
cathartica is non-clonal shrub or small tree that may sucker (Gleason and Cronquist 1993) from its base if cut, but is not clonal. It is dioecious and produces fruit that is bird-dispersed (Godwin 1943) potentially as far as 6 km (Haas 1995). It is found in both high and low light habitats but its growth rate is faster in high light (Harrington et al. 1989). *Celastrus orbiculatus* is a vine that reproduces by seed (Gleason and Cronquist 1993) and vegetatively via root suckers (Dreyer et al. 1987). The fruit are dispersed by birds (Robinson and Handel 1993) or small mammals (Dreyer et al. 1987). This species is imperfectly dioecious with some hermaphroditc and monoecious plants (Hou 1955, Dreyer et al. 1987). While this species germinates readily in low-light environments, growth is slower than plants in high light (Patterson 1975, Greenberg et al. 2001).

Study Site and Design. Sixteen sites in West Virginia, each with roadside populations adjacent to a closed-canopy forest, were sampled in June and September of 2005 and again in 2006. Sampling took place along three perpendicular transects 50 m into the forest interior and two additional transects with a plot located whenever *Microstegium vimineum* was present (Figure 2). Cover of all plant species within each 1 m^2 plot was determined in June. Cover of *M. vimineum*, height of the tallest stem of *M. vimineum* within each plot, and number of chasmogamous (CH) and cleistogamous (CL) inflorescences on that same stem were determined in September. Seed were collected from each flower type from interior and roadside populations near the sample sites. Seed were weighed and tetrazolium viability tests were conducted. Soil (0.001 m^3) was

Figure 2. Sampling design. Circles represent 1 m^2 plots. Circles with nested smaller circles represent plots with *Microstegium vimineum* present. Two transects between the main three were used to estimate the spread front as well as furthest distance, placing plots only where *M. vimineum* was present.



collected just outside of each plot at the same distance and analyzed for its seed bank. Sticky traps were placed just outside of each plot in late September and remained until plant senescence along the main transects in order to determine actual seed dispersal. Light measurements and canopy densiometer readings were taken at every plot over a three hour period of a generally sunny day for each site, and percent bare ground and litter depth were determined. Soils were analyzed for total N and C. Logistic regression analysis was used to determine which variables defined the presence or absence of *M. vimineum* under forested canopies.



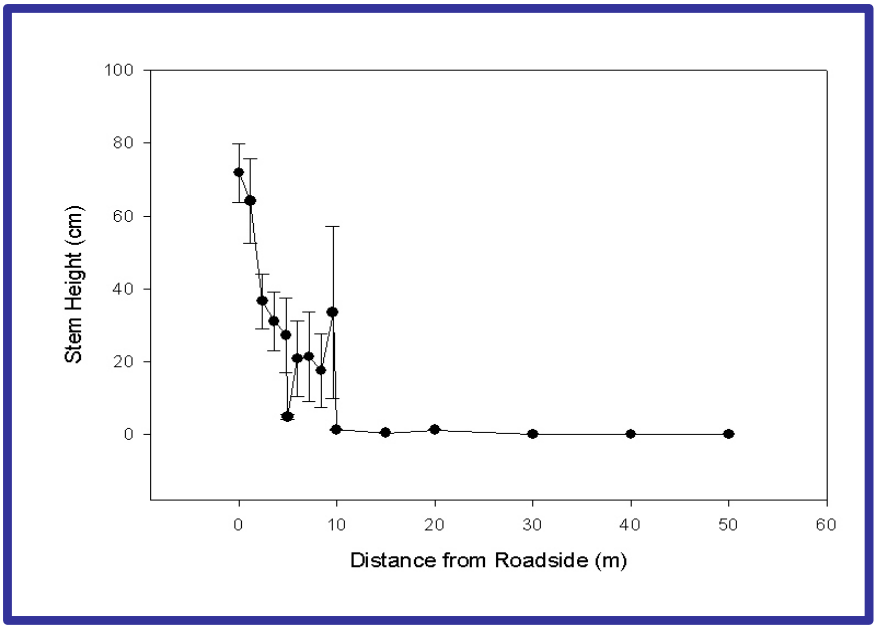


Figure 3. a. Percent cover of *Microstegium vimineum*; b. stem height along the roadside to forest interior gradient (averaged over 16 sites).

Results and Discussion

While *M. vimineum* plants were found as far as 30 m from the road in 2005, most cover was found within 10 m of the roadside (Figure 3a), and stem heights were significantly taller along the roadside compared to distances greater than 5 m into the forested sites (Figure 3b). Seed production, stem height, and cover were significantly correlated with light (possible threshold of 30 ˜molm^{-2}s^{-1}) and canopy opening; these findings agree with other studies (Claridge 2002, Gibson et al. 2002). Full CH inflorescences only occurred between 0 - 5 m (Figure 4a). Seeds were not found beyond 1.5 m on the sticky traps and an

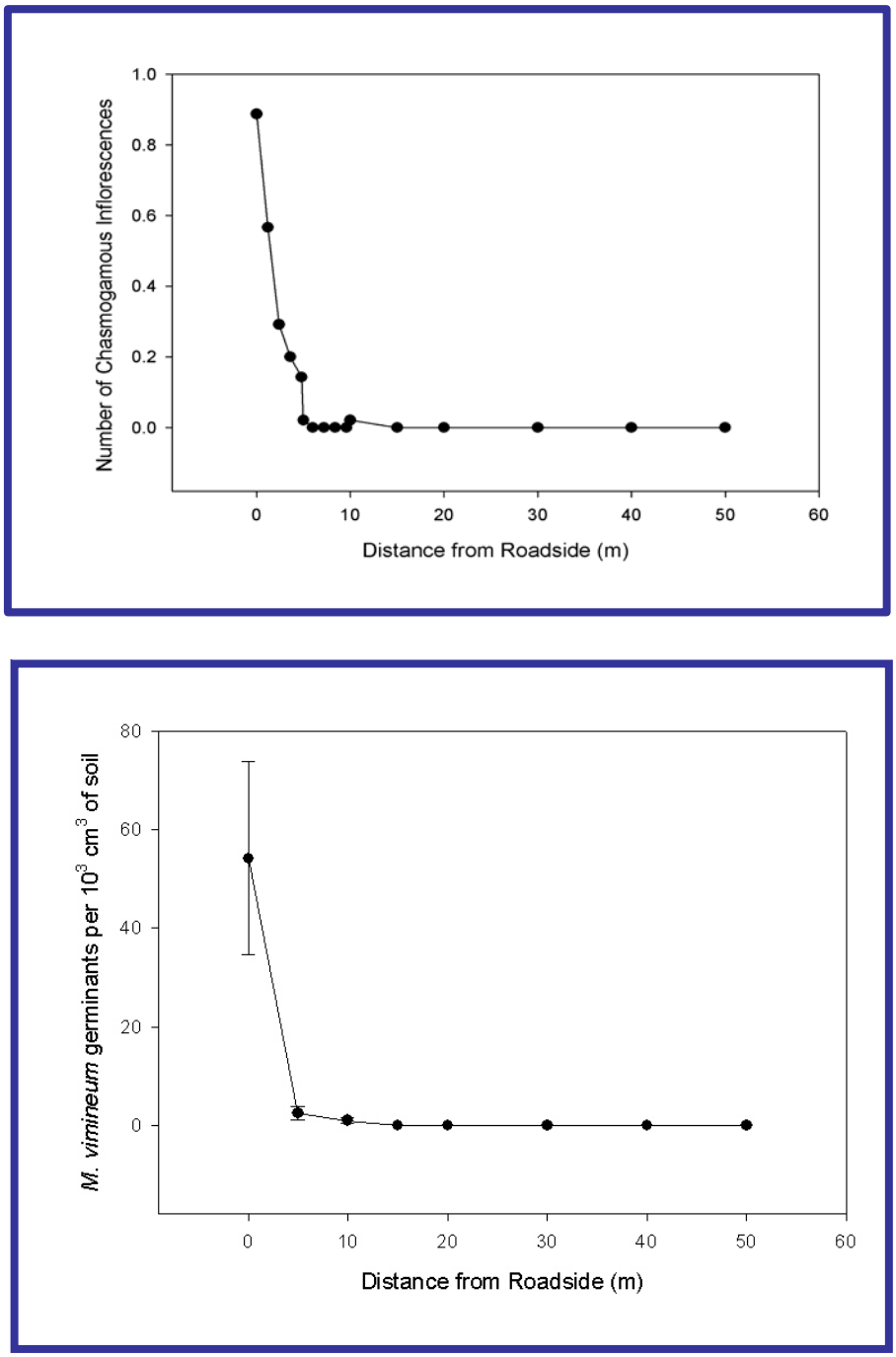
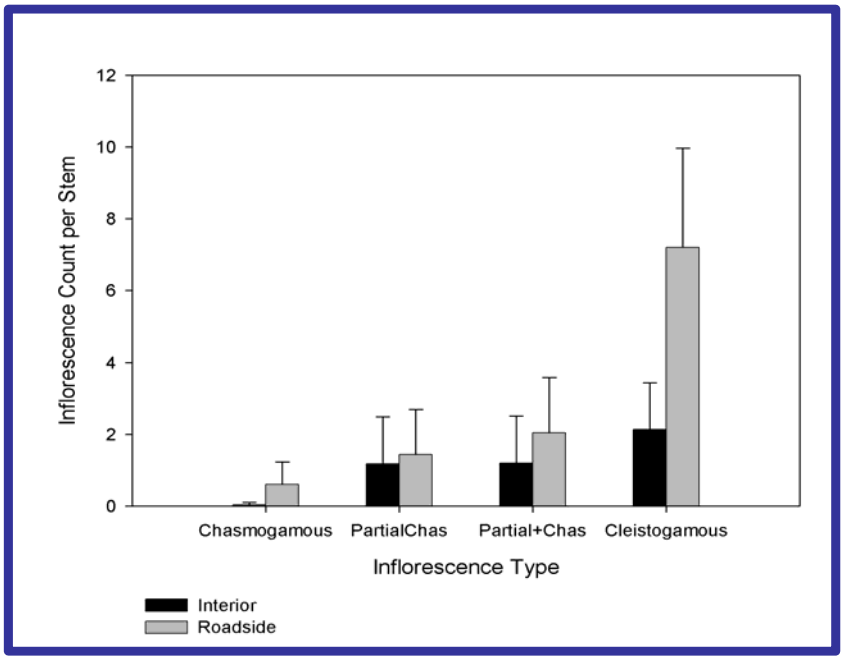


Figure 4. a. Number of chasmogamous flowers and b. germinants from the seed bank of *Microstegium vimineum* along the roadside to forest interior gradient.

average of 24 seeds per site were deposited on the traps, suggesting that most seed remains on or near the plants and that the seed are not wind dispersed. Seed did not germinate from distances beyond 10 m in the seed bank, with a mean of 54 germinants at distance 0 m, 2.44 at 5 m and 0.98 at 10 m (Figure 4b). While interior populations (beyond 5 m) produced far less seed of each type (Figure 5a), the ratio of partially CH+ CH inflorescences to CL inflorescences was larger (Figure 5b). Viability tests showed that interior population



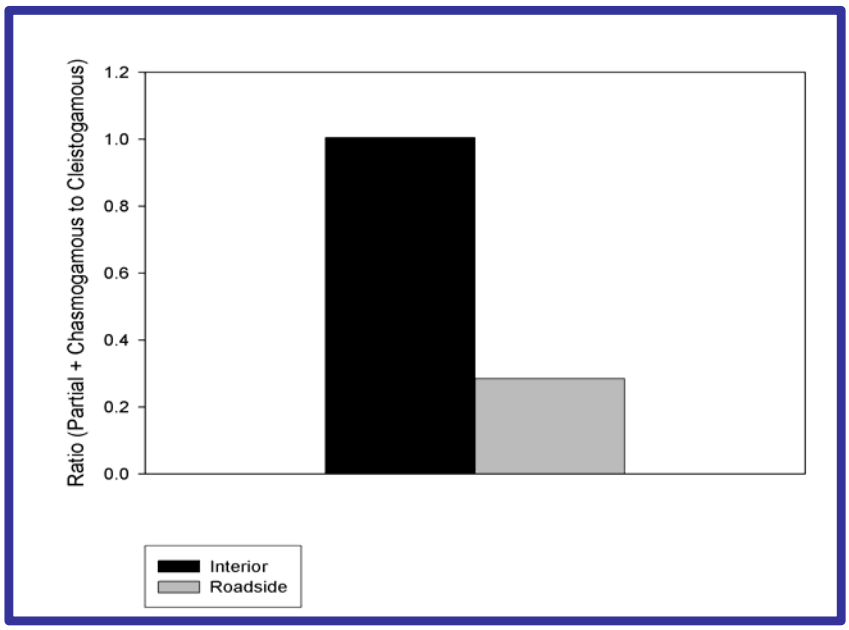


Figure 5. a. Comparison of inflorescence types and b. ratio of inflorescence types between interior (located beyond 5 m into the forest interior) and roadside populations. Partially chasmogamous refers to inflorescences with a few open flowers but mostly closed flowers. PartialChas refers to partially chasmogamous flowers. Partial + Chas indicates a sum of both partially chasmogamous and chasmogamous flowers.

seeds were equally viable to roadside population seeds (CL or CH), and that seeds from CL and interior flowers weighed less than seeds from CH and roadside seeds, respectively. Presence or absence of *M. vimineum* at distances beyond 5 m was significantly related to species richness (richer plots were 39% more likely to have *M. vimineum*), but not to litter depth, amount of bare ground, C:N ratio, or increased canopy opening or light. Because plant spread, cover, and seed production are significantly lower under shaded environments (<30 ~mol$m^{-2}s^{-1}$), such populations may experience an Allee effect and decrease in size (with no new disturbance) or increase more slowly than roadside populations. The Allee effect is a positive relationship between population density and a population's per capita growth rate; i.e., individuals of smaller populations exhibit a decrease in reproduction and a survival. These results suggest that management efforts should focus on roadside populations. The invasive front decreased slightly in distance (0.53 m on average) from the roadside populations between 2005 and 2006 (Table 2). However, the number of individuals found beyond 5 m increased (1.81 new plots on average) as well as the distance of the furthest individuals (47.2 m being the furthest distance and 5.6 m being the average increase), but not significantly. The potential importance of long-distance dispersal by deer and water flow events needs to be determined.

Based on these data, most *Microstegium vimineum* spread will occur along non-forest corridors over short distances; potentially a meter a year. Movement into forested areas will be in cms/yr with greater movement after flood events and via people and deer. The rate of spread by deer, water and other similar sources may increase as the corridor and encroaching understory population increases in size. Focusing on the corridor populations early should reduce directed spread into gaps and the rate of coalescence of interior populations. In contrast, spread of the herb *Alliaria petiolata* along corridors or into forests is likely to be at approximately equal rates (1 – 1.5 m/yr based on average plant heights). Because plant size and seed production do not appear to differ between roadside and interior populations (but this needs confirmation), it is unlikely to suffer from any Allee effect and its spread into forest interiors may be faster than that of *M. vimineum*. Flood events and relatively rare dispersal by people and deer may result in small outlying populations, which are predicted to coalesce more rapidly than those of *M. vimineum*. While all five species may respond positively to forest canopy gaps and other disturbances that increase light, species with long-distance dispersal are likely to benefit the most. Gap formation (due to tree mortality) is estimated to occur at a rate of 1% of the trees per year in temperate forests (Runkel 1985), which could be used to estimate spread rate, especially for plant species whose seed are dispersed by animals attracted to such openings. The shrub *Rhamnus cathartica* will not spread by clonal

Table 2. Comparison of spread of *Microstegium vimineum* (MV) into forest interiors of West Virginia in 2005 and 2006. Numbers are averages over 16 sites. Differences were not significant; standard errors are in parentheses.

Year	Number of plots with MV located beyond 5 m from the roadside	Furthest (m) plot with MV from the roadside	Distance (m) of MV the front from the roadside
2005	2.94 (1.04)	10.9 (2.72)	4.73 (0.85)
2006	4.75 (1.22)	16.3 (2.95)	4.20 (0.55)

growth and is solely dependent on birds/animals for dispersal. However, dispersal distances will be greater and more selective (to other similar sites and forest gaps), which may make establishment of new reproducing individuals more likely, although research to confirm this is needed. Some studies indicate that bird travel between abundant food sources (open fields and forest edges) is greater than that into forest gaps (Bartuszevige and Gorchov 2006). The spread of *R. cathartica* will also depend on the presence of female trees. Much of *Ailanthus altissima*'s spread will occur along non-forest corridors and slowly into the forest interior. Dispersal will occur over long distances, but seeds may be less likely to find optimal sites. *Ailanthus altissima* will be able to respond quickly to a disturbance and these new populations, if female trees are present, will produce seed that can travel long distances. *Celastrus orbiculatus* will spread along corridors, slowly into forest interiors, and by birds or mammals over long distances into forest gaps and along new corridors. The directed dispersal, its ability to spread clonally, and its use of perfect and monoecious reproductive strategies may make this species the fastest spreading of the five taxa. However, more information about bird preferences for the fruit and successful establishment after dispersal is needed to confirm this prediction.

Microstegium vimineum and *Alliaria petiolata* may have greater local impacts on native forest vegetation despite being relatively limited in initial spread rates, but this impact is likely to be reduced more effectively by focusing on the corridor populations. However, because of the possible local Allee effect in *M. vimineum* populations, it may be possible not only to slow the spread of this species but eradicate it locally. The apparent lack of such an Allee effect in *A. petiolata* and the long distance dispersal of the other three species make focusing both on the source populations and the satellite populations a must if eradication is sought. Slowing the spread of the species with long-distance dispersal may be best done at a landscape scale, focusing on the satellite populations and new safe sites (new disturbances) where plants are more likely to become established. Unless all corridors over a very large area can be treated, focusing on the corridors will not prevent spread of species with long-distance dispersal into forests and back into the previously treated sections of corridor.

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Glossy Buckthorn management of Irwin Prairie State Nature Preserve

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Abstract. Rhamnus frangula (glossy buckthorn) is a shrub native to Eurasia that is often used in landscaping in the eastern United States. Like many ornamentals it has escaped cultivation and has spread throughout our native plant communities. In Ohio, glossy buckthorn has negatively impacted many areas of northern Ohio including sensitive areas like bogs, fens, and wet prairies.

In the last 25 years, glossy buckthorn has become established at Irwin Prairie State Nature Preserve and has replaced approximately 100 acres of species diverse wet prairie into a monoculture of glossy buckthorn. Since 2001 we have worked extensively to eradicate glossy buckthorn from the preserve and re-establish the native wet prairie community.

Many methods have been used all with varying degrees of success. We have found that no one species of fighting glossy buckthorn will eradicate it but that a combination of methods has allowed us to be successful in fighting glossy buckthorn. The methods used are dependent on different factors including water depths, plant size, time of year, and location in the preserve to name a few. We continue today to adjust our methods and efforts to meet the challenge of eradicating this aggressive shrub.

Introduction

Irwin Prairie State Nature Preserve lies in Spencer Township, Lucas County, in the heart of the Oak Openings region of northwest Ohio. One of Ohio's 130 state nature preserves, Irwin Prairie contains the largest preserved area of the rare twigrush-wiregrass wet prairie community (Schneider and Cochrane 1997). Known only from northwestern Ohio, this wet sedge meadow community, dominated by *Cladium mariscoides* (twigrush) and *Carex lasiocarpa* (wiregrass), is considered globally imperiled by NatureServe (Faber-Langendoen 2001).

Irwin Prairie is a remnant of a once larger wet sedge meadow that extended approximately 7 miles (11.3 km) long and 1 mile (1.6 km) wide. The increase of human activity in the area, including urban sprawl, drainage, habitat fragmentation, and fire suppression has led to the conversion of sedge meadows to impervious surfaces of shrubs and trees. Successional processes have been accelerated at Irwin Prairie by the invasion of the non-native shrub, *Rhamnus frangula* (glossy buckthorn). In 2001, the Ohio Department of Natural Resources Division of Natural Areas and Preserves (DNAP) undertook a large-scale ecological effort to restore native prairie to

areas where Glossy Buckthorn had become the dominant species. These efforts are critical to ensuring the future viability of the twigrush-wiregrass wet prairie community.

Methods

Prior to 2001, Glossy Buckthorn management consisted of small groups of DNAP staff cutting buckthorn stems with loppers and treating the stump with herbicide. This was done a few times each year with very little overall success. Glossy buckthorn became established in new areas faster than it could be removed. Before large-scale restoration began in 2001, existing Glossy Buckthorn stands were mapped using GPS units and ArcView GIS software. The following methods were used in Glossy Buckthorn removal depending on factors like size of plants, density of stems, and location in the preserve.

Method 1: Backpack Foliar Spraying

Using backpack sprayers, DNAP staff foliar sprayed stands of Glossy Buckthorn during the growing season with a 5-10% solution of the broadleaf herbicide Garlon 3A and a surfactant. Re-sprouts and seedlings were sprayed after they reached 2-3 feet (0.6-0.9 m) in height. 2-3 years of spraying was required to kill the plants and the seedlings. Garlon 3A is the preferred herbicide because it does not affect grasses and sedges. However, Garlon 3A should not be sprayed over areas of standing water.

Method 2: High-volume Industrial Foliar Spraying

In 2002, DNAP contracted a private vegetation control company to spray a large stand of Glossy Buckthorn consuming nearly 4 acres (1.6 ha) of the prairie. During the growing season, the contractor applied a 0.75% solution of Garlon 3A herbicide with a surfactant. The Glossy Buckthorn was then allowed to stand for one full year to fully assess the effects of spraying. In 2003 the contractor reapplied the solution to areas of re-sprouting.

Both backpack and industrial foliar spraying is effective in killing Glossy Buckthorn. However, you need to remove the dead stands of Glossy Buckthorn to encourage the growth of prairie species. Areas that we did not remove the dead buckthorn resulted in new Glossy Buckthorn growth.

Method 3: Mowing

In addition to foliar spraying, DNAP staff mowed the dead Glossy Buckthorn with tractors to allow for follow up spraying and to promote native plant growth. This was accomplished only when the prairie was completely dry, about every other year.

Method 4: Cut and Stack

Using chainsaws and trimmers with steel circular saw blades, DNAP staff cut and stacked Glossy Buckthorn shrubs too large to mow and spray. If the temperature was above freezing then the cut stump was treated with a 25% Garlon 4 and 75% Penevator oil solution. Stump treatment was only effective in plants that had a diameter larger than one inch or roughly the size of a quarter. The piles of cut buckthorn were then burned in the winter months or as part of a controlled burn. Stacking the brush piles in areas of heavy buckthorn infestation and then burning them has been a great way of eliminating buckthorn seedlings and encouraging native seed germination.

Method 5: Controlled Burn

Controlled burning was used when weather conditions and water levels permitted. To kill young Glossy Buckthorn seedlings, remove the stacked piles of cut buckthorn, which will encourage native prairie plant seeds to germinate in the restored areas. Unfortunately, larger plants and clumps of Glossy Buckthorn

are not affected by the fire due to the lack of fuel around their base.

Results

Management efforts at Irwin Prairie have, in the short term, been extremely successful. Large areas that were once stands of Glossy Buckthorn are now reverting back to wet prairie. They now have a majority of transitional species with *Carex cryptolepis* (little yellow sedge) being the first dominant species followed by various annual grasses and rhizomatous sedges.

The climax species appear to depend on water levels. In areas of deeper, longer lasting standing water, the twigrush-wiregrass wet prairie is returning. Areas that are dryer throughout the growing season are returning to a more mesic sand prairie community with *Liatris spicata* (spiked blazing star), *Coreopsis tripteris* (tall coreopsis) and *Andropogon gerardii* (big blue-stem grass) becoming dominant.

Discussion

A variety of methods are needed to control Glossy Buckthorn and continued improvements to our methods will be needed to ensure prolonged success. Reseeding has not been an issue due to small "islands" of prairie surviving within the clumps of buckthorn and an influx of seed from the seasonal flooding of the preserve. However, in areas where there is not a seed bank, we may need to address this issue.

Hand cutting with loppers, hand pulling of seedlings, chipping of cut stems, and hauling away cut Glossy Buckthorn stems have all been used at Irwin Prairie. These methods do work in eradicating Glossy Buckthorn; however, they have proven to be very time consuming. These methods would be useful in a smaller infestation but with the vast amount of Glossy Buckthorn present at Irwin Prairie they have proved to be inadequate.

Through multiple techniques we have restored much of the wet prairie at Irwin Prairie State Nature Preserve. As with any invasive species management program, a strong commitment is necessary to achieve success. DNAP is dedicated to maintaining and monitoring these efforts into the future.

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Evaluation of control techniques for *Phragmites australis* in coastal wetlands, Ottawa National Wildlife Refuge

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Case Study Summary

Crane Creek is a drowned-river-mouth wetland along the shore of western Lake Erie within the boundaries of Ottawa National Wildlife Refuge (ONWR) in which wetland plant communities have been degraded by high lake levels and anthropogenic effects. As a result of recent low lake levels, emergent plant communities are becoming established along portions of the shoreline with exposed wetland sediments, and invasive species such as Common Reed (*Phragmites australis*) are dominating localized areas. Existing literature and previous research in ONWR suggest that early attention to these invasive species could have long-lasting effects on the success of restoration. This study evaluated the effectiveness of five management treatments targeting *Phragmites* that were applied following aerial spray of herbicide. In one growing season, treatments of cutting, raking, and hand-sprayed herbicide for control of *Phragmites* were assigned to fifty plots in the *Phragmites*-dominated areas. Quadrat sampling of wetland vegetation within the plots was performed prior to and following treatments. Soil samples were collected for bulk-density and seed-bank analyses. Preliminary results indicated that raking was a key component for promoting the growth of native species, especially at shallow water depth, and the herbicide had the greatest effect at middle water depths. Long-term success maintaining native species richness, however, will depend on a combination of cutting, raking, and herbicide application.

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Cultivars of Purple Loosestrife threaten wetlands

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Abstract. Morden Pink, a supposedly sterile cultivar of *Lythrum salicaria* (purple loosestrife), and *Lythrum virgatum* L. (European wand loosestrife), can be fertilized by insect-carried pollen from wild naturalized *L. salicaria*. The seeds produced by those crosses germinate and produce viable seed when backcrossed against themselves, their parents, and other cultivars available in the horticulture marketplace. The next generation of these hybrids is likewise fertile showing that horticultural specimens of this plant can establish seed-producing populations when grown in gardens open to natural pollinators. While the number of viable seeds in some of these crosses was lower than crosses between male and female *L. salicaria*, all produced over 50% germination and robustly growing plants. These results suggest that all state governments should ban both the sale and propagation of these cultivars and of naturalized purple loosestrife as a way to enhance efforts to manage this alien invasive plant.

Introduction

Lythrum salicaria (purple loosestrife, LS), and *L. virgatum* (European wand loosestrife), a likely subspecies of LS are non-native invasives that threaten wetlands (Thompson et al 1987). In Ohio, it is illegal to sell LS, but legal to sell cultivars that have been dubbed sterile. Reports of crosses between the wild and horticultural forms of this plant are well known and some are published (Lindgren and Clay1993, Ottenbreit and Staniforth. 1993, Rachich and Reader 1999). This paper extends those earlier findings and calls for the all states to prohibit the sale of the cultivars.

Purpose

Provide convincing evidence that LS cultivars represent a threat to wetlands similar to that of *L. salicaria*.

Objectives

1. Demonstrate that LS cultivars (Table 1) produce viable seed when naturally fertilized with *L. salicaria* pollen by wild insects.
2. Demonstrate that progeny are fertile.
3. Show progeny can produce viable seed when crossed against their siblings, parents and naturalized *L. salicaria*.
4. Show that a molecular genetic analysis of cultivars might show them to be similar, if not identical to naturalized *L. salicaria*.

Methods

The essential flow of experimental process is shown in Figure 1. Fertilization, in all cases, was carried out by natural communities of insects such as bees, wasps and flies on plants in experimental groups well isolated (over 1 km) from one another. Some experiments were

Table 1. Horticultural cultivars of *Lythrum* spp. in the U.S. as of 1993.

***Lythrum salicaria* L.**	***Lythrum virgatum* L.**
Atropurpureum	Columbia Pink
Beacon *	**Dropmore purple * (DP)**
Brightness	**Morden Gleam [b] * (MG)**
Gypsy Blood (aka	**Morden Pink * (MP)**
Zigeunerblut) *	Morden Rose [b] *
Firecandle *	Pink Spires*
Flashfire (aka Fueukerze) *	Purple Dwarf [c] *
Happy *	Purple Spires *
Lady	Rose Queen *
Sackville	Rosyglow
Loosestrife	Rocket
Mr. Roberts	
Robert [a,c] *	
Rose Gleam	
Rosy Gem	
Unknown var.	
var. tomentosum	
var. roseum superbum *	
var. *gracilior*	

Modified from Anderson & Ascher 1993; Strefeler 1996
Bold indicates types used in present experiments.
[a] Disputed parentage; also classified as *L. virgatum*.
[b] Interspecific hybrid cultivars derived from the cross 'Morden Pink' x *L. alatum*.
[c] Dwarf cultivars with *L. alatum* in their pedigrees.
*Hybrids used by Strefeler (1996).

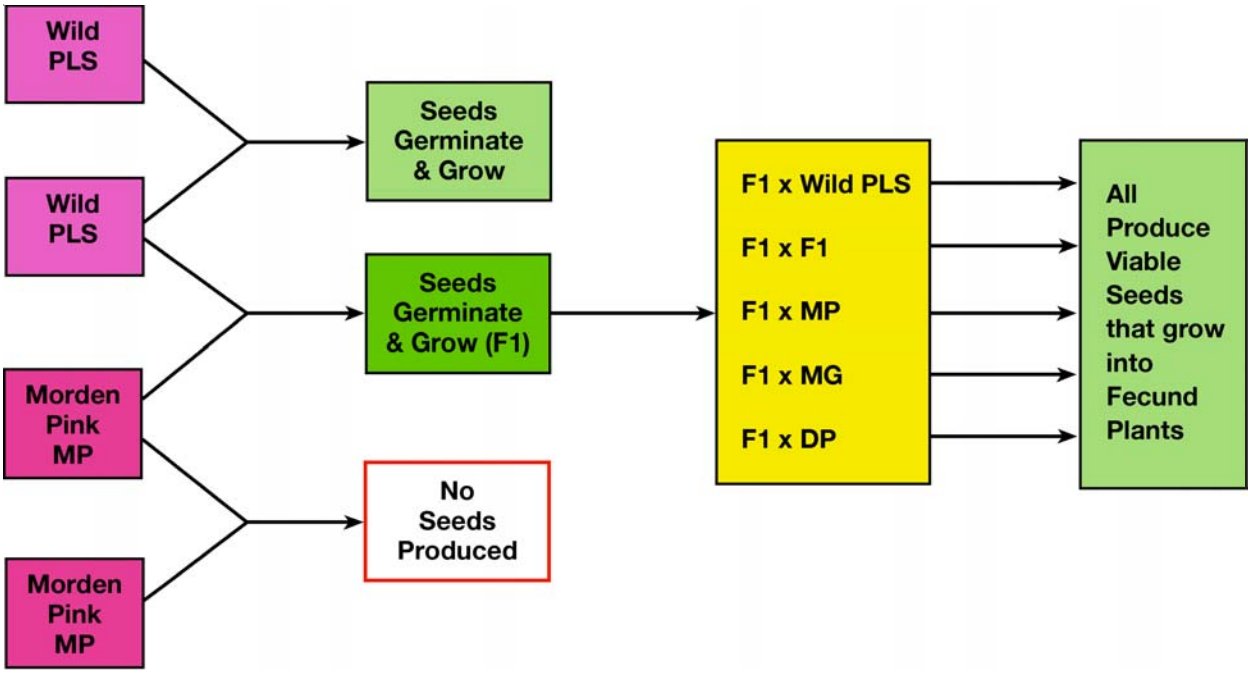
completed around or near the Wright State University greenhouse and other isolated experiments were conducted throughout the Dayton, Ohio Metropolitan area. Seeds gathered from pods were stored dry at 4°C then placed in moist sand at 4°C for a few weeks prior to germination tests in the greenhouse. All plant material was controlled and killed before disposal. DNA analysis (RAPDs) followed the methods of Williams et al (1990).

Seed size, number per pod, and average weight were recorded based on harvest of hundreds of pods from each plant then 25 pods were then selected randomly from the combined harvest. Seed length was based on 5 seeds per plant and germination based on 25 seeds per plant. Weight of individual seed was determined by counting the number in 50mg of mingled seed from the 25 pods. Germination testing was under fluorescent grow lamps on a 15-hour light cycle at 21ºC.

Results

Figure 1 shows that cultivars Morden Pink (MP), Morden Gleam (MG), and Dropmore Purple (DP) all could form viable hybrids with either LS or the progeny (F1) of the first MP x LS cross. MP crossed with other MP cultivars was unable to set any seed (Table 2). There was a small variation in the size of seeds and the percent germination in the first set of experiments using LS and MP (Table 2). When crosses were extended to MG and DP, more variation was observed but the overall result was strong seed production and subsequent

Figure 1. Experimental flow diagram and results



In the left column wild Purple Loosestrife (PLS) and the Horticultural Purple Loosestrife are subjected to initial crosses. Column two shows the result. Wild x Horticultural progeny (F1) are crossed with self, wild and horticultural types in column three and in column four all produce viable seeds. All crosses are done in isolated clusters of plants and fertilized by wild insects.

Table 2. Success of 2000 and 2001 hybrid crosses between (MP) 'Morden Pink'and (LS) wild *L. salicaria*

Cross	Average length of seeds (mm)	% Germination
MP female x MP male†	0 (0)	0%
LS female x LS male	0.728 (0.083)	76%
LS female x MP male	0.938 (0.094)	59%
MP female x LS male‡	0.852 (0.099)	51%

Standard deviation of seed size for single plants is indicated by parentheses.
† No seed produced for this cross. ‡ Cross that produced F1 progeny.

Table 3. Success of the 2003 hybrid crosses between (LS) wild *L. salicaria*, (MP) 'Morden Pink', (MG) 'Morden Gleam', and (DP) 'Dropmore Purple'.

Cross*	Number of plants	Average # of seeds per capsule	Average length of seeds (mm)	Average weight of 1000 seeds (mg)	% Germination	Reproductive potential per capsule (# seeds x % germination)
LS f x LS m	3	96.70	0.890	88.7	92%	88.96
LS f x MP m	3	61.52	0.900	115.4	60%	36.90
F1 x F1	3	30.41 (20.60)	0.987 (0.090)	326.0 (113.8)	77% (8)	23.42
F1 f x LS m	2	49.22 (9.72)	0.922 (0.082)	186.4 (40.2)	70% (10)	34.45
F1 f x MP m	7	54.45 (8.40)	0.921 (0.034)	202.5 (31.4)	87% (4)	47.37
F1 f x MG m	4	37.55 (4.88)	0.894 (0.034)	213.6 (27.4)	94% (3)	35.30
F1 f x DP m	5	14.25 (3.62)	1.011 (0.021)	399.2 (61.9)	62% (5)	9.40
LS f x ∑		39.40	0.890	193.7	100%	39.40
MP f x ∑		15.50	1.015	334.7	60%	9.30
MG f x ∑		18.44	0.785	296.8	68%	12.51
DP f x ∑		14.05	1.080	378.8	72%	10.15

Standard error of variation between plants is indicated by parenthesis when n > 1.
∑ Indicates open random crosses with either naturalized *L. salicaria* L., MP, MG, or DP.
F1 denotes the progeny produced from Morden Pink (female) x naturalized *L. salicaria* (male).

germination of that seed (Table 3). By using leaves gathered over much of Ohio and Pennsylvania for molecular analysis by RAPDs, we found that the DNA variation among plants of LS overlapped with the variation among LS compared to MP. This suggests that they are likely to be the same species. Native *Lythrum alatum* was less related to MP or LS than MP was to LS as expected.

Discussion & Conclusions

It is clear that cultivars of *L. salicaria* and *L. virgatum* are able to reproduce and are not appropriate for commercial or retail trade anywhere in the USA where they represent a significant threat to wetland communities. LS is known to compete for pollination (Grabas and Laverty1999, Brown and Mitchell 2001) with the native species *L. alatum* and it is likely that these hybrids will do the same. More recently, habitats of amphibians were shown to be at risk in LS invaded pools (Brown et al 2006). The hybrid vigor that may result from these crosses may also increase their threat to wetland ecosystems. While not an exhaustive study, the evidence from the RAPDs suggests that some of the vigor seen in naturalized LS (Chun et al. 2007) may be related to its genetic mixing with *L. alatum* or *L. virgatum* or both.

Table 4. Percent PCR-RAPD bands in common with various species.

A. Percent in Common with Morden Pink	
Lythrum salicaria (PLS)	65
Morden Pink (MP within sample, n=4)	71
Lythrum alatum (LA)	52
B. Common within or between species	
LA to LA (within W. Central OH)	75
PLS to PLS (over several states)	61
PLS to LA	49

A similar situation appears to be occurring in the horticultural pear species (*Pyrus calleryana)* thought to be "sterile". Vincent (2005) has shown that these cultivars are spreading and is likely due to crosses with introduction of multiple pollination compatible cultivars. Individual State Departments of Agriculture need to produce regulation that prevents distribution of such cultivars of these invasive species.

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Use of Roundup Ready® soybeans to reduce Chinese Lespedeza *(Lespedeza cuneata)* competition during establishment of high diversity prairie

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Abstract. During mine reclamation in southeast Ohio, Chinese lespedeza *(Lespedeza cuneata)* and other invasive species were used extensively for the purpose of erosion control and revegetation due to their ability to grow in a wide variety of conditions. *L. cuneata* is a highly aggressive perennial able to form extensive root systems and is able to create a persistent seed bank that remains viable for decades, making removal challenging. These competitive advantages have resulted in large areas of *the Wilds* being dominated by this plant. The objective of this project was to explore the use of Roundup Ready® soybeans as a cover crop, in combination with glyphosate applications, in order to effectively control *L. cuneata* and proceed with a high diversity prairie planting.

The trial was conducted at *the Wilds* on a 23-acre site dominated by *L. cuneata*. Roundup Ready® soybean cultivars Asgrow AG5605 and AG5501, inoculated with a standard soybean *Rhizobium sp.*strain, were planted at a rate of 100 lb ac^{-1} (112 kb ha^{-1}) during spring of 2005 and 2006, respectively. *Lespedeza cuneata* and other weeds were controlled with five applications of glyphosate herbicide over a two-year period. During the growing seasons, the soybeans were unaffected by the herbicide and served as a nitrogen fixing cover crop. In addition, low level applications of N-P-K fertilizer were applied in May 2005 and again in March 2006 to improve soil fertility. In November following the 2006 soybean crop, the site was planted with a high diversity prairie species mix.

Results show that following a two-year cover crop period, *L. cuneata* was not observed in the interior of the site and <5% persisted in the perimeter of the site. Our study suggests that using a herbicide resistant cover crop for two consecutive years can be effective in controlling *L. cuneata* prior to prairie establishment. Further results will be based on the successful establishment of the high diversity prairie ecosystem and whether there is continued suppression of *L. cuneata*.

Introduction

Native to Asia and introduced into the U.S. in the late 1800s, *Lespedeza cuneata* is a semi-woody long-lived perennial and is an extremely aggressive invader of open areas, out competing native vegetation. It has been widely planted for wildlife habitat, erosion control and mine reclamation. Its deep taproot enables it to obtain water and nutrients even during periods of drought, and its ability to shade-out native plants is proposed as its primary mechanism for

dominance (Brandon et al. 2005). *Lespedeza cuneata* contains allelopathic compounds such as tannins, which inhibit the growth of other plants while also making it unpalatable to animals. Once it is established, it is very difficult to remove due to the seed bank, which can remain viable for decades. It was one of the many invasive species intentionally introduced at *the Wilds* as part of the mine reclamation process to control erosion and provide ground cover.

Also known as the International Center for the Preservation of Wild Animals, Inc., *the Wilds* is a large-scale, non-profit center for conservation research and education occupying 9,239 ac (3,739 ha) of Muskingum County in Southeastern Ohio. *The Wilds* has experienced over half a decade of disturbance due to surface mining for coal. Following mining activities, the land was reclaimed into a mixture of cool-season non-native grasses and legumes. Many invasive species were introduced as part of the reclamation process including *Eleagnus umbellata* and *L. cuneata*. The reclaimed grasslands have been found to be resistant to forest encroachment due to soil characteristics such as compaction, poor permeability and low organic matter. (Steiger 1996, Ussiri and Rattan 2005). *Lespedeza cuneata* established rapidly and has now become one of the dominant species in the grasslands areas of *the Wilds*. The large land base of *the Wilds* provides ideal circumstances to study the potential restoration and recovery of these disturbed habitats.

The Restoration Ecology Department of *the Wilds* is exploring different management techniques that will reduce the presence of invasive, low quality grasslands and replace them with higher diversity prairie systems that will help rebuild soil while providing habitat for wildlife. Prairie establishment is often challenging, even in non-degraded sites, due to the competitive nature of non-native grasses and other annual weeds (Kilde 2000). Removal of the non-native vegetation, especially aggressive species such as *L. cuneata*, either by means of mowing, tilling or fire, has proven to be only the initial stage in preparation for replanting with native prairie species. These challenges have led the program to explore the judicious use of herbicide, in combination with a resistant nitrogen fixing cover crop, to increase the likelihood of success in prairie establishment.

The objective of this project was to explore the use of Roundup Ready® soybeans as a cover crop in combination with glyphosate applications to effectively control *L. cuneata,* prior to planting a high diversity prairie on reclaimed mine land at *the Wilds.*

Methods

The Roundup Ready® soybean trial was conducted at *the Wilds* on a 23-ac (9 ha) site dominated by *L. cuneata.* Soil nutrients at the site and in the grassland areas in general are low, especially N and P, which often fall below 5 ppm. The pH range is from 7.3 to 8.1. The 1996 USDA Soil Survey of Muskingum County defines the mined soils in the project area as Morristown silty, clay loam with slopes ranging from 1 to 15 percent. Permeability is moderate to poor and available water capacity is low, resulting in a soil type that is wet in the spring but droughty during the growing season (Steiger 1996).

The method used to conduct the vegetation survey was a stratified random design, using 20 1 m^{-2} plots. Species canopy cover and stem densities were recorded within each plot. Dominance of *L. cuneata* was estimated at <90% canopy cover based on both stem densities and visual estimates of cover class for species within each plot (Barbour et al. 1998). Estimates of species abundances are given in Table 1.

Table 2. Species presence before treatment, throughout trial period, and species planted for prairie establishment

Species present: Spring 2004 before treatment			Species present: Spring 2005 during treatment			Dominant species seeded: Fall 2006 following treatment		
>90%:	*Lespedeza cuneata*	sericea lespedeza	>90%:	*Glycine max*	Roundup Ready soybeans	~50%:	**Grasses**	
<10%:	*Dipsacus sylvestris*	teasel	<10%:	*Festuca elatior*	tall fescue		*Andropogon gerardii*	big bluestem
	Festuca elatior	tall fescue		*Daucus carota*	wild carrot		*Bouteloua curtipendula*	sideoats grama
	Aesclepias syriaca	common milkweed		*Trifolium pratense*	red clover		*Elymus virginicus*	Virginia wild rye
	Circium arvense	Canada thistle		*Chrysanthemum leucanthemum*	ox-eye daisy		*Panicum virgatum*	switchgrass
	Trifolium pratense	red clover		*Ambrosia artemisiifolia*	artemesia		*Schizachyrium scoparium*	little bluestem
	Chrysanthemum leucanthemum	ox-eye daisy		*Meliotus officinalis*	yellow sweet clover		*Sorghastrum nutans*	indian grass
	Ambrosia artemisiifolia	common ragweed		*Trifolium campestre*	hop clover	~50%:	**Forbes**	
	Circium altissimum	thistle		*Trifolium repens*	white clover		*Asclepias syriaca*	common milkweed
	Meliotus officinalis	yellow sweet clover		*Barbarea verna*	winter cress		*Aster lowrieanus*	aster
	Trifolium campestre	hop clover		*Galium aparine*	cleavers		*Aster novae-anbliae*	New England aster
	Trifolium repens	white clover		*Lactuca sp.*	wild lettuce		*Aster pilosus*	frost aster
	Trifolium campestre	hop clover		*Aesclepias syriaca*	common milkweed		*Baptisia alba (leuc/lactea)*	white wild indigo
	Veronica officinalis	common speedwell		*Circium arvense*	canada thistle		*Bidens polylepis*	tickseed sunflower
	Barbarea verna	winter cress		*Veronica officinalis*	common speedwell		*Chamaecrista fasciculata (nictitans)*	partridge pea
				Dipsacus sylvestris	teasel		*Coreopsis tripteris*	tall coreopsis
				Circium altissimum	thistle		*Eriyngium yuccifolia*	rattlesnake master
				Lespedeza cuneata	sericea lespedeza		*Eupatorium maculatum*	joe pye Weed
							Helenium autumnale	sneezeweed
							Helianthus maximiliani	maximilian sunflower
							Heliopsis helianthoides	ox-eye / early sunflower
							Liatris spicata	marsh blazing star
							Monarda fistulosa	wild bergamot
							Ratibida pinnata	yellow coneflower
							Rudbeckia triloba	brown eyed susan
							Silphium terebinthinaceum	prairie dock
							Solida rigida	stiff goldenrod
							Tradescantia ohiensis	Ohio spiderwort

Site preparation began May 1, 2005 when the area was mowed and sprayed with a 2.5 % solution of Roundup WeatherMAX™ herbicide (glyphosate 5.5 lb active ingredient gal^{-1} [0.7 kg a.i. l^{-1}]) sufficient to thoroughly wet the vegetation. All subsequent glyphosate herbicide applications were at this concentration and rate. The area was tilled and planted with Roundup Ready® soybean cultivar Asgrow AG5605 at a rate of 100 lb ac^{-1} (112 kg ha^{-1}). The soybeans were inoculated with a standard soybean *Rhizobium sp.* strain to improve crop establishment. In May, 0-14-42 (N-P-K) fertilizer was applied at a rate of 300 lb ac^{-1} (336 kg ha^{-1}) to improve soil fertility. Glyphosate herbicide was applied for weed control in July and again in September 2005.

The site was sprayed with glyphosate herbicide on June 1st, 2006 prior to planting. Following the herbicide application, the area was surface tilled on June 8 and planted for the second growing season on June 13 with Roundup Ready® soybean cultivar Asgrow AG5501 at a rate of 100 lb ac^{-1} (112 kg ha^{-1}). The soybeans were inoculated with a standard soybean *Rhizobium sp.* strain. The site was sprayed with glyphosate herbicide on August 1 to control *L. cuneata* and other weed species. Over the two year period, a total of five applications of glyphosate were applied; one application each year prior to planting and three applications to the soybean cover crop (Table 2).

Prairie Planting

The interior 19 ac (8 ha) were planted between November 22 and November 28, 2006 with a seed mixture containing a 1:1 ratio of perennial grasses and forbs (based on seed weight) at a rate of 12 lb ac^{-1} (13.5 kg ha^{-1})

Table 2. Management activity using Roundup Ready (RR) Soybeans to prepare site for prairie planting.

Date	Activity	Description
5-01-05	Glyphosate treatment	2.5 % solution of glyphosate (Roundup WeatherMAX™) at a rate of 2.5 gal / 100 gal using a tractor mounted spray boom
5-11-05	Fertilizer treatment	Pelletized N-P-K (0-14-42) fertilizer applied at a rate of 300 lbs / acre using a standard utility tractor
5-17-05	RR Soybean crop planted (1st year)	Roundup Ready Soybeans® varieties Asgrow AG5501 ('06) and AG5605 ('05) at a rate of 100lbs/acre using no-till drill
7-07-05	Glyphosate treatment	2.5 % solution of glyphosate (Roundup WeatherMAX™) at a rate of 2.5 gal / 100 gal using a tractor mounted spray boom
9-06-05	Glyphosate treatment	2.5 % solution of glyphosate (Roundup WeatherMAX™) at a rate of 2.5 gal / 100 gal using a tractor mounted spray boom
3-15-06	Fertilizer treatment	Pelletized N-P-K (6-24-24) fertilizer applied at a rate of 300 lb. / acre using a standard utility tractor
6-01-06	Glyphosate treatment	2.5 % solution of glyphosate (Roundup WeatherMAX™) at a rate of 2.5 gal / 100 gal using a tractor mounted spray boom
6-08-06	Surface till treatment	Site lightly tilled site using a standard utility tractor
6-13-06	RR Soybean crop planted (2nd year)	Roundup Ready Soybeans® varieties Asgrow AG5501 ('06) and AG5605 ('05) at a rate of 100lbs/acre using no-till drill
8-01-06	Glyphosate treatment	2.5 % solution of glyphosate (Roundup WeatherMAX™) at a rate of 2.5 gal / 100 gal using a tractor mounted spray boom
11-22-06– 11-28-06	Prairie seed mix planted	Site planted with seed mixture that included a 1:1 ratio of perennial grasses and forbs (see fig. 2 species list) at a rate of 12 lb. per acre using no-till drill

using a 7' End Wheel Great Plains no-till seed drill (model 706NT) at a planting depth of < ¼ in. The outer perimeter of the site (4 ac [1.6 ha]) was hand-seeded with a combination of mixed short grass and forb species. A total of 47 native species were included in the prairie planting (see Table 1 for dominant species planted).

Results and Discussion

Results show that the use of an herbicide resistant cover crop facilitates application of a broad-spectrum herbicide, effectively controlling *L. cuneata* during the early phase of habitat restoration. Following a two-year cover crop period, *L. cuneata* was not observed in the interior of the site and <5% persisted in the perimeter of the site, as compared to >90% canopy cover prior to treatment (Table 1; Table 2). Our data suggests that using an herbicide resistant cover crop for two consecutive years can be effective in controlling *L. cuneata* prior to prairie establishment.

This trial sought to reduce soil disturbance that could trigger successive waves of *L. cuneata* seed germination by using a conservation tillage system. The no-till drill method allowed the soybean crop residue to remain on the soil surface, which has shown to improve tilth, increase the content of organic matter, and conserve moisture (Steiger, 1996). In addition to helping prevent undesired seed germination, employing the no-till drill technique also helped to reduce soil erosion.

The estimated costs for implementing this project averaged ~\$175 ac^{-1} (~\$432 ha^{-1}). This estimate is based on two crops of Roundup Ready® soybeans and five glyphosate treatments over the course of two years. Additional costs include the purchase of native prairie seeds, which range in price from \$250 to \$1200 ac^{-1} (\$618 to \$2964 ha^{-1}) applied at 10 lb ac^{-1} (11 kg ha^{-1}). Seed costs can vary greatly depending on supplier, grass to forb ratio, and species diversity. The seed used in this trial was acquired through a combination of local collections, donations and purchases. The need for fertilizers will also vary depending on site conditions and may range in cost from \$5 to \$10 ac^{-1} (\$12 to \$27 ha^{-1}).

The use of an herbicide resistant cover crop that facilitates application of a broad-spectrum herbicide has shown to be effective in controlling *L. cuneata* and other invasive species during the early phase of habitat restoration. Competition from the cover crop in combination with herbicide applications controlled *L. cuneata* while also protecting the soil from erosion. Further experimentation is required to fine-tune the application of this method and asses its long-term effectiveness. Further results on the effectiveness of this method will be based on the successful establishment of the high diversity prairie and continued suppression of *L. cuneata*.

Acknowledgments

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Reproductive biology and habitat-dependent pollinator services in *Lonicera maackii*

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Abstract. Most invasive exotic plants require seeds to colonize new locations. Some invasive plants require pollinators to set seed. Variation in interactions with local pollinating mutualists, then, can contribute to rate of spread. Because the extent of pollen-limitation of seed set will depend on the mating system, the reproductive biology of the invasive species could help predict responses to different mutualist scenarios. Pollination services can vary among habitats and locations resulting from variation in abundances or community compositions of pollinators. Individuals or populations that suffer pollen limitation of seed set due to poor pollination services will factor less potently in the spread of that population via seed. On the other hand, individuals or populations that enjoy adequate pollinator services will contribute more to local and long distance colonization. Habitat-dependent reproductive output could focus management strategies on those individuals that make the greatest contribution to spread.

In this study, we investigated the mating system and pollination of *Lonicera maackii* (Rupr.) Herder (Caprifoliaceae), Amur Honeysuckle, in Central Ohio. We also examined how pollination services varied across habitats within populations and across populations. In two Columbus urban forests we studied forest edge and understory invasions of *L. maackii*. In a third rural forest site *L. maackii* was only present in the understory. Because pollinators prefer to forage in light environments, we expected lower pollinator services and lower fruit set in understory habitats. In each site, we located eight experimental plants in each of the invaded habitats (edge or understory). We investigated mating system at one urban site by comparing the proportion of flowers that set seed among bagged flowers, self-pollinated flowers, outcrossed flowers, and open pollinated flowers. We tested for pollen limitation at all three sites by comparing fruit and seed set of open pollinated flowers with outcrossed flowers. We also conducted pollinator observations and collected stigmas from senescent open pollinated flowers to count the stigmatic pollen loads.

Lonicera maackii set little seed (< 3%) when isolated from pollinators, indicating the need for pollinators. Self-pollination resulted in a significantly lower fruit set (21%), compared to outcross-pollinated flowers (63%). Fruit set of *L. maackii* was pollen limited in both urban sites, but not in the rural site. Hand pollination with outcross pollen increased seed set by 1.5-fold and 1.4-fold in the two urban populations, but there was no difference in the degree of pollen-limitation across habitat types. Pollinator visitation per flower and the number of pollen grains deposited per stigma over the lifetime of the flower, however, were higher for plants in edge habitats than understory habitats. In the rural site, understory habitats had visitation rates comparable to those in edge habitats of urban sites, but much higher stigmatic pollen loads.

Lack of concordance between patterns of visitation and pollen-limitation can be explained by variation in the composition of pollinators and their foraging patterns. Urban edge plants were visited primarily by honey bees, which visited more flowers per plant than the native bees dominating the understory habitats. Honey bees probably transfer more geitonogamous (self) pollen than other visitors, and therefore contribute less to fruit production.

Introduction

Invasions of most exotic plants require seeds to colonize new locations. For plants whose seed set depends on pollinators, variation in interactions with local pollinators can influence rate of spread. Plant species that require pollinators to reproduce are expected to have a lower probability of invading (Baker 1955). Self-incompatible plants also face genetic obstacles to establishment and spread (Elam et al. 2007) that are exacerbated by poor pollination services. Because the extent of pollen-limitation of seed set will depend on mating system, knowledge of the reproductive biology of the invasive plant can help predict responses to different mutualist scenarios.

Pollinator services can vary among habitats and location resulting from differences in pollinator abundances or community compositions (Schemske and Horovitz 1984, Herrera 1988, Thomson 2001, Burgess et al. 2006). Inadequate pollination that results in poor seed set could inhibit population growth in the absence of other limiting factors (Parker 1997). Furthermore, the initial spread of all non-clonal invasions and their expansion at the margins of populations are ultimately limited by seed (Parker and Haubensak 2002). Local-scale variation in pollination and seed production among individual invasive plants within a population could result from small-scale habitat heterogeneity. These differences in seed production could be exploited to leverage control efforts by targeting the most productive individuals in the population. Most invasive woody perennials reproduce via seed, prompting us to focus our study of habitat-dependent reproduction on the shrubby invader *Lonicera maackii* (Rupr.) Herder.

A native of China, *L. maackii* is invasive in disturbed forests and fields of the Eastern US (Luken and Thieret 1996). It often dominates the forest understory where it has demonstrated negative impacts on native flora (Miller and Gorchov 2004) and fauna (Leston and Rodewald 2006). The presence of *L. maackii* can reduce growth and flowering of perennial forest herbs in both disturbed and undisturbed deciduous forest (Miller and Gorchov 2004). These effects translated into reduced seed and fruit set of native perennial herbs in disturbed forest only (Miller & Gorchov 2004). *Lonicera maackii* produces large numbers of fragrant flowers that offer copious nectar and pollen (personal observation) and are attractive to a wide variety of insect species. Because *L. maackii* spreads by seed, pollinators may play a large role in its rate of spread. Despite great interest in its control and its potential to strongly interact with resident pollinator communities in the invaded region, little is known about the reproductive biology and pollination ecology of *L. maackii.* Here we report on the pollination and mating system of *Lonicera maackii*, Amur Honeysuckle, in Central Ohio, USA.

Our research addressed four questions: 1) what is the breeding system of *L. maacckii?* 2) Is seed set of *L. maackii* pollen limited? 3) How do pollination services to *L. maackii* vary among populations and between forest edge and forest interior habitats within popu-

lations? 4) Does pollinator species composition or foraging behavior on *L. maackii* differ among habitats in ways likely to affect pollination?

Methods

Our study sites were two Columbus urban forests where *L. maackii* had invaded both forest edge and interior habitats: Ohio State University's Waterman Agricultural Experiment Station and Kenney Park, a Metro Park along the Olentangy River on High Street, and one rural site in Granville, Ohio, where only interior habitats were invaded: Denison University's Biological Reserve. All forests studied consisted of secondary hardwood forest. In each site, we located eight experimental plants in each of the invaded habitats (edge or interior). Because rural habitats likely support larger pollinator populations than urban sites, we expected higher pollinator visitation rates and lower pollen limitation of fruit set in the rural site. Low light habitats in forest interiors likely support lower densities of pollinators because many pollinators prefer to forage in light environments. Pollinator composition may also differ between edge and interior habitats depending on species-specific nesting and foraging preferences. If these differences in pollinator visitation and composition exist and translate into differences in seed set, then edge and interior plants may differ in their contribution to population growth and spread. We hypothesized that pollinator services and fruit set were lower in interior habitats resulting in greater pollen limitation of fruit and seed set than in edge habitats.

Mating system of *L. maackii* was studied only at the Waterman site during spring of 2006. Each of the eight experimental plants were subjected to four treatments applied to whole branches and designated before anthesis (bagged, self, open, and outcross); each branch contained 100 - 200 buds. Fine mesh fabric bags were placed on self and bagged treatments to exclude pollinators. Bagged treatments remained closed throughout the duration of the study. Hand pollinations of all open flowers with pollen from non-experimental flowers on the same plant were performed on self treatments at least every other day. Similarly, hand pollinations were performed for outcross treatments at least every other day, using pollen from a plant at least 5 meters away from the experimental plant. Hand pollinations commenced when the first bud opened and ended when all flowers on the branch had fallen off the experimental branch. Every flower in the respective treatment (either self or outcross) was pollinated each time hand pollinations were performed. Open treatment branches remained un-bagged until all flowers matured and senesced. An additional open pollinated treatment branch was designated for each experimental plant on an adjacent, similarly sized plant as a control for resource allocation. Once all treatment flowers had fallen off, open, outcross, and control branches were also bagged until fruits were collected to protect them from deer browsing or other damage. We collected and counted the fruits in November once they had matured. We counted seeds in ten randomly selected fruits per treatment per plant to estimate the ratio of seeds per fruit.

We assessed pollen limitation on all plants at all sites during spring of 2006 by comparing fruits per flower and seeds per fruit between open, outcross, and control treatments that were applied as described above. Greater fruit or seed set in outcross flowers compared to open pollinated flowers indicated significant pollen limitation.

We assessed pollinator visitation to *L. maackii* at all sites through pollinator observations conducted May 17 through May 27, 2006 on sunny or partly sunny days.

Plants were randomly selected for observation, and approximately 100 adjacent flowers were then selected on the plant. These flowers were observed for 5 minutes during which time all visitors data were recorded to species, if known. If a species was unknown, a voucher specimen was collected along with detailed written description used for later identification. The number of flowers visited by each visitor was also recorded.

An additional measure of pollinator services is pollen deposition on stigmas. We collected ten stigmas from mature, open pollinated flowers on all experimental plants (but from non-experimental branches) at all sites to assess stigmatic pollen load. Stigmas were placed in microcentrifuge vials containing a 70% ethanol solution for storage. Stigmas were soaked in a solution of alcohol and basic fuschin to stain the pollen grains, mounted onto a microscope slide in a drop of glycerine gel, and covered with a coverslip. The number of pollen grains deposited per stigma were counted with a compound microscope.

Analysis

Mating system data were analyzed using mixed model analysis of variance with pollination treatment (bagged, self, and outcross) as a fixed independent variable and plant identity as a random independent variable. Dependent variables were the number of fruits per flower and the mean number of seeds per fruit (from10 fruits per plant). The sample size was sixteen experimental plants at the Waterman site. We used the variance components covariance structure and the maximum likelihood estimation method. Planned mean contrasts tested for specific differences among treatment levels.

Pollen limitation of fruit and seed set was assessed using mixed model analysis of variance on the proportion of flowers that set fruit and the mean number of seeds per fruit. Treatment (open, supplemental pollen, and control), site and their interaction were the fixed independent variable and plant the random independent variable. We also tested for effects of treatment, habitat (edge vs. interior) and their interaction on fruit and seed set for the Waterman site only using mixed model with treatment, habitat and their interaction as fixed independent variables and plant and habitat x site interaction as a random independent variables. Dependent variables were as listed above. The Denison site had only interior plants and the Kenney site experienced considerable mortality of branches in the interior, so both were omitted from the analysis that considered habitat.

Pollen deposition on stigmas was compared across sites and habitats (for Waterman only) using analysis of variance with plant and site as random independent variables. We also tested the effect of habitat on pollen deposition for the Waterman and Kenney sites with a mixed model analysis of variance. Habitat was a fixed independent variable and plant a random independent variable. The number of pollen grains deposited on each of ten stigmas per experimental plant was the dependent variable.

Pollinator visitation was measured as the proportion of observed flowers that received visits and analyzed using mixed model analysis of variance with habitat as the fixed independent variable and site as a random independent variable.

Results and Discussion

Mating system

The results of our mating system study indicated that *Lonicera maackii* is partially self compatible and requires pollinators for full seed set. Bagged flowers set little fruit (< 3%),

indicating the need for pollinators (Fig.1). Self-pollination resulted in a seven-fold greater fruit set than bagged flowers. Outcrossed flowers set three-fold more fruits than selfed flowers (Fig.1A, Table 1). The number of seeds per fruit was significantly greater for outcrossed flowers than self flowers, but bagged and self fruits did not differ significantly in the mean number of seeds per fruit (Fig. 1B, Table 1). The lack of difference in seeds per fruit between bagged and self treatments probably reflects the fact that any fruit set in bags resulted from autogamous self pollination within flowers or

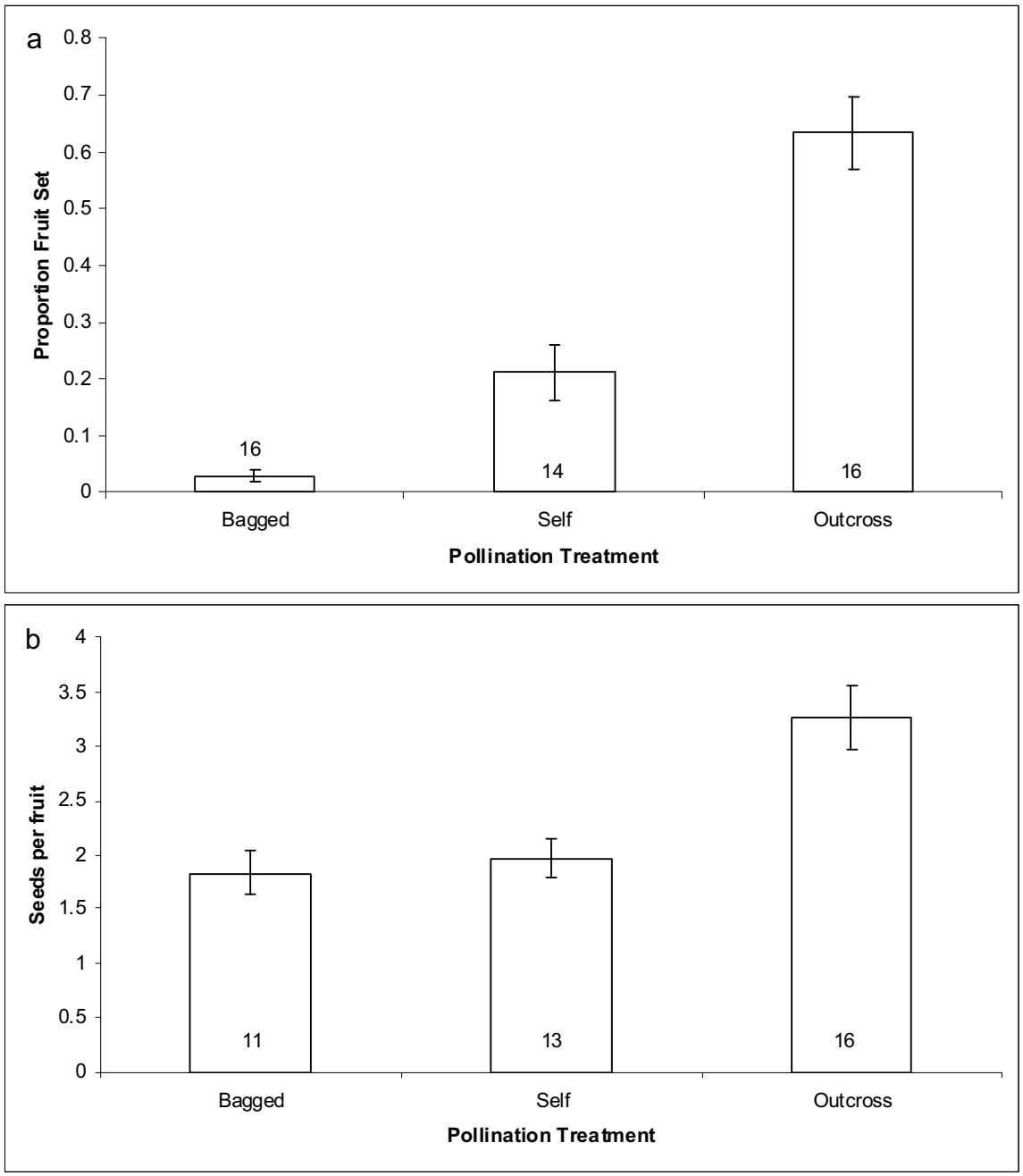


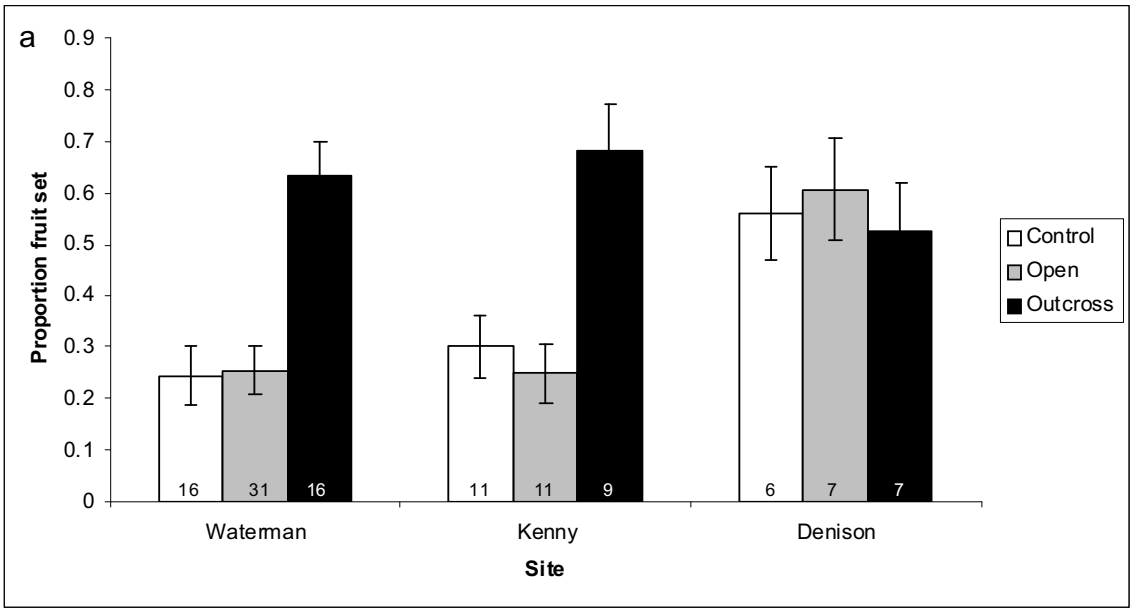
Figure 1. a. Mean fruits produced per flower for three pollination treatments, and b. Mean seeds produced per fruit for three pollination treatments. The data were collected at the Waterman Agricultural Station of Ohio State University, Columbus, Ohio. Error bars are ±1SE. Numbers at base of bars indicate the sample size (number of plants).

Table 1. Mating system analysis. Mixed model analysis of variance table testing the effect of pollination treatment (Bagged, Self, and Outcross) on the proportion of fruits set per flower (arcsine-square root transformed) and the mean number of seeds per fruit. These data were collected from 16 plants at the Waterman Agricultural Station of The Ohio State University, Columbus, OH. Plants that did not set fruits are not included in the seed set analysis.

I. Fruit set			
Effect	DF (Num, Den)	F	p
Treatment	2, 27	79.05	< 0.0001
Contrasts			
Bagged vs. Self	1, 27	17.15	0.0003
Self vs Outcross	1, 27	61.47	< 0.0001
II. Seed set			
Effect	DF (Num, Den)	F	p
Treatment	2, 22	11.53	0.0004
Contrasts			
Bagged vs. Self	1, 22	0.13	0.72
Self vs Outcross	1, 22	15.08	0.0006

Table 2. Pollen limitation of fruit and seed set. Analysis of variance table showing effects of pollination treatment (Open pollinated, supplemental pollination, and control plant) on the proportion of flowers to set fruit (arcsine square root-transformed) and the mean number of seeds per fruit.

I. Fruit set			
Effect	DF (Num, Den)	F	p
Treatment	2, 55	15.64	< 0.0001
Site	2, 34	2.02	0.15
Treatment x site	4, 55	5.31	0.001
Denison			
Treatment	2, 11	0.29	0.75
Kenney			
Treatment	2, 15	29.97	<0.0001
Contrasts			
Open vs control	1, 15	1.13	0.31
Open vs supplement	1, 15	52.65	<0.0001
Waterman			
Treatment	2, 29	21.02	<0.0001
Contrasts			
Open vs control	1, 29	0.00	0.97
Open vs supplement	1, 29	31.41	<0.0001
II. Seed set			
Effect	DF (Num, Den)	F	p
Treatment	2, 55	1.86	0.1646
Site	2, 31	1.16	0.3255
Treatment x site	4, 55	0.94	0.4464



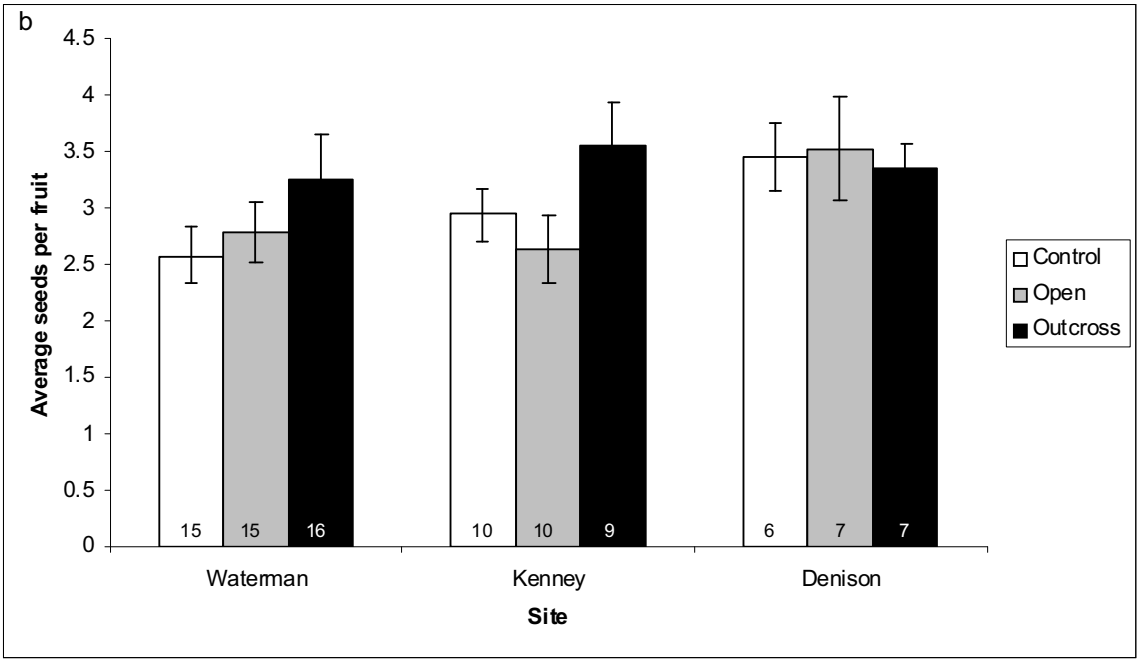


Figure 2. Pollen limitation expressed as a) mean fruits produced per flower and b) mean number of seeds per fruit for three pollination treatments. Control branches were on neighboring plants. Error bars are ±1SE. Numbers at base of bars indicate n.

self pollen transferred between flowers on the same branch when flowers were knocked together by wind.

Pollen limitation

Control and open treatments did not differ in fruit or seed set, indicating that *L. maackii* pollen limitation is not a result of internal reallocation of resources (Table 2, Fig. 2). Analysis of the data for pollen limitation of fruit set indicated a significant site by treatment interaction (Table 2) so sites were analyzed separately for effects of treatment on the proportion fruit set. Fruit set of *L. maackii* was significantly pollen limited in both urban sites with proportion of fruit set per flower for outcross flowers 2.5 and 2.7 –fold greater than that for open pollinated flowers for Kenney

Table 3. Pollen limitation of fruit and seed set in edge and interior habitats. Analysis of variance table showing the effects of pollination treatment and habitat on the proportion of flowers to set fruit (arcsine square root-transformed) and the mean number of seeds per fruit. The data were collected at the Waterman Agricultural Station.

I. Fruit set			
Effect	DF (Num, Den)	F	p
Habitat	1, 27	31.78	< 0.0001
Treatment	2, 27	18.78	< 0.0001
Treatment x Habitat	2, 27	0.27	0.77
Contrasts			
Open vs control	1, 27	0.05	0.65
Open vs supplement	1, 27	25.47	< 0.0001
II. Seed set			
Effect	DF (Num, Den)	F	p
Habitat	1, 26	7.04	0.01
Treatment	2, 26	3.19	0.06
Treatment x habitat	2, 26	1.02	0.37
Contrasts			
Open vs control	1, 26	0.13	0.10
Open vs supplement	1, 26	15.08	0.02

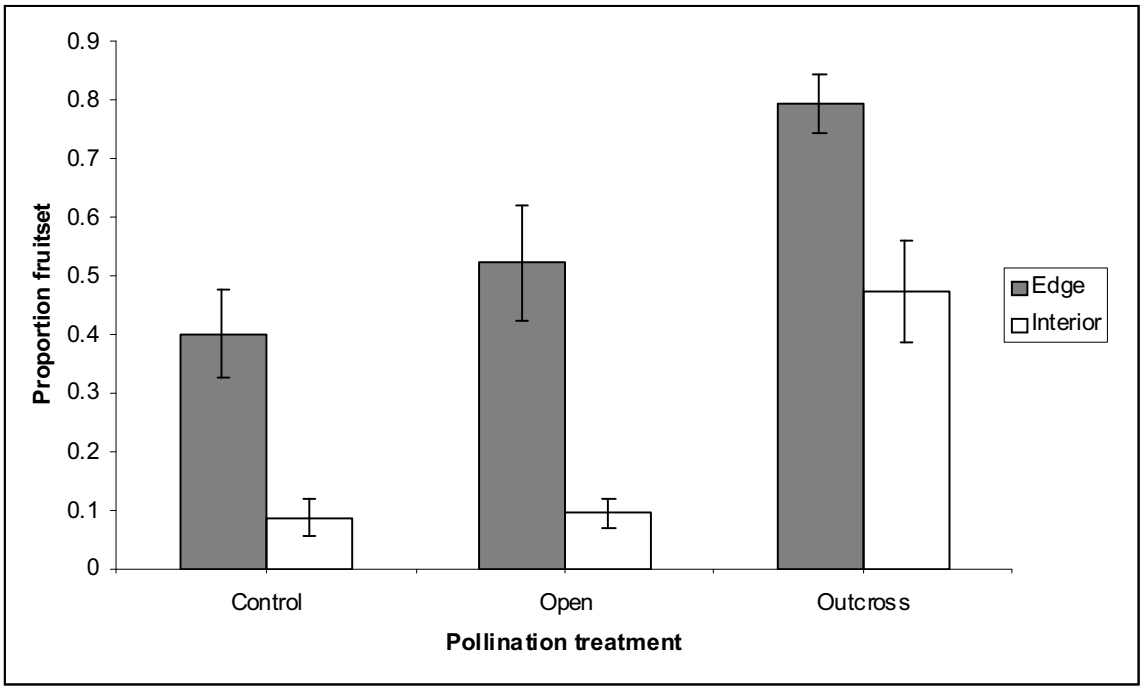


Figure 3. Pollen limitation across habitats. The proportion of fruits set per flower for plants in interior and edge habitats shown for control, open pollinated, and outcrossed branches at the Waterman Agricultural Experiment Station of Ohio State University. Error bars are ±1SE. Sample sizes (number of plants per habitat per treatment) are n = 8 for all but the Edge/open treatment, for which n = 7.

and Waterman, respectively (Table 2, Fig 2a). The rural site, Denison, showed no evidence of pollen limitation of fruit set (Table 2, Fig 2a). The number of seeds per fruit did not differ significantly between outcross and open treatments, nor were there significant effects of site or site by treatment interaction (Table 2, Fig 2b). The effect of habitat on pollen limitation could only be evaluated for the Waterman site because insufficient outcross branches survived for the interior habitat at Kenney. There was no difference in pollen limitation between the edge and interior habitats, as indicated by the non –significant treatment x habitat interaction (Table 3, Fig. 3). Interestingly, there was a significant effect of habitat on proportion of fruit set. Flowers on edge plants were 2.6 times as likely to set

Table 4. The average number of bees observed foraging on *L. maackii* flowers and the proportion of bees that were *Apis*. See Fig. 5 for sample size. The remaining bees observed were native species of 6 genera.

		Proportion *Apis*	
Site	Bees/min	Edge	Interior
Waterman	0.14	0.80	0.00
Kenney	0.13	0.33	0.00
Denison	0.15		0.22

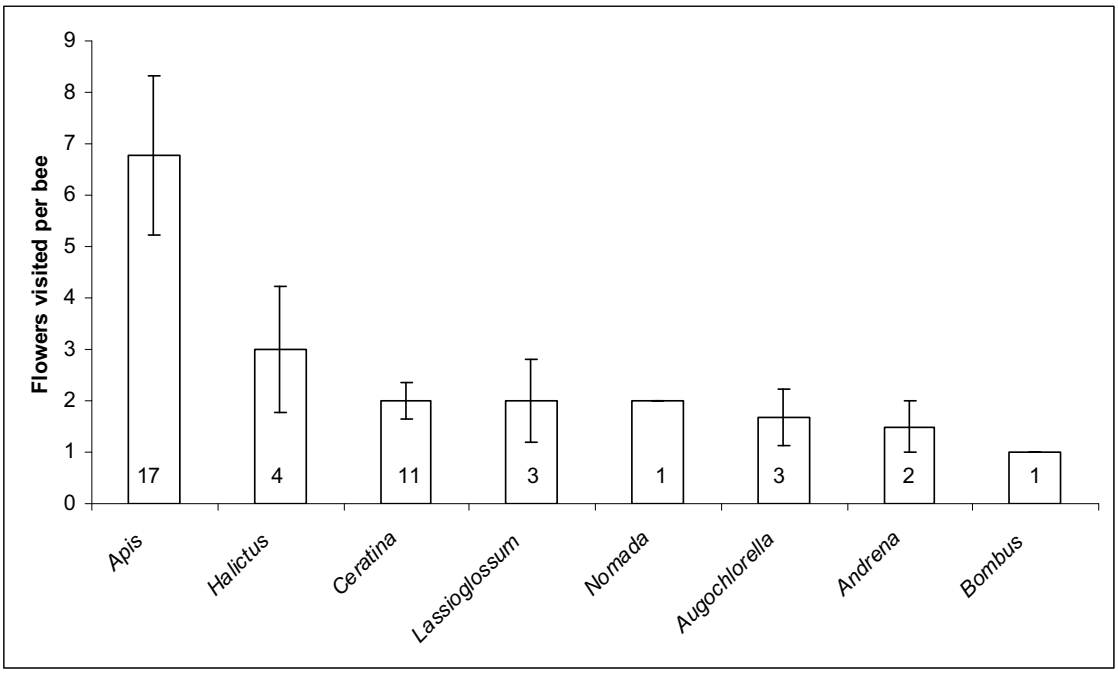


Figure 4. The average number of flowers visited on an observed branch per bee during the 5 min observation period shown for eight genera. Error bars represent ± 1 SE. Numbers at the base of bars are the number of bees observed.

Table 5. Analysis of variance table showing effects of site and habitat on the proportion of observed flowers that received visits.

Effect	DF (Num, Den)	F	p
I. All sites			
Site	2, 54	1.88	0.16
II. Urban sites only			
Site	1, 80	4.64	0.03
Habitat	1, 80	1.57	0.21
Site x Habitat	1, 80	1.50	0.22

Table 6. Analysis of variance table showing effects of site and habitat on the number of pollen grains deposited on stigmas of *Lonicera maackii* flowers at I. All sites and II. Urban sites where *L. maackii* grew in both edge and forest interior habitats.

Effect	DF (Num, Den)	F	p
I. All sites			
Site	2, 339	47.15	<0.0001
II. Urban sites only			
Habitat	1, 260	15.60	0.0001
Site	1, 260	0.20	0.65
Site x Habitat	1, 260	0.14	0.71

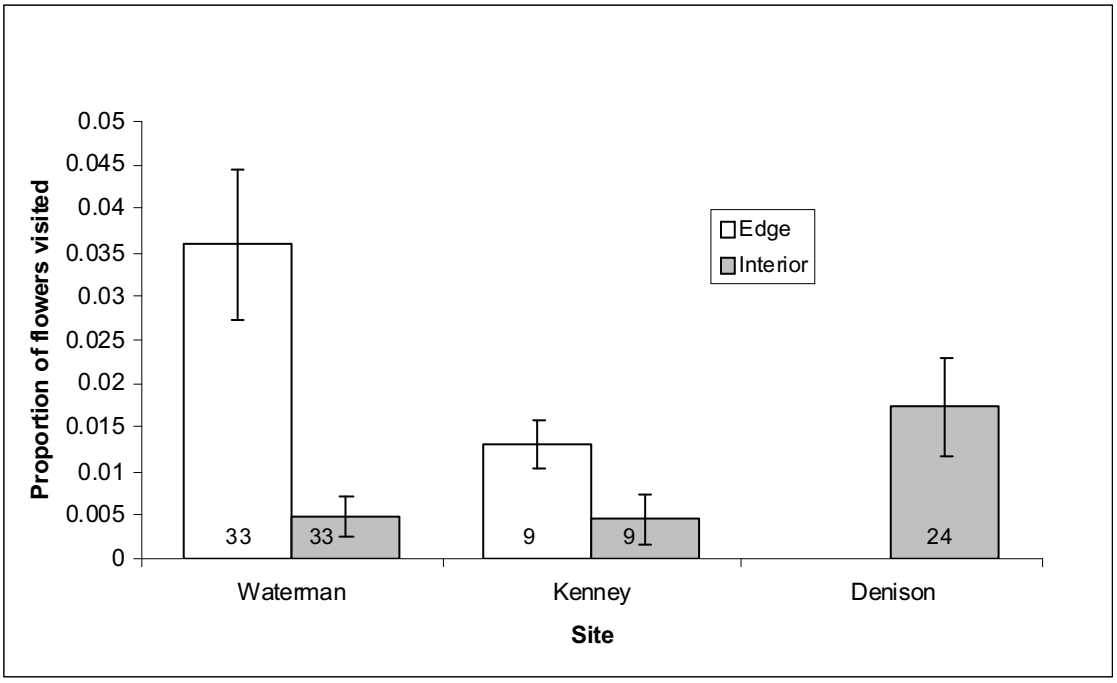


Figure 5. The proportion of observed *L. maackii* flowers that received visits during 5 min observation series.. The error bars are ± 1 SE. The numbers at the base of the columns represent sample sizes (number of observation series).

fruits than those in the interior. This result suggests underlying differences in resource limitation of fruit set between edge and interior habitats irrespective of pollination treatment. These differences could reflect the much lower light availability in the understory environment than the edge during the flowering period of *L. maackii* (KG personal observation).

Pollinator visitation

Representatives of eight bee genera visited *L. maackii* flowers during a total of 330 minutes of observations, and native bees tended to visit fewer flowers per plant than *Apis* (Fig. 4). There was no significant difference among sites in the proportion of flowers visited over 5 minutes, but there was a significant difference among habitats (Table 5). Flowers in edge habitats were more than 6 times as likely to be visited as those in interior habitats (Fig. 5).

Numbers of pollen grains deposited per stigma over the lifetime of the flower were also higher for plants in edge habitats (Fig. 6, Table 6). We found significant differences among sites in the number of pollen grains deposited as well (Table 6). Higher visitation rates corresponded with larger pollen loads on stigmas in forest edge habitat at urban sites. The rural site, Denison, had a 2 – 2.5-fold higher pollen deposition on stigmas than the urban sites. The urban sites did not differ in pollen deposition. Urban edge plants were visited primarily by honey bees (Table 4), with honey bees comprising 80% of total visitors at Waterman. In the rural site, understory habitats had visitation rates comparable to those in edge habitats of urban sites, but much higher stigmatic pollen loads (Fig. 5). Our data suggest that differences in the pollinator abundances and potentially composition between the urban sites and the rural site lead to differences in visitation, pollen deposition and pollen limitation of seed set. Because we only have data for one rural site and two urban sites, we can not make general statements yet about the pollination of *L. maackii* along a rural to urban gradient.

Although pollination is important to *Lonicera maackii* reproduction because it requires pollinators to set seed, identity of pollinators is also important because of variation in their foraging behavior. Honey bees were the most abundant visitors, but tended to visit more flowers per plant than other bee species (Table 4). Therefore, honey bees probably transferred more gietonogamous (self) pollen than other species. Differences in foraging patterns among bee species likely explains the lack of concordance between patterns of visitation and pollen-limitation between forest edge and interior habitats at the Waterman site. The predominantly native bees visiting interior plants may have delivered better quality pollen (higher proportion of outcross pollen) than the predominantly honey bee visitors along the edge. The honey bee visits, albeit more numerous than the native bee visits to interior plants, likely contribute less to fruit production. Similarly, higher pollen quality could compensate for lower frequency of visitation in the interior habitat and lead to similar fruit and seed set. Although exotic bees have been hypothesized to contribute to plant invasions, these data suggest that native bees are better quality pollinators for this exotic plant. In sites with a low abundance of native bees however, exotic honey bees may facilitate the invasion of *L. maackii.*

From a management perspective, despite similar levels of pollen limitation across habitats, plants invading the interior of forests tend to have fewer branches and flowers, and produce fewer fruits per flower than edge plants. Therefore, edge plants may contribute more to overall spread and population growth of this species. Focusing control and eradication programs on plants growing along edges or in open areas could enhance control efforts.

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Genetic analysis of Callery Pear cultivars to determine the origin of invasive populations

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Abstract. Studies of invasive species usually occur only after extensive ecological or economic impacts are noticed. Introduced species in the beginning stages of invasion are rarely studied, mainly because they are difficult to identify. To understand why species become invasive, it is necessary to examine introduced species that are beginning to form invasive populations. The ornamental tree, *Pyrus calleryana*, appears to be such a case. Originally introduced into the United States from China, the Callery Pear is represented today by at least 25 different horticultural varieties (cultivars) frequently planted within residential and commercial areas. Within the last ten years, this ornamental species has begun to spread and wild populations are now present in many states. It has been suggested that wild populations result from crossing between various Callery Pear cultivars. The objectives of this study were to genetically identify commercially available cultivars and perform a preliminary analysis to determine if they are parents of wild individuals. Using nine microsatellite loci, cultivars and one wild population were genetically characterized. All individuals within a cultivar were genetically identical and most cultivars were genetically different from one another. Parentage analysis revealed that a mixture of cultivar genotypes was present in the wild population, indicating direct parentage from neighboring cultivars. This is consistent with predictions that wild populations of *Pyrus calleryana* originate from cross-pollinations between genetically different ornamental cultivars planted nearby.

Introduction

Pyrus calleryana is a popular ornamental tree originally from China that is now spreading into disturbed and early successional areas across the United States. The species was originally introduced to breed fire blight resistance and provide compatible rootstock for *P. communis*, the common edible pear. It is now one of the most common ornamental trees in the United States, and is currently

Figure 1. Callery Pear cultivar planted in a residential area near Cincinnati, OH. These trees bloom profusely in the early spring and typically give off a malodoriferous fragrance that attracts many types of generalist pollinators.

represented by over 25 different cultivated varieties (cultivars) frequently planted in residential yards and commercial areas (Fig. 1; Culley and Hardiman, in press). The original 'Bradford' cultivar, developed in the 1960's, is still sold throughout the United States, as are other cultivars such as 'Chanticleer' which was named the 2005 Urban Tree of the Year (Phillips 2004). The species itself was originally thought to be of limited invasive potential and produced few fruits due to self-incompatibility. Within the last 10 years, however, fruit set on cultivars has often been observed and wild populations of *P. calleryana* have been identified in natural areas across 23 states (Vincent 2005). Currently, the Callery pear is listed by the U.S. Fish and Wildlife as a plant invader of Mid-Atlantic natural areas (Swearingen *et al.* 2002) and is considered a problem invasive weed of the Northeast (Bravo and Curran 2003). It is also listed as an invasive, exotic species in many states, and has recently been observed in many areas of Ohio including parks, restored prairies, and along transportation corridors (N. Hardiman & T. Culley, pers. observ.).

Individual trees of *Pyrus calleryana* are self-incompatible, meaning that they cannot produce fruit on their own but must rely on pollen from a genetically different tree. Consequently, wild populations of *Pyrus calleryana* have been suggested to result from cross-pollination between different cultivars planted in the surrounding area (Vincent 2005). Thus *P. calleryana* may serve as an ideal system of intraspecific hybridization, defined as crossing between genetically different populations of the same species. Although intraspecific hybridization is a mechanism that may contribute to the formation of invasive species (Ellstrand and Schierenbeck 2000), it has not been well studied until now. Hybridization may benefit introduced species by increasing genetic variation in hybrid populations, from which novel genotypes may have increased fitness or establishment ability (Lee 2002). Because fruits can be produced in crosses between cultivars of *P. calleryana* (Hardiman and Culley, unpubl. data), intraspecific hybridization may be key in the production of wild populations of this species.

To understand if crossing between Callery Pear cultivars has contributed to the spread of the species in the United States, the cultivars must first be genotyped. Thus the purpose of this study was to genetically characterize commercially available cultivars of Callery Pear using microsatellite markers and ultimately to create a cultivar reference database. This information can then be used to examine the parentage of wild individuals to determine if they result from crossing between nearby cultivars. This study tested the following hypotheses: (1) individuals within a given cultivar are genetically identical; (2) cultivars are genetically distinct from other cultivars; (3) planted trees in residential neighborhoods consist of a variety of cultivars; (4) wild individuals are the hybrid offspring of cultivars planted in the surrounding neighborhood.

Methods

Microsatellite markers are repeated nucleotide sequences of DNA that can be found throughout the genome and consequently are extremely useful for genetic studies. To examine genetic variation and differentiation among *P. calleryana* cultivars, this study used nine microsatellite primer sequences initially developed for related species and genera (Table 1; Guilford *et al.* 2001, Yamamoto *et al.* 2002, Gianfranceschi *et al.* 1998, Mnejja *et al.* 2004).

To first create the cultivar reference database, leaf tissue samples were obtained from several cultivars ('Bradford', 'Cleveland Select',

Table 1. Microsatellite primers used in *Pyrus calleryana*. Given are the repeat, reference and original species for which primers were developed (denoted by asterisks) or tested for amplification.

Primer Name	Repeat	Reference	Species Developed In
KA16	$(ct)_4t(tc)_{17}$	Yamamoto *et al.* 2002	*Pyrus pyrifolia*, P. bretschneideri, P. ussuriensis, P. communis**
KU10	$(ct)_{20}$	" "	
CH01F02	$(ag)_{22}$	Gianfranceschi *et al.* 1998	*Malus x domestica*, M. floribunda*
CH01H01	$(ag)_{25.5}$	" "	
CH01H10	$(ag)_{21}$	" "	
CH02B03b	$(ga)_{22}$	" "	
CH02B10	$(ga)_{19.5}$	" "	
CH02D11	$(ag)_{21}$	" "	
CH02D12	$(ga)_{19}$	" "	

'Aristocrat', 'Redspire', 'Capital', 'Chanticleer', and 'Autumn Blaze') that were being sold commercially or planted in a Callery Pear planting at Heritage Park, Westerville, OH. Samples were also acquired from the National Clonal Germplasm Repository in Corvallis, Oregon ('Stonehill', 'Early Red', 'Avery Park', 'Valzam', 'Princess', 'Grant St. Yellow', and 'Whitehouse'). The cultivar 'Faurie' was also included even though it is a separate species (*P. fauriei*) because it is sometimes marketed as a Callery Pear cultivar. For each of five cultivars ('Bradford', 'Cleveland Select', 'Redspire', 'Aristocrat' and 'Capital'), it was possible to sample at least 10 individuals which were used in additional analyses. To test the use of the cultivar reference database, leaf samples were also obtained from a wild pear population located at the Harris M. Benedict Botanical Preserve (known locally as Hazelwood) in northeastern Cincinnati, OH. Pears at this site are growing in an area of the preserve that was formerly a mature deciduous forest but was destroyed in 1999 by a tornado. Samples were also collected from cultivated trees in the residential and commercial areas immediately surrounding the preserve.

DNA was extracted from leaf tissue and amplified in the laboratory with the Polymerase Chain Reaction (PCR) using the Qiagen Multiplex kit. PCR products were then analyzed on a sequencing machine to generate the genetic information. Alleles for each of the nine loci were first compared within and among cultivars to identify cultivar-specific genotypes and create the cultivar reference

database. Standard measures of genetic variation for each cultivar were then quantified. Genetic differences among cultivars with multiple sampled individuals was examined with θ (Weir and Cockerham 1984), a statistic of genetic differentiation that ranges from 0 (no genetic differences) to 1 (all cultivars completely different from one another). To visualize genetic relationships among cultivars, a Principle Coordinates Analysis (PCoA) was constructed using the GenAlEx software program (Peakal & Smouse 2006). Tightly-grouped clusters indicate individuals and/or cultivars that are genetically similar. Finally, the utility of the cultivar reference database was tested by determining the parentage of the wild Callery Pear population using the Cervus software program. Callery Pear trees planted in the residential area surrounding Hazelwood were identified to cultivar type using GeneClass2 software.

Results

Using the nine microsatellite primers, descriptive population genetic statistics were generated for all cultivars (Table 2), indicating substantial genetic variation across cultivar types. The average percentage of polymorphic loci (i.e. those that contained more than one allele) was 0.77 (range: 0.40-1.00) and the average number of alleles was 1.78 (1.40-2.10). The mean proportion of individuals who were heterozygotes (H_o) was 0.74 (0.40-1.0), and mean expected heterozygosity under conditions of equilibrium (H_e) was 0.52 (0.20-0.80). Eight loci did not statistically deviate from Hardy-Weinberg equilibrium in any of the groups.

Table 2. Descriptive population statistics for *Pyrus calleryana* cultivars. Shown is the number of individuals (*N*), percent polymorphic loci (*P*) in which more than one allele is present, average number of alleles per locus (*A*), observed heterozygosity (H_o), and expected heterozygosity based on Hardy-Weinberg equilibrium (H_e).

Cultivar	*N*	*P*	*A*	H_e	H_o
Cleveland Select	22	0.9	1.9	0.4	0.8
Aristocrat	19	1.0	2.1	0.5	1.0
Bradford	11	0.8	1.8	0.4	0.8
Redspire	11	0.9	1.9	0.2	0.4
Capital	10	0.8	1.8	0.4	0.8
Chanticleer	4	0.8	1.8	0.4	0.8
Autumn Blaze	3	0.9	1.9	0.5	0.7
Stonehill	2	0.8	1.8	0.5	0.8
Avery Park	1	0.6	1.6	0.6	0.6
Early Red	1	0.7	1.7	0.7	0.7
Valzam	1	0.8	1.8	0.8	0.8
Princess	1	0.4	1.4	0.4	0.4
Grant St. Yellow	1	0.7	1.7	0.7	0.7
Whitehouse	1	0.7	1.7	0.7	0.7

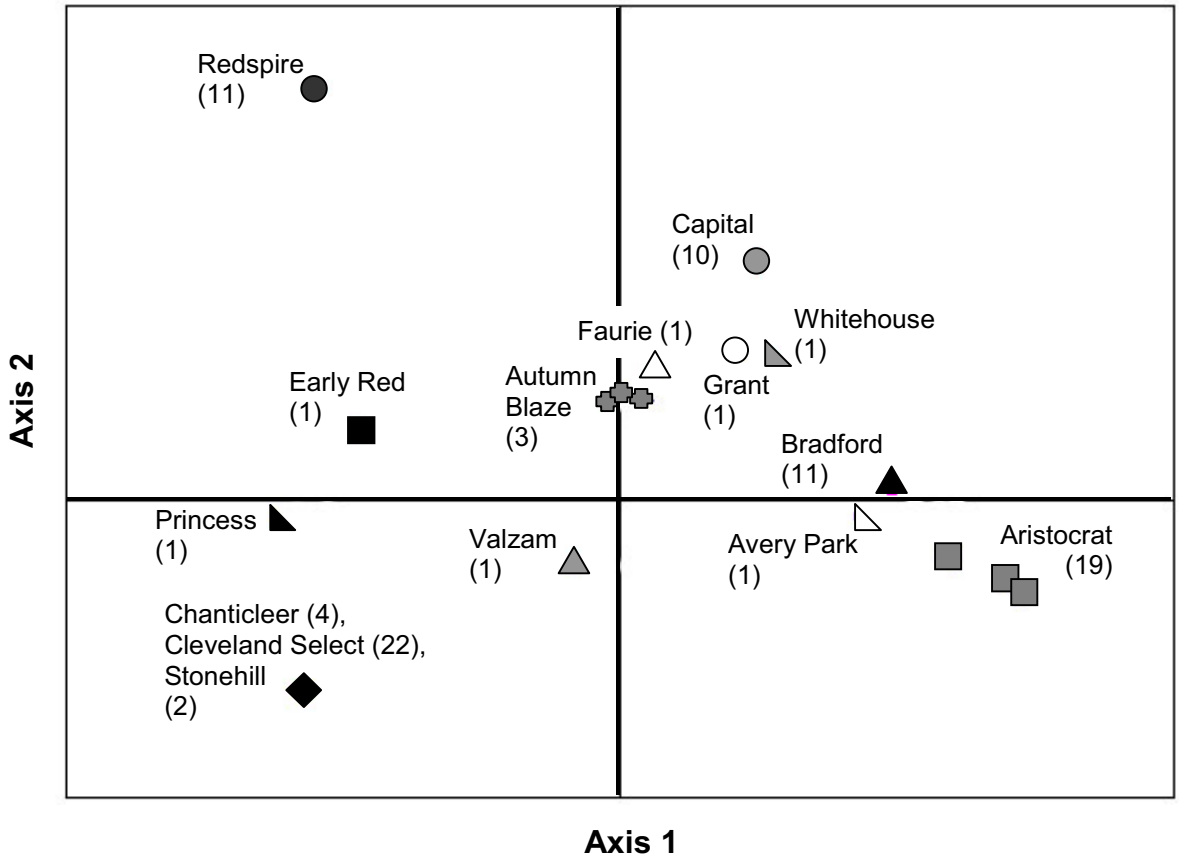

Figure 2. Principle Coordinates Analysis of all available cultivars. The number of individual samples of each cultivar is shown in parentheses below the name, and the first and second axes represent 45.55% and 16.73%, respectively, of the variation. Distance between cultivars, along with the tight grouping of individuals of the same cultivar, indicates that cultivars are genetically distinct.

All cultivars of *P. calleryana* differed genetically from one another at several loci, with three exceptions. 'Chanticleer', 'Cleveland Select', and 'Stonehill' were all genetically identical, which is consistent with a suggestion that these cultivars originated as clones of the same street tree in Cleveland, OH (Santamour and McArdle 1983). The genetic data did not support the reported parentage of certain cultivars; for example, 'Redspire' is reported to have had a 'Bradford' parent but they shared an allele at only two of the nine loci. Our analysis also indicated that mislabeling had occurred in 11.5% of the five cultivars for which multiple individuals had been obtained from commercial sources. For example, two trees marketed as "Redspire' were actually 'Chanticleer' and three trees being sold as 'Bradford' were genotyped as 'Aristocrat'.

The subsample of cultivars represented by multiple individuals ('Bradford', 'Cleveland Select', 'Redspire', 'Aristocrat' and 'Capital') were genetically differentiated from one another, as indicated by an overall θ of 0.514, which was significantly different from zero (upper and lower CI = 0.579-0.445). θ generated for individual pairs of these cultivars ranged from 0.418 to 0.661 (Table 3), indicating that all cultivars were genetically different from one another. PCoA showed that cultivars exhibited tightly grouped clusters (Fig. 2), indicating little to no variation within individuals of the same cultivar (with the exception of the mislabeled individuals noted earlier). Conversely, different cultivar types were distinct from each other, a further indication of genetic differences among cultivars.

Table 3. Coefficients for θ (above diagonal), a measure of genetic differentiation, for five *Pyrus calleryana* cultivars with multiple samples. 'Cleveland' refers to the cultivar 'Cleveland Select' and also includes 'Chanticleer' and 'Stonehill'. *represents a statistically significant difference from zero at P<0.05.

	Aristocrat	Bradford	Cleveland	Capital	Redspire
Aristocrat	-	0.418*	0.512*	0.455*	0.596*
Bradford		-	0.542*	0.535*	0.661*
Cleveland			-	0.526*	0.463*
Capital				-	0.629*
Redspire					-

Of the Callery Pear trees planted in the residential and commercial areas surrounding the Harris M. Benedict Botanical Preserve, 96% could be identified to cultivar type. More importantly, a mixture of cultivars was present (Fig. 3), consisting primarily of 'Bradford', followed by 'Aristocrat', 'Redspire', and 'Chanticleer'. Many of these cultivar genotypes were also represented in the nearby wild population (Fig. 3) and 44% of wild individuals had at least one identified cultivar parent. For example, most of the residential and commercial trees were the 'Bradford' cultivar, which also was represented in the majority of wild trees for which a parent would be identified. Additional analyses have revealed that the majority of wild individuals are F_1 hybrids whose parents are cultivars planted in the surrounding residential area. It is likely that the remainder of wild individuals

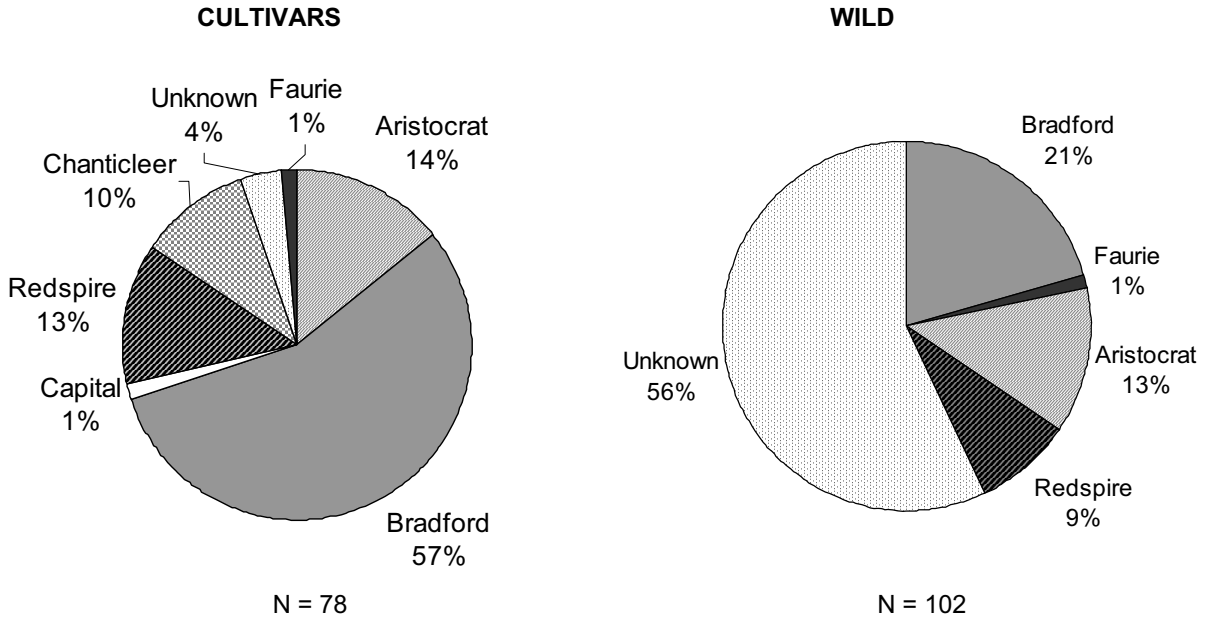

Figure 3. Percentage of Callery Pear cultivars identified in residential and commercial areas surrounding the Harris M. Benedict Botanical Preserve (left) and the proportion of cultivar genotypes identified as one parent of each of the wild individuals sampled in the nearby wild population (right).

may have a different unsampled cultivar parent or may represent F_2 or more advanced generation hybrids (Culley and Hardiman, unpubl. data). A more extensive genetic analysis of this population is still ongoing.

Discussion

Evidence from our study supports the suggestion that wild populations of *Pyrus calleryana* have originated from crossing between genetically distinct Callery Pear cultivars (Vincent 2005). This is likely because of the widespread popularity of this ornamental tree and the tendency for it to be planted in mixed populations of cultivars. The mislabeling of cultivars being sold to the general public will also contribute to the variety of cultivars being planted in a given location. It is also possible that cross-pollinations may involve other related species, as suggested by the appearance of the 'Faurie' genotype (*P. fauriei*) in the wild Ohio population and reports of possible hybridization with *P. betulifolia* or *P. bretschneideri* (Vincent 2005). A third contribution to the formation of wild populations not examined here is possible cross-pollination between the upper cultivar genotype (the scion) and the lower rootstock of grafted cultivated individuals, if the rootstock is allowed to sprout and flower; in these cases, the rootstock is usually an unrelated *P. calleryana* genotype (Culley and Hardiman, unpubl. data). All of these scenarios are now being examined in greater detail.

While specific cultivars of *P. calleryana* are difficult to identify based on morphological measures, it has been shown that they can be identified with substantial reliability using genetic markers and only a single leaf sample. The genetic variation observed among cultivars may reflect the fact that they represent different Chinese genotypes selected because of specific traits (floral production, branching pattern, environmental tolerance). These individuals were then cloned, grafted onto rootstock, and sold as separate cultivars. Based on the cultivar reference database, it is now apparent that Callery Pear trees planted in residential and commercial areas today represent a mixture of cultivars. This is true for the Ohio population of wild individuals described here, as well as several other wild populations across the United States (Hardiman and Culley, in preparation).

It is noteworthy that very little variation was found among individuals within the same cultivar. In the few cases where intra-cultivar variation was observed, these trees were later identified as either mislabeled cultivars or if not, they were always obtained from a single nursery. This could possibly indicate the presence of a somatic mutation that occurred during the clonal propagation process of this particular stock, which was then distributed to the nursery. Future research will focus on understanding the circumstances under which variation within a cultivar can arise.

This study has also shown that microsatellite markers can be applied across related genera and species. Because the work involved in developing new microsatellite markers can be expensive and time-consuming, the possibility of using primers already developed in related taxa offers a distinct advantage and appeal. Thus researchers interested in a new species may be able conduct a genetic project much more quickly and take advantage of existing research.

Future analysis using these microsatellite loci will focus on a greater number of wild populations to determine the parentage of each wild individual and the relative contribution of the various cultivars to the populations as a whole. Hybridization, either through reproduction between different populations or multiple introductions of exotic species, may

be a key mechanism for the evolution of invasiveness following introduction (Ellstrand and Schierenbeck 2000). Identifying the potential for hybridization and the evolution of invasiveness will be critical for the prediction and prevention of new and future invasive species.

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Investigating the possible role of roadways in the dispersal of the invasive shrub Amur Honeysuckle (*Lonicera maackii*) in southwestern Ohio.

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Abstract. The Amur Honeysuckle (*Lonicera maackii*) was introduced into North America in arboreta from its native northeast Asia approximately one hundred years ago. However, with time, it escaped cultivation and became invasive, spreading and displacing native flora in the eastern United States and parts of Canada. Today, it is considered noxious in 24 states in the eastern United States. This study was undertaken to determine the mode of spread of the honeysuckle in southwestern Ohio, focusing mainly on the role of disturbed areas, particularly roadways, in aiding its dispersal. Surveys of the presence and the density of the shrub were conducted along major and minor roadways radiating out from the city of Dayton. Locations of discrete fronts (sites along a roadway beyond which the shrub was no longer seen), were determined by recording the geographical coordinates with a Global Positioning System (GPS) unit. Fronts were found to the north, northeast, east and southeast of Dayton. The distribution was very extensive to the south and southwest of Dayton, showing no readily discernible front in a survey radius of over 80 km (50 miles) in these directions. This pattern of distribution is consistent with the reported dispersal of the shrub from centers of origin in the Oxford, Ohio area and southern Hamilton County, where *L. maackii* was first introduced in horticultural plantings in the 1960s. Several authors have suggested that invasiveness was probably enhanced by the release of genetically selected varieties such as Rem-Red and Cling-Red in the early 1970s. In order to determine whether the phenotype of these cultivars is indeed enriched in populations of bush honeysuckle at the leading edges of the invasion, and to determine gene-flow patterns along the roadways, leaf-samples were gathered in this study for further molecular studies.

Introduction

A plant species introduced from its native habitat to a foreign location often appears to pass through an initial lag period growing under unfamiliar conditions. If it survives this phase and reproduces with a sustained fitness, it is said to be naturalized. However, with time, some exotic species manage to acclimatize so well that they begin to out-compete those that are native, displacing them and thus, decreasing biodiversity in plant communities. Amur Honeysuckle (*Lonicera maackii,* (Rupr) Herder), native to northeastern Asia, was introduced to North America by way of Europe in the late 1800s. It escaped cultivation from the Morton Arboretum in Chicago in the mid-1920s (Luken and Thieret 1996) and was so successful in becoming established that the invasive nature of this plant was recognized as early as 1960 (Braun 1961). In 1970, naturalized plants were collected in Maryland and a genetically selected strain of *L. maackii*

was introduced as "Rem-Red" by the U.S. Department of Agriculture Soil Conservation Service (Natural Areas Conservation Service), Cape May (New Jersey) Plant Materials Center. This selection was actively promoted as a multipurpose landscape shrub with beneficial properties such as cover to help preserve the environment, a windbreak, and a source of nesting sites and food for songbirds (Sharp and Belcher 1981). In 1971, another selection from Asia was introduced by the Elsberry Missouri Plant Materials Center as "Cling-Red" with similarly advertised uses. Some researchers have suggested that selection of vigorous cultivars such as Rem-Red and Cling-Red could conceivably have increased the likelihood of invasiveness of Amur honeysuckle (Hutchinson and Vankat 1997).

In the 1960s, *Lonicera maackii* was introduced in horticultural plantings in the Oxford Ohio area and southern Hamilton County. Studies indicate that the shrub is spreading from multiple foci (Hutchinson and Vankat 1997, Bartuszevige 2004). It has been reported to show high density along well-developed roads (Gelbard and Belnap 2003, Flory and Clay 2006). A recent study concludes that density of Amur honeysuckle is enhanced by the amount of connectivity, spatial arrangement of corridors, and the amount of edge in the landscape (Bartuszevige 2004). Construction and maintenance of roadways disturb land on either side of the road, forming a corridor of edges that frequently contain bush honeysuckle. Overhead power lines and trees along many roadsides provide attractive bird perches. These factors make roadways a logical agent for the distribution of Amur honeysuckle from foci where the shrub is abundant. Addressing this lead, the present research conducted a series of surveys to study patterns of distribution of Amur honeysuckle along roadways in south-western Ohio.

Material and Methods

Surveys were conducted to study the presence and density of Amur honeysuckle along both major arteries and rural roadways radiating from the city of Dayton, including Interstate highways I-75 and I-70, United States routes U.S. 22, U.S. 35, U.S. 42, U.S. 68, and U.S. 127, and State Routes OH 48, OH 49, OH 63, and OH 73. The roads radiating in all directions from Dayton were selected to map the distribution of honeysuckle. From June to December 2005, and July through November, 2006, a total of 38 trips were made along different roadways by car. Amur honeysuckle plants were readily identified by the unique nature of the growing tips in the summer and the red berries in the fall. A front was defined as the last sighting of a single plant or stand along a given roadway and the subsequent absence on that route for a distance of at least 15 km (9.4 miles) in the direction traveled. Geographic coordinates on these fronts were recorded with a Global Positioning System (GPS) unit. Coordinates for established stands of the Amur honeysuckle were also recorded for stands selected along the way. Additional data were also recorded such as plant size, stage of development, location with respect to the road, the surrounding plant community and signs of possible disturbance in the soil at the sites. Leaf samples were collected from each front and from established stands along the way for later DNA-extraction and gene-flow studies.

Results

A total of 38 samples were collected and nine fronts were noted on roadways leading out of Dayton. Distinct fronts were found to the north, northeast and southeast of the city (Table 1, Figure 1). The area east of Dayton after about 40 km (25 miles) all the way to the Columbus area, about 75 km (46.9 miles) due east of Dayton, is largely free of Amur

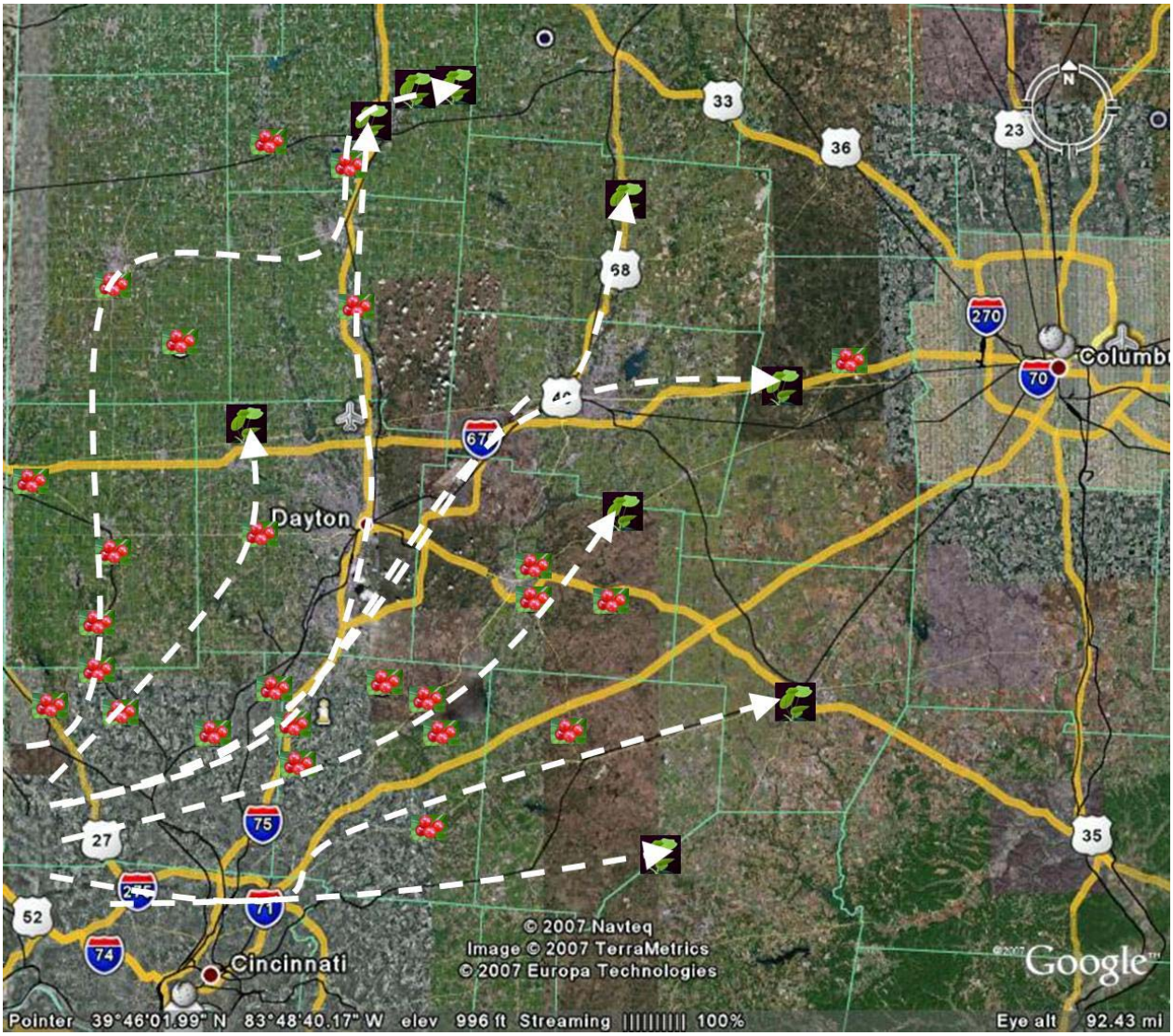

Figure 1. Sites in southwestern Ohio sampled for established stands or fronts of the Amur honeysuckle. Established stands are indicated by an image of the red berries of the mature plant and fronts are shown by an image of a growing shoot-tip. Speculations about possible routes and directions for the spread of the honeysuckle are indicated by dashed lines and arrows, respectively. The location of each stand or front was determined by means of a Global Positioning System (GPS) unit.

honeysuckle. A front was found east of the city of Dayton approximately 40 km (25 miles) away on U.S. 42 before South Charleston. After the front, there was a span of about 30 km (18.75 miles) where honeysuckle was not seen and then, near Columbus, it was noted again in forest stands. These results suggest that the second front might be moving from a source to the east (e.g., Columbus), such that the two fronts could eventually meet somewhere in the middle.

Survey data show that roadways to the south, southwest, and west of Dayton have quite dense stands of Amur honeysuckle and contained no front within the 80 km (50 miles) radius survey area west and south of Dayton. These results suggest that the source of the dispersing Amur honeysuckle along roadways in southwest Ohio is the Oxford, Miami area, especially in light of the deliberate introductions there in the 1960s. However, roadways OH 63, OH 73, US 22, and US 127

Table 1. A list of the location, roadway, direction and distance from the city of Dayton of Amur honeysuckle fronts.

Location	Roadway	Direction	Distance in km (miles)
Sidney	I-75	North	55 (34.375)
Urbana	US-68	Northeast	50 (31.25)
South Charleston	US-42	East	40 (25)
Beyond the front	US-42	East	75 (46.875)
New Vienna	OH-73	Southeast	65 (40.625)
Washington Court House	US-35	Southeast	65 (40.625)
Southwestern Ohio	various	West, South, & Southwest	not found > 80 (50)
Sidney	East of I-75	North	65 (40.625)
Port Jefferson	East of I-75	North	63 (39.375)
London	US-40	East	64 (40)

intersect roads radiating out of Dayton, and contain stands of Amur honeysuckle, thus demonstrating that the invasive shrub is extending between main roadways leading out of Dayton. The pattern of dispersal synthesized from the surveys, including information on the fronts, is shown in Figure 1.

Discussion

This invasive shrub was introduced in southwestern Ohio in the Oxford area around 1960 (Hutchinson and Vankat 1998) and the present study indicates that it appears to be spreading from there, suggesting that roadway surveys may be useful for locating invasion source regions. Bartuszevige (2004) concluded that Amur honeysuckle spreads out from multiple foci, taking advantage of sites of disturbance, chance animal vectors and other means of distribution. Roadways particularly appear able to favor dispersal of invasive plant species because they are corridors that provide connectivity and form habitat edges with periodic disturbance. The pattern of fronts obtained from the present survey of roadways identified southwest Ohio as a major center from which the Amur honeysuckle appears to have radiated out. Leaf samples have been collected at sites along the survey route in order to confirm this speculation by means of a gene-flow study using appropriate DNA markers such as microsatellites.

Roadway surveys may also have strategic value for choosing sites for controlling the spread of bush honeysuckle. Because roadways are favorable sites for invasion (Gelbard and Belnap 2003, Flory and Clay 2006), the roadside fronts likely mark the present extent of the invasion. Therefore, roadsides can provide a strategic location to apply control measures to contain the spread. Similarly, they offer a favorable sampling site for the purpose of studying gene flow. Gene-flow studies with DNA

markers should provide a more conclusive picture of the dynamics of the spread of the Amur honeysuckle in this area. DNA analysis may also reveal whether a particular variety of Amur honeysuckle, e.g., Rem-Red, is more common at the invasion front, perhaps because of an enhanced weediness brought about by genetic selection.

Acknowledgments

We would like to thank Shuang-Ye Wu and Donald Pair (Geology Department, University of Dayton) for loaning GPS units and help with data analysis. This research was supported by a University of Dayton Learn, Lead, and Serve Award to MKR and a University of Dayton Research Council Seed Grant to SRG.

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Population genetics of the invasive shrub Multiflora Rose (*Rosa multiflora*) in northeastern Ohio

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Abstract. Multiflora Rose (*Rosa multiflora*) is a highly invasive shrub native to East Asia. It was widely introduced to North America in the 1930s and 1940s for use as erosion control, wildlife cover, crash barriers, and living fences. Populations of colonizing plants spread rapidly in disturbed areas throughout much of the US, resulting in declared "noxious weed" status in 11 states. We used allozyme variation to study the genetics of three hedgerow and five naturalized populations in northeastern Ohio, finding low levels of genetic differentiation among the sampled populations (F_{ST} = 0.08). Dendrograms based on Nei's (1972) genetic distance indicate that three hedgerow populations are the most similar among the eight sample units. This genetic similarity among hedgerow populations is likely due to common ancestry of hedgerow individuals rather than to gene flow between sites. Observed heterozygosity among progeny samples is slightly lower than that reported for species with similar reproductive characteristics (Hamrick and Godt 1989). Since data are unavailable for the genetic diversity of Multiflora Rose in its native range, our results cannot assess bottleneck effects in U.S. populations. Biological control as a management tool could prove effective if our results hold for populations across the invasive range.

Introduction

Multiflora Rose is a prickly shrub native to Japan, Korea, and eastern China. Plants are highly fecund, producing a persistent seed bank, and also reproduce asexually. Widely introduced throughout the US in the 1930s and 1940s, naturalized populations are now found throughout most of the US. Multiflora Rose is considered a noxious weed in 11 eastern and midwestern states.

Three agents are currently being investigated for potential use as biocontrol for multiflora rose: the rose seed chaclid *Megastigmus aculeatus* var. *nigroflavus* Hoffmeyer, rose rosette disease and its eriophyid mite vector, *Phyllocoptes fructiphilus* Keifer, and the rose stem girdler *Agrilus aurichalceus* (Amrine 2002). Effectiveness of biological controls will likely be related to the degree of genetic variation within and among populations. Our investigation seeks to estimate levels of variation in planted hedgerows and compare that variation with the variation found in colonizing populations. Namely, are colonizing stands a random subset of the hedgerows, or is there a pattern of genetic variation associated with successful invasiveness?

Methods

Seed collection and germination: Rosehips were collected from three planted hedgerows and five naturalized populations within 50 miles (80 km) of Oberlin, Ohio: Camden Bog,

Kipton Reservoir, Malabar Farms, the Firelands Boy Scout Camp, Caley Reservation, and Charlemont Reservation. Seeds were cleaned, subjected to a cold treatment (minimum 30 days at 4°C [39°F]) to break dormancy, and germinated in a growth chamber (14-hr days at 25°C [77°F]/10-hr nights at 20°C [68°F]). Seedlings were used in allozyme studies to estimate genetic diversity within and among populations.

Allozyme analysis: Protein extracts prepared from young leaf tissue were absorbed onto filter paper wicks and used in starch gel electrophoresis. Gels were stained for nine different enzyme loci: triose-phosphate isomerase (TPI), fluorescent and colorimetric esterase (FEST & EST), glutamate oxaloacetate transaminase (GOT), shikimic dehydrogenase (SKD), phosphoglucomutase (PGM), isocitrate dehydrogenase (IDH), phosphogluconate dehydrogenase (PGD), and cathodal peroxidase (c-PER). Gels were scored for heterozygosity at eight loci. Buffer and stain composition, electrophoretic conditions, and general procedures follow Wendel and Weeden (1989).

Table 1a. Sample size (n), percent loci polymorphic (P), average number of alleles per locus (A), observed heterozygosity (Hobs), and expected heterozygosity (Hexp) for all populations for "progeny included" data set. See Fig. 1 for site locations.

Population	n	P	A	H_{obs}	H_{exp}
1	72	62.50	1.63	0.045	0.062
2	40	50.00	1.50	0.094	0.135
3	189	62.50	1.63	0.054	0.107
4	81	50.00	1.50	0.011	0.108
5	60	62.50	1.62	0.125	0.127
6	152	50.00	1.50	0.036	0.049
7	82	62.50	1.62	0.070	0.071
8	72	62.50	1.63	0.066	0.089
Mean	**93.5**	**57.8**	**1.58**	**0.063**	**0.094**

Table 1b. Sample size (n), percent loci polymorphic (P), average number of alleles per locus (A), observed heterozygosity (Hobs), and expected heterozygosity (Hexp) for all populations for "minus progeny" data set. See Fig. 1 for site locations.

Population	n	P	A	H_{obs}	H_{exp}
1	22	50.00	1.500	0.034	0.056
2	10	50.00	1.500	0.125	0.192
3	42	62.50	1.630	0.080	0.123
4	26	37.50	1.375	0.095	0.100
5	20	50.00	1.500	0.088	0.084
6	20	25.00	1.250	0.050	0.066
7	26	37.50	1.375	0.039	0.038
8	18	37.50	1.375	0.056	0.078
mean	**23**	**43.75**	**1.438**	**0.071**	**0.092**

Statistical analyses: All statistical analyses were performed with Popgene v. 1.31: Chi-square tests for Hardy-Weinberg equilibrium, Observed (Hobs) and expected (Hexp), *F*-coefficients (F_{IS}, F_{IT}, and F_{ST}), gene flow estimate (Nm = $(1-F_{ST})/4F_{ST}$), and Nei's (1972) genetic distance. All analyses were performed on two data sets:

1. "Progeny included" - a complete set, which included a significant number of sibling groups, and
2. "Minus progeny" - which included only one offspring per parent plant.

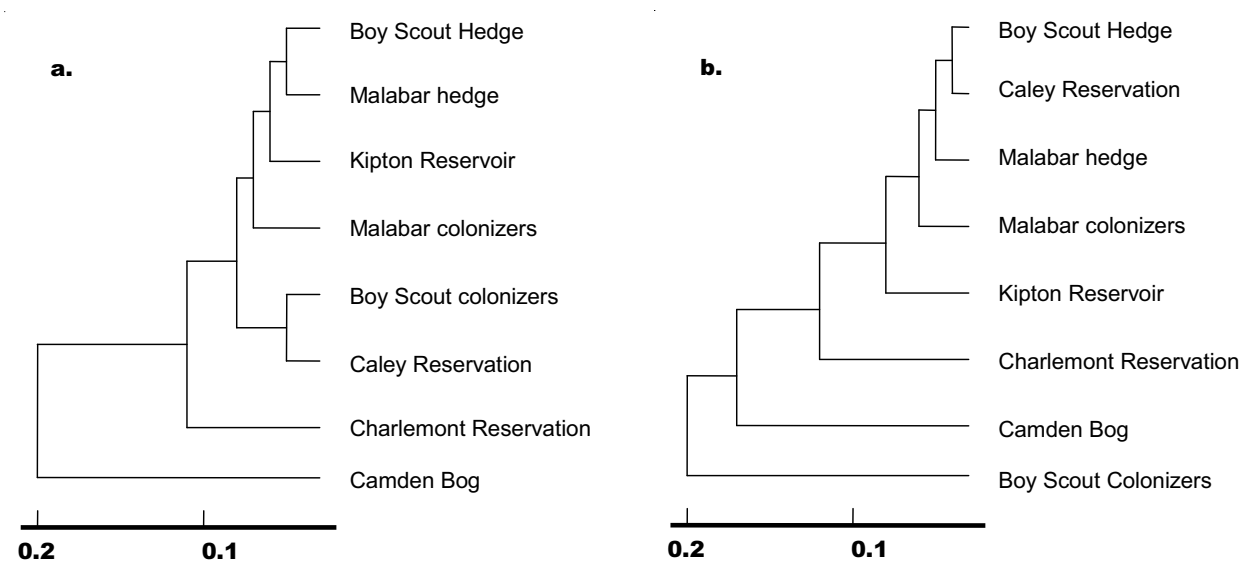
Results

"Progeny included"

We used six polymorphic loci that revealed average observed heterozygosity (H_{obs}) = 0.063 and average expected heterozygosity (H_{exp}) = 0.094 (Table 1a), with low differentiation among the populations (F_{ST} = 0.089, and only slightly non-zero Nei's distances, Fig. 1a). Most of the variation can be explained by the FEST locus, which had an F_{ST} = 0.165, and the corresponding gene flow estimate of Nm = 1.27. Among the polymorphic loci, PGM-2 had the largest effect on genetic homogeneity across all populations, with the lowest among-population differentiation (F_{ST} =0.031) and the highest estimated gene flow (Nm = 7.793). Inbreeding was evident, with F_{IS} = 0.228 and F_{IT} = 0.296, with the FEST locus having the highest contribution.

"Minus progeny"

Five polymorphic loci were used, revealing H_{obs} = 0.071 and H_{exp} = 0.092, with an overall F_{ST} = 0.076, slightly lower than the "progeny included" data set (see Table 1b). Nei's values were also lower in these data (Fig. 1b). Highest variation was observed in PGD-1 (F_{ST} = 0.124; Nm = 1.775), followed by FEST (F_{ST} = 0.088; Nm = 2.604). The lowest variation was at TPI-1 (F_{ST} = 0.032; Nm = 7.539). The overall inbreeding coefficients (F_{IS} = 0.203 & F_{IT} = 0.264) were slightly less than in the above data set, again with the FEST locus having the highest contribution.



Figures 1. UPGMA dendrograms for **(a)** "progeny included" and **(b)** "minus progeny" data sets, based on Nei's (1972) genetic distances.

Discussion and Conclusions

Allozyme data indicate little genetic differentiation among the populations. Genetic distances between populations are nearly zero in all cases ($D < 0.02$) as allele frequencies were very similar among the populations. Observed homogeneity among populations is likely due to genetically similarity of the planted populations. We observed no pattern of correlation between genetic and geographic distances. The three hedgerow populations are the most closely related, which fits with both the history introduction and the life history characteristics of this species. Lacking information about genetic variation in the native multiflora range, it is impossible to know what level of genetic bottleneck may result from the transfer from Asia to North America. However, it appears that the invasive populations surveyed lack the diversity expected for their life history traits (Hamrick and Godt 1990). Given the genetic similarity among hedgerow and escaped populations, there is no evidence to support the hypothesis that escaped plants represent a distinct subset of the parent populations. Since observed heterozygosity is lower than expected, the inbreeding reflects non-random mating that may result from a genetic bottleneck. Differences among the data sets suggest that outcrossing individuals may be more successful. The "progeny included" data set has more polymorphic loci than does the "minus progeny" data set. The relative diversity of progeny arrays suggests that seeds result from outcrossing, which may also result from higher germination rates. Should our results be representative, then the overall genetic similarity among populations indicates that biological controls should have similar results over the species range in North America.

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Effect of deer and *Lonicera maackii* (Amur Honeysuckle) removal method on restoration of understory plant communities

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Abstract. Invasive *Lonicera maackii* (Amur Honeysuckle) is frequently removed from natural areas due to its negative impact on native communities. However, after removing honeysuckle it may be necessary to replant or reseed to increase the success of the restoration project, especially in fragmented or heavily impacted sites. Deer may also thwart restoration efforts in some areas. Using a split-split plot design, we investigated the success of native understory plants (from natural colonization and from planted seedlings) after removal of honeysuckle, using two common control methods (cut/paint and garlon basal application) in the presence and absence of deer. We measured light levels and amount of honeysuckle biomass removed from our experimental plots. We removed an equal amount of biomass from our two removal treatments; however, there was a significantly greater amount of honeysuckle biomass in our control (no removal) plots. Light levels in the cut/paint plots were significantly higher than light levels in the garlon plots which were significantly higher than light levels in the control plots. In 2005, we locally transplanted 6 seedlings of *Impatiens capensis* into our experimental removal plots. In 2006, we measured the amount of *Impatiens* seedlings in these plots, presumably from seeds from the previous year's transplants. The number of *Impatiens* seedlings was significantly higher in the cut/paint removal plots than in garlon removal plots. There was a trend towards a greater amount of seedlings in the fenced deer exclusion plots than in the unfenced plots. In adjacent removal and control plots, we measured the frequency and number of plants which naturally recruited into the site in both the spring and fall. Spring and fall naturally-recruiting native species richness was significantly higher in cut/paint and garlon plots than control plots. Spring species richness was also significantly affected by the interaction of removal treatment and fencing. When fenced, there were more species and number of individuals in cut/paint plots than in garlon plots. When unfenced, cut/paint plots and garlon plots had similar species richness, indicating a protective effect of the standing biomass against deer herbivory in garlon removal treatments. In both the spring and fall, there were significantly more individuals of the invasive species *Alliaria petiola* in cut/paint plots than control plots, with the amount of *A. petiolata* in garlon plots intermediate between the two. Our results indicate that there are differences between removal methods in successful understory restoration and that the effects of the removal method and the effect of deer must be weighed together in devising restoration strategies. In addition, our results indicate that control of honeysuckle may be beneficial to not only the native species of interest, but also to garlic mustard, another invasive species.

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Native plant and mycorrhizae establishment after Garlic Mustard removal: Implications for restoration

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Abstract. Garlic Mustard (*Alliaria petiolata*), a Eurasian native that has invaded North America, produces compounds that inhibit growth of neighboring plants and their associated mycorrhizae. We explored the effects of three Garlic Mustard removal techniques (RoundUp treatment, total plant removal, shoot removal only) on the growth of introduced pale jewelweed (*Impatiens pallida*) and its associated mycorrhizae. Garlic Mustard plants were grown in root-viewing chambers for four months, then removed and replaced with a newly germinated *I. pallida* seed. Half the chambers contained activated carbon to absorb allelopathic organic compounds. We added field collected mycorrhizal inocula to the surface of half the chambers when planting *I. pallida* seeds. Weekly, we monitored *I. pallida* plant height, and the status of mycorrhizae in the root system using epifluorescence microscopy. We calculated root colonization and soil colonization indices and quantified the fluorescence of several root segments within each chamber. Every three days we monitored root development, and measured total length and area covered, and calculated the fractal dimension of the root system. Adding activated carbon or mycorrhizal inocula was largely unimportant for the health of *I. pallida* plants, but the method used to kill *A. petiolata* was highly significant. *I. pallida* plants and their mycorrhizae were healthiest when exposed to a minimum of dead Garlic Mustard root tissue; plants in chambers where the entire *A. petiolata* plant was removed were healthiest and plants in chambers where RoundUp was used to kill *A. petiolata* plants were least healthy.

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Physiological characterization and potential air quality impacts of the exotic invasive tree *Ailanthus altissima* (Tree of Heaven) communities

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Abstract. Tree of Heaven (*Ailanthus altissima*) is an exotic invasive tree that has negatively impacted both urban and natural landscapes in the United States. In some areas, *Ailanthus* has the potential to be a stand dominant tree. What might be the biogeochemical effects of increasing stand dominance of this invasive, at the cost of displacement of native forest? Little published research exists on the physiological function of *Ailanthus altissima*. To address the gap in the literature, two undergraduate research projects are underway in the Tree Ecophysiology lab at Ohio University. The first project addresses the question "what are the forest carbon consequences of *A. altissima* invasion?" To do this, a forest carbon model (PnET) will be used to model *A. altissima* net primary production. PnET uses, among many inputs, the physiological relationship between leaf nitrogen content and maximum photosynthesis to estimate stand productivity. Leaf nitrogen is the parameter to which the model is the most sensitive. It is commonly observed that many invasive plants have higher rates of maximum photosynthesis and greater leaf nitrogen content than native species. To parameterize the model for *A. altissima*, photosynthesis data taken *in situ* during September 2006. Leaf nitrogen and carbon content were measured on the same leaves from 32 trees from various populations in Athens County OH. Maximum photosynthetic rates were found to be 10.98 μmol CO_2 m^{-2} s^{-1} (+/- 3.80 sd) and leaf mass per unit area (LMA) were found to be 59.0 g m^{-2}, (+/- 11.6 sd). Nitrogen data and PnET model estimates will be presented. The goal of the second project is to examine the isoprene emitting potential of *A. altissima.* Air pollution, particularly surface level ozone, is a major human health issue currently affecting many major urban areas. One of the primary sources of ozone is the reaction of anthropogenic nitrous oxides with volatile organic carbons (VOC's), primarily isoprene. Isoprene is produced in some species of plants during photosynthesis under conditions of high light and temperature. Isoprene emitting potential of *A. altissima* will be examined by utilizing a leaf cuvette enclosure system of a modified Li-COR-6400 to sample gasses produced by photosynthesizing leaves of individuals of *A. altissima.* Results will be compared between seedlings grown in growth chambers under high light and low light conditions. Gas samples will be analyzed for the presence and quantity of isoprene using a gas chromatograph. Isoprene flux rates will be scaled to the canopy level using a forest canopy process model, allowing for the estimation of potential impact of this invasive on air quality, as it potentially increases in density in this region. The outcomes of these two projects will inform us about the biogeochemical impacts of *Ailanthus altissima* invasion with regard to forest carbon cycling and local air quality.

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Effects of Amur Honeysuckle on soil microbial activity

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Abstract. Amur Honeysuckle (*Lonicera maackii*) has been shown to reduce abundance, diversity, growth, and reproduction of herbaceous and woody species in native plant communities. There is less information, however, on the effects of honeysuckle on soil microbial communities that contribute to litter decomposition and nutrient availability. Soil microbial activity is vital to litter decomposition and nutrient turnover. Altered microhabitat (e.g., temperature, humidity, chemistry) in soil under Amur Honeysuckle shrubs might affect microbial activity. The current project determined whether decomposing Amur Honeysuckle leaves altered soil microbial activity in greenhouse and field experiments.

Topsoil from a non-invaded mature forest in the Cincinnati Nature Center, Milford, Ohio, was sieved into twenty 15 x 15 x 15cm pots (350 g each) in a greenhouse at the University of Cincinnati. Two grams Amur Honeysuckle and Sugar Maple (*Acer saccharum*) leaves were placed on the soil surface of separate pots (n = 10 each). Three 2-g soil samples were taken from each pot biweekly for 20 weeks and triphenyltetrazolium chloride (TTC) reduction was used to estimate microbial activity.

Microbial activity was also quantified from soil samples (500 g) taken in August and October 2006 from three forested habitat types at Woodland Mound, Cincinnati, Ohio: honeysuckle-invaded, honeysuckle-killed and uninvaded. In each habitat, three 500-g soil samples were collected and subsamples (n = 5) of each were analyzed as above.

Amur Honeysuckle leaves in greenhouse pots completely decomposed within 8 weeks. During this time, microbial activity was higher in soil from honeysuckle-treated than native pots. However, there was no significant difference between soils during the 12 weeks following honeysuckle decomposition nor was there any difference over the entire 20 week study period. Similarly, there were no significant differences in microbial activity in soil from the three field sites. These data suggest that time since invasion of Amur Honeysuckle may be critical in determining effects on soil microbial activity. Microbial communities appear to respond positively just after appearance of honeysuckle (first 8 weeks of greenhouse study), but appear to be inhibited after prolonged exposure (field soil). Mechanisms by which this could occur are not clear.

Native plant communities may not respond to honeysuckle control if steps are not also taken to assess effects on the soil community. Although this study did not find that microbial communities *in situ* were affected by honeysuckle, it did show that communities in previously uninvaded soil responded positively to the decomposition of honeysuckle leaves. Future studies should examine the time frame of invasion effects on soil microbial communities.

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Garlic Mustard: The beginning of its demise?

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Abstract. Garlic Mustard (*Alliaria petiolata*) is an invasive biennial herb that is reducing native species diversity in deciduous forests and disturbed areas in North America. To date, long-term controls of Garlic Mustard are lacking, while conventional removal methods are costly and temporary. Throughout Ohio, many Garlic Mustard populations are infected with the powdery mildew fungus *Erysiphe cruciferarum*, although population and environmental variation in attack rates exists. The direct and indirect impacts on the herb have not been examined, but *E. cruciferarum* may serve as a potential biocontrol agent for *A. petiolata*. Effects of the fungus on plant growth, as well as responses of both the plant and fungus to altered environmental conditions were examined on greenhouse-grown first-year Garlic Mustard plants from local Ohio populations. Also, the effects of the fungus on growth and fitness were studied in the field on a local population of second-year plants. Results indicated significantly reduced growth on heavily infected first-year plants. Furthermore, moisture limitation significantly reduced disease development, while light limitation significantly increased it. Field plants protected from the fungus grew significantly taller with significantly more siliques and twice as many seeds produced. These results suggest that plants that incur infection during their first year will have overall reduced growth, which may negatively affect their second-year growth and fitness. Also, unprotected second-year plants will likely produce significantly less seed, which may steadily reduce future populations of Garlic Mustard over time. These effects will likely be more evident in moist, shady sites that favor fungal development.

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Can forest understories invaded by *Microstegium vimineum* be easily restored?

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Abstract. Habitat restoration following the invasion of a non-native invasive species can be difficult. *Microstegium vimineum* (Trin.) *A. Camus* is an annual invasive grass threatening much of the eastern United States. *M. vimineum* has been observed to overtake native species and dominate the understory of many hardwood forests. In such forests, regeneration often plummets to very low levels with a nearly absent regeneration layer. While information regarding the invasion, spread, and general ecology of *M. vimineum* is available, few data exist regarding habitat restoration. We assessed the efficacy of various control methods and examined the effects of *M. vimineum* on planted hardwood seedling survival and growth. Two-year old native hardwood seedlings (*Acer saccharum*, *Quercus rubra*, and *Liriodendron tulipifera*) and one-year old invasive tree seedlings (*Ailanthus altissima*) were planted in a split-plot (open vs. closed forest canopy) block (replicates) design at Crummies Creek Tree Farm, Calhoun County, West Virginia. Within each block, three control treatments were employed: chemical (Sethoxydim), mechanical (hand pulling), and no removal (control). The growth (height and basal diameter) and survival of planted seedlings was assessed within each treatment over a 2-year period. Hemispherical photographs were taken to quantify canopy cover and soil was sampled to determine soil nutrients and moisture. Hardwood seedling height and diameter responded significantly by forest canopy type and by species ($P < 0.05$); however, no significant treatment effect was detected ($P = 0.64$). Mechanical removal of *M. vimineum* increased the survival of native hardwood seedlings compared to the control but did not differ from chemical treatment. The lack of treatment effect as well as high rate of survival and growth is promising in regards to restoring habitats invaded by *M. vimineum*. Data suggest that restoration of invaded forested habitats can be achieved by a top down approach that utilizes planted native hardwood seedlings. Restoring a midstory regeneration layer will likely shade *M. vimineum* and further erode its dominance in the stand. The impact of *M. vimineum* on natural regeneration and seedling recruitment still warrants further investigation. Natural regeneration from seed will not likely occur until *M. vimineum* reaches some minimum critical threshold.

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The use of native plants to restore fire suppression lines on Wayne National Forest

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Abstract. Forest wildfires are a common occurrence in the spring and fall in southern Ohio. These fires are either caused by arson or escaped from private land owners burning debris. Between fall of 2005 and spring of 2006 the Ironton District of the Wayne National Forest responded to and suppressed more than 150 wildfires. On four of these fires, a bulldozer was used to create fire breaks to stop the fire from advancing further. Two wildfires were used to test the effectiveness of planting native graminoids and forbs for re-vegetation of the bull dozer lines. Re-vegetation of bulldozer lines is an important aspect of post-fire rehabilitation because bulldozer lines can lead to erosion problems on steep slopes are prime location of the establishment and spread of non-native invasive species.

We planted 17 species of native plants on dozer lines; 11 grasses, 1 sedge, 1 wood rush and 4 forbs. We also used composted mulch in planting holes. Nursery grown plants were planted at the County Line Fire (November 2005, 156 ac) and the Red Bud Fire (April 2006, 43 ac). The plants ranged in size from 1" plugs to 4" pots. Approximately 7000 ft of dozer line was constructed and planted. We emphasized plantings on slopes greater than 20% and spaced plants 4-6 ft apart.

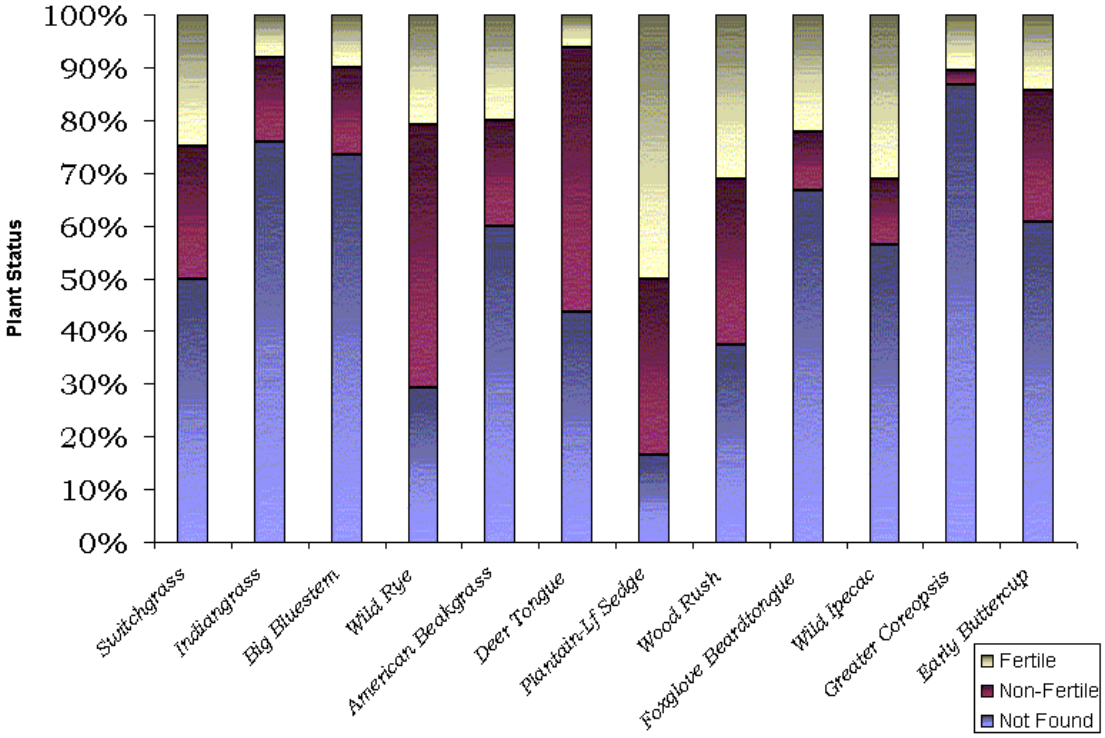
138 of 358 planted plants were re-located. In some areas it was difficult to re-locate planted plants due to natural re-vegetation. On average 30% of the dozer lines naturally re-vegetated from colonizing seeds or root sprouts. The success of the plantings and natural re-vegetation varied by slope and aspect. However, even after 1 year many areas along dozer lines had exposed bare soil. Infestations of two invasive species, Asiatic Stiltgrass and Tree-of-Heaven were spread unintentionally along dozer lines. These were treated in the summer of 2006 and will be monitored and treated as needed.

Some plant species fared better than others when planted on the dozer line. The following is a list of suggested species to use on bulldozer line plantings:

Full Sun: Switch Grass (*Panicum vigatum*), Wild Ipecac (*Porteranthus stipulatus*)
Filtered Sun: Wild Rye (*Elymus canadensis*), Deer Tongue *Panicum clandestinum*
Shade: Plantain-Lf Sedge (*Carex plataginea*), Wood Rush (*Luzula acuminata*), Beakgrass (*Diarhhena americana*) Early Buttercup (*Ranunculus fasicularis*)

In the future we would like to improve on our monitoring and re-vegetation results by 1) tracking survival by flagging plants, 2) documenting physiographic variable of planting areas. 3) using native seeding treatments 4) trying other species such as White Snakeroot (*Eupatorium rugosum*), White Wood Aster (*Aster divaricatus*), and White Grass (*Leersia virginica*).

Example of bulldozer line with burned (right) and unburned areas (left) of the Red Bud Fire.



Survival and fecundity of species planted along bulldozer lines.

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Possible herbicide translocation to non-target plants following Tree of Heaven injection

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Abstract. Ailanthus altissima Miller [Swingle] (Tree of Heaven) can be easily managed with herbicide injection. However, the potential herbicide translocation to neighboring trees must be evaluated prior to widespread recommendations for herbicide injections. We assessed the non-target translocation of imazapyr (Arsenal ™), an herbicide commonly used to manage woody vegetation in forests, from injected Tree of Heaven trees to neighboring non-injected stems. Canopy defoliation was used as an indicator of stress and/or mortality. Targeted imazapyr injections not only killed all injected Tree of Heaven trees, but also killed 17.5% of neighboring non-injected Tree of Heaven and eight other woody species 62 weeks after treatment. Non-target mortality from herbicide translocation decreased as the distance from injected Tree of Heaven increased (up to 3 m) and as stem diameter of non-injected plants increased. Each Tree of Heaven stem injected with imazapyr produced a mean mortality of 2.5 neighboring non-injected stems, with a maximum mortality of eight neighboring non-injected stems, per 28.3 m^{-2}. The plausible modes of the inter- and intraspecific herbicide translocation include root grafts, mutually-shared mycorrhizal fungi, root exudation and absorption, and/or leaf senescence. Because Tree of Heaven is clonal, patch size and vegetation heterogeneity will be an important determinant of herbicide injection protocols. In forest environments with many small patches (i.e., high edge to interior ratio), or mixed species stands, non-target hardwoods are at an increased risk of mortality. In isolated large patches (with lower edge to interior ratio), or dense monospecific clones, injection risk to non-target species will be relatively low.

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Phenotypic plasticity as an indicator for exotic noxious plants

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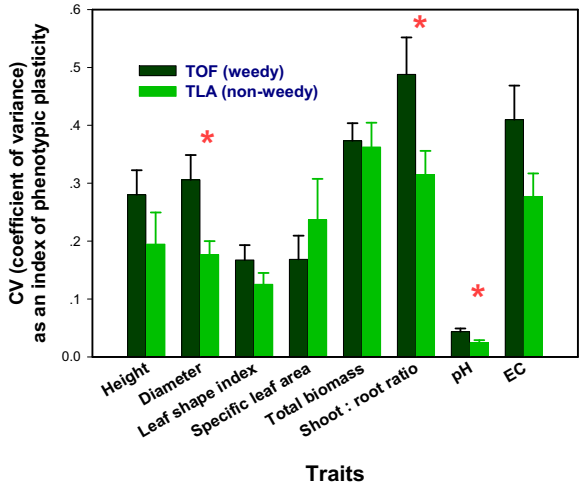
Abstract. Exotic noxious plants, including invasive plants and exotic weeds, have cost huge economic loss and ecological damage around the world. To prevent further introductions of such species as crops or ornamental plants, biological and ecological traits associated with invasiveness and weediness need to be identified so that prediction can be made on the potential of being noxious for proposed species. It was suggested that weeds were usually generalists which can survive and reproduce in a wide range of environments, *i.e.* they were quite "plastic" in response to different environments. In accordance to this idea, phenotypic plasticity has been recently proposed as an indicator and predictor for weeds and invasive plants.

This hypothesis is tested using two exotic dandelion species: *Taraxacum officinale* (common dandelion), a notorious wide-spread weed, and *T. laevigatum* (red-seeded dandelion) which occurs in a much lower frequency, at least in Ohio. A greenhouse experiment was conducted in which the two species were grown in two soil moisture levels (dry vs. wet) combined with two light exposure levels (full sun vs. light competition). Various traits were measured to see whether *T. officinale* is more plastic than *T. laevigatum* in these four environments. The results show that, when using coefficient of variance (CV) as a measurement of plasticity, *T. officinale* has significantly larger CV than *T. laevigatum* in plant diameter (P=0.02), shoot:root ratio (P=0.04) and soil pH (P=0.02). This indicates that *T. officinale* is more plastic in some of the resource-capture-related traits such as leaf morphology and biomass allocation, and presumable also in root exudates which alter the soil pH.

This result shows that higher phenotypic plasticity might be one of the reasons why *T. officinale* is much more successful than *T. laevigatum* as an exotic weed. Further studies in other life



Mean CV (+ 1 SE) of *Taraxacum officinale* (TOF) and *T. laevigatum* (TLA) for eight traits. CV works as an index for phenotypic plasticity: larger CV indicates higher plasticity. Asterisk indicates significant difference between two species (P<0.05, type 3 t-test). Notice that TOF, the weedy species, had larger CVs for most traits than TLA, the non-weedy species.

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Exploring management methods for *Botumus umbellatus* on Ottawa National Wildlife Refuge

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Abstract. Flowering Rush, or *Botumus umbellatus*, is an exotic invasive species threatening native wetland plant diversity and habitat quality. Flowering Rush has been present on Ottawa National Wildlife Refuge (NWR) for years. In the past, this invasive was of lesser concern than other more aggressive invasives (i.e. *Phragmites australis*) that were compromising wetland quality. But in recent years, flowering rush appears to have taken a more aggressive hold on Ottawa NWR's wetlands and is increasing in density and distribution. It was decided action was needed in order to set back the aggressive plant and to encourage germination of native vegetation. However, currently there are few resources available describing control techniques. The Ottawa NWR decided to explore a variety of techniques to control infestations of flowering rush and to determine which is most effective and efficient.

Five different control techniques were evaluated. Control methods were implemented in the 2006 field season and only preliminary field observations can be reported. The methods used and the results achieved are listed in the table below. During the 2007 field season, monitoring of test plots will determine the ultimate success of the control methods. In addition, Ottawa NWR will continue to combat this invasive species by exploring a variety of techniques.

Control methods for Flowering Rush and current treatment results.

Method	Treatment	Results (to date)
1	Backpack spraying a 1% solution of Habitat herbicide to budding plants	Killed approximately 50% of the infestation in 2 months with no regrowth the remainder of the growing season
2	Backpack spraying a 1% solution of Habitat herbicide to flowering plants	100% of flowers wilting and plants dying in 2 months with no regrowth the remainder of the growing season
3	Broadcast spraying 6 pints/acre of Glypro to second growth budding plants	Stressed plants, but an early freeze prevented complete evaluation of spray effectiveness
4	Broadcast spraying 3 pints/ac of Habitat herbicide to flowering plants and then dewatering, disking, and then flooding	Spraying stressed the plants, but did not kill. The combination of dewatering, disking, and then flooding prevented any new regrowth this season.
5	Dewatering flowering rush then disking and flooding	Regrowth of flowering rush during the flooding phase

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Separating effects of allelopathy and shading by *Alliaria petiolata* (Garlic Mustard) and *Lonicera maackii* (Amur Honeysuckle) on growth, reproduction and survival of *Impatiens capensis* (Touch-me-not)

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Abstract. Ways that invasive plant species can exert their effects on native species include allelopathy and light competition. In a fully factorial field study, we examined the effects of activated carbon application to the soil (to ameliorate allelopathic effects) and removal of aboveground biomass (to ameliorate light competition) on the survival, growth, and reproduction of transplanted *Impatiens capensis* seedlings in understory habitats dominated by either *Lonicera maackii* (Amur Honeysuckle) or *Alliaria petiolata* (Garlic Mustard). Removal of light competition by Garlic Mustard significantly increased pod production of transplanted *I. capensis* plants. The effects of carbon application to the root zone of transplanted *I. capensis* on both height and pod production depended upon the light environment. In plots where Garlic Mustard was not cut, application of carbon increased plant height and pod production. In plots where Garlic Mustard was cut, application of carbon decreased height of *I. capensis* and had no effect on pod production. Carbon application increased survival of *I. capensis* in Garlic Mustard-dominated plots, while cutting increased survival of *I. capensis* in both Garlic Mustard and honeysuckle-dominated plots. Removal of light competition by honeysuckle tended to increase pod production of transplanted *I. capensis*, but carbon had no significant effect on height or pod production. Across treatments, there was a significant positive correlation between height and pod production. Overall, *I. capensis* survived longer and reached larger sizes when in competition with Garlic Mustard than with honeysuckle, but both proved to be effective competitors for light. Allelopathic effects of Garlic Mustard were capable of being ameliorated by carbon application, while those of honeysuckle were not. While carbon application may benefit native plant growth in the presence of allelopathic invaders, the addition of activated carbon to alleviate allelopathic effects after removal of aboveground biomass (a source of both allelochemicals and light competition) may have little benefit.

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Prioritizing management of *Ailanthus altissima* at the Edge of Appalachia Preserve: A study of competitive abilities and abiotic interactions.

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Problem: *Ailanthus altissima*, an invasive exotic tree from China, threatens several rare plant communities in the Edge of Appalachia Nature Conservancy preserve in Adams County, Ohio. In some areas it exists as a canopy dominant tree, and is clearly increasing in number and area at the site.

By mapping populations of *Ailanthus altissima* and characterizing abiotic factors correlated with these populations, a management plan will be created for *Ailanthus altissima* at The Edge of Appalachia Preserve. This plan will prioritize management areas based upon the threat level of *Ailanthus altissima* spreading into sensitive plant communities. A survey of juvenile growth rates of *Ailanthus altissima* will also be conducted to investigate the competitive ability of this invasive tree to outpace the vertical growth of native hardwood saplings.

During the summer of 2006, modified-Whittaker plots (10×50 m) were placed in 12 *Ailanthus altissima* populations and 12 randomly-selected control plots. The purpose of the control plots was to test differences in abiotic factors between invaded and non-invaded sites. In test plots, mature *Ailanthus altissima* trees were counted and the age of five mature trees in each population was assessed by aging tree cores. Subplots within each larger plot were used to measure *Ailanthus altissima* sapling and seedling density, percent cover by other species, soil samples, and canopy structure. Additionally, 10 saplings were collected to determine the age and rate of vertical extension based upon bud scars and annual growth rings. Native sapling growth rates were determined from non-destructive field-measurements. The locations of all plots were mapped with GPS and polygons were drawn around each studied population. Future output will include more laboratory-based analysis of maps, juvenile saplings, and soils. Results will be examined with the site history of each area and the proximity to roads, ATV trails and land features. Information from soils maps, geology maps and aerial photographs will also be analyzed for possible effects on *Ailanthus altissima* populations.

Preliminary data suggest minor differences based on plot data between the *Ailanthus altissima* (test) plots and control plots for soil moisture (35.8% and 29.6% respectively) and for soil pH (6.5 and 6.2 respectively). *Ailanthus altissima* density for canopy dominant trees was 87 stems ha -1. Based on the five largest dominant trees per plot, the average diameter was found to be 18.3 cm (n = 48). Results of tree size distributions, leaf area index data, soil texture, tree age, and disturbance will also be shown. In addition, GIS

maps showing distribution and abundance of *Ailanthus altissima* throughout the preserve, and how this relates to slope, aspect, proximity to roads, etc. will also be presented.

Management Implications: This project will lead to a better understanding of *Ailanthus altissima* and will facilitate solutions to protect rare species and community types that at the Edge of Appalachia preserve.

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Hybrids between *Typha angustifolia* and *T. latifolia* – are they more invasive than their parents?

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Abstract. Spontaneous crosses between common cattail species in North America has resulted in hybrid populations of *Typha x glauca* that may be more invasive than their parents. *Typha latifolia* L. (Broad-leaved Cattail) is native to North America, while *T. angustifolia* L. (Narrow-leaved Cattail) is considered to be invasive and is thought to have been introduced from Europe in the mid-nineteenth century. Their first-generation hybrids are mostly sterile, yet they appear to be common in Ohio and elsewhere. Hybrids can be difficult to identify based on morphological traits, but RAPD DNA markers have been used successfully to distinguish hybrids (*T. x glauca*) from their parent taxa. Despite concerns about the competitiveness and rapid spread of *T. x glauca*, little quantitative data has been published on its genetics, ecology, or relative invasiveness compared to its parent species. Previously published studies indicated that *T. x glauca* are first generation (F1) progeny between *T. latifolia* and *T. angustifolia*. However, in a pilot study, we found evidence for backcrossing in a population in Cheboygan, Michigan. We screened 26 putative hybrids and 40 putative nonhybrids for the presence of three species-specific RAPD markers in 2004. Individuals were sampled at intervals of at least 10 m apart to reduce the likelihood of sampling more than one ramet from each clone. Two plants were genetically distinct, advanced-generation hybrids, one of which had been designated as *T. x glauca* and the other as *T. angustifolia* based on morphology. The other 26 putative hybrids had a full complement of markers from both parents, as expected for F1 hybrids. We hypothesize that infrequent and partially fertile F1 hybrids can backcross with their parent taxa or produce self-pollinated F2 progeny. Furthermore, we suggest that genes from T. *latifolia* may be introgressing cryptically into *T. angustifolia* (RAPD data for the two advanced-generation plants are consistent with this hypothesis). This process could have important implications for the evolution and invasiveness of both *T. x glauca* and *T. angustifolia* if the above scenario is supported further by our ongoing research. The next step in our research is to compare the growth and competitive ability of F1 hybrids, backcrossed plants, and the two parent taxa to test for differences in potential invasiveness. We also plan to survey wetlands in Ohio and Michigan to gain a better understanding of the frequency of hybrid populations, including possible advanced-generation "hybrid swarms" with intermediate genotypes. In 2006, we found our first hybrid plant at the Olentangy River Wetlands Research Park at Ohio State University, so this site will be studied further in detail. We welcome suggestions from the Ohio Invasive Plants Council and other interested groups as we begin this phase of our research.

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Isolation, identification and characterization of allelochemicals present in Bush Honeysuckle (*Lonicera maackii*)

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Abstract. Amur Honeysuckle (*Lonicera maackii*) is an invasive shrub that was first introduced in the United States in 1846 for use as an ornamental plant. The species has escaped cultivation due to high seed production and the disbursement of their seed by birds. The shrub is commonly found along forest edges, road sides, fence lines or any other disturbed area where they are capable of out competing native plant species for available light by direct shading. Aqueous extracts from honeysuckle plant tissue have also been shown to inhibit the growth and germination of *Arabidopsis thaliana* and other native plant species. The identification, characterization and isolation of the allelochemicals responsible for these effects have never been examined, but the results from these procedures may play a role in the development of new pre-emergent herbicides. The results could also generate controls that may be used to halt the growth and spread of this invasive plant. Chemical analysis of crude methanolic extract by high-performance liquid chromatography coupled to electrospray ionization tandem mass spectrometry revealed that this honeysuckle species was dominated by the presence of chlorogenic acid and 2 flavones: apigenin and luteolin and their glycosides, apigenin-7-glucoside and luteolin-7-glucoside. The unequivocal identification of these 5 phenolic compounds was based on comparison of retention times, UV spectra and mass fragmentation data with those of authentic standards. A fractionation protocol for honeysuckle was performed by sequentially extracting ground leaf tissue with ethyl ether, methanol, distilled water and 1-butanol. Results suggest that the phytotoxic constituents of honeysuckle reside in the ethyl ether and methanol fractions due to the level of seed germination inhibition that was observed. The identification of phenolic compounds present in a crude methanol extract coupled with the activity observed in the methanol fraction suggests that these chemicals may play a role in native plant suppression. Also, aqueous leaf extracts from several native shrubby trees were examined to determine if allelopathy was common in species indigenous to the area. The results indicate that Eastern Redbud (*Cercis canadensis*) and Ohio Buckeye (*Aesculus glabra*) exhibit germination suppression characteristics that are similar to that of Amur Honeysuckle (*Lonicera maackii*) and Tree-of-heaven (*Ailanthus altissima*).

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Effects of disturbance on Japanese Stiltgrass dispersal and recruitment

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Abstract. Microstegium vimineum (Poaceae), or Japanese Stiltgrass, is an invasive C4 grass adapted to living in shaded environments. Since its first sighting in Tennessee in 1919, *M. vimineum* has spread rapidly across the eastern United States. This species has only recently invaded Ohio and is now commonly found along roads and trails within forest matrices. It also has the ability to establish beneath closed forest canopies, and is often found in moist forests such as floodplains and stream banks. Once established, *M. vimineum* forms dense monocultures within the herbaceous community, likely displaces native species, decreases forest regeneration, and ultimately changes soil properties. Previous studies have suggested that bare soil microenvironments are critical to the establishment of new *M. vimineum* populations. This observation combined with the proclivity of *M. vimineum* for commonly disturbed areas suggests a tight relationship between disturbance regime and the establishment of *M. vimineum* populations. However, despite this apparent correlation, few studies have attempted to go beyond observational patterns and tease apart the specific mechanisms that might be driving this relationship. With the aim of overcoming this shortcoming, the objectives of this research were to determine the effects of vegetation, litter and soil disturbance on *M. vimineum* dispersal and recruitment along roadsides. We conducted an experiment during the growing season of 2006 where we utilized six roadside study sites with established populations of *M. vimineum* in the Wayne National Forest near New Floodwood, Ohio. Within each site (statistical block), we established four experimental partitions (treatments) in a 2 × 2 design by keeping/removing understory vegetation and disturbing/not-disturbing understory litter/soil. We then monitored each treatment for *M. vimineum* recruitment, cover, species richness, as well as the movement of the edge community into the forest understory. After one year of observation, our results indicate that, although a combined effect might occur (between vegetation and soil disturbance), disturbance of the litter layer was the most significant effect influencing the germination and establishment of *M. vimineum*. However, none of the treatments appeared to affect the rate of migration of the road edge community into the forest understory, or species richness. This apparent inconsistency is likely the result of the short duration of our study. Despite its prevalence within the literature, the importance of light availability to the establishment of *Microstegium vimineum* populations was shown to be overestimated within our experiment. Disturbance to the forest floor was the overwhelmingly important effect in determining the rate at which *M. vimineum* becomes pervasive within the understory. Additional study, extending past one year, will assist in better understanding the dynamics and dispersal of this species.

USDA Forest Service, State and Private, Northeastern Area