

# Ohio Invasive Plant Research Conference:

Bridging the Gap  
Between Land Management and Research

Proceedings



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Ohio Agricultural Research and Development Center



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**John Cardina, Editor**

Department of Horticulture and Crop Science  
Ohio Agricultural Research and Development Center  
The Ohio State University



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Cover Photos: Invasive plants examined as part of this conference include purple loosestrife (*Lythrum salicaria*), garlic mustard (*Alliaria petiolata*), and tatarian honeysuckle (*Lonicera tatarica*).

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# Preface

Weedy and invasive plants are recognized as significant contributors to — and symptoms of — global environmental change. Due to diverse topography, natural resources, geography, history, and current land use, it should come as no surprise that Ohio is a state that is severely impacted by invasive plants. Human traffic along its northern and southern borders has long been a significant mode of introduction of invasive species.

In a state comprising some 44,830 square miles, there are 41,000 miles of rivers and streams, and 33 physiographic regions that vary in glacial geology, bedrock geology, topography, soils, and geologic history. With this favorable topography and negotiable waterways, Ohio has been a cross-roads for human migration for thousands of years.

The development of the interstate highway system has made Ohio the fifth most trafficked state, with more miles of road than any other state in the Midwest. This high level of human activity superimposed on a state where four ecoregions intersect makes Ohio a crucible for mixing of plant genotypes and the adaptation and selection of weedy growth habits.

Invasive plants have been a subject of interest in Ohio since at least the late 1800s, when A. D. Selby (1897) produced *A First Ohio Weed Manual*, one of the earliest weed identification books published in the United States. His book described 279 species considered to be weeds of agriculture. Soon after that, Kellerman and Kellerman (1900) published *The Non-Indigenous Flora of Ohio*, a list of 430 non-indigenous woody and herbaceous plants, comprising just over 20 percent of the known flora.

These books are the best historic record of invasive species in the state. It is remarkable

to read them and recognize how many species that we now consider serious invaders were not apparent at the end of the 1800s. These include *Alliaria petiolata* (garlic mustard), *Lythrum salicaria* (purple loosestrife), *Lonicera maackii* (amur honeysuckle), *Phragmites australis* (common reed), *Rosa multiflora* (multiflora rose), *Polygonum cuspidatum* (Japanese knotweed), and others that today would rank at the top of any list of serious invasive plants in the state.

In the more agriculturally oriented book by Selby (1897), a modern reader finds that species such as *Abutilon theophrasti* (velvetleaf) and *Ambrosia trifida* (giant ragweed), which are now serious broadleaf weeds, were considered merely occasional components of roadside flora. Some species that Selby characterized as very important weeds in the late 1800s, such as *Rumex acetosella* (red sorrel) and *Verbascum blattaria* (moth mullein), are barely recognized as weeds today. Reading this older literature helps to illustrate that our flora is in flux along with our concept of what constitutes a weed or a troublesome invader.

The Ohio Invasive Plant Working Group was initiated in 2002 to bring together expertise and interested parties throughout the state to address issues associated with invasive plants. A sub-group focusing on research and science initiated the idea of a state-wide conference to bring researchers and land managers together to discuss current understanding of the extent of the invasive plant problem, the ecology of specific species, effective control and restoration methods, and efforts to halt the establishment of potentially destructive species.

To this end, the Ohio Invasive Plant Research Conference was held on March 5, 2004, at the Ohio Department of Transportation Auditorium in Columbus, under the theme *Bridging the Gap*

*Between Land Management and Research.* Eight oral presentations and more than 20 poster presentations covered a wide diversity of topics represented in the papers and abstracts contained in this volume.

The highly successful conference resulted from the work of many people. I would like to acknowledge the ad-hoc committee that was primarily responsible for planning and running the conference:

Nicole Cavender  
The Wilds

Catherine Herms  
The Ohio State University  
Ohio Agricultural Research and Development Center  
Department of Horticulture and Crop Science

Marleen Kromer  
The Nature Conservancy

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Cleveland Museum of Natural History

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The Garden Club of Cleveland

Little Garden Club of Columbus.

*John Cardina*

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Selby, A. D. 1897. *A First Ohio Weed Manual*. Ohio Agricultural Experiment Station. Bulletin 83. 400 pgs.

# Introduction:

## Bridging the Gap Between Land Management and Research in Ohio

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### History of Invasion Biology

Over the last two decades, invasive species have gained attention as an important cause of species decline and native habitat degradation (Vitousek *et al.*, 1997; Wilcove *et al.*, 1998). The scientific recognition of biological invasions, however, dates back as early as the 1850s when Charles Darwin in his writings noted the issue of movement of species across geographic boundaries and recognized the potential threats that introduced species may have on native ones.

Although there were studies done in the years since Darwin, the seriousness of the threat of invasive exotics was brought to the attention of the scientific community in 1958 by Charles Elton's book, *The Ecology of Invasions by Animals and Plants*. Elton's book sounded an early warning about an environmental catastrophe caused by the invasion of non-native species.

Although the book is amazing in its prescience, it was largely under-appreciated until the 1980s when invasion biology emerged as a formally recognized field of study, and it became widely acknowledged that invasion by non-native species was one of the biggest threats to naturally occurring species and ecosystems.

A host of conferences and symposia resulted in books documenting the seriousness of the problem (Macdonald *et al.*, 1986; Mooney and Drake, 1986), and since then, there is mounting evidence of severe environmental degradation due to non-native invasions.

Invasive species comprise about 10 to 20% of the non-native species, or more than 650 to 1,750 species of plants, animals, and plant pathogens in the United States that have become established outside of human cultivation in free-living populations (Williams and Mefee, 1996; Chornesky and Randall, 2003). Furthermore, it is now estimated that more than 60% of the species listed under the Endangered Species Act are threatened indirectly or directly because of invasive species (Wilcove *et al.*, 1998; D'Antonio *et al.*, 2001).

There are some 2,500 species of naturalized non-native plant species. Of these non-native plants, only a small percentage threaten native biodiversity, but the small number that do can be significant in determining floristic, structural, and dynamic community properties.

### Invasive Plant Awareness in Ohio

Invasive plants in natural areas in Ohio gained increasing awareness from public land agencies in the mid 1980s. The many rare plant species and communities of Ohio were becoming more important as vegetation surveys were compared to early records from the state. Park districts, state agencies, and The Nature Conservancy began small-scale land management projects in targeted areas to hold back succession. At the time, however, intervention in natural areas was controversial. The common consensus was that natural areas were best left alone, and little research was available to assist with local land-management decisions.

In the mid 1990s, the Natural Areas Council, whose members were appointed by the governor, began seriously acknowledging the problems of invasive plants in Ohio and appointed the Division of Natural Areas and Preserves, part of the Division of Natural Resources, to formally lead a campaign to address the issues to the public. With funding provided by the Ohio Environmental Protection Agency's (EPA) Environmental Education Grant program, educational materials were produced in partnership with the Division of Natural Areas, the Nature Conservancy, and the Columbus and Franklin County MetroParks. These materials included brochures, a series of fact sheets describing the most invasive plants in Ohio's natural areas, and a list that provides alternative plants to use in the landscape (Windus and Kromer, 2001).

Inevitably, the Ohio landscape industry was pulled into the discussion. Several of the plants listed as the most invasive plants of Ohio were once common in the nursery trade and some still remain today. The point should be made, however, that not all non-native plants are invasive pests. Furthermore, non-native plants may be of ecological value in some systems, playing important roles in recovery after disturbance or being surrogates for extirpated species, especially in human-dominated or severely human-impacted systems (Williams, 1997).

In order for the issue of invasive plants in natural areas to be addressed successfully at all levels, the participation of land managers, scientists, members of the landscape industry, and government officials must all be a part of the ongoing discussion and decision making.

Public workshops were presented around Ohio between 1996 and 2000 to promote public awareness of the threats and management issues of invasive plants. This was successful in bringing more awareness to the public and land managers, but there was a need to involve a larger constituency.

The Ohio Invasive Plant Working Group formed in 2002. The working group is an informal network represented by a diverse group of partners interested in many aspects of invasive plant issues. Organized committees are focused on education, communication, public relations, research and science, control methods, and restoration of natural communities. The working group recognizes the need for ongoing discussion between scientists and land managers as necessary to effectively tackle problems caused by invasive species.

## **Impacts of Plant Invasions in Natural Areas**

Evidence of ecological damage from plant invasions can be found just about everywhere in the United States, except maybe Alaska, and their impacts can range from local suppression of a single native species to significantly impairing the functioning of entire systems (Williams and Meffe, 1996; Cox, 1999; Ewel *et al.*, 1999; Mack *et al.*, 2000; Randall, 2000; Chornesky and Randall, 2003). Plant invasions can displace native species from native plant communities by competition (see McCarthy, Gorchov, Blossy, Mal, and others in these proceedings), hybridization (Vila *et al.*, 2000), and by altering the abundance or behavior of native plant pollinators (Grabas and Laverty, 1999; Brown *et al.*, 2002).

At the ecosystem level, invasive plants can directly or indirectly alter soil chemistry, soil biota, hydrology, and water availability, as well as alter natural disturbance regimes such as fire (Schmitz *et al.*, 1997; Gordon, 1998; Kilronomos, 2002). Invasive plants also can have impacts on food-web dynamics. For instance, plants have been shown to reduce reproductive success of birds and other types of wildlife indirectly by altering the architectural characteristics of a system (see Rodewald in these proceedings).

Ohio's forests, as well as the Midwestern and Eastern forests in general, have had tremendous negative impacts from a variety of invasive plants including garlic mustard (*Alliaria petiolata*), amur honeysuckle (*Lonicera maackii*), Japanese honeysuckle (*L. japonica*), multiflora

rose (*Rosa multiflora*), and tree-of-heaven (*Ailanthus altissima*).

Garlic mustard, a European biennial, with its rapid seedling growth and its ability to grow in both shade and sun as well as in wet and dry sites, is finding a strong foot-hold in our forests and is readily out-competing native species (see McCarthy, Gorchov, Cipollini, Banker, Slaughter, Sosnoskie and Cardina, and Frey in these proceedings).

Amur honeysuckle can establish a dense shrub stratum in forests, reducing diversity of herbs and woody seedlings and leading to a suppression of native canopy replacement (see McCarthy, Geiger, Lieurance and Brown, Banker, Fuchs and Geiger, Dorning and Cipollini in these proceedings).

Wetlands have been altered dramatically by invasive plants such as purple loosestrife (*Lythrum salicaria*) and glossy buckthorn (*Rhamnus frangula*). As these plants replace the native vegetation, there can be negative repercussions to wildlife habitat and natural ecosystem processes (see Blossey, Mal, and Moser in these proceedings).

Introduced species frequently interact with one another and may have synergistic interactions that may lead to accelerated impacts on native ecosystems. Two or more harmful exotic species may act in consort so that their joint impact is more severe than that of the several species acting separately. This has been referred to as invasional meltdown as presented by Simberloff and Von Holle (1999). Purple loosestrife may be interacting in such a way in Ohio's natural areas (see Mal in these proceedings).

Tackling these types of issues is often a daunting task for land managers and conservationists. In many natural areas, the number of established invasive species or the area infested already far exceeds local management resources. The term "overwhelmed" is often what you hear in the same sentence as controlling invasive plants. The lack of money and staff forces land managers to set priorities and address what they determine

as the most important problems first. Although informal or formal monitoring programs may be in place within some organizations, most land managers are not able to implement formal research programs, and they rely on partnerships to assist with research.

It is important to address these invasive plant issues at many levels. The land that we are managing not only includes classic Ohio landscapes but also land that has already been highly disrupted by agriculture, industry, suburban sprawl, and other human impacts. We must understand the best approaches to managing and restoring land at the urban and suburban level (see Quigley in these proceedings) as well as addressing invasive plant issues in some of our large tracts of lands, such as The Wilds, that have been severely altered from their original state due to mining. These areas may be heavily established with invasive plants but still have tremendous value for the support of native wildlife.

As scientific research progresses and information exchange with land managers becomes more fluent, priority decisions will become easier and management at species and ecosystem levels will become more effective.

## **Research Priorities for the Preservation of Ohio's Biodiversity**

Based on conversations with land managers, researchers, and other land conservationists in Ohio, I have outlined some of our priority research areas:

### **Applied Research Designed To Address Specific Management Problems**

- Develop target control methods specific to natural areas.
- Develop more efficient control strategies, including chemical and non-chemical techniques.
- Examine costs and benefits of control strategies.
- Conduct formal research on control methods in a peer-reviewed setting.

## Research on the Economic and Environmental Impact of Invasive Plants

Managers have expressed the need to have more examples of indirect and direct impacts of invasive plants in order to substantiate their land-management decisions.

- Loss of native and beneficial species.
- Impacts to community-level dynamics.
- Economic and social implications.
- Thresholds — when do restoration and economics strike a balance?

## Impacts of Control Methods and Restoration

- Consequences of various management actions, including no management (*i.e.*, the difference between chemical vs. physical mechanisms).
- The re-recruitment process.
- The restoration process after control.
- Strategies of keeping invasive plants from coming back after initial control.
- Research on species interactions in plant communities, including plant-plant interactions, plant-microbial community interactions, and synergistic interactions (*i.e.*, effects of deer and their interactions with invasive plants).

## Basic Research on the Invasion Process

Controlling invasive plants without understanding the causes of the invasions is likely to only be a temporary solution.

## Basic Ecology of Species That Are Potentially an Invasive Threat to Natural Areas

Many of the papers and abstracts presented in these proceedings have begun to address some of these research priorities.

## Bridging the Gap

Simply preserving land is not enough to maintain its value for the future. One of the goals of the Ohio Invasive Plant Research Conference (March 2004) was to facilitate collaboration

and discussion between researchers and land managers in order to develop more effective control and restoration methods, set realistic targets, and curb further potentially destructive invasions of natural areas before they happen.

By publishing these proceedings, it is our intention to further assist in communicating the research that is currently being done in Ohio so that it can be applied by land managers as well as other researchers.

## Acknowledgments

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# Pre-Emptying Establishment of Exotic Invasives Through Ecologically Sound Landscape Practices

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## Abstract

Plant invasions are fostered by empty ecological niches in converted, disturbed, and recovering landscapes, in both woody and herbaceous plant communities. In millions of farmed acres, intensive monospecific cultivation leaves soils without cover crops in the off-season. Waterways are denuded of plant cover and channeled to accelerate runoff. These examples of deficient stewardship values have precluded directional management of recovering woody plant communities in the wake of widespread field and pasture abandonment.

I propose that many invasions of undesirable exotics — and undesirable native plants as well — can be slowed, if not completely precluded, by conscious landscape management that fills all potential plant niches (strata) with desirable plants. Areas left unplanted in the constructed landscape — bare soils, covers of permanent decorative mulch, ornamental canopy tree zones without an understory component, or bare plowed fields that sit for months at a time — are literally nursery beds for invasive plants to exploit. Various lines of research in my lab attempt to demonstrate the value, practicality, and utility of filling vacant areas in the urban, suburban, and rural landscapes.

## Introduction

The flow of plant species between the Old World (Eurasia) and the New (the Americas) has been strangely unidirectional. While many important domesticated and wild plants from the Americas were introduced to Eurasia both intentionally

and accidentally, and some have naturalized there, very few American plants have actually become invasive there. Contrarily, hundreds of plant species from the Old World have become widely established in American landscapes, and scores of them are now significant components of regional ecosystems. Many are actually invasive in the sense that, once established, they displace native species.

How can such a wide variety of habitats be so vulnerable to invasion on this continent, when a very similar array of conditions in Europe and Russia has not seen comparable invasions? I hypothesize that the difference is at least partially attributable to differences in land management, both in methods and intensity.

In brief, the European colonists in America “converted” (a euphemism for clearing native plant communities) the landscape at a scale and a rate unprecedented in human history. As they subsequently moved from the rocky coast of New England and the thin-soiled coastal plains of the Southeast into the fertile soils of the upper Midwest, vast tracts of old fields and degraded forested lands were left to the processes of succession. These millions of acres are rich in sites for colonization by new species.

In addition, extremely large tracts of homogeneous plant communities, forests, grasslands, and wetlands were fragmented by clearing for agriculture and other human uses, so that ratios of edge to interior habitat increased exponentially. These conditions of continual disturbance and increased “edge” are ideal for

ruderal or r-selected plant species to arrive, establish, and proliferate.

In Europe, a much smaller land mass had a much denser human population for many centuries; landscape conversion was significantly completed before the new American propagules were introduced there. Historically, in most of Europe and vast areas of Asia, land has been more intensively managed, with negligible abandonment of farmlands.

Industrial annual monoculture is not under discussion here; our society would not support perennial polyculture for its production of cereals, vegetables, fruits, and fiber. The installed or “ornamental” landscape, however, is very amenable to a change in methods that will result in more attractive plantings with much less maintenance than now performed with the agricultural mentality. The landscape production, installation, and maintenance industries now earn billions of dollars a year — more than \$3 billion in Ohio alone. A more sustainable approach to design and landscape management would not only mean greater profits, but also increased environmental health, including greater resistance to exotic invasive plants.

Landscape function should aim for “sustainability” — that is, obviating the need for frequent or intensive mechanical and chemical inputs. The recent awareness that “native” plants are often better adapted for local conditions has not been complemented by public understanding that native plants are adapted to specific environmental conditions — many of which no longer exist in a converted landscape. Native plants also have native pests and pathogens, while many introduced plants have escaped the pests of their original habitats.

Use of native plants must be combined with knowledge of appropriate planting combinations, substrate, density, and nutrition, just as for any introduced plants. “Native to Ohio” is meaningless; “native to mesic lowland forest understory” is meaningful. Mixed communities are almost always more resilient than limited or monospecific plantings. More horticultural research should focus on the

interactions — both competitive and beneficial — of multiple stratum plantings of various habits, whether native, introduced, or a combination of plants.

Even when investigations are focused on a small component of a landscape system, it should be borne in mind that these findings have a distributive effect — that is, when applied over a regional scale, improvement of watershed function and plant-community health can be promoted even by small landowners. No garden is too small, no forest too large, to be independent of ecological management principles.

Whether for expanding suburban development, extractive forestry, or row-crop farming, all land planning should occur in watershed units. Unlike most political boundaries, drainage reaches delineate land areas, at all scales, that are connected not only by stream flows but also by biotic migrations, groundwater and surface pollutant movement, and contiguous or fragmented plant communities. For example, many plant invasions have first occurred along riparian corridors, gradually reaching entire ecosystems.

## Recent Research

I report briefly on some of my work and that of former students at Ohio State. While the individual lines of research were not simultaneous or even coordinated, they share a common thread of sustainable (perennial) landscape function, productivity, and aesthetic value.

Three lines of research deal explicitly with invasive plant species in the Ohio landscape. Populations of the exotic shrubs Amur honeysuckle (*Lonicera maackii*) and common privet (*Ligustrum vulgare*) were described in the Glen Helen Reserve, a tributary of the Miami River in southwestern Ohio. We found that they had a distinct gradient of habitat preference and that the bottomlands, where the native plant community was relatively dense, had far fewer and smaller exotic plants.

We are also working on the control of garlic mustard (*Alliaria petiolata*) in central Ohio woodlands and in the greenhouse. Preliminary findings are that the spray application of gibberellic acid in a winter warm spell, when native plants are completely dormant and not vulnerable to the treatment, will cause existing rosettes of this biennial pest to bolt into flowering shoots which are then killed by the next hard frosts.

Other research is focused on the apparent inability of the native woody plant community to re-establish on the vast re-contoured mine spoils of southeastern Ohio. In many areas, the only woody plants to colonize are autumn olive (*Elaeagnus umbellata*) and tree-of-heaven (*Ailanthus altissima*). Studies are needed on the role of mycorrhizal symbionts in the success of these exotics and methods for facilitation of native forest recovery.

Other researchers are examining very different components of wetlands and watersheds in plant communities ranging from rural old-fields to downtown roof systems. In six very large wetland basins constructed on former fields, we are studying the rate of native wetland community recovery, the species sequence of arrival and competitive establishment, and methods of accelerating the successional trajectory while pre-empting the establishment of invasive exotics that are a particular nuisance in Midwestern wetlands.

Studies are quantifying the absorption and adsorption of petrochemical pollutants from paved areas in urban and suburban Columbus, Ohio, through varying levels of organic amendments to the soils of "bioswales." These landscape amenities are intended to detain and filter polluted runoff while using natural processes of decomposition and plant uptake. An added benefit is that bioswales appear as wetland gardens rather than polluted or litter-filled ditches that are too common in our populated areas.

In other research, we are taking the distributive nature of runoff treatment to the tops of urban buildings, in a demonstration of the ecological

services provided by green roofs. Even conventionally designed buildings can benefit from the added insulation of a maintained plant community, and the whole watershed benefits from the decreased intensity of urban runoff and the filtration of air-borne pollutants at the point of contact.

I have demonstrated that planting of groundcovers in ornamental landscapes will dramatically reduce the weed load even in the first year and improves with time. More significantly, the use of two or more groundcover species simultaneously will provide much more rapid establishment and even greater reduction of weeds. An undergraduate researcher found that the native medicinal plant goldenseal can be optimally cultivated under the filtered shade of existing woodland communities; such findings may offset the dangers of goldenseal's extirpation from over-collection in the wild, as has happened with ginseng throughout its Appalachian range.

Work at Ohio State University's Chadwick Arboretum has assembled the most comprehensive collection of willow (*Salix*) trees, shrubs, and groundcovers in North America, including eight Ohio natives. These versatile and resilient plants can sequester heavy metals from polluted soil and water, can withstand the damage caused by elevated ozone in city environments, and can actually grow preferentially in heavily compacted soils. The introduction of (non-invasive) alpine species from eastern Europe also holds great promise for the nursery trade. The potential for willow use in biomass production in the upper Midwest is a future line of research.

Four urban gardens have been installed to demonstrate inputs and potential outputs of intensive residential gardens in Ohio. Recent publications show the disparity of productivity from typical lawn/ornamental border landscapes, to "organic" vegetable and fruit gardening, to the novel idea of "forest gardening" in which edible plants are interplanted in a dense, seemingly random layout through a very thick layering of organic mulches. The tidier the landscape (in the sense of

simple layout and minimal diversity of plants), the greater the weed load.

We have also tested the interspecific competition of under-planting of apple trees with four species of berry shrubs, in a rare demonstration of perennial polyculture in the temperate landscape. Two of the four berries did very well in the partial shade of the apple trees, without significant effect on the apple yield. While this kind of fruit production is more suited for the residential rather than the commercial scale, it is another encouraging display of the benefit of planting more than one species in a single space and time.

## Conclusions

The diverse studies outlined previously point to several principles of landscape management that can be followed to reduce the vulnerability to, and impact of, invasive plants:

- Manage landscapes so that desirable or appropriate plants occupy all strata and niches.

- Mingle edible plants with other ornamentals.
- Accelerate wetland community succession.
- Create small wetlands and filtration systems, as even these small areas can benefit a regional watershed.
- Diversify landscape habit and structure.
- Provide mixed groundcovers — including ephemerals. Mulch alone will facilitate invasive weeds.
- Manage abandoned field and disturbed woodlands.
- Target invasives not just for elimination but pre-emption.

## Acknowledgments

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# Ecology of Invasive Species in Southern Ohio: A Tale of Four Species

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## Introduction

Ecologists, environmentalists, and land managers have noticed a dramatic rise and spread of invasive species throughout the landscape over the last several decades. The arrival of these species has been believed to dramatically alter community and ecosystem level processes, although many of these processes have not been studied in detail.

Elton (1958) was the first to tackle the issue of invasive species in a comprehensive manner with his landmark publication, *The Ecology of Invasions by Animals and Plants*. Soon after, Baker (1965) attempted a synthesis of the characteristics of invasive species. Many of their insights were excellent; however, this proved to be a difficult endeavor as there was considerable variation in plant life-history attributes and there were ultimately many exceptions to any generalizations that could be wrought.

The pioneering work of Elton (1958) and Baker (1965) was limited by the fact that their observations were all qualitative in nature. Their goal was to evaluate process through the derivation of the pattern of life-history traits. Initial, broad-based analyses such as these are certainly necessary, but it now appears unlikely that this will yield much useful insight. However, there are other approaches to this search for pattern in species characteristics. Moreover, focusing on specific functional or phylogenetic groups appears to be quite a useful approach. Rejmánek and Richardson (1996) tackled the general question of what traits make a species invasive by restricting themselves to 24 species

in the genus *Pinus* (12 that are non-invasive, 12 that are highly invasive). In this manner, they were able to control for phylogenetic divergence. They analyzed 10 life-history traits and found that seed size and seed-crop size were extremely important in predicting which species would most likely become invasive.

The study of invaded habitats has proved equally difficult in that the information from the opposing side of the coin has always been absent — what patterns and processes resulted in failed invasions, which are not evidenced in the landscape? Elton (1958) suggested that species-poor communities characterized by disturbance were most susceptible to invasion.

Levine and D'Antonio (1999) reviewed those studies in the literature linking species diversity and invasibility. While many studies agreed that species-rich communities were more resistant to invasion, they found considerable conflicting information. Likewise, while most ecologists continue to believe that disturbance makes a community vulnerable to invasion, there is considerable conflicting data. For example, Smith and Knapp (1999) found that grasslands subjected to annual burning resulted in a decreased abundance of invasive species. Thus, the ability to predict species invasiveness and habitat invasibility has been fraught with problems (Mack *et al.*, 2000).

Given the lack of predictability within and among species and habitats, the approach necessitated is a careful examination of individual species observed across a range of

environments and disturbance regimes. Thus, I have examined the ecology of four species that are either invasive or have the potential to be invasive in southern Ohio — garlic mustard (*Alliaria petiolata*), princess tree (*Paulownia tomentosa*), Amur honeysuckle (*Lonicera maackii*), and tree-of-heaven (*Ailanthus altissima*).

Ecologists have long recognized that “no species can become established and hold its place in a community unless the seedlings can survive and grow to maturity” (Keever, 1950). Thus, the overriding goal of my research has been to examine how the aspects of early regeneration ecology relate to habitat invasion. I present here a précis of selected studies on each of these four species as it relates to seed and seedling ecology.

## Garlic Mustard

Garlic mustard (*Alliaria petiolata*; Brassicaceae) is a bit unusual in that it is a strict biennial throughout most of its invaded U.S. range (Cavers *et al.*, 1979). Thus, populations of this species often alternate between rosette and mature form from year to year (McCarthy, 1997). The species has a xenogamous breeding system, so only one plant is required to establish a population. Copious seeds are produced, and a viable seed bank is maintained for four to five years (Baskin and Baskin, 1992). Populations are extremely plastic (Byers and Quinn, 1998), and the species is able to invade both upland and lowland forested habitats, often associated with edges (Nuzzo, 1993).

Many invasive species, including garlic mustard, have long been believed to cause a community- or ecosystem-level effect in the communities in which they invaded. For example, invasion by garlic mustard was often touted by managers as causing a loss of species within the community. Alternatively, others argued that species might just be entering communities and filling empty niches.

By 1990, this question still had not received any empirical attention, so in 1991 I began a garlic-mustard-removal study to test this hypothesis. Species abundance and diversity were monitored

following garlic-mustard removal in paired plots in a floodplain in western Maryland (McCarthy, 1997). Results in the second year showed a dramatic response to release. Many species emerged from the seed bank that had not been previously prominent in the community (Figure 1). Further, the patterns of diversity were inversely related to the phase of garlic mustard in the community (*i.e.*, effect was greatest when the population contained primarily rosette plants). Thus, garlic mustard was indeed having empirically defined negative impacts on plant community diversity and abundance.

In a separate experiment, we wished to understand how garlic mustard invaded stable forest communities. Observations suggest that a number of potential factors are involved. First is habitat. While garlic mustard has been observed to enter both lowland and upland communities, we’ve noticed that it always appears to be greater in yield and reproductive potential in lowland habitats. Moreover, we often have seen it enter woodlands in association with edges (as also observed by Nuzzo, 1993).

Lastly, recognizing that the seeds need an appropriate seed-bed in which to germinate, we were uncertain what the role of litter disturbance would be on recruitment and establishment. We designed an experiment to examine these factors (Meekins and McCarthy, 2000). Seeds were sown into plots in upland/lowland, habitat edge/interior, and in plots where leaf litter was disturbed/undisturbed in an Athens County, Ohio, hardwood forest.

We examined a whole suite of potential regeneration and growth variables over several years, ranging from rosette establishment, survival, growth, and biomass to mature plant height, biomass, and fecundity. We discovered that, indeed, habitat was most important in explaining the patterns of garlic mustard growth and reproduction (Figure 2) and that plants growing on edges were also bigger and more fecund than those in forest interiors (Meekins and McCarthy, 2000). Litter disturbance was found not to be significant. Thus, land managers need to pay closest attention to lowland edge

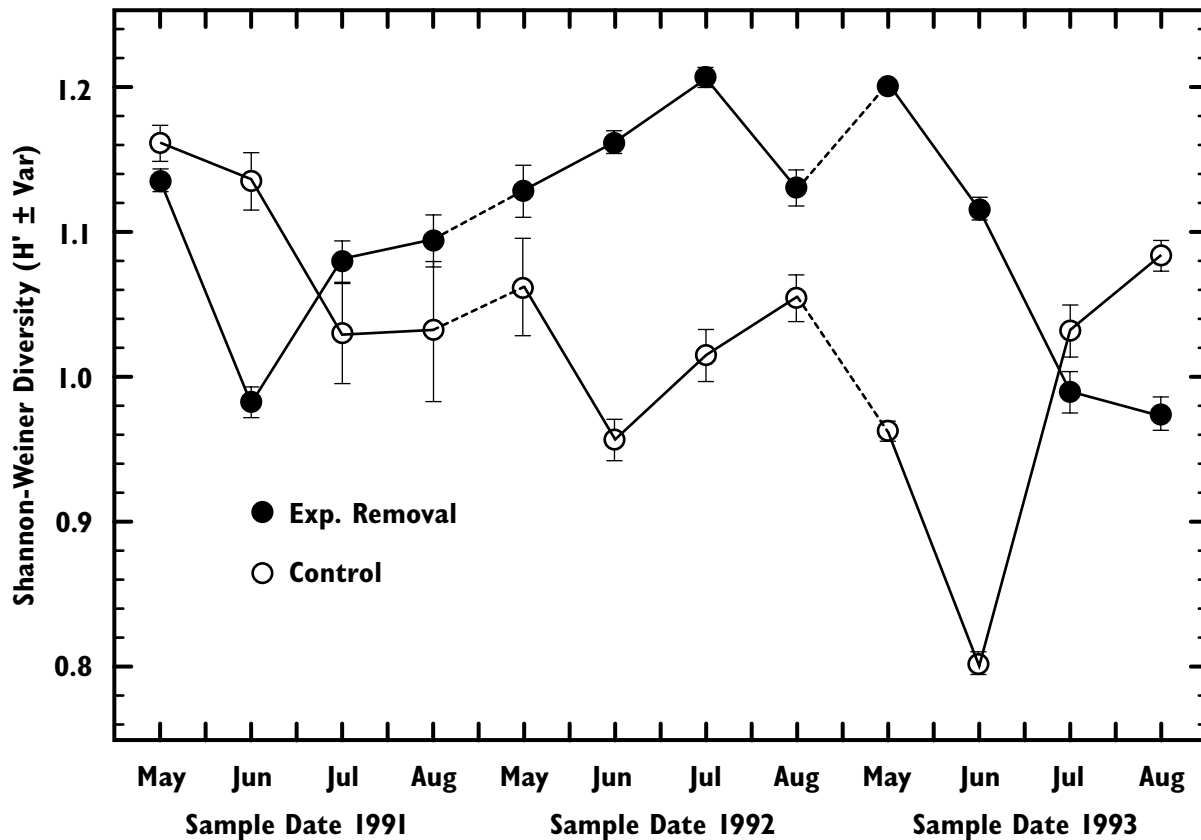


Figure 1. Mean  $\pm$  var ( $n = 9$ ) Shannon-Weiner ( $H'$ ) diversity in control vs. experimental plots, where garlic mustard was continually removed by hand weeding for the duration of the study.  $H'$  was significantly (adjusted  $P < 0.05$ , paired t-test) greater in removal plots. During the greatest release period (1992), most of the garlic mustard plants existed as rosettes. Alternating years of rosettes vs. mature plants may provide brief windows of release for native species. See McCarthy (1997).

habitats as a source of entry and proliferation in areas that have not yet been invaded.

## Princesstree

Princesstree (*Paulownia tomentosa*; Scrophulariaceae) is a perennial tree that has long been a problem species in the southern Appalachians where it has invaded high quality forests and poses a threat to forest biodiversity. The species is a prolific seed producer, out-producing many hardwoods 100 to 1 at maturity. Dispersal of the seeds is by wind, and the presence of a seed bank is unknown.

The species has been constrained below the Ohio

River for many years, as it is not winter hardy. Above-ground portions die back at subzero temperatures during the establishment phase. As regional climates warm, this species has now moved north of the Ohio River and will pose an immediate threat to the mixed oak forests of southern Ohio. I have now observed the species in several forest canopy gaps in Athens and Vinton Counties.

The species is also known to invade easily following forest fire in the southern Appalachians. Ironically, this species is being touted as a commercial crop species in neighboring states.

Given that this species has been found in gaps

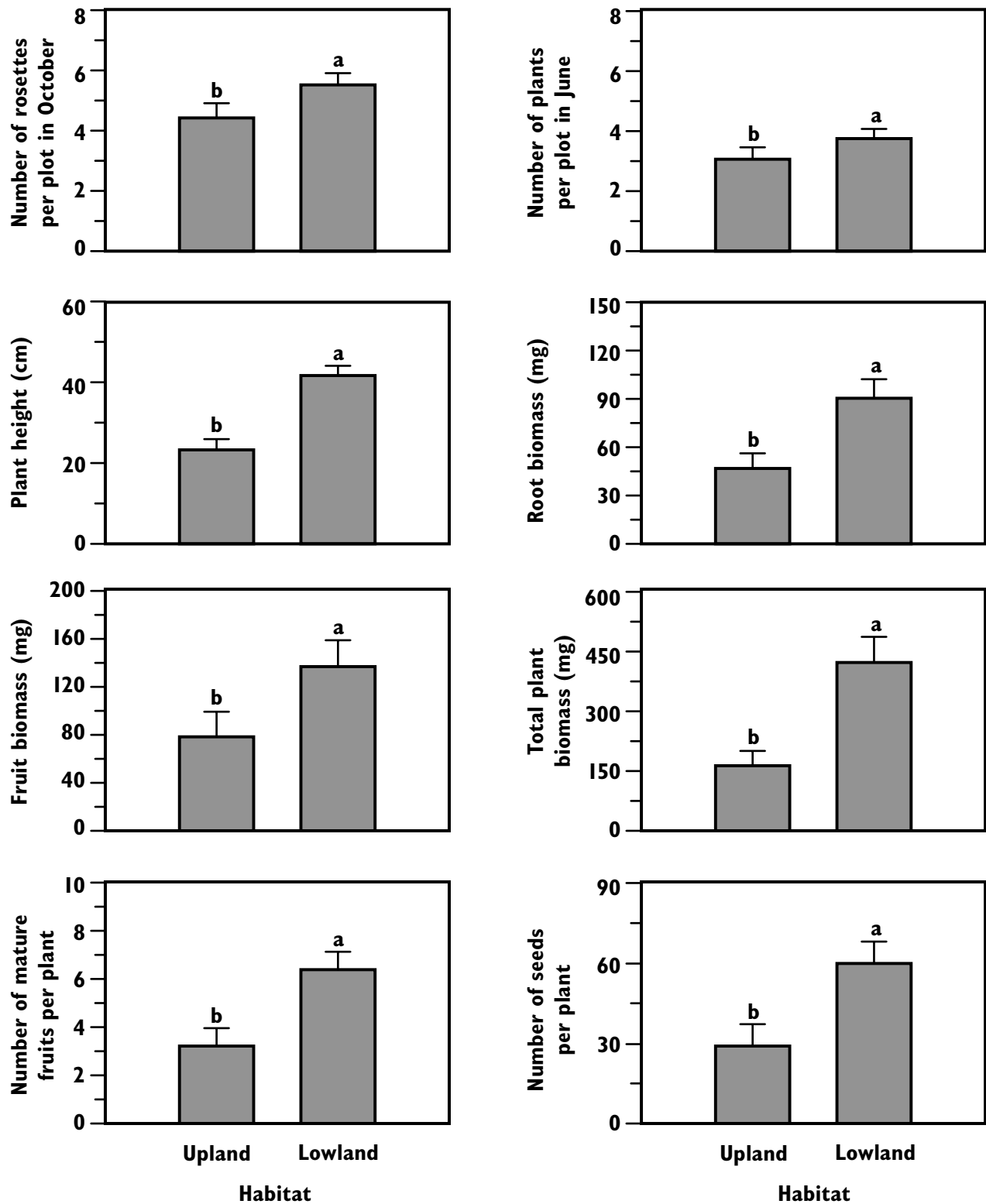


Figure 2. Mean  $\pm$  SE of height, biomass, survival, and reproductive output of mature *Alliaria petiolata* plants growing in upland and lowland plots of a mature deciduous forest in southeastern Ohio. See Meekins and McCarthy (2001).

and that it is an extremely fast-growing light-loving species, we became concerned about the effects of princess tree in managed landscapes. In particular, what happens in clear-cuts that have very high light availability and a diversity of microsites and disturbed soil? We established a study using five clear-cuts in southeastern Ohio and ran replicate transects perpendicular to the cut so that they included open clear-cut, exposed edge, and forest interior (Longbrake and McCarthy, unpublished).

Seedlings were planted in replicate plots and evaluated for survival and biomass allocation patterns, following partial harvests from 1998-2000 (cf. Longbrake and McCarthy, 2001). While winter kill was evident in our study (winters still exhibit sub-zero temperatures), as was localized browsing by rabbits and deer, survival of this species ranged from 20 to 45%, with final survival being greatest in clear-cuts (Figure 3). It may prove that the abundance of princess tree in a landscape may be very important as it relates to what type of forest management is practiced. Should regional climate changes proceed along the same trend, princess tree has the potential to become a highly problematic species in our managed forest landscape.

Because the seed bank of this species has yet to be investigated, we designed an experiment whereby seeds were planted in replicate mesh bags below the litter layer (between O- and A- horizons) as well as 5 cm into the soil (A-horizon). Replicate bags were placed in the same three managed landscape treatments (clear-cut, edge, forest interior) and harvested at six-month intervals for a period of three years. Seeds were removed from their bags, placed on Petri plates, and transferred to a seed germinator and monitored for germination. Ungerminated seeds were checked for viability using a tetrazolium test. After six months, germination remained very high (>95%) in all habitats (Figure 4).

By three years, germination of seeds from leaf litter had fallen to 60 to 70%, but remained high (ca. 90%) when buried in the soil. Thus, our experiment proves that this species does, indeed, keep a persistent viable seed bank. This is particularly problematic for land managers

because — like garlic mustard — the species will never be removable with a single treatment application.

Lastly, we wished to investigate what microsite substrates permitted the easiest establishment of princess tree. We chose six substrates on which the species has been observed to grow in southeastern Ohio — cobble, gravel, sand, bare soil, and litter; we added potting mix as an organic soil with increased water-holding capacity for the sixth substrate. Microsites were set up in 25 × 25 cm samples and arranged in a 6 × 6 Latin Square design in a common garden (Figure 5).

We found that germination, establishment, and survival were best on bare soil. In rank order, the species preferred bare soil > potting soil > cobble > gravel > sand > leaf litter (Longbrake and McCarthy, unpublished). The terminal opposing ranks of bare soil and leaf litter flags a very important issue as it relates to forest management. Patches of bare soil are at a much greater risk of princess tree establishment compared to undisturbed litter beds.

## Tree-of-Heaven

Tree-of-heaven (*Ailanthus altissima*; Simaroubaceae) is a perennial tree with a dioecious breeding system. This species represents a bit of an anomaly in that species with separate sex individuals generally do not make good invasive species because both genders must be dispersed to a site for it to become established. This species produces copious wind-dispersed seeds and is a denizen of typically disturbed or waste areas (brown ways) along road corridors. The species is increasingly escaping into intact forests in southern Ohio. Whether or not the species keeps a seed bank is unknown.

Tree-of-heaven is another species that does appear to be strongly disturbance mediated. As such, it may prove to be a particularly problematic species to deal with in our landscape. The Fire and Fire Surrogate study (<http://www.fs.fed.us/ffs/>) of the U.S. Forest

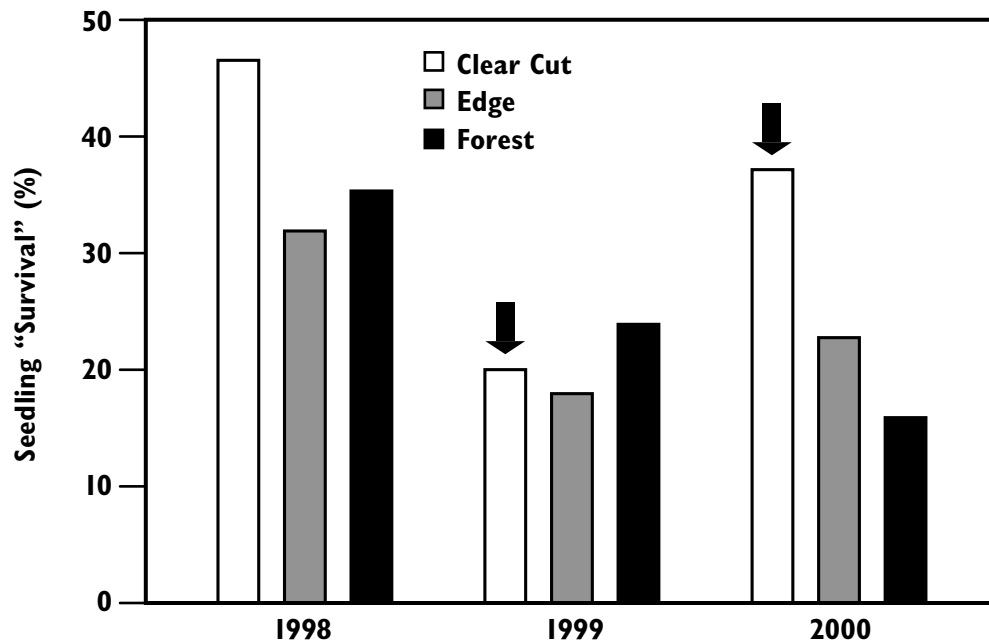


Figure 3. Above-ground stem "survival" for *Paulownia tomentosa* planted in three landscape elements and tracked over a three-year period. Because stem-kill was used instead of true survival, resprouting permits stems to return to the population in subsequent years (arrows). The reaction of *P. tomentosa* to light and its sprouting have been examined in further detail by Longbrake and McCarthy (2001).

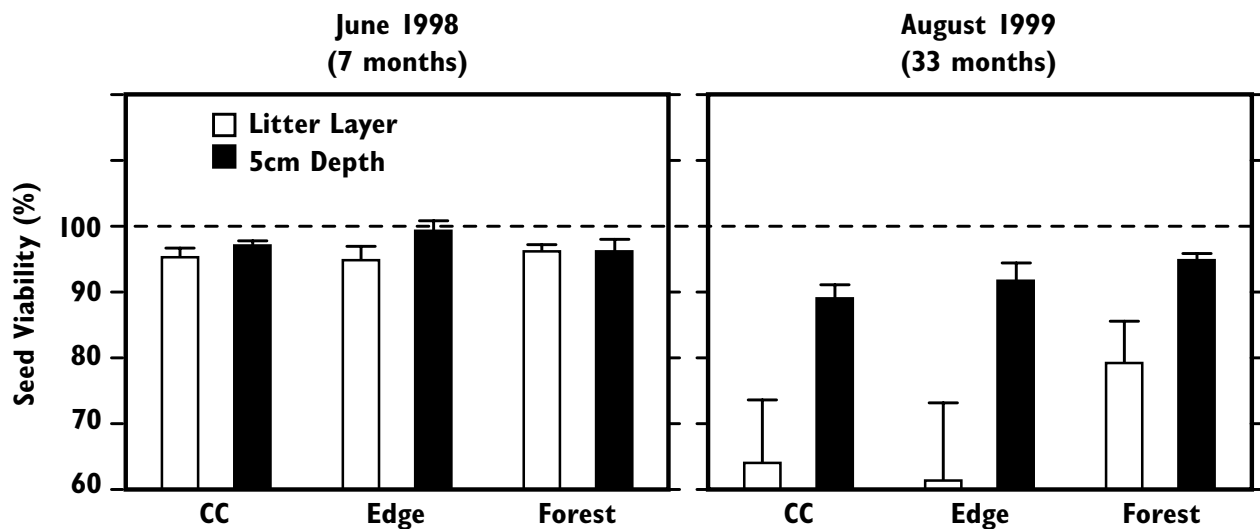


Figure 4. Results of a seed-bank experiment with *Paulownia tomentosa*. Seeds were placed in mesh bags and planted at the soil-litter interface as well as within the A-horizon in three different landscape environments (CC = clearcut). After seven months, most seeds were still viable. After almost three years, viability was greatest for seeds buried in the soil, but still in excess of 60% viability for seeds at the soil-litter interface (which suffered from greater fungal decomposition).



Figure 5. Photograph of Latin Square field design used to evaluate six different types of substrates on seed germination of *Paulownia tomentosa*. Seeds germinated from best to worst: bare soil > potting soil > cobble > gravel > sand > leaf litter.

Service being conducted in southeastern Ohio is an ecosystem-level study designed to examine the interactive effects of forest thinning and prescribed fire. One of the sites for this study is Tar Hollow State Forest, where Hutchison *et al.* (2004) provide some interesting data to suggest that the combination of cutting and fire may dramatically increase the density of this species in our woodlands. A similar result has been discovered in the Great Smoky Mountains following a prescribed burn. Both tree-of-heaven and princess tree invaded from nearby seed sources.

Recently, we have begun investigating different ways to control this species. Preliminary experiments are being conducted at The Wilds in central Ohio, where a fairly healthy population of tree-of-heaven exists. We are employing the EZ-Ject lance as our primary control method, as this has proved quite effective on woody plants and is environmentally safe. We are experimenting with three different herbicides (glyphosate, imazapyr, and triclopyr) to generate dose response curves to kill trees of different sizes. Preliminary data suggest that large trees

may be difficult to kill using this method, and triclopyr does not appear to be particularly effective for the eradication of this species.

We have also just begun experiments equivalent to our prior studies with princess tree to determine the seed bank of this species. Seeds are being placed out in multiple environments in a managed forest landscape and will be retrieved at regular intervals to determine germinability of the seeds.

### Amur honeysuckle

Amur honeysuckle (*Lonicera maackii*; Caprifoliaceae) is a widely recognized problem species in southern Ohio, especially the southwestern part of the state. It is a perennial shrub of great plasticity that invades forested understories. The dispersal unit is a small red berry consumed by birds, which subsequently disperse the seeds. Whether this species maintains a seed bank has not been empirically evaluated, but seed longevity is believed to be four to five years.

We have done various experiments with ecology,

control, and restoration following the removal of this species (Hartman and McCarthy, in press; Hartman and McCarthy, unpublished). I will focus here on some seed-related work that we have done in my lab. Miller and Gorchov (in press) have clearly demonstrated that the explosion of Amur honeysuckle in forest understories has dramatically impacted perennial herbs.

As Amur honeysuckle has been well established in southwestern Ohio for some time now (Hutchinson and Vankat, 1998), perhaps 40 years in some areas, we have become concerned that the effect of this species will be to deplete the seed bank of native species in the understory. Amur honeysuckle forms monospecific stands and allows little to no light penetration to the forest floor. Most forest herbs disappear under these circumstances.

We selected a chronosequence of stands invaded by honeysuckle using 16 metroparks from around Cincinnati and Dayton, Ohio. We felled multiple honeysuckle plants in each park to determine the linear relationship between biomass and age (older stands have a greater biomass of honeysuckle per unit area). We used the oldest honeysuckles in each stand to determine the approximate time of invasion. Interestingly, we noticed that honeysuckle was causing a significant decline in tree growth in stands in which it had invaded, and we report this in a separate poster in this symposium. This is the first recording of this of which I am aware.

Replicate soil samples were collected from each stand and arrayed in pans within a greenhouse to examine germination and species identification from the seed bank. While we did not see a significant decline in herb species diversity, we did observe a significant decline in woody seedling species diversity with increasing honeysuckle abundance (Figure 6). Clearly, honeysuckle has the capacity to reduce understory species richness. It may pass a threshold, if left uncontrolled for a sufficient period of time, where simple removal does not allow restoration and recovery of the habitats from the seed bank, and plantings will be required to restore the habitat. This is a much

more time-consuming process and expensive proposition for land managers.

## **Conclusions and Management Implications**

The ecology of each species is intimately linked to the details of its life-history characteristics and the environment in which the species finds itself. A simple alignment of habitat characteristics and invaded habitats will not likely prove feasible. Empirical studies are necessary to evaluate the population, community, and ecosystem-level impacts of specific invasive species, and they should be managed accordingly.

Life-history characteristics associated with seed production, longevity in the soil, and germination ecology seem to be key in understanding how many of these species enter and remain in forested communities of southern Ohio. Empirical studies should probably focus on these details first. In particular, it does not appear that the seed-bank ecology of many of these species is particularly well studied, with the exception of garlic mustard (Baskin and Baskin, 1992) and princess tree (Longbrake and McCarthy, unpublished). Knowledge of the seed-bank dynamics is critical in considering control and restoration of any invasive species.

Moreover, there appears to be little development in the literature regarding the relationship between forest-management practices and invasive species ecology and invasion. Much of this information is scattered and known only to local forest managers, if at all. Most of the forested landscape of southern Ohio, and much of the Appalachian region, for that matter, is in production. As such, we need to better understand the relationship between silvicultural practices and invasive species.

## **Acknowledgments**

I would like to extend special thanks to my graduate students J. Forrest Meekins, A. Christina Longbrake, Kurt M. Hartman, and Kevin Lewis who collected most of the data discussed in this paper. They were assisted by many other undergraduate and graduate

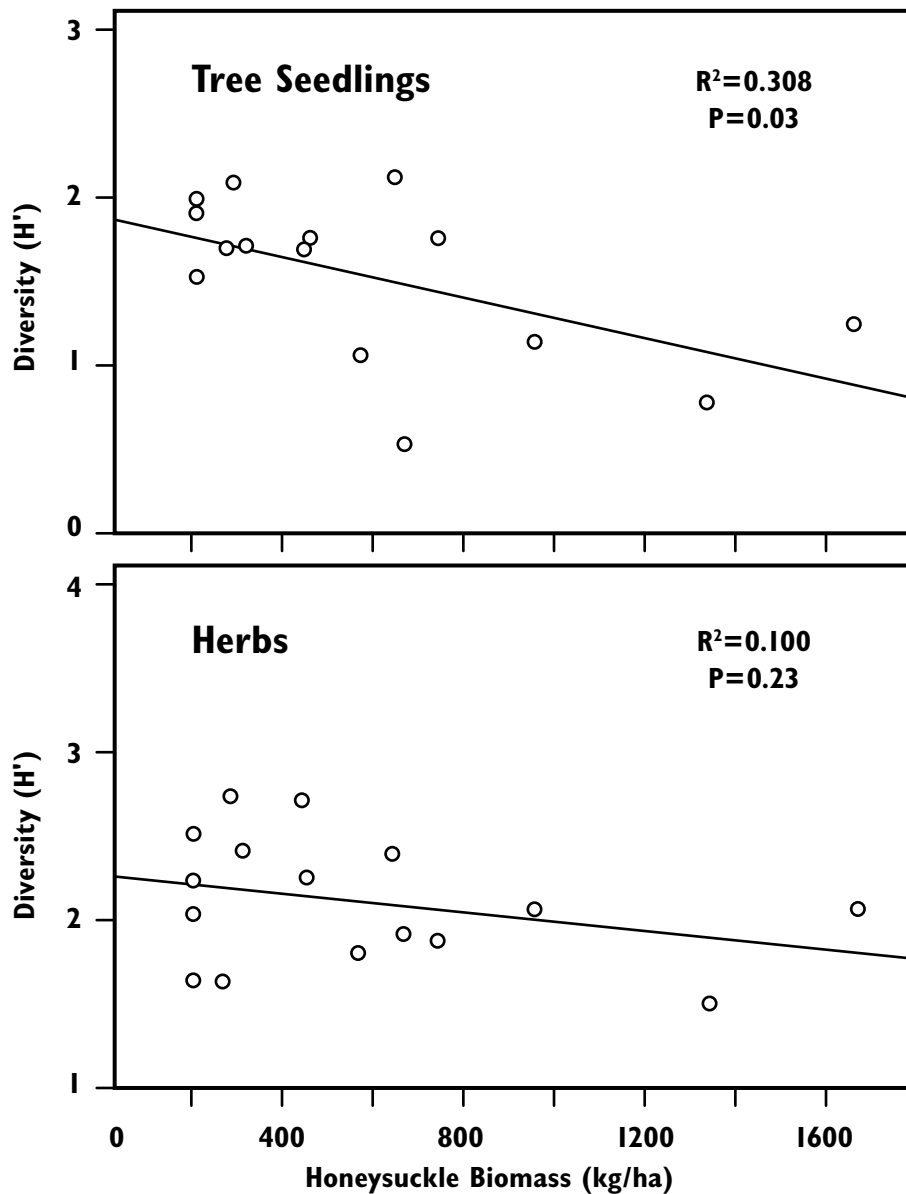


Figure 6. Least squares regression analysis relating Amur honeysuckle biomass to understory diversity ( $H'$ , Shannon-Weiner) for herbs and woody seedlings. Data are taken from 16 metroparks around Cincinnati and Dayton, Ohio.

students from Ohio University, for which I am also grateful. Mary Droege assisted with my early studies of garlic mustard in Maryland. The Hamilton County Park Commission, Ohio Department of Natural Resources, and The Wilds all graciously provided access to various field sites for study.

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# Control of Forest Invasives and Responses of Native Forest-Floor Plants: Case Studies of Garlic Mustard and Amur Honeysuckle

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## Introduction

Many protected areas of deciduous forest, as well as other communities, are infested with or threatened by invasive non-native plants. In order to assess whether control or eradication efforts are warranted, one needs to know what effects the invasive species are having on the community and what control methods will be effective. In this paper, I will address these questions, focusing on two non-native plant species that are invasive in many deciduous forests of the eastern United States and adjacent Canada — garlic mustard, *Alliaria petiolata*, and Amur honeysuckle, *Lonicera maackii*.

The observation that invasive plants often become abundant in plant communities has led to the assumption that they have significant negative impacts on native species, including native plants. Clearly, there are well-documented examples of an invasive plant changing an ecosystem's disturbance regime (D'Antonio and Vitousek, 1992) or nutrient cycling (Vitousek *et al.*, 1987), with consequent effects on other species.

However, in cases where the invasive inserts itself into the community without such transformational effects, there have been surprisingly few studies of direct competitive effects on native species. Thus, we find general statements in review papers that reach seemingly opposite conclusions. For example, in their *Issues in Ecology* review, Mack *et al.*

(2000) concluded, "Plant invaders can...greatly diminish the abundance or survival of native species..." whereas Davis (2003), citing personal communication from J. T. Kartesz, stated, "Yet there is no evidence that even a single long-term resident species has been driven to extinction, or even extirpated within a single U.S. state, because of competition from an introduced plant species."

These statements are not really contradictory, as an invasive species may significantly reduce individual survival, growth, or reproduction, or population density, structure, or dynamics, of a native plant species, without having caused (yet) its extinction. Nevertheless, we have remarkably little information on the negative effects of invasive plants on native plants in many systems, including the deciduous forests of the eastern United States.

Where does the inference that invasive plants harm native plant species and communities come from? To a large extent, it comes from observations that stands with a high density of one or more invasive species also have low diversity of native species. For example, among forest stands in southwestern Ohio forests, abundance of Amur honeysuckle is negatively correlated with density and species richness of tree seedlings and with herb cover (Hutchinson and Vankat, 1997). Although one logical inference from such a "comparative approach" is that the invasion caused a decline in native

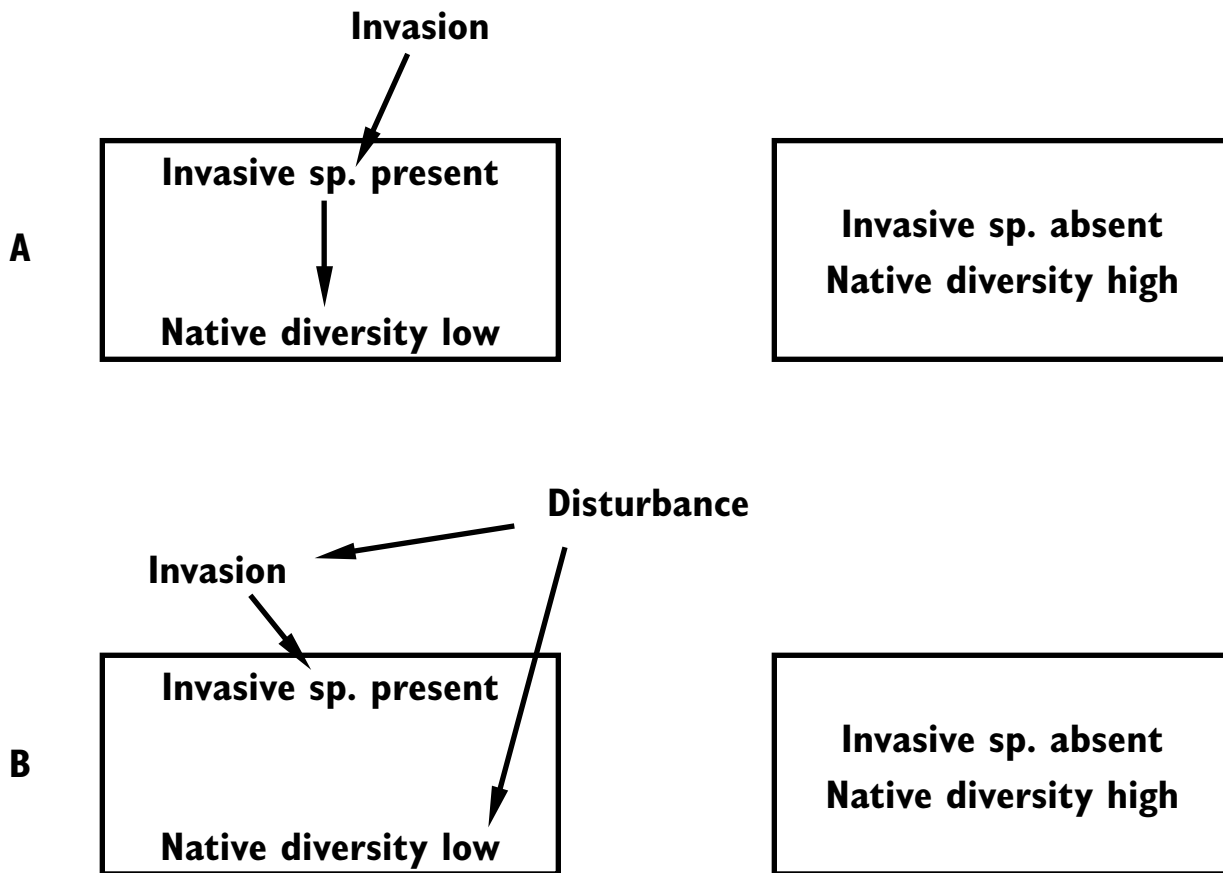


Figure 1. Comparative studies often reveal that stands with invasive plants have lower diversity and/or density of native plants. This is often attributed to negative effects of the invasives on natives (A). However, the same pattern could be due to some other factor, such as past disturbance, both enhancing invasion and reducing natives (B).

species density and richness (Figure 1A), this is not the only logical inference.

An alternative hypothesis to explain the negative correlation between density of an invasive and density/diversity of natives is that some difference among stands predisposes or causes some to both be invaded and have low native diversity.

The likely candidate in many deciduous forest systems is disturbance (Figure 1B). Disturbance has been shown to facilitate plant invasions in a variety of systems (Hobbs and Huenneke, 1992; Davis *et al.*, 2000) and can independently result in the decline or local extinction of species sensitive to that particular disturbance

(Luken, 1997). For example, grazing of cattle in woodlots might be expected to cause reductions in seedling density of preferred tree species, and perhaps local extinctions of preferred species of herbs, as would over-browsing by high populations of white-tailed deer (Rooney and Dress, 1997; Russell *et al.*, 2001).

Thus, the hypothesis that invasive plants harm native plant species, though supported by comparative studies, requires further testing. Surprisingly few experiments have been done to quantify impacts of invasive plants on native plants (*e.g.*, Witkowski, 1991; Midgley *et al.*, 1992; Dillenburg *et al.*, 1993; Huenneke and Thomson, 1995; McCarthy, 1997; Meekins and McCarthy, 1999).

We have taken such an experimental approach, generally involving the addition or removal of an invasive species, and comparing the response of individual plants and plant communities in treatment plots vs. control plots. Specifically, we have removed Amur honeysuckle shrubs and compared the survival, growth, and reproduction of transplanted individuals of selected tree, annual, and perennial herb species to that of individuals on control plots. These experiments fall within the neighbor (competitor) - target design first proposed by Goldberg and Werner (1983) and widely applied in recent decades.

For *Alliaria petiolata* we have taken a different approach, reducing the abundance of this invasive biennial with an accepted management treatment (annual herbicide application), and monitoring the response of the in-situ plant community, including cover measures of individual species and growth-form groups, in comparison to control plots.

To evaluate the direct effects of treatment on the target invasive species and on non-target plants, we highlight the relevant literature on Amur honeysuckle and summarize our own findings for garlic mustard.

## Garlic Mustard

Garlic mustard, *Alliaria petiolata* (Bieb.) Cavara and Grande (Brassicaceae), is native to Eurasia; the first record in North America was on Long Island in 1868 (Nuzzo, 1993). It is now naturalized in 33 U.S. states as well as adjacent Canada and has become one of the most prevalent invasive plants in forests of the eastern United States, invasive in wet to dry-mesic deciduous forests as well as disturbed areas (Nuzzo, 1993; Byers and Quinn, 1998; PlantsDatabase).

Garlic mustard is an obligate biennial. Seeds germinate in early spring, and first-year plants (rosettes) either remain green through the winter or become leafless at the end of the season (Anderson *et al.*, 1996). Early in the second spring, the one-year-old plants (adults) bolt and flower. Seeds mature in late spring

and are dispersed over the summer, following senescence of adults. Seeds either germinate the next spring or remain dormant, with disturbance promoting germination (Figure 2).

We investigated the effects of the herbicide glyphosate in an old-growth beech-sugar maple stand and in a second-growth tulip-tree-dominated stand in Hueston Woods State Nature Preserve, Oxford, Ohio (Carlson and Gorchoy, in review). In each stand, we selected 50 1-x-1 m plots that had high density of garlic mustard in spring 2000 but were not near gaps, trails, or drainages. We randomly assigned plots to be treatment (herbicide) or control until we had 25 of each treatment in each stand. Treatment consisted of spot-application of 1% glyphosate (Round-Up®) on garlic mustard rosettes within the plot and a 1-m buffer on a warm day each November from 2000 through 2003. Fall application of glyphosate had been shown to reduce garlic mustard with minimal impacts on native plants (Nuzzo, 1991) and had been approved by the Ohio Department of Natural Resources for control of this invasive in this Nature Preserve.

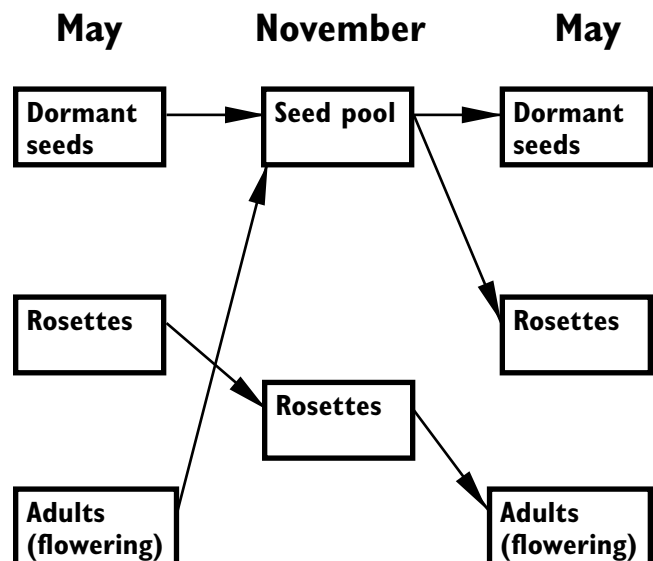


Figure 2. A representation of the life history of garlic mustard, emphasizing the stages of plants in May, the month of census data reported here, and November, the month of glyphosate application.

Among other measures, we recorded the density of garlic mustard rosettes in May and October 2000 (before spray) and rosettes and adults each May 2001 – 2003, in each plot. We expected adult densities to be greatly reduced by the fall herbicide application, as these plants were in the rosette stage and thus susceptible in the fall. Rosettes visible in May would not be directly affected by fall herbicide application, as they germinated after glyphosate application (Figure 2). But if reduced adult densities result in reduced seed production, then densities of rosettes would be expected to be lower in herbicide plots beginning in the spring following the second year of treatment (May 2002).

Among our measures of the response of non-target plants, we determined the percent cover of each species < 80 cm in each plot using a point frame each May and late June, from 2000 (before treatment) to 2003. For each species, we

analyzed peak cover (either May or June), and for analyses, species were grouped into growth forms (tree seedlings, shrubs, vines, annuals, spring perennial herbs that senesce by late May/early June, summer perennial herbs, graminoids, and ferns).

Complete results for the first two years are in Carlson and Gorchov (in review), but are summarized here, along with summaries of more recent data. Glyphosate treatment significantly reduced the density of garlic mustard adults in both stands in both 2001 and 2003 (Figure 3a, b). In 2002, adult density was low in both stands, but there was no treatment effect on density (Figure 3a, b). In the old-growth stand, rosette density of garlic mustard was reduced in herbicide plots (Figure 3c) in 2002 but not 2003. In the second-growth stand, herbicide reduced rosette density in both 2002 and 2003 (Figure 3d).

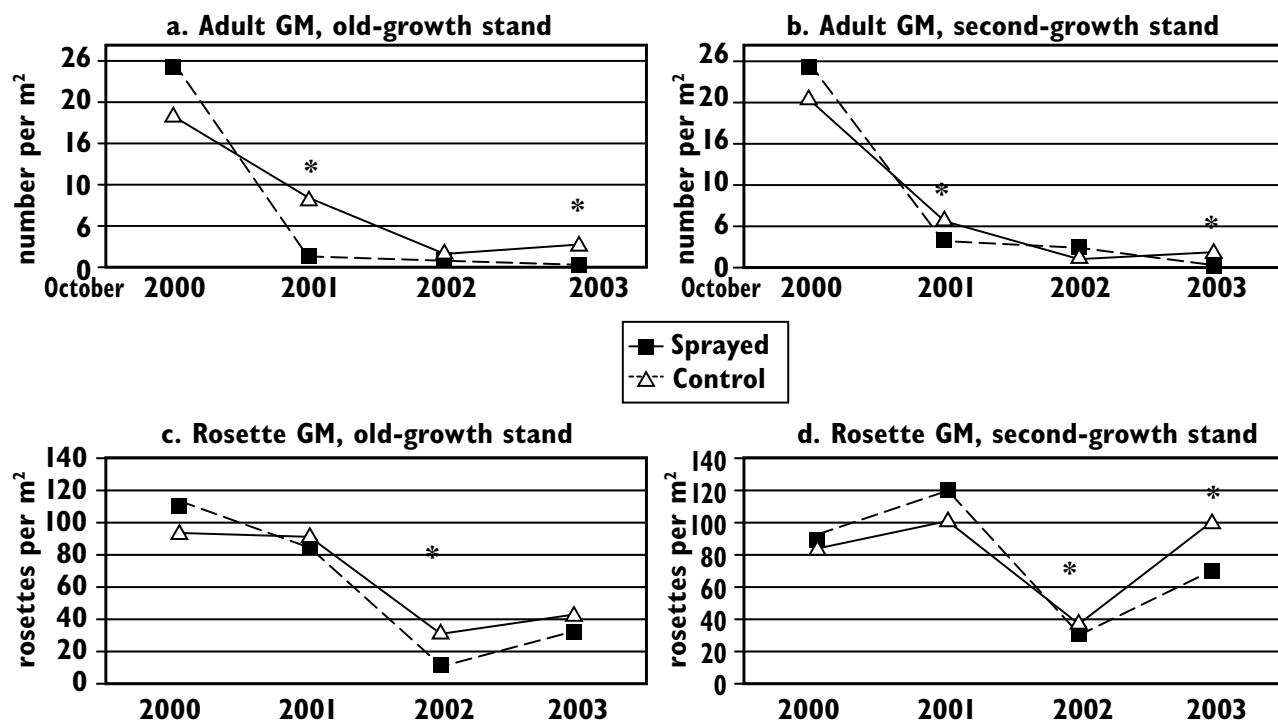


Figure 3. Mean + SE density of garlic mustard adults (a, b) and rosettes (c, d) in glyphosate-treated and control plots in old-growth (a, c) and second-growth (b, d) stands in Hueston Woods, Oxford, Ohio. Glyphosate was applied each November beginning 2000. No adult density is available for May 2000, so October 2000 rosette density is reported. All other data are for May of the year indicated. Data from 2000-2002 are from Carlson and Gorchov (in review); data from 2003 are from Slaughter and Gorchov (unpublished). Asterisks indicate significant ( $P < 0.05$ ) treatment effects based on ANOVA, or on the Kruskal-Wallis test where low densities resulted in violation of ANOVA assumptions.

In the old-growth stand, the cover of all species (summed) of spring perennial herbs was greater in glyphosate-treated plots in 2001, the first spring after treatment, but not in the two subsequent years (Figure 4a). In the second-growth stand, spring perennials had greater cover in treated plots in 2003, but not in other years (Figure 4b). There were no significant treatment effects in either stand in any year for summer perennial herbs, annuals, tree seedlings, shrubs, or vines (Carlson and Gorchoy, in review; Slaughter, Saunders, and Gorchoy, unpublished).

## Amur Honeysuckle

Amur honeysuckle, *Lonicera maackii* (Rupr.) Herder (Caprifoliaceae), is native to northeastern Asia and was introduced to the United States in 1897 and subsequently promoted for ornamental and other uses (Luken and Thieret, 1995). It has since become naturalized in at least 24 eastern states (Trisel and Gorchoy, 1994; USDA Plants Database). Its success in habitats ranging from old fields to closed canopy forests has been attributed to its ability to establish in sun or shade, plasticity in biomass allocation, and high photosynthetic rates in full sun (Luken, 1988; Luken *et al.*, 1995, 1997). It also expands

leaves earlier and retains them later than native deciduous woody plants (from Trisel, 1997, Figure 5).

We assessed the effect of Amur honeysuckle on native tree seedlings, annual forest herbs, and perennial herbs by comparing their performance in plots where this invasive shrub was removed to that where the shrub was present. In the spring of 1992, we planted one bare root seedling of each of four tree species — sugar maple (*Acer saccharum*), white ash (*Fraxinus americana*), black cherry (*Prunus serotina*), and red oak (*Quercus rubra*) — in each of 160 plots in Gregg's Woodlot (GW), a disturbed hickory-ash-oak stand (Gorchoy and Trisel, 2003). Plots had been randomly assigned to four treatments:

- Honeysuckle shoots removed (shoot competition removed).
- Soil around planted seedlings trenched to remove roots of honeysuckle and other forest plants (root competition removed).
- Honeysuckle shoots removed and soil trenched.
- Un-manipulated control (Figure 6).

An additional sugar maple seedling was planted

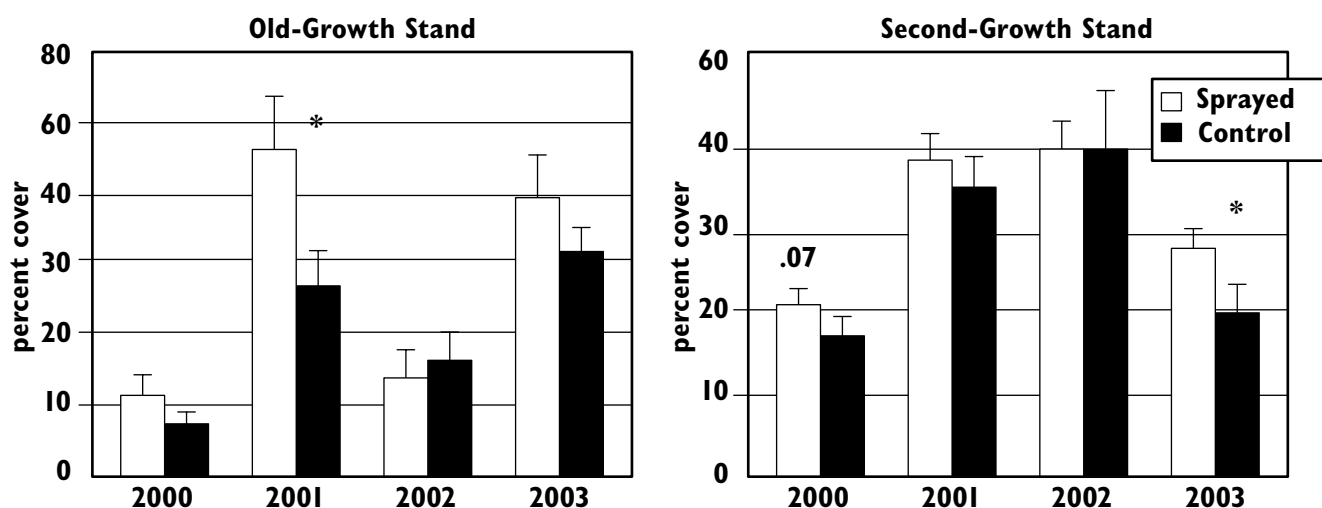


Figure 4. Mean + SE cover of spring perennial herbs in glyphosate-treated and control plots in old-growth and second-growth stands each May. Glyphosate was applied to garlic mustard each November beginning 2000. Data from 2000-2001 are from Carlson and Gorchoy (in review), data from 2002 and 2003 are from Slaughter, Saunders, and Gorchoy (unpublished). Asterisks indicate significant ( $P < 0.05$ ) treatment effects, based on Kruskal-Wallis test.

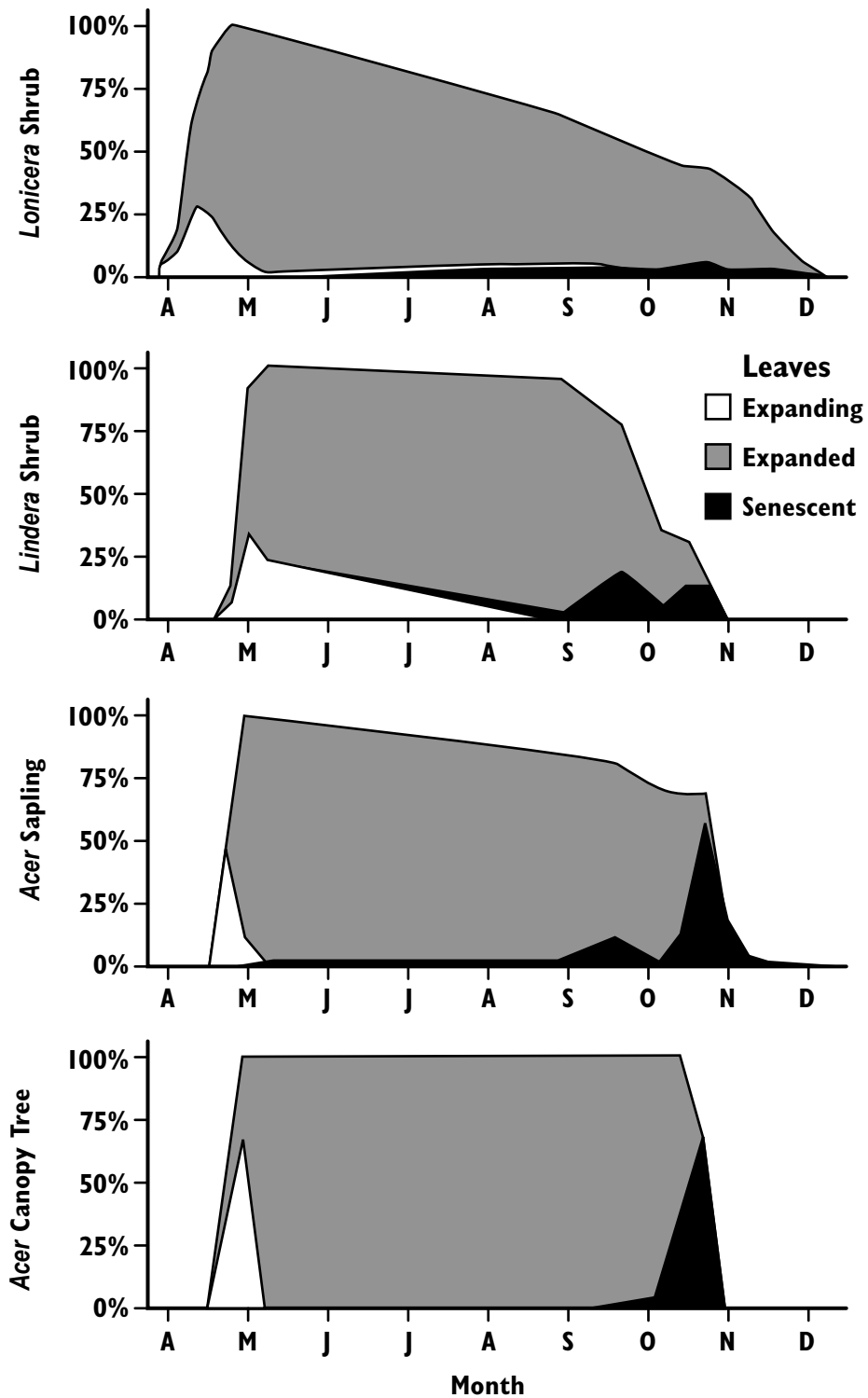


Figure 5. Amur honeysuckle (*Lonicera maackii*) extends leaves earlier in the spring and retains them later in the fall than spicebush (*Lindera benzoin*), a common native shrub, and sugar maple (*Acer saccharum*), a common tree (from Trisel, 1997).

in each plot in the spring of 1993 and protected from deer browse with poultry wire. Because the effects of trenching were weaker than those of shoot removal (Gorchov and Trisel, 2003), I report here pooled data for treatments 1 and 3 as “*Lonicera* shoot removal” and treatments 2 and 4 as “*Lonicera* present.”

We found that seedling survival of three out of the four tree species (sugar maple, white ash, and red oak) was significantly higher where honeysuckle shoots had been removed (Figure 7, Trisel and Gorchov, 2003).

To test the effect of Amur honeysuckle on annuals and perennial forest herbs, we compared the performance of transplanted individuals in a blocked design field experiment consisting of three treatments — Honeysuckle Present, Honeysuckle Absent, and Honeysuckle Removed at Western Woods (WW), a 40 ha relatively undisturbed oak-ash-sugar maple-dominated stand in Oxford, and consisting of Honeysuckle Present and Honeysuckle Removed treatments at GW.

For the annuals, we transplanted seedlings of three species with distinctive phenologies, *Galium aparine*, *Impatiens pallida*, and *Pilea pumila*. The presence of honeysuckle reduced the survival of the two species with earlier leaf phenologies, *G. aparine* and *I. pallida*, at GW, and reduced the seed production of surviving individuals of all species at both sites (Figure 8, Gould and Gorchov, 2000).

For forest perennials, we transplanted bulbs or rhizomes of three species (*Allium burdickii*, *Thalictrum thalictroides*, and *Viola pubescens*) into the same treatments at GW and WW, and monitored survival, growth, and flower and fruit production over five years (Miller and Gorchov, 2004). We also planted seeds of these three herb species into Honeysuckle Present and Honeysuckle Removed plots at both sites and monitored seedling emergence, survival, and growth over three years. While honeysuckle did not significantly reduce adult survival of any of the three species, it reduced growth (*e.g.*, number of leaves or leaflets) and size of survivors at the end of the experiment, for all three species.

Honeysuckle also reduced flowering and seed production of all species, including cumulative seed production over the five-year period.

Negative effects on herb demography were manifest sooner and more pronounced at GW, the disturbed stand (Miller and Gorchov, 2004). While first-year seedling emergence did not differ significantly between treatments for any species at either site, survival of seedlings from 2001 to 2002 tended to be higher in the removal treatment for *Viola* at both sites and for *Thalictrum* at WW. Second-year *Viola* emergence was also greater in the removal treatment, resulting in more total seedlings (Miller *et al.*, 2004).

## Control of Bush Honeysuckles

Trisel (1997) compared mechanical vs. chemical control methods for Amur honeysuckle in 20 x 20 m plots at Richardson Preserve, Hamilton County, Ohio. He found the most effective method was to grub out the entire crown, including the burl at the base of the shoot, using a polaski (Table 1). However, this method was very labor intensive (Table 1). A more efficient tool for severing the roots from the burl and prying out the burl plus shoot is the Honeysuckle Popper (misterhoneysuckle.com). Simply cutting shoots is not effective due to the impressive resprouting ability of bush honeysuckles. Even monthly (June – October) removal of sprouts (following April cutting) killed only 7% of shrubs (Table 1).

Several chemical methods are effective in killing bush honeysuckles. Trisel (1997) found cutting and painting stumps in late April with 33% glyphosate was more effective than foliar spray of 2.5% glyphosate during the same period and avoided negative effects on non-target plants (Table 1). However, cutting and painting consumed nearly four times as much labor as foliar spray. Current recommendations for foliar spray specify a 2% solution of glyphosate or triclopyr plus a 0.5% non-ionic surfactant (Conover and Geiger, 1993; Miller, 2003; Southeast Exotic Plant Pest Council). Because these herbicides are most effective > 65°F, it is difficult to schedule spraying when native plants are not in leaf.

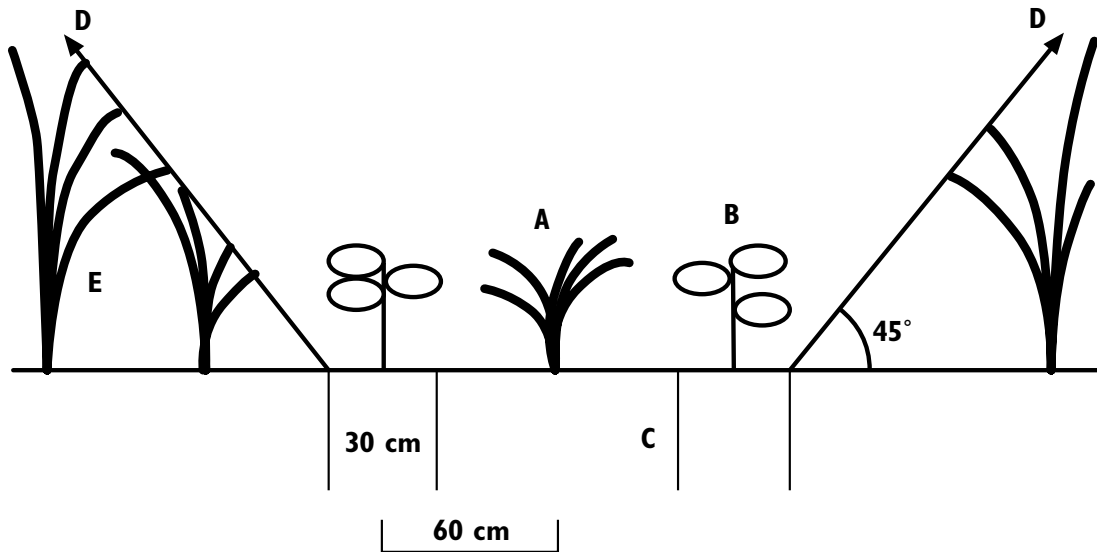


Figure 6. Diagram showing shoot removal and trenching treatments and location of planted tree seedling and focal Amur honeysuckle in Greggs Woodlot (from Trisel, 1997).

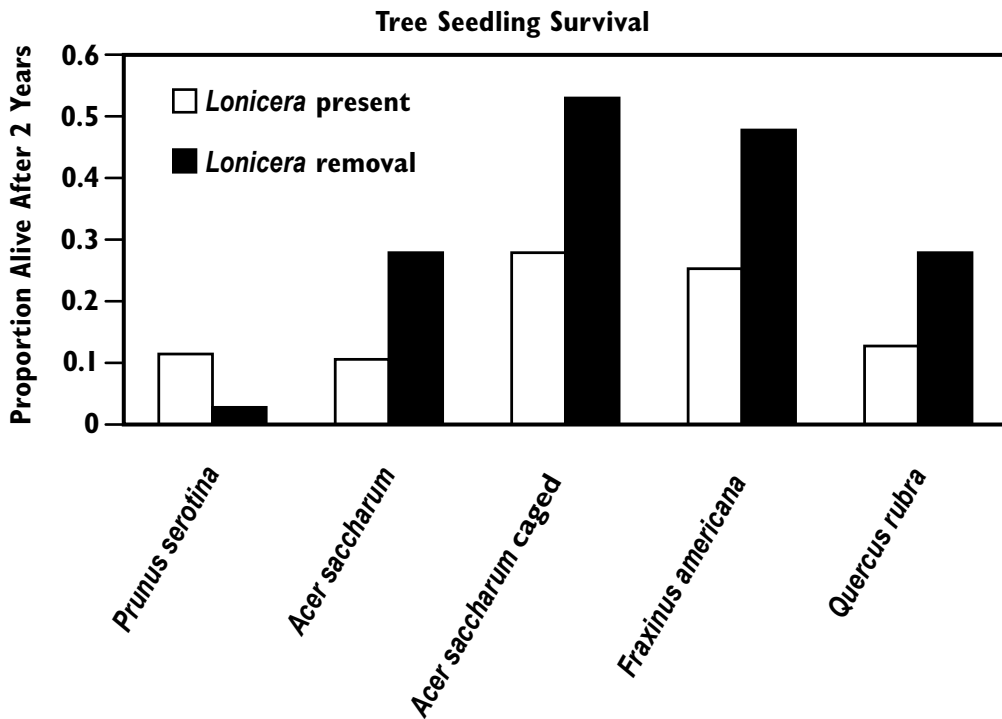


Figure 7. Effect of Amur honeysuckle shoot removal on survival of seedlings of four species of native trees (N = 160 seedlings/species) in Greggs Woodlot, Butler County, Ohio (from Trisel and Gorchoy, 2003). Survival was monitored over two years, except for the “caged” cohort of *A. saccharum*, which was protected from deer browse by poultry wire and monitored over one year. “Lonicera removal” refers to treatments where the shoots of the focal honeysuckle shrub were removed (Figure 6). All species except *Prunus serotina* had significantly higher survival in the removal treatment, as determined by log-linear contingency tests.

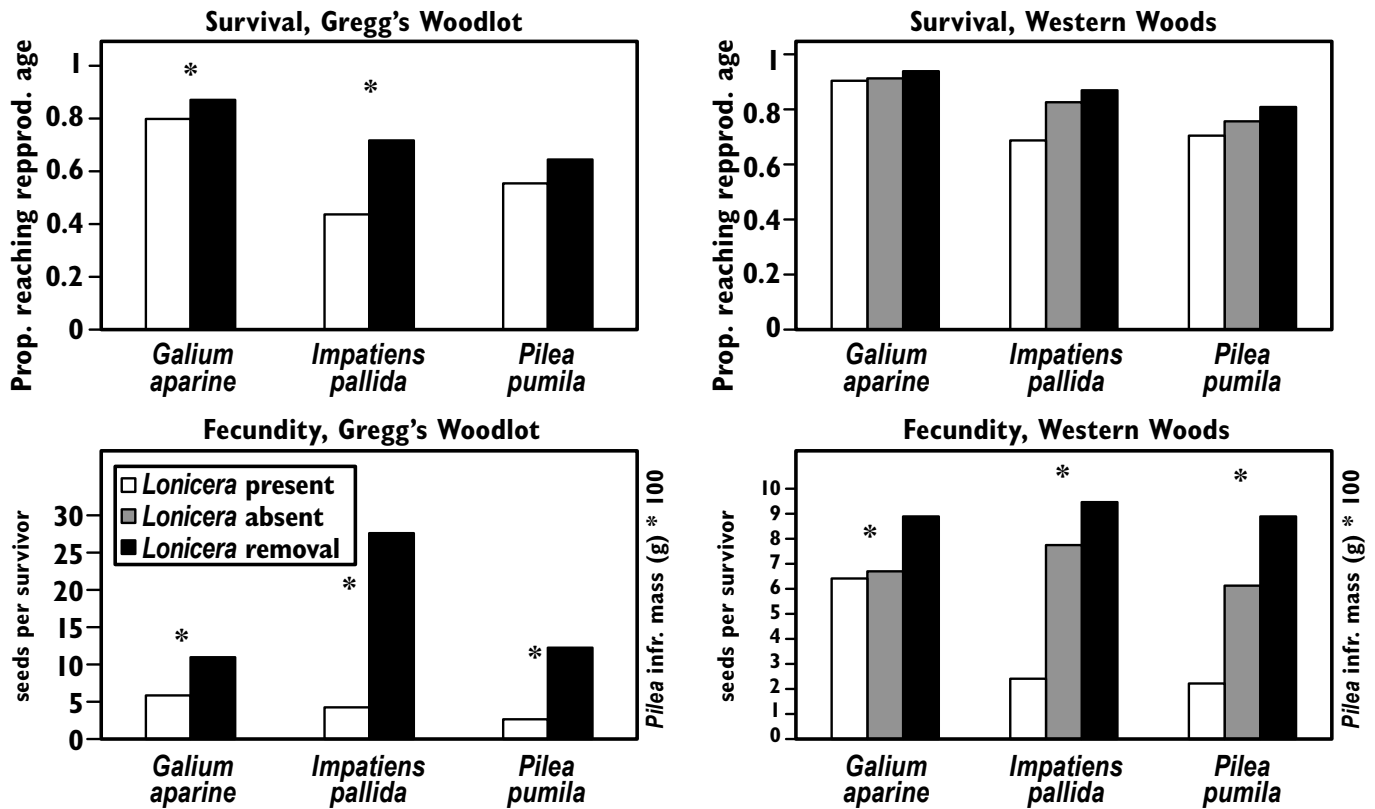


Figure 8. Effect of Amur honeysuckle treatment (removal, present, absent) on survival from seedling transplant to flowering and reproduction of surviving individuals of three species of native annuals in Gregg's Woodlot (GW) and Western Woods (WW), Butler County, Ohio (from Gould and Gorchov, 2000). Treatment effects on survival were tested by logistic regression; treatment effects on fecundity were tested by log-linear models.

	Cut and Paint	Foliar Spray	Crown Removal	Monthly Sucker Removal
% shrubs controlled	66	38	100	7
Effect on herb layer	+	-	+	+
Start-up cost	\$172	\$165	\$ 42	\$126
Time (hours)	5.75	1.25	4.5	9
Physical effort	4.5	1	4	5
Ease of movement through area	2	5	1	2

Cut and paint used 33% glyphosate; foliar spray 2.5% glyphosate. Scale for physical effort was 1 (low) to 5 (high). Scale for ease of movement through area (following treatment) was 1 (easy) to 5 (difficult).

Damage to non-target plants can be minimized by applying herbicide directly to stems, and this is effective at cool as well as warm temperatures. Application to stems cut near the ground is most effective when done immediately after cutting. Current recommendations are to apply 20% – 25% glyphosate or 25% triclopyr to the outer 20% of the stump (Southeast Exotic Plant Pest Council; Miller, 2003). Basal spray application to the lowest 30–38 cm of stems is also effective, with the Southeast Exotic Plant Pest Council recommending 25% triclopyr and 75% horticultural oil, and Miller (2003) recommending 20% Garlon 4 in basal oil with penetrant.

In recent years stem injection of glyphosate with the E-Z-Ject® lance has been found to be effective in killing Amur honeysuckle (Franz and Keiffer, 2000). Hartman and McCarthy (2004) compared the costs and effectiveness of stem injection with cut-and-paint with 50% glyphosate. While both were effective, killing 94% of Amur honeysuckle, stem injection required less labor and involved less exposure of the operator and non-target plants to herbicide.

## Discussion

Spot application of garlic mustard rosettes with 1% glyphosate in the fall significantly reduced both the density of sprayed cohorts and the recruitment of new rosettes in subsequent years. However, the latter effect was observed for only three of the four year-by-site combinations, and even after three years of spraying, treated plots still had some garlic mustard. It is not clear to what extent the persistence of garlic mustard in the sprayed plots is attributable to incomplete mortality vs. recruitment from the seed bank or seed dispersal. Mortality was incomplete because of some combination of individuals escaping the herbicide application (because they were leafless or covered by leaf litter during the early November applications) and individuals surviving the application (which might be remedied by higher concentrations of glyphosate).

Even if herbicide application prevented any garlic mustard seed production on the treated plots, rosettes can recruit both from the seed

bank (Anderson *et al.*, 1996) and from seeds disseminated from outside the treated plots. Since herbicide application extended only 1 m around each 1 x 1 m plot, seed input from unsprayed areas may be much more important in this experiment than it would be if herbicide had been applied to entire stands. Thus, an assessment of whether repeated fall application of herbicide is sufficient to eradicate garlic mustard will require larger plots.

Fall application of glyphosate avoids the negative effects on native plants associated with spring application (Nuzzo, 1991). On the other hand, the response of the native plant community to the herbicide-caused reduction in garlic mustard has been modest. Spring ephemeral herbs, which have the greatest phenological overlap with garlic mustard, did have higher total percent cover in treated plots, but this effect was significant in only one of three post-spray years at each of the two sites. Other functional groups of forest floor plants have not shown significant effects of treatment yet, nor has species richness been affected (Carlson and Gorchoy, in review; Slaughter, Saunders, and Gorchoy, unpublished).

These findings contrast with McCarthy's (1997) finding that vines, tree seedlings, and the annual *Impatiens* sp. all increased in response to garlic mustard removal. Although one might interpret the modest responses of the native plant community as evidence that garlic mustard has only weak effects on native plants, I think it is more likely a reflection of the modest difference in garlic mustard density between treated and control plots over the first three years of the study (Figure 3).

We have found clear effects of Amur honeysuckle on native plants, including reduced survival of tree seedlings and annuals, reduced growth of perennial herbs, and reduced reproduction of annual and perennial herbs. Similarly, Hartman and McCarthy (2004) found significantly higher survival of transplanted tree seedlings in plots where Amur honeysuckle had been killed by herbicide. While honeysuckle does not kill forest herbs, it is expected to reduce population sizes over time by limiting

individual growth, reproduction, and seedling recruitment. Short-term studies that record only measures of abundance or cover, as in our garlic mustard research, are unlikely to detect these demographic effects that may cause population declines and even extinction in the longer term.

While the early leaf expansion of this shrub led us to expect that the effects would be greatest on those native species most dependent on light before canopy leaf-out, our findings do not support this. Although the two annuals with earlier development were more affected than the one with the later phenology, this pattern did not hold for perennials. The three perennial herb species had similar responses to honeysuckle, perhaps reflecting that all three have substantial dependence on irradiance before canopy leaf-out, despite the later senescence of *T. thalictroides* and *V. pubescens*.

Similarly, tree species with earlier leaf expansion were not more affected by honeysuckle. In fact, the species with the earliest leaf expansion, black cherry, was the only one that did not increase survival in response to honeysuckle shoot removal. Hartman and McCarthy (2004) did not find significant differences among the six tree species in the responses of their seedlings to honeysuckle control.

One question raised by these field experiments is whether the competitive effect of the invasive species is any greater than a comparable native species. While this question ought to be addressed in future studies, it also must be recognized that invasive plants often reach high densities and biomass in invaded communities. For example, the densities of Amur honeysuckle in our sites are more than 100 times the densities of the most common native shrub, spicebush (*Lindera benzoin*), in an old-growth stand in nearby Hueston Woods (Foré *et al.*, 1997). Such densities, combined with the effects documented in studies like those summarized here, establish that the effects of invasive plants are important.

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# Interactions Between Exotic Shrubs and Breeding Birds in Riparian Forests

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Ecologists have long known that exotic plants are among the most serious conservation threats to native biodiversity (Wilcove *et al.*, 1998). Exotic plants have disrupted ecosystem processes, such as hydrological and nutrient cycles (Vitousek, 1990) and altered floristic composition (*e.g.*, Luken, 1988; Woods, 1993; Hutchison and Vankat, 1997; Mack *et al.*, 2000). However, the influence of exotic plants on higher trophic levels or predator-prey interactions is less understood.

Bird communities, in particular, should be strongly influenced by habitat modifications caused by exotic plants (Reichard *et al.*, 2001). Exotic plants change the structure and floristic composition of habitat and thereby affect foraging and nesting resources. For example, exotic shrubs can affect the type, quality, and temporal availability of food resources (*e.g.*, Southwood, 1961; Ingold and Craycraft, 1983; Mills *et al.*, 1989; White and Stiles, 1992).

Recent studies have suggested that exotic plants can indirectly affect breeding birds by increasing vulnerability to nest predation. Because exotic shrubs often grow most densely at habitat edges (*e.g.*, Matlack, 1993; Woods, 1993; Luken and Goessling, 1995; Hutchison and Vankat, 1997), they may entice birds to nest at edges, where they are likely to experience greater rates of nest predation (*i.e.*, “ecological trap” *sensu* Gates and Gysel, 1977). Many exotic shrubs exhibit early leaf flush (Trisel and Gorchov, 1994), which may further attract nesting birds.

Nest predation also may be increased if invasion by exotics reduces the diversity of nesting

substrates, thereby increasing nest resource overlap of breeding birds (Martin, 1993). Finally, exotic shrubs may induce changes in nest placement (*e.g.*, closer to the ground) and make nests more visible or accessible to predators (Schmidt and Whelan, 1999; Borgmann, 2002).

Understanding interactions between exotic shrubs and breeding birds is particularly important in urbanizing landscapes where exotics, such as honeysuckles (*Lonicera* spp.), are common invasive plants. Although mechanisms of invasion are not completely understood, urban land uses seem to facilitate invasion, probably due to the combination of abundant seed sources and high levels of disturbance in forested areas (Rejmanke, 1989; Matlack, 1994; Luken and Goessling, 1995; Hutchison and Vankat, 1997, 1998; Rottenborn, 1997).

In addition to altering the ecological, hydrological, and biogeochemical roles ordinarily served by riparian forests (Vitousek, 1990), exotic shrubs may seriously diminish the capacity of a forest to provide quality habitat to birds. In this paper, I review recent research conducted in my graduate lab and discuss how *Lonicera* spp. affect the abundance and nesting success of birds in riparian forests of Ohio.

## Study System

Since 2001, my graduate students and I have studied bird communities in 34 riparian forest sites within the Scioto River Watershed in central Ohio. This watershed is dominated by agricultural and urban land uses, and relatively

little forest cover remains (<10% cover at the county level). Our study sites are composed of maple (*Acer* spp.), black cherry (*Prunus serotina* Ehrh.), white ash (*Fraxinus americana* L.), American elm (*Ulmus americana* L.), cottonwood (*Populus deltoids* Bartr. ex Marsh.), Ohio buckeye (*Aesculus glabra* Willd.), and boxelder (*Acer negundo* L.). Common understory shrubs include honeysuckle (especially *Lonicera maackii* [Rupr.] and *L. tatarica* L.), multiflora rose (*Rosa multiflora*), and to a lesser extent native shrubs including spicebush (*Lindera benzoin* L. Blume), and hawthorn (*Crataegus* spp.).

Bird species at our study sites can be classified as residents (reside at sites year-round), short-distance migrants (spend winter in southern United States), or long-distance or Neotropical migrants (spend winter in Mexico, Caribbean, or Central/South America). Common breeding resident and short-distance migratory birds include American robin (*Turdus migratorius*), Northern cardinal (*Cardinalis cardinalis*), downy woodpecker (*Picoides pubescens*), red-bellied woodpecker (*Melanerpes carolinus*), Carolina chickadee (*Poecile carolinensis*), tufted titmouse (*Baeolophus bicolor*), white-breasted nuthatch (*Sitta carolinensis*), and blue jay (*Cyanocitta cristata*).

Examples of long-distance migratory birds on sites include Acadian flycatcher (*Empidonax virescens*), great crested flycatcher (*Myiarchus crinitus*), red-eyed vireo (*Vireo olivaceus*), blue-gray gnatcatcher (*Poliophtila caerulea*), yellow-throated warbler (*Dendroica dominica*), and indigo bunting (*Passerina cyanea*).

Study sites were located in mature riparian forests (50–550 m wide and > 250 m long) along rivers 20–40 m wide. From 2001–2003, my students and I surveyed bird communities annually and measured habitat characteristics (e.g., stem density, plant species composition, canopy cover) along a 40-m wide x 250-m long transect located adjacent to the river's edge at each site.

As part of three master's theses (Borgmann, 2002; Bakermans, 2003; Leston, in progress),

we also studied the nest placement and nesting success of common understory birds, such as Northern cardinal and Acadian flycatcher, on a subset of 12 sites from May–August 2001–2003. Nests were located primarily by observing adult behavior (e.g., carrying nesting material or food) and secondarily by systematic searching for plants with nests. We collected a series of measurements describing the placement (e.g., nest height, percent concealment) and vegetation surrounding the nest for the most common species, Northern cardinal.

## Results and Discussion

Honeysuckles (*Lonicera* spp.) were dominant shrubs in many riparian forests, especially in urbanizing landscapes. Understory woody vegetation of 13 sites was dominated by honeysuckles (i.e., honeysuckle was the most abundant woody plant in the understory), whereas 21 sites were primarily comprised of native species in the understory. Habitat measurements showed that 73% of forest sites within urbanizing landscapes were dominated by honeysuckle compared to 10% of forests in rural landscapes (Figure 1, A. D. Rodewald, unpublished data). Indeed, Borgmann (2002) found that volume of honeysuckle in riparian forests of central Ohio was best explained by type of land use in the landscape matrix rather than local variables.

Forests dominated by honeysuckles supported different bird communities than those containing a more native understory. Resident and short-distance migrants were 25–50% more numerous in honeysuckle-dominated sites, but long-distance migrants were over 40% less abundant than in sites containing greater cover by native plants (Wilks' Lambda  $F_{3,30} = 7.32$ ,  $P < 0.001$ ; Figure 2). Species richness was marginally greater in forests with native understory vegetation (28.4 species + 0.91 SE) than those dominated by honeysuckles (26.2 species + 0.67 SE;  $F_{1,32} = 2.85$ ,  $P = 0.101$ ). The underlying reasons for these differences remain unclear, but possibilities include changes in floristics, fruit abundance, and habitat structure that are associated with honeysuckles.

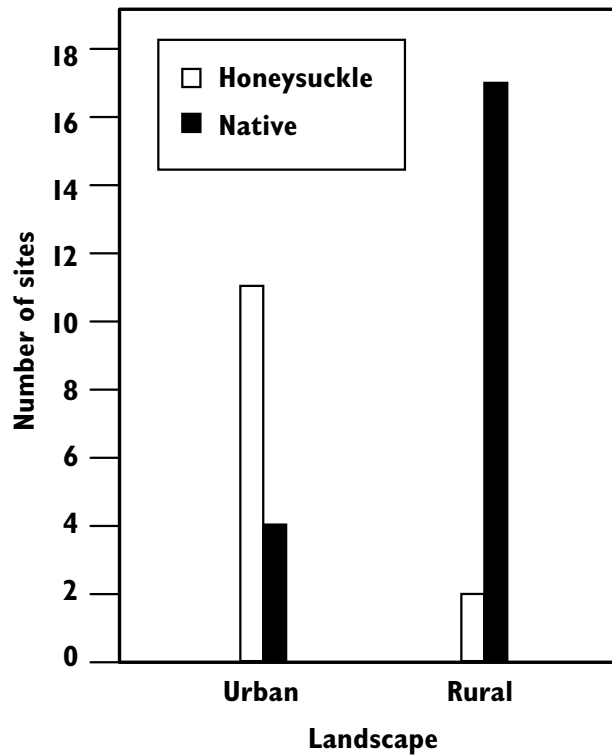


Figure 1. Number of sites with understory woody vegetation dominated by honeysuckle or a native species in 34 riparian forests within urban and rural landscapes in central Ohio, 2001 (A. D. Rodewald, unpublished data).

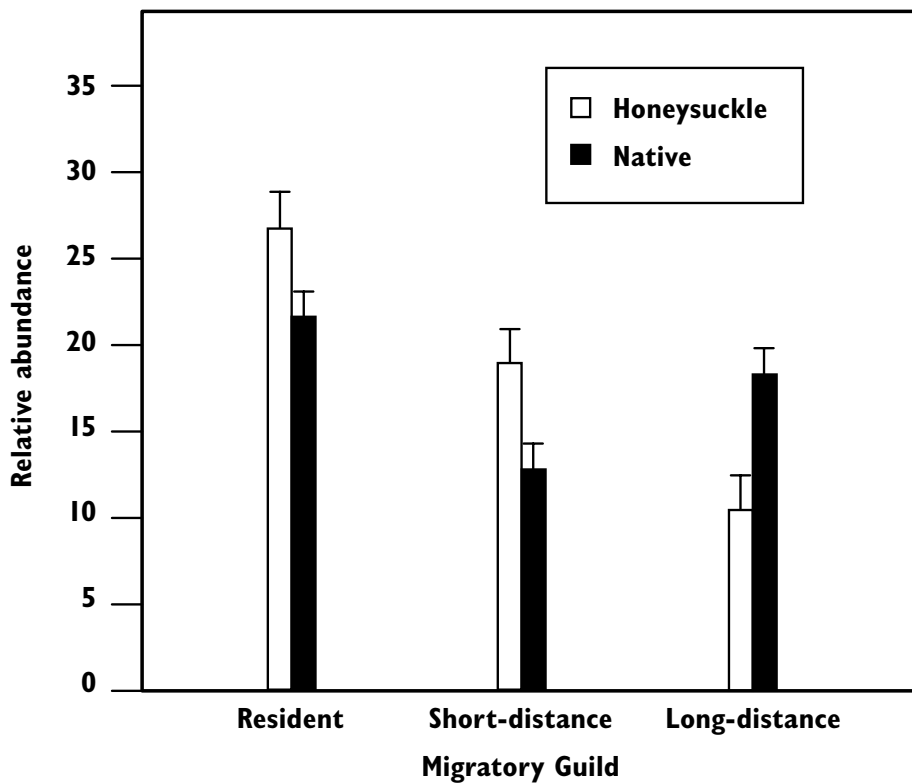


Figure 2. Relative abundance of avian migratory guilds across 34 riparian forest sites dominated by either honeysuckle or native woody vegetation in the understory, 2001–2003.

Honeysuckles may promote high densities of certain resident species by providing additional food and nesting resources, and this may carry negative consequences for other birds. Preliminary data collected by L. Leston (M.S. student) suggest that Northern cardinals actively seek out honeysuckle as a nesting substrate, and they seem to prefer nesting in patches of exotic shrubs. Thus, the abundant nesting substrate provided by honeysuckles may promote high densities of this common bird. Data also indicate that honeysuckle fruits provide the vast majority (>90%) of available fruits in urban riparian forests during late fall and winter, and sites lacking honeysuckles tend to have very low availability of late-season fruit.

Because many residents and short-distance migrants rely on fruits for energetic needs during the non-breeding season, the abundant fruit provided by honeysuckles also may act to increase densities of these species in forests dominated by honeysuckles. Indeed, urban forests with abundant honeysuckles tended to support more wintering birds than other sites (Atchison, 2003). High densities of cardinals, robins, and associated species could negatively impact less common birds in riparian forests by increasing competition and density-dependent predation (*i.e.*, high densities of prey attract predators to the area, which increases overall predation rate at the site). We are only beginning to investigate these possibilities in our lab.

Habitat modification associated with honeysuckles may contribute to absence of certain sensitive riparian species, such as Acadian flycatcher. Bakermans (2003) found that Acadian flycatchers preferentially selected breeding territories with 2.5x lower densities of understory vegetation than random plots, which suggests that they avoid areas with dense understory vegetation. Vegetation density may be an important habitat cue for Acadian flycatchers because they prefer an open understory for foraging, nesting, and aerial defense (Wilson and Cooper, 1998; Whitehead and Taylor, 2002). Furthermore, of 81 nests located, only one Acadian Flycatcher nest was found in honeysuckles.

As a nesting substrate, honeysuckles were associated with increased risk of nest predation. From 2001–2003, more than 500 nests of common breeding birds were monitored, including 135 nests of American robin and Northern cardinals. Robin and cardinal nests in exotic shrubs within urbanizing landscapes were twice as likely to be depredated as nests in native substrates (Borgmann and Rodewald, in press; Figure 3). Borgmann (2002) found that cardinal nests in honeysuckles were 1.5–2 m lower to the ground and within patches containing six to nine more exotic shrub volume than nests in native substrates. These differences in nest placement coupled with greater numbers of certain mammalian predators (*e.g.*, cats) in urban landscapes may account for differences in nest mortality rates.

## Conclusions

Our research suggests that invasion of riparian forests by honeysuckles may carry negative consequences for the breeding bird community, as sites dominated by the exotic shrub supported fewer species and long-distance migrants (*i.e.*, Neotropical migratory birds). Even for species that seemed to benefit from the abundant fruit and nesting substrate provided by the exotic shrub (*e.g.*, Northern cardinal and American robin), honeysuckle was linked to greater nest depredation than native substrates in urbanizing landscapes. Thus, honeysuckle may seriously diminish the quality of habitat available to birds in urban forests and may limit the capacity of urban forests to contribute to conservation of breeding birds.

At the same time, our findings have a number of important caveats. First, the strong positive association between honeysuckles and urbanizing landscapes makes it difficult to completely separate the landscape-scale effects of urbanization from the local-scale effects of exotic plants on avian community structure. However, patterns in community structure persisted even when I examined data from urban forests alone.

Second, increased nest depredation may not impact population recruitment of some species if birds successfully re-nest following depredation.

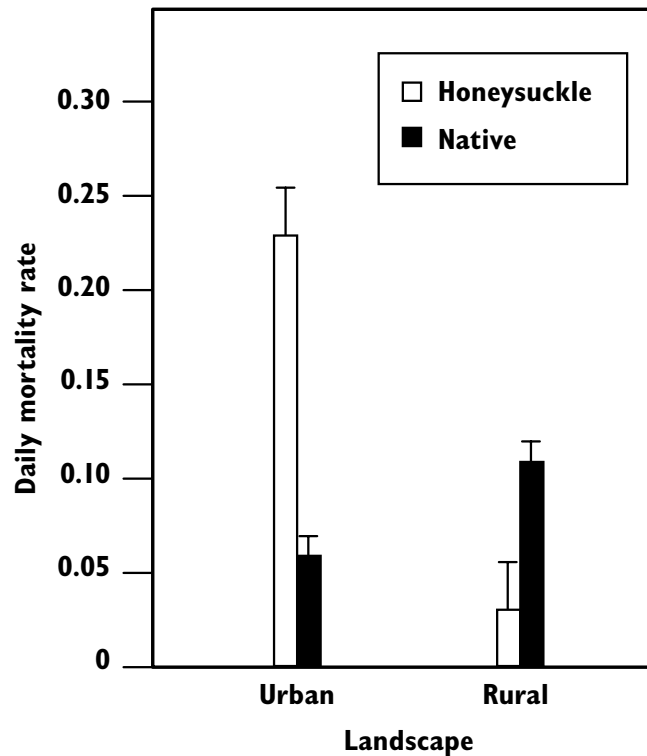


Figure 3. Daily mortality rates of Northern cardinal and American robin nests located in either honeysuckle or a native woody plant in 12 riparian forests in central Ohio, 2001-2003.

Detailed studies of annual productivity are necessary to fully elucidate the effects of honeysuckles on reproductive performance.

Third, increased honeysuckle fruits may sufficiently increase bird condition or survival to the point where they compensate for reduced nesting success. My students and I are currently investigating these issues with the ultimate goal of understanding the complex interactions among exotic plants, urban development, and bird communities.

### Acknowledgments

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# Ecological Indicators of Detrimental Impacts of Invasive Plants

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## Introduction

The invasion of non-indigenous plants is one of the main threats to ecosystems in North America. The danger to rare and endangered plant species can be observed by changes in plant communities. But the threat invasive plants pose to the integrity and function of ecosystems has been more difficult to quantify. There are many reports that plant invasions are primary threats to native species and ecosystems in terms of loss of diversity, disruption of food webs by altering community composition, and species interaction. These changes in primary producers are thought to alter crucial ecosystem processes, such as nutrient cycling. However, experimental evidence for such threats is minimal. The purpose of this paper is to explore some of the approaches being used at Cornell University to detect and quantify ecosystem impacts of invasive plants.

It is sometimes difficult to determine which species are being introduced. A variety of species arrives, but few of these become established. Of those that establish in natural areas outside of human cultivation, few will become invasive. Not all invasive species are actually introduced; native species can be invasive (expand locally and regionally and dominate plant communities; *e.g.*, goldenrods in old fields and cattails in wetlands). Ecosystem functioning can be affected by species identity, and it becomes increasingly clear by the genotype of a species. In addition, the evolutionary history of a species can affect how it functions within an ecosystem. It is important to examine a species within the context of its community and to define it by

identifying communities to which it does not belong. When a native species such as goldenrod dominates during succession, it is referred to as a thriving successional species. When an introduced species dominates in the same situation, it is referred to as an invasive species.

The program that I direct at Cornell studies how introduced plants become invasive, as well as the impacts of invasive plants on an ecosystem. This is considered from the initial establishment of a plant to when it dominates the biomass of a system to when it declines through chemical, mechanical, or biological control. We look to the use of biocontrol programs to restore or create systems that resemble original communities.

How can we assess broad-scale impacts of invasives that can be measured in any ecosystem? If we can develop simple protocols to be implemented by many different people, we may be able to generate large datasets that then could describe general invasion impacts more reliably than even sophisticated experiments that are necessarily restricted to the few places that we are able to study as individual investigators.

## Bioindicators in Wetlands

We have conducted studies to determine if we can identify a test animal that will tell us what impacts invasive plants are having in wetlands. Invasive plants are often present in amphibian habitats, and as the populations of these plants increase, the populations of the amphibians decline. The question is whether plant invasions and amphibian declines are linked.

We examined this question by studying the impact of purple loosestrife (*Lythrum salicaria*) on ecosystem function by examining the larval development of the American toad (*Bufo americana*). When purple loosestrife replaces common cattail (*Typha latifolia*) in an aquatic environment, the American toad serves as an indicator of the introduced plant's effect on the food web.

In our experiments, communities of cattails and purple loosestrife were planted in cages. Tadpoles at stage 28 (one-week old) were released into these cages. The tadpoles were recaptured at stage 42, shortly before they metamorphosed. Two different enclosure designs were used — one cage had a screen bottom that prevented predators (dragonfly larvae, predaceous beetle larvae, etc.) from encountering the tadpoles, and the other had an open bottom that allowed tadpoles access to the sediments, which had been cleared of predators. Gut analysis was used to determine the algal genera that were available to the tadpoles in each community.

The survival rate for tadpoles released into the open-bottom enclosure was half of that for those in the screened cage. Tadpoles in loosestrife cages barely grew from stage 28 to 34 over a period of 40 days; those in cattails, however, were ready to metamorphose, regardless of their cage design. Gut analysis revealed that 15 algal genera are unique to the purple loosestrife community, 19 occur only in cattail, and 23 occur in both ecosystems.

As purple loosestrife replaces cattail in wetlands, a shift in the algal composition occurs, which provides food for developing tadpoles. Shifts in the quality of available food result in differences in tadpole development rates.

To determine more mechanistically how invasive plants might be impacting higher taxa, American toads and yellow-spotted salamanders (*Ambystoma maculatum*) were raised in environments stressed only by a plant extract. To each system, extracts from one of the following plant species were added: native *Typha*

*latifolia*, introduced *Typha angustifolia* (narrow-leaved cattail), native *Phragmites* (common reed), introduced *Phragmites*, reed canary grass (*Phalaris arundinacea*), or purple loosestrife. Results showed species-specific interactions. Salamanders were heavily affected by introduced *Phragmites*, but not by native *Phragmites*. Toads were unaffected by *Phragmites*, but heavily influenced by purple loosestrife.

Introduced *Phragmites* contain high levels of saponins, which probably act as a poison by blocking oxygen exchange via the external gills of salamander larvae. Toads do not have external gills, but they are sensitive to tannins, which occur in high concentrations in purple loosestrife. The native *Typha latifolia* and the native *Phragmites* contain fairly low levels of tannins. Tannins act as digestive inhibitors that bind to proteins and render nutrients unavailable.

To understand the differences in survival between toads raised in screen-bottomed cages and those in bottomless cages, we created systems that exposed toads to plant extract alone (either cattail or purple loosestrife), and plant extract along with leaf litter. Results showed that in the presence of purple loosestrife extract alone, the survival rate of the toads dropped to 50%. Adding the corresponding leaf litter caused the survival rate to decline to 5%. The explanation of this effect is unknown. While the extract delivered a direct toxicity effect, the leaves may contain microorganisms that confer toxicity once tadpoles ingest them.

## **Bioindicators in Terrestrial Habitats**

The terrestrial salamander *Plethodon cinereus* (eastern red-backed salamander) establishes its territories in the Northeastern forest. The abundance of this species and its function within the forest floor ecosystem make it an ideal indicator for the effects of invasive plants on the forest ecosystem. We monitored sites containing garlic mustard (*Alliaria petiolata*) in the Finger Lakes Region of New York, barberry (*Berberis thunbergii*) in the Poconos in Pennsylvania, and Japanese stiltgrass (*Microstegium* spp.) around Philadelphia, Pa. Plots measuring 1 square meter

were evaluated for invasive plants and volume of leaf litter present.

Salamanders were collected from the invasion front every few weeks and marked by injecting spots of fluorescent dye beneath the skin. This technique allows labeling of small individuals and juveniles from the same year. We examined the stomach contents of these salamanders and found that most had a diverse diet, but we observed some uniformity, including individuals that foraged completely on an introduced European root-feeding weevil.

The major influence on forest floor ecosystems is the invasion of Eurasian earthworms. It is hard to judge the effect that plants have on the system since invasive plants always were found associated with earthworm invasions. Salamanders naturally consume small creatures such as many detritivores.

Results showed that invaded areas had lower levels of leaf litter. Decreasing volumes of leaf litter have a detrimental effect on the salamander population. As the leaf litter levels decline, invertebrates that live on the forest floor and provide food for the salamanders disappear. Accelerated declines were observed in areas that contain invasive plant species compared to areas that have only earthworm invasions.

Salamander abundance varied geographically but not in relation to any native plant or invasive plant. Salamander abundance declined as leaf litter levels declined, and leaf litter levels were always lower under invasive plants. We believe that nonnative earthworms are the driving force affecting salamander populations by reducing the leaf litter level. With disappearing leaf litter, small invertebrates that live in the litter and constitute food for immature salamanders disappear, followed by a crash in the salamander populations.

## **Conclusion**

Our research has demonstrated detrimental ecological impacts of selected invasive plant species on higher order taxa that serve as sensitive indicators of ecosystem change. The ubiquity of these organisms and their sensitivity to alterations in habitat quality suggest that they might serve as effective indicators of invasive plant impacts over large spatial scales. Long-term monitoring of these indicator species populations before and during an invasion could provide valuable ecological information to help prioritize management efforts. In addition, monitoring of these species before and after control attempts would be critical in determining success of efforts to preserve and stabilize sensitive habitats. The continued use of biological control programs to manage invasive plants should be explored as a way to keep invasives in check while minimizing the overall impact on ecosystem function.

# Do Species and Communities Matter in Wetland Invasions?

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## Abstract

Native North American ecosystems have been affected by the introduction of exotic plant species. In this paper, I present a brief overview of the invasiveness of exotic species and invasibility of native plant communities. I discuss why some exotic species are aggressive, using an example of a classic invasive species, *Lythrum salicaria* (purple loosestrife), and how we can manage species introduction using a multiple trait-based decision tree.

Often, invasive species are able to outcompete native species; however, when two or more invasive species interact synergistically, this can lead to *invasional meltdown*. Species-rich native communities have been suggested to be more resistant to invasion. Other studies have suggested that diverse communities are more likely to contain highly resistant species; this is referred to as the *sampling effect*. In order to develop management strategies, however, it is desirable to maintain diversity of native plant communities to reduce the chances of colonization by invasive species.

## Introduction

*“Nowadays we live in a very explosive world, and while we may not know where or when the next outburst will be, we might hope to find ways of stopping it or at any rate damping down its force. It is not just nuclear bombs and wars that threaten us, though these rank very high*

*on the list at the moment: there are other sorts of explosions....”*

— C. S. Elton (1958)

Charles Elton (1958), in his classic book, *The Ecology of Invasions by Animals and Plants*, referred to the invasion and sudden spread of alien species as the other sorts of explosions, which he termed *ecological explosions*. As human activities are becoming more and more widespread, organisms capable of colonizing habitats close to humans are thriving, through introduction and reintroduction of these species beyond their native ranges.

Native North American ecosystems have been affected seriously by a series of invasions, as animals and plants have been brought in either accidentally or on purpose. Colonizing weeds, for example, have been invading our natural areas at an unprecedented rate and forming extensive monocultures while eliminating native species from the habitats. Plant invasions can be ecologically as well as economically devastating. More species have been driven to extinction by anthropological biological invasions than by human-caused climate change (Antonio and Vitousek, 1992).

In economic terms, the estimated loss in the United States from harmful non-indigenous species was approximately \$100 billion by 1991 (U.S. Congress Office of Technology Assessment, 1993; Pimentel *et al.*, 2000). The development of appropriate management strategies for invasive species depends on understanding the ecological processes of colonization by invasive species.

## Invasiveness of Introduced Species

Herbert Baker (1965) coined the term *general purpose genotype* to describe colonizing species that thrive in a wide range of environmental conditions through phenotypic and developmental plasticity, autogamous reproduction, and clonal growth (Parker *et al.*, 2003; Figure 1).

Often the spread of non-native species is preceded by an initial lag period when the introduced species are present but have not become invasive. During this lag period, evolution could play an important role because natural selection can act powerfully on organisms to overcome limits to self-sustained population growth (Parker *et al.*, 2003).

High levels of genetic variation in outcrossing species may allow rapid responses to selection and may help create novel genotypes, thus facilitating widespread invasion. It is clear that

not all exotic species are equally invasive, and successful exotic species may have traits that are responsible for their invasiveness. Identifying those attributes may help us detect the potential for a species to be invasive.

### *Lythrum salicaria* (purple loosestrife) — A Case Study

Purple loosestrife is a very important invading and colonizing weed in North American wetland habitats. It forms extensive monocultures, eliminating native wetland species. Many traits can contribute to the remarkable success of this weed. Purple loosestrife usually occurs in low-lying coastal areas, wet, marshy places, stream banks, and ditches. It is a perennial herb ranging in height from half a meter to 2.5 m. One of the major attributes of purple loosestrife is its ability to grow clonally. It produces up to 30 to 50 annual shoots arising from root buds on its rootstock. The inflorescence is terminal and consists of a dense spike with numerous small

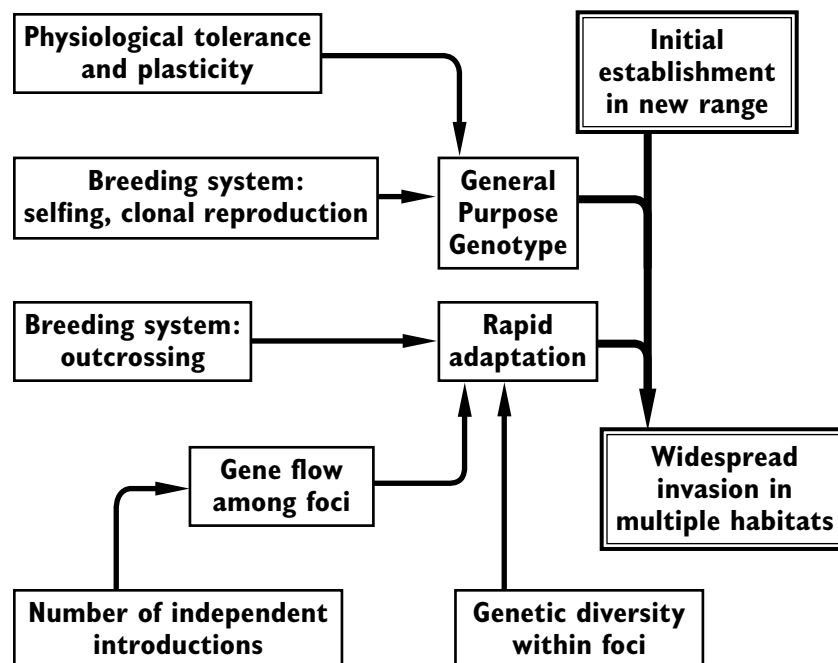


Figure 1. Factors that influence colonization success of introduced species (after Parker *et al.*, 2003).

flowers. Besides clonal growth, the species is also a prolific seed producer and produces more than two million seeds a year (Mal *et al.*, 1992).

### Breeding System

Purple loosestrife has an interesting breeding system. The species is heterostylous and has trimorphic flowers; that is, three different flower types can exist in a population (Darwin, 1877). An individual plant bears flowers of a single type (Mal *et al.*, 1992). They are either long-, mid-, or short-styled morphs (Figure 2, after Darwin, 1877). A long-styled flower has mid and short stamens, a mid-styled flower has long and short stamens, and a short-styled flower has long and mid stamens. Flowers thus separate male and female reproductive organs in space, facilitating cross pollination. A biochemical self-incompatibility system is also associated with this morphological differentiation. Only pollen produced from stamens of the same height as the pistils can fertilize ovules successfully. Darwin (1877) called this *legitimate pollination*.

### Population Dynamics in Native and Introduced Habitats

Heuch (1979) showed that the frequencies of the three morphs should be 1:1:1 for the most efficient functioning of the elaborate mating mechanism of purple loosestrife. He suggested that this ratio can be achieved in a population if no selective factors are operating, and disassortive mating and legitimate pollinations are occurring. In native European populations of purple loosestrife, the three style morphs are often present in equal frequencies, a condition called *isoplethy* (Eckert and Barrett, 1992).

Frequencies of the three morphs of purple loosestrife in introduced populations, however, were found to differ. We conducted a survey of 74 purple loosestrife populations from Windsor, Ontario, to Gaspé Peninsula in Quebec to understand its population dynamics and the extent of invasion (Mal and Lovett-Doust, 1997). We found that 67% of the surveyed populations were significantly anisoplethic, *i.e.*, all three morphs were not present in equal frequencies (Figure 3). Monomorphic and dimorphic populations were also documented; however, these populations were restricted to

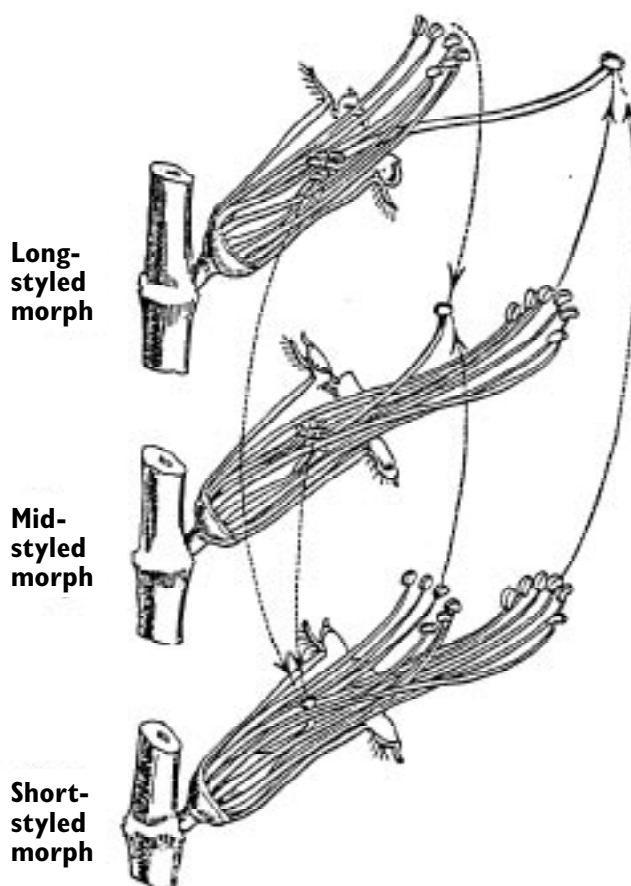


Figure 2. Trimorphic flowers of *L. salicaria* showing three different levels of anthers and stigmas and 'legitimate' pollen flow among them (after Darwin, 1877).

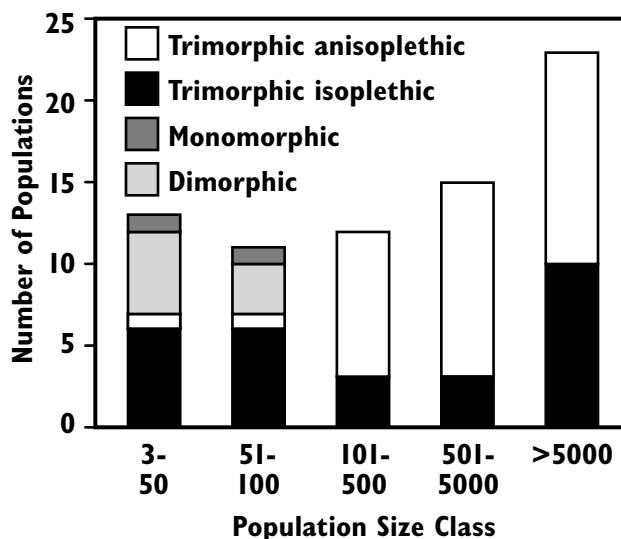


Figure 3. The relationship between population size (number of individuals) and morph structure (after Mal and Lovett-Doust, 1997).

smaller populations with 50 to 100 individuals. Populations containing more than 100 plants were resistant to morph loss, but they were not resistant to skewed morph ratios. In European populations, however, most populations are trimorphic isoplethic. These unequal morph frequencies and morph-loss may be found in a population during initial colonization stages due to founder events.

### **Heterostyly and Colonization**

Colonization of new sites is often associated with periods of low starting density. The difficulties of mating under these circumstances may be expected to impose severe restrictions on reproduction and population growth. In other tristylous colonizing species, heteromorphy and bio-chemical self-incompatibility have been reported to break down. For example, in studies of the mating system of water hyacinth, *Eichhornia*, a progressive change from trimorphism to dimorphism to monomorphism has been documented (Barrett, 1992).

In order to observe such a phenomenon in purple loosestrife, we sampled flowers from 49 populations and measured different floral structures, such as size of perianth and length of pistils and stamens (Mal and Lovett-Doust, 1997). We calculated stigma-anther separation (the distance between the stigma and the closest anther) for each of 3,804 flowers. Stigma-anther separation was significantly greater in the long morph than in the short morph. The mid-morph has the least stigma-anther separation. Therefore, the mid morph may have more potential for evolution of its breeding systems. In fact, we have identified several populations with individuals of mid morph bearing variant flowers in which stamen positions overlap with those of stigma (Mal and Lovett-Doust, 1997).

### **Phenotypic Plasticity**

We have been conducting controlled manipulative experiments to study morph-specific behavior of growth and reproduction in purple loosestrife. High variability in the vegetative and reproductive characters, particularly among sites, prompted us to conduct a replicated and cloned experiment involving different soil-moisture treatments.

The environment plays two important roles in the evolutionary process. First, the environment establishes the relationship between the phenotype of an individual and its fitness. Second, the environment interacts with developmental processes and plays a role in determining the phenotype (Scheiner, 1993). This interaction is termed phenotypic plasticity, the change in the expressed phenotype of a genotype as a function of the environment.

We investigated the relationship between genotypes and phenotypes, and the amount of phenotypic variation attributable to genotype, environment, and to their interactions in different phenotypic traits. From all the traits observed, we calculated a plasticity index that differed significantly among morphs, and, in fact, it was significantly greater in the mid morph compared to that in the long and short morphs (T. K. Mal, unpublished). We also found that the plasticity index is significantly greater in the vegetative traits compared to that in the reproductive traits. Phenotypic plasticity in this species may have provided sufficient ammunition for adaptation in new environments.

### **Predicting Invasiveness of Introduced Species**

How can we take preventive measures to avoid future introduction of an invasive species? Agriculturists, horticulturists, and foresters often introduce new plant species for commercial purposes. Reichard and Hamilton (1997) proposed a decision tree for accepting a particular species based on discriminant analysis (DA) and classification and regression-tree (CART) analysis.

These authors used simple attributes of different introduced woody plants in North America in the analysis and attained overall predictive rates of 76.5% using CART to 86.2% using DA. The decision tree they proposed allows users to divide the species into three categories: admit (low risk of invasiveness), deny admission (high risk of invasiveness), or delay admission for further analysis and/or monitor intensively (*i.e.*,

the risk cannot adequately be assessed based on the included attributes).

### Colonization of Invasive Species and Community Invasibility

Invasive species often can induce changes in the community structure and impact organisms at higher trophic levels. They can also affect ecosystem processes such as nutrient cycling, hydrology, and fire regimes (Levine *et al.*, 2003). Indeed, we have demonstrated that purple loosestrife can out-compete *Typha angustifolia* (narrow-leaved cattail) in a long-term competition experiment (Mal *et al.*, 1997). In the first year of the experiment, the rate of ramet production in *Typha* was greater than that in *Lythrum*. However, the rate of ramet production in *Typha* was much lower than *Lythrum* from the second year onward, and by the fourth year, *Lythrum* gained an overall competitive advantage in mixtures of *Lythrum* and *Typha* and formed virtual monocultures (Figure 4). The study

reinforced the need to follow the competitive behavior of study species over several years.

### Invasional Meltdown

Often two or more harmful alien species may interact, leading to a more severe combined impact than their individual impacts. These interactions can be both detrimental as well as facilitative. Simberloff and von Holle (1999) considered *invasional meltdown* as a process by which a group of exotic species facilitates one another's invasion in various ways, increasing the likelihood of survival and/or of ecological impact, and possibly the magnitude of impact. For example, the introduced honey bee (*Apis mellifera*) is a major pollinator of purple loosestrife, facilitating its reproductive success and spread (Mal *et al.*, 1992).

Recently, O'Dowd *et al.* (2003) provided a classic example of invasional meltdown caused by crazy ant, *Anoplolepis gracilipes*, in an island

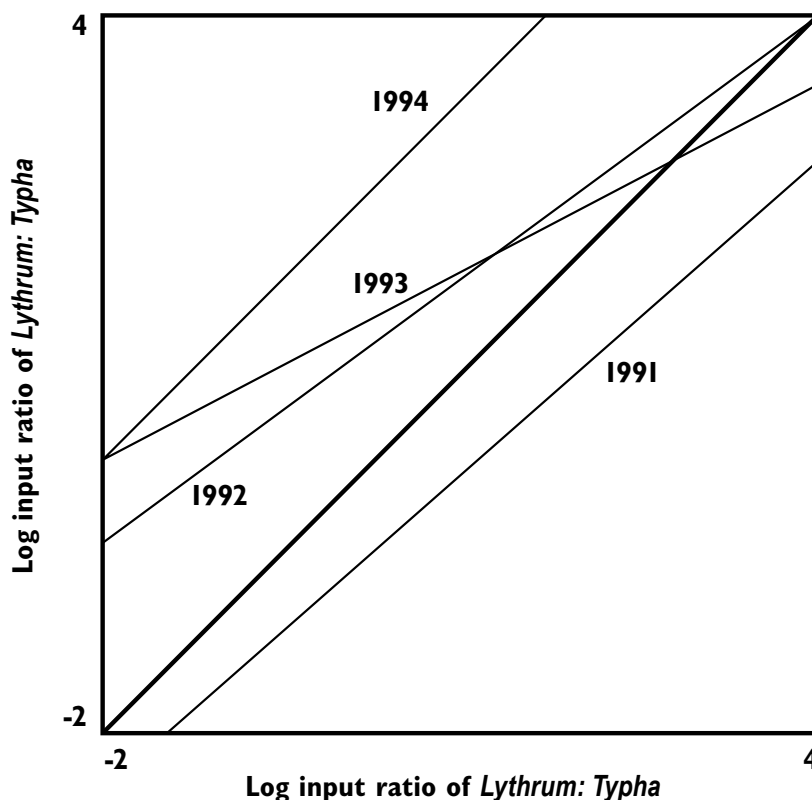


Figure 4. Log of output ratios plotted against the log of input ratios of each treatment, density, and year. Each ratio indicates the line of best fit for each of the four years; the diagonal line (bold) indicates the 45° threshold connoting coexistence (after Mal *et al.*, 1997).

rainforest in the northeastern Indian Ocean. In invaded areas, crazy ants extirpated the red land crab, the dominant endemic consumer on the forest floor. Crazy ants indirectly released seedling recruitment, enhancing species richness of seedlings, and slowing litter breakdown. In the forest canopy, new associations between the invasive ant and honey-dew secreting scale insects accelerate and diversify impacts.

### Species Richness and Community Invasibility

Several authors, including Charles Elton (1958), suggested that the species richness of biological communities may have a role in influencing their susceptibility to invasion. It has been suggested that species richness increases resource use complementarity and thereby increases the proportion of resources used, leading to the low availability of resources for invaders and decreasing the invasibility of a biological community.

Several recent studies present experimental evidence from synthesized plant communities (Knopps *et al.*, 1999; Levine, 2000; Naeem *et al.*, 2000; Kennedy *et al.*, 2002). However, the sampling effect has been suggested as a plausible explanation for the resistance of species-rich communities to invasion (Aarssen, 1997; Huston, 1997; Wardle, 2001). They propose that the increase of species richness in a synthetic community also increases the probability of

including the most competitive species, leading to an increase of overall competitive ability and thereby reducing its invasibility (Figure 5).

### Species Richness and Sampling Effect

Wardle (2001) evaluated eight experimental studies from recent publications to identify whether a sampling effect is responsible for the observed invasion resistance of the experimental communities (Crawley *et al.*, 1999; Knopps *et al.*, 1999; Levine, 2000; Naeem *et al.*, 2000; Prieur-Richard *et al.*, 2000; Symstad, 2000). Wardle (2001) explained how experimental studies on community invasibility can differ from the observational studies.

In observational studies, species diversity generally demonstrates a hump-back relationship with productivity (Figure 6a, after Wardle, 2001). That is, the diversity increases initially with productivity, and then decreases with further increases in productivity. "Invasibility should be positively correlated with diversity over the productivity range 'a' because conditions are less adverse for invaders as productivity increases, and positively correlated with diversity over range 'b' because the resident plant community exerts a greater competitive effect against invaders with increasing productivity" (Wardle, 2001). Experimental studies, however, show increasing diversity and more complete resource utilization leading to

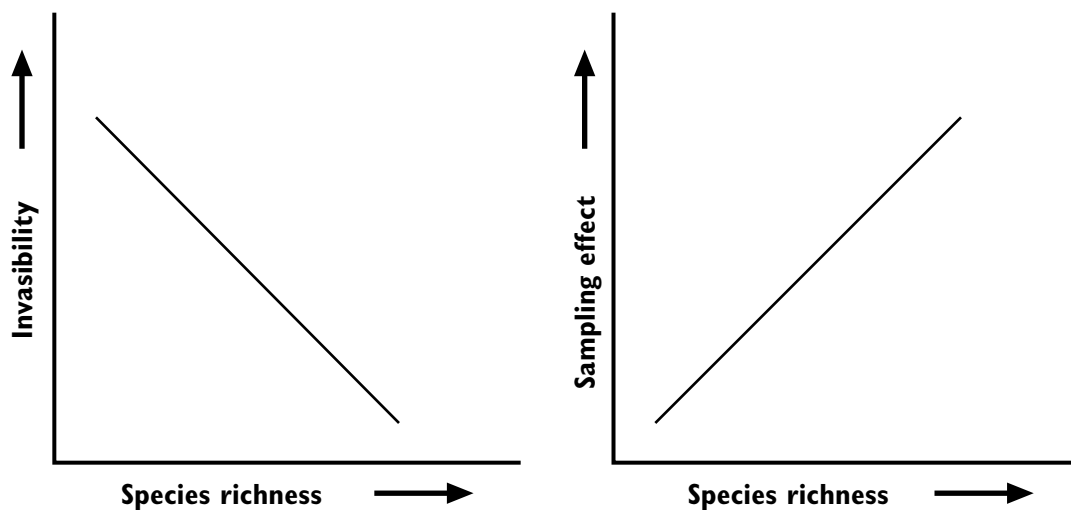


Figure 5. Plausible relationships among species richness, sampling effect, and invasibility.

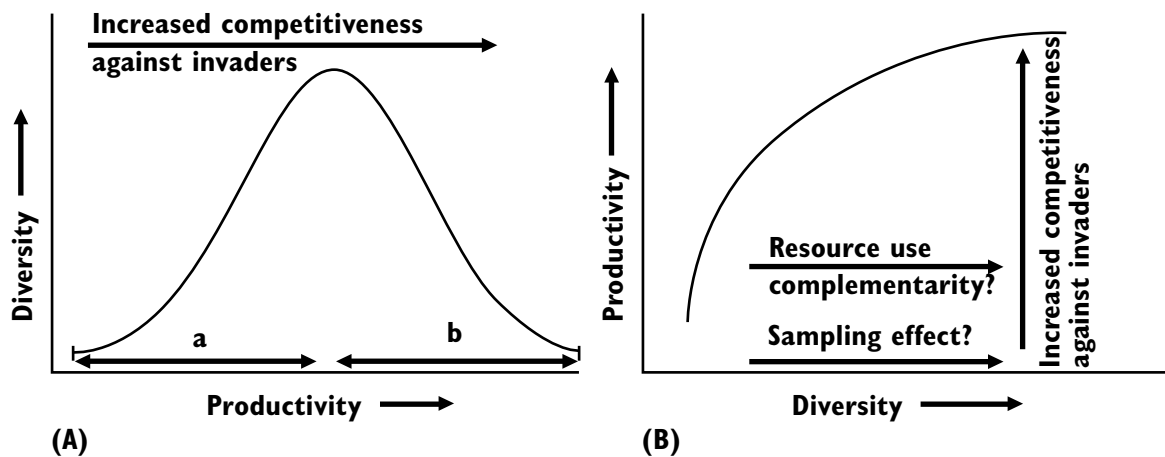


Figure 6. Diversity and invasibility relationship may differ between (A) observational and (B) experimental studies (after Wardle, 2001).

reduced invasibility (Wardle, 2001; Figure 6b). More recently, Meiners *et al.* (2004) demonstrated that invasions by native and exotic species do not differ, and the control of species invasion is primarily individualistic.

**Species Richness and Spatial Scales:** Shea and Chesson (2002) suggested that a positive relationship between native species richness and the number of exotic species may be found with varying spatial scales and extrinsic factors (Figure 7, after Shea and Chesson, 2002). Extrinsic factors can vary considerably across broad spatial scales, and factors that favor high numbers of native species may also increase niche opportunities for the invasive species. However, within any cluster, higher numbers of native species lead to poorer niche opportunities for invaders, generating a negative relationship between the two (Shea and Chesson, 2002).

#### Diversity Indices and Community Invasibility

An important aspect of the numerical structure of communities is completely ignored when the composition of the community is described simply in terms of the number of species present. Intuitively, a community of 10 species present in equal proportions seems more diverse than another community with 10 species, with 60% of individuals belonging to one species and less than 5% in each of nine other species. Therefore, we need to account for the proportional

abundance of each species in the community as well. Simpson and Shannon's diversity indices quantify just that. Therefore, we need to consider maintaining higher diversity indices (and not just species richness) in our communities, which may increase the resource use complementarity and decrease resource availability leading to fewer species invasions.

In our wetland mesocosm experiment, we intended to synthesize native communities with 4, 8, 16 different species and then simulate invasion by *Lythrum salicaria*, *Phragmites australis* (common reed), and *Lythrum* and *Phragmites* together to examine community invasibility. We found at the end of our first growing season that the native species *Echinochloa muricata* (American barnyard grass) out-competed all other native species irrespective of the treatment (T. K. Mal, personal observation; M. Parsons, personal communication). This may be considered an example of the sampling effect. We will be following this study for several years and would like to see whether these synthetic communities can resist colonization by purple loosestrife and/or common reed.

#### Conclusions

Exotic species often differ in their invasiveness, and appropriate management strategies can be more easily adopted if we can determine which

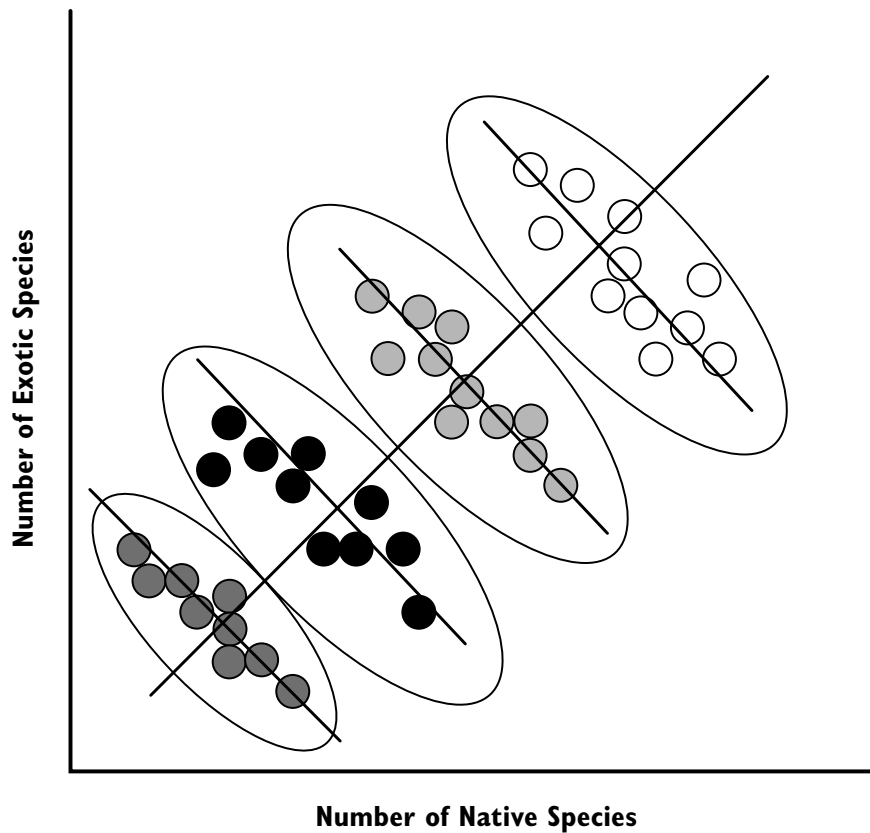


Figure 7. Effects of spatial scale on species richness and invasion success (after Shea and Chesson, 2002).

species has greater invasive potential. A decision tree may be used in regulating future plant species introduction. Often an invasive species can out-compete a native species; however, when two or more exotic species interact, their synergistic effects often can lead to invasional meltdown. The relationship between species richness and community invasibility is not a straightforward one and may be confounded by sampling effect, the spatial scale of the study, and by the measure of diversity used.

### Acknowledgments

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# Woodland Restoration: Applied Science, Natural History, and Technology

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## Abstract

The comprehensive program for management of the invasive Amur bush honeysuckle, *Lonicera maackii* (Rupr.) Maxim., on the Nature Reserve at Mt. St. John, includes not only control of the invasive species but also management to restore the disrupted plant communities and prevent re-invasion by the shrub. Research on the natural history of the site, the invasive traits of bush honeysuckle, its susceptibility to control measures, characteristics of glyphosate that promote control, and restoration practices that lessen re-invasion provide the basis for adaptive management practices. The site at Mt. St. John varies greatly in moisture and soil conditions and the wide diversity of habitats and associated communities. The area was part of the Native American hunting grounds and later was impacted by farming.

Amur honeysuckle, probably introduced on the Nature Reserve in the 1970s, found a favorable habitat for invasion on disturbed areas. Control of bush honeysuckle by glyphosate-based herbicides was helped by taking advantage of the shrub's seasonal cycle of carbohydrate storage and mobilization and the delayed senescence. Glyphosate is transported readily to active storage tissues, including the stem tissues involved in carbohydrate storage and mobilization. Killing these stem storage tissues by foliar glyphosate applications prevents formation and translocation of xylem sap needed for bud break and leaf growth in spring. Because bush honeysuckle is an opportunist that invades disturbed areas readily, it is important to avoid management of weedy species by methods that

disturb the soil and open it to invasion by plants such as bush honeysuckle and garlic mustard. Studies of the reestablishment of the herbaceous layer and tree seedlings following honeysuckle removal indicate that at least some areas of woodland soil still have a seed bank that is able to initiate recovery once the honeysuckle has been removed. Current studies show a trend in the recovering woodland toward an increasing proportion of shade-tolerant species, such as sugar maple and ash, among the seedlings and young trees.

## Introduction

Ridding the woodland at Mt. St. John Nature Reserve of the Amur bush honeysuckle (*Lonicera maackii*) that was established more than two decades ago involves more than killing the invasive plants. With time, our goal was expanded to include not only control of the invasive shrub, but also restoration of the disturbed woodland ecosystem to a less vulnerable state.

The campaign to control bush honeysuckle brought a number of questions to the fore. Why was the invasion so successful? After all, there are some woods in our area that have far less Amur honeysuckle. When did the invasion start and what triggered it? It will not suffice to tackle the increasing population if the causes are not identified. What must one do to succeed in such a vast task? What traits of Amur honeysuckle make it susceptible to control and can we capitalize on weaknesses to control it more readily? Once honeysuckle is removed, how can we prevent reentry?

## Profile of Background Research

Research on the natural history of the site provided us with the early history of our local ecosystems and landscapes (Ludlow, 1802; Braun, 1961; Gordon, 1969; Nolin and Runkle, 1985). Our efforts to understand the invader and its establishment were helped by those who investigated the natural history of the invasive Amur honeysuckle, from its collection in the Amur River region of Manchuria, through its many transfers, to its distribution in our area (Luken and Thieret, 1996).

Development of control strategies was aided by our studies of the biochemistry and physiology of glyphosate in plants and mechanisms of its distribution and mode of action (Gougler and Geiger, 1984; Geiger and others, 1999; Geiger and Fuchs, 2002). Data from these studies, together with practical experience and field observations, enabled us to develop methods for effectively controlling large areas of bush honeysuckle.

Finally, studies by a number of students documented recovery of the understory vegetation of the woods and the succession of the plant community on the restored areas after control of the honeysuckle.

## Natural History of the Woodland Site

The Wisconsin glacier was a key factor in habitat formation on the Mt. St. John Reserve. Much of the woodland is on a large esker deposited some 17,000 years ago, providing a landscape with a rich variety of moisture and soil regimes on the site, including a woodland fen, hillside springs, and well-drained hilltops with thin clay soils on thick glacial till deposits. Israel Ludlow (1802), who surveyed the site in June of 1802, noted the thin, well-drained soil and the wide variety of moisture regimes in a landscape dominated by an oak-hickory-dogwood woodland. With European settlement, the diverse habitats on the site were disrupted, first by a 19th century Shaker community farm and then, in the early 20th century, by the Marianist community that occupies the present Mt. St. John site. From the 1940s to 1965, pigs and cattle ranged over a sizeable portion of the wooded area and

adjacent fields of Mt. St. John, contributing to the disturbance of the land on which alien bush honeysuckle thrived.

Growth rings of the oldest bushes suggest that rows of Amur bush honeysuckle were planted along roads and fence lines in the early 1970s. These early bush honeysuckle shrubs appeared to be the result of a program for distribution of the USDA-patented Rem Red Amur bush honeysuckle promoted by the Soil Conservation Service (Luken and Thieret, 1996).

## Natural History of Introduced Amur Bush Honeysuckle

Amur bush honeysuckle, which was introduced into North America in the late 19th Century and distributed in the 1970s and 80s, was well suited genetically to invade disturbed areas, responding to a wide variety of conditions with a high degree of adaptability and plasticity (Hutchinson and Vankat, 1997). An important factor in its success appears to be the favorable climate in the temperate deciduous forest biome, which provided environmental conditions that were more favorable for reproduction than in the gardens of Europe. Conditions in eastern North America resulted in high rates of seed production and very high levels of primary productivity (Hutchinson and Vankat, 1997).

The early years of observation and experience with control measures yielded knowledge that was useful in devising control procedures. Early attempts to control the shrub by foliar application of the original formulation of glyphosate revealed seasonal variations in effectiveness that we attributed to plant susceptibility. Our initial working hypothesis was that this difference may result from seasonal differences in glyphosate uptake by leaves and, possibly, seasonal cycles of carbohydrate metabolism.

We hypothesized that the deciduous shrub would show a cycle of carbohydrate storage and mobilization characteristic of woody perennials (Nelson and Dickson, 1981; Larcher, 1995; Sauter and Wellenkamp, 1998). Storage cells in the

wood parenchyma and wood rays mobilize stored starch and sugars in spring as a basis for sap flow and nutrition for bud break and leaf growth. These cells were seen as potential targets for the glyphosate herbicide.

Another trait was the delayed loss of leaves in fall until long after the native deciduous shrubs and trees, a consequence of its being from the Amur River region of northern Manchuria, on the border with Russia. This trait made Amur bush honeysuckle a highly visible target and provided an element of selectivity because native shrubs and trees are dormant and so easier to avoid spraying and less vulnerable to the herbicide.

### Mode of Action of Glyphosate

Our studies of the physiology of glyphosate, dating from the mid 1980s, revealed characteristics that make it a highly effective herbicide, ideal for use in controlling widespread populations of bush honeysuckle. Glyphosate enters the fine leaf veins and is transported with nutrients from photosynthesis to the growing and metabolically active parts of the plant (Gougler and Geiger, 1984). Within a few days

to a week, the target tissues die. For herbicidal success, it is important that the tissues killed are essential to the life of the plant. In our case, we chose to verify that tissues essential for supplying carbohydrates to nourish bud break and leaf development were killed by the herbicide.

### Seasonal Accumulation of Starch and Sugars in Woody Stems

To refine our control methods, we focused our attention on the seasonal cycle of carbohydrate accumulation that supports leaf renewal, a characteristic of deciduous woody perennials (Nelson and Dickson, 1981). The timing of the steps in a typical cycle of storage and mobilization of carbohydrates in a woody stem is shown in Figure 1. Starch accumulation in the woody stem begins in April with the development of the canopy and continues until September in willow (Sauter and Wellenkamp, 1998). Sugar accumulation begins in fall and continues until sap flow in February. Spring sap flow derived from the stored carbohydrates is well known from maple syrup production and is a critical part of this cycle that supports the nutrition of the new leaves that emerge in

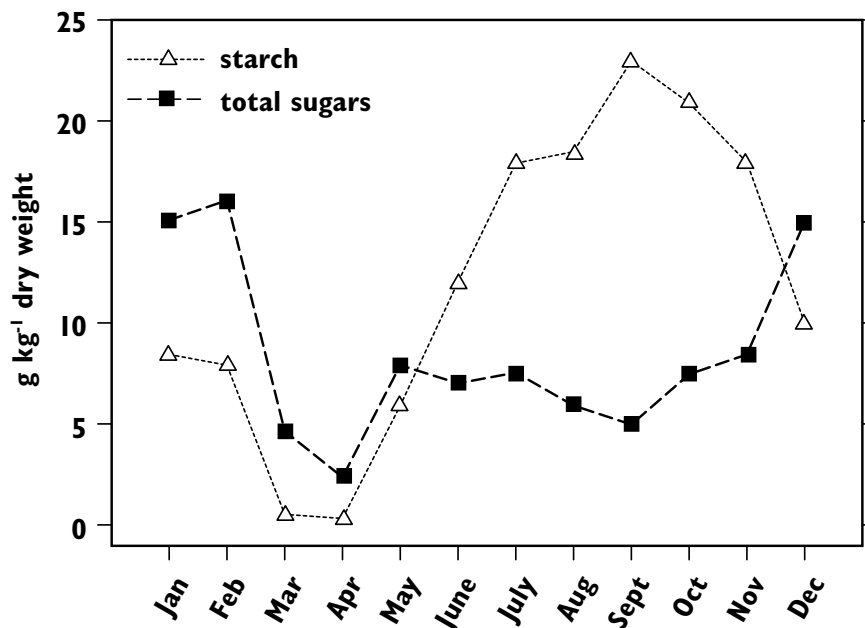


Figure 1. Seasonal accumulation and mobilization of carbohydrate in woody stems of willow (from Sauter and Wellenkamp, 1998). Starch accumulates during the growing season and is converted to sugar during the fall and early winter, prior to initiation of sap flow up the xylem.

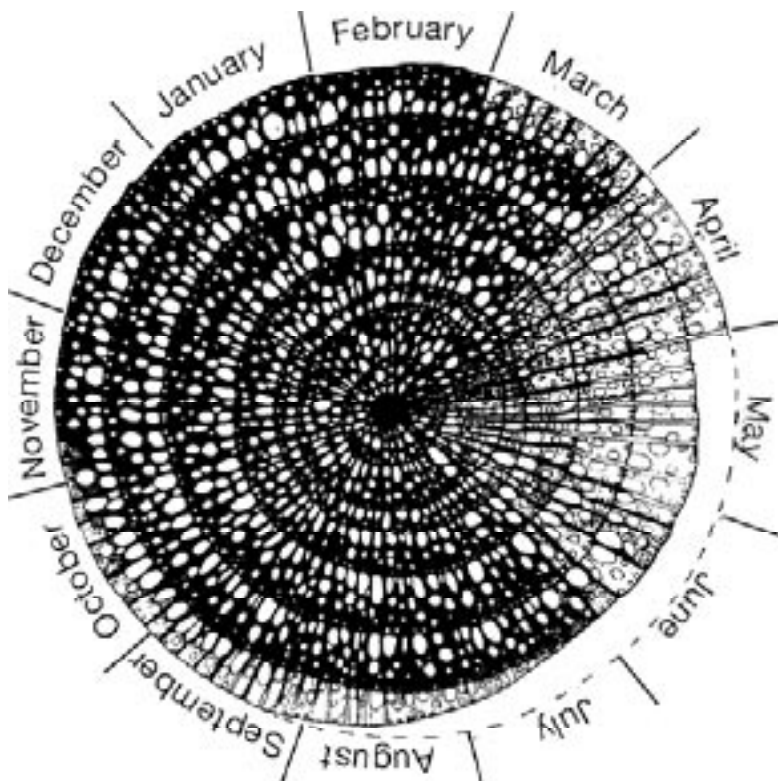


Figure 2. Tissue location of seasonal accumulation of carbohydrate in a woody stem (from Larcher, 1995). Annual ring formation starts at the end of June and continues until September. Starch accumulation (black) begins in the season of active photosynthesis.

spring. Localization of carbohydrate storage in a woody stem is shown in Figure 2. The new annual ring develops in July and August, and the accumulation of starch in the new ring continues until October in this plant (Larcher, 1995). By the end of February, the starch mobilization to sugar is seen.

In designing our strategy for controlling bush honeysuckle, we concluded that killing the stem cells that store and mobilize carbohydrate would prevent opening of buds and development of leaves. Transport of glyphosate to these tissues is a critical part of the control strategy. During the latter part of the growing season, sugar from the leaves is transported to these cells, and this continues into the fall when the nutrients are being reclaimed from the senescent leaves. We reasoned that foliar application of glyphosate at this time would deliver a lethal dose of the herbicide to these cells.

### Localization of Tissue Death Following Foliar Application of Glyphosate

To verify the death of the critical tissues, we developed a vital staining method to observe

which tissues are killed and when this occurred. Figure 3 shows the killing of cambium and of the starch-storing xylem and phloem cells by foliar spraying with glyphosate, as revealed by vital staining. The xylem rays and parenchyma store and mobilize the nutrients for formation of the sap needed for bud break, while the vascular cambium is needed to transport nutrients from the new leaves to the plant.

### Control of Bush Honeysuckle

Removal of bush honeysuckle generally is done in two stages. First, the mature well-established plants are controlled by foliar application of 1.3% Roundup Pro-Dry, aiming for nearly full to complete coverage (Figure 4). The advantages of foliar spray application in late September to early November include ease of spotting the green honeysuckle leaves, the accumulation of glyphosate in wood parenchyma cells of the stems, the ease of avoiding native plants, and their reduced susceptibility at this time of year.

Often we control the very large plants by cutting the trunk with a chain saw just above ground level and applying 20% Roundup solution to the

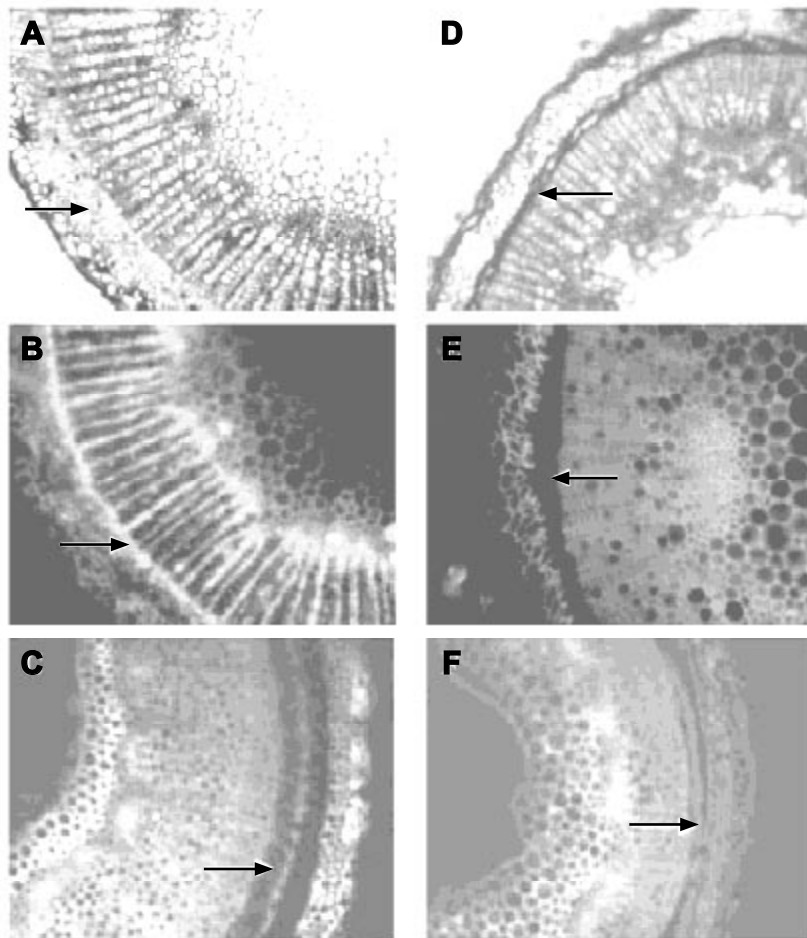


Figure 3. Sites of tissue death resulting from foliar application of Roundup. Cross sections of young woody stems of *Lonicera maackii* shown in the left panels (A-C) are from stems collected prior to application of glyphosate, while those in panels on the right (D-F) are from tissue collected one month after foliar application of a 1.3% solution of Roundup Pro-Dry. Top panels (A and D) are unstained tissue. Note the collapsed state of the phloem layer (arrow) in treated plants. Middle panels (B and E) show sections that have been stained with the fluorescent dye 6-carboxyfluoresceine diacetate, CFDA. Note the areas of bright tissue that indicate living phloem and cambium (outer ring), phloem rays (radial strips), and pith parenchyma (irregular inner ring) in tissue from the control plants on the left (B). Brightness in the tissues in sections from treated plants are the very dim fluorescence from the dead tissue (E). Bright areas indicated by arrows in the bottom panel on the left (C) show the fluorescence of chlorophyll in the living stem and absence of the pigment in the stem from the treated plant (F).

cut stump immediately. This method of control can be used throughout the year.

The presence of bush honeysuckle seedlings the season following spraying with herbicide suggests that honeysuckle seeds generally sprout the next year after they develop. The number of seedlings that become established depends on the amount of shading by the leaf litter and early growth of herbaceous layer plants where the

honeysuckle was removed. It is best to wait until these seedlings are two to three years old before controlling them by foliar spray. These seedlings do not flower for the first several years so there is no harm in waiting until the plants are large enough to be readily targeted.

Generally, we do not remove the dead bushes because the associated physical disturbance encourages invasion by garlic mustard and bush



Figure 4. Mature honeysuckle stand (A) and cleared area (B) in the Mt. St. John woodland, spring 1990. The height and density are typical of the stands present at the start of the campaign to control the invasive shrub.

honeysuckle. After the second control session, the area is essentially free of the honeysuckle and ready for any further restoration that is needed (Figure 4).

### **Restoration and Enhancement of the Woodland Ecosystems**

Next, we carry out any procedures necessary to restore the site to a stable, functional woodland habitat. This step entails continued spot removal of seedlings that are introduced from other sites to allow continued development of the herbaceous layer and planting of shrubs to

replace those displaced by honeysuckle. Prior to the foliar spraying, the density of the invading honeysuckle excluded most ground cover and seedlings of woody plants, even seedlings of bush honeysuckle (Figure 5).

Following the control of bush honeysuckle, we generally find that there is a sufficient seed bank or seed dispersal from adjacent areas to establish a herbaceous layer. The return of a herbaceous layer offers some protection to lessen reinvasion by bush honeysuckle and invasion by garlic mustard.



Figure 5. Comparison of herbaceous layer under living (A) and killed (B) honeysuckle. Herbaceous layer ground cover is visible on the site in June following a fall spraying of the shrub (B).

A list of species present in the herbaceous layers is given in Table 1. In mesic to moist habitats, the renewal of ground cover in the herbaceous layer and recruitment of tree seedlings generally is good and diverse, especially following the second removal of honeysuckle. In areas where the restored herbaceous layer is more mature, white snakeroot, common clearweed, and violets

are common. In the moist low areas, spring ephemeral species are present early in the season and later, green dragon (*Arisaema dracontium*), Jack-in-the-pulpit (*A. triphyllum*), and several species of waterleaf flower are present.

Control of bush honeysuckle changes the aspect of the woodland sharply from that associated

<b>Table 1. Plant composition of the reestablished woodland herbaceous layer.</b>		
Plants listed are commonly found in the recovering woodland herbaceous layer following control of Amur bush honeysuckle. Frequency gives the proportion of transects with a given type of plant.		
<b>Common Name</b>	<b>Scientific Name</b>	<b>Frequency</b>
Avens	<i>Geum</i> spp.	0.94
White snakeroot	<i>Ageratina altissima</i>	0.87
Violets	<i>Viola</i> spp.	0.63
Wild grape	<i>Vitis</i> spp.	0.56
Garlic mustard	<i>Alliaria petiolata</i>	0.56
Virginia creeper	<i>Parthenocissus quinquefolia</i>	0.38
Common clearweed	<i>Pilea pumila</i>	0.38
Bedstraw	<i>Gallium</i> spp.	0.31
Sweet cicely	<i>Osmorhiza claytonii</i>	0.25
Lopseed	<i>Phryma leptostacha</i>	0.06
Waterleaf	<i>Hydrophyllum</i> spp.	NR
Wood-nettle	<i>Laportia canadensis</i>	NR
Clustered snakeroot	<i>Sanicula gregaria</i>	NR
NR: Not recorded in the transect survey but a rather common species in certain transects.		

with the invading bush honeysuckle (Figure 6). Our inventory of mature trees, saplings, poles, and seedlings in areas where honeysuckle has been removed revealed an interesting trend. The recovering woodland shows a pattern characteristic of a woodland that is undergoing a transition in succession. The trend favoring more shade-tolerant tree species is similar to that described in a recent report (Kennedy *et al.*, 2003). Particularly on mesic sites, the proportion of shade tolerant species, such as sugar maple, blue ash, and green ash, present in a given age class decreases with age. Currently, we are examining whether factors ascribed to increased effects of human activity — longer growing season, higher temperatures, higher carbon dioxide, and acid rain fertilization — might be increasing woodland canopy density and changing succession.

### Acknowledgments

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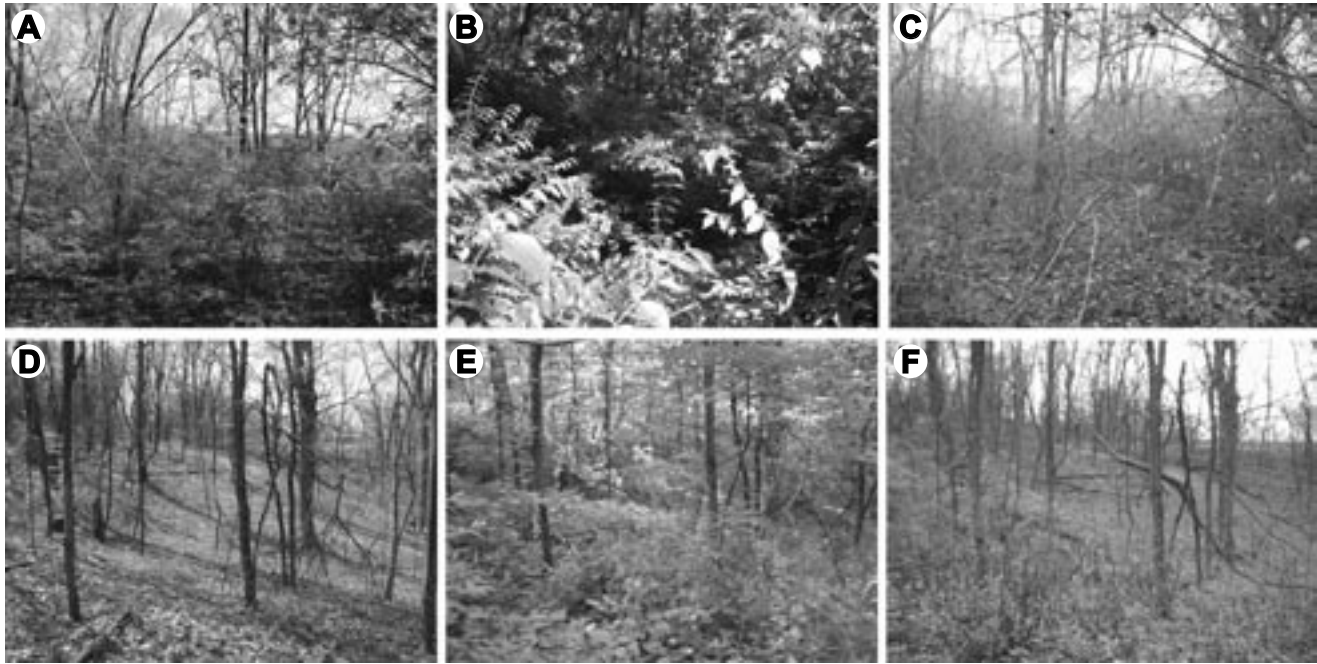


Figure 6. Seasonal comparison (spring, summer, fall) of two sites in a woodland that were either managed by removal of bush honeysuckle (D-F) or left unmanaged (A-C). Bush honeysuckle in the managed site (D-F) was given a foliar application of Roundup the previous fall. The high density of vegetation in the unmanaged area in summer (B) essentially precludes a herbaceous layer and recruitment of tree seedlings.

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# Invasive Species and Aquatic Macrophyte Diversity in the Cuyahoga River Watershed in Northeastern Ohio

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## Abstract

We surveyed aquatic macrophyte diversity at 20 sites along the main channel of the Cuyahoga River and its tributaries. These sites included 12 progress indicator sites in the watershed whose observed Index of Biological Integrity (IBI, a fish diversity index) values deviated significantly from predicted IBI values. These sites were classified as Best of the Best, Worst of the Best, Best of the Worst, and Worst of the Worst for site type.

To characterize a site, we collected data on the physical features of the stream and quantified species abundance of aquatic macrophytes along a 100-m transect. Site characterization also included physical measurements of stream width, bank full width, stream depth, and canopy coverage, as well as analysis of water samples for nitrate, phosphate, and ammonia concentrations. A Qualitative Habitat Evaluation Index (QHEI) for each site was quantified.

Aquatic macrophytes were discovered at seven of the 20 sites, with an overall diversity of 11 species among sites. The most common aquatic macrophytes were *Elodea canadensis* (common waterweed), *Sparganium americanum* (bur-reed), and *Sagittaria latifolia* (arrowhead). *Potamogeton crispus* (curly pondweed), an introduced invasive species, was discovered in low numbers at multiple sites. *Potamogeton crispus* has an ability to spread rapidly and can affect the growth of native aquatic macrophytes. Numerous states have already added *P. crispus* to their invasive species lists. The initial finding of only a few

*P. crispus* individuals indicates that widespread invasion has not yet occurred. However, steps should be taken to reduce its spread to prevent formation of monocultures and loss of native aquatic macrophytes in the watershed. Further surveys should be undertaken at additional sites within the Cuyahoga River watershed for a comprehensive assessment of aquatic macrophytes and identification of sites with invasive species such as *P. crispus*.

## Introduction

The Cuyahoga River in northeastern Ohio has played a major role in policy and public awareness surrounding water quality. In the 20th Century, following years of industrialization and point-source and non-point-source pollution in the watershed, the Cuyahoga River caught fire numerous times, leading to the passage of the Clean Water Act in 1972. However, abiotic pollution is not the only source of environmental problems. Biotic pollution can also affect the health of an aquatic ecosystem. The Cuyahoga River may have also been playing a significant role in the transportation and spread of unwanted nonnative plant and animal species through the ballasts and propellers of boats. These invasive species can cause extraordinary ecological and economic damage to the watershed, as well as to the entire Great Lakes Basin.

Running-water environments harbor diverse and unique ranges of species, habitats, and ecosystems, including some of the most threatened species and ecosystems on earth

(Allan and Flecker, 1993). Aquatic macrophytes are an important component of biological communities and serve as structural elements. These macrophytes provide primary food production, nutrients, and habitat for a wide range of organisms living in and around lotic sites (Gregg and Rose, 1982). Native aquatic macrophytes in the Cuyahoga River watershed have played a vital role in its ecosystem function for thousands of years. However, it is possible that invasive species that outcompete native macrophytes could cause catastrophic changes in the composition and species diversity of the Cuyahoga River watershed.

A qualitative survey of aquatic macrophytes in the Cuyahoga River watershed was last conducted 35 years ago. The purpose of this study was to quantify diversity of aquatic macrophytes within the Cuyahoga River watershed in northeastern Ohio and determine the extent of colonization by invasive species. Physical stream characteristics were also recorded in an attempt to correlate water quality, macrophyte diversity, and the physical layout of stream and river sites.

## Materials and Methods

The study area is located in the Cuyahoga River watershed in northeastern Ohio (Figure 1). The watershed drains 813 square miles and includes 37 named tributaries. In its upper reaches, the Cuyahoga River consists of an East and West Branch, which eventually meet to form a main channel that subsequently empties into Lake Erie. The geology of the watershed was heavily influenced by the region's preglacial and glacial history. The study area is also significantly urbanized with two major cities, Cleveland and Akron.

Twelve of the 20 stream sites surveyed were selected based on data from a joint project involving the Ohio EPA and Cleveland State University. The joint project aimed at identifying sites whose projected Index of Biological Integrity (IBI, a fish diversity index) scores deviated significantly from their actual IBI scores following field surveys. Sites were divided into four categories depending on the degree

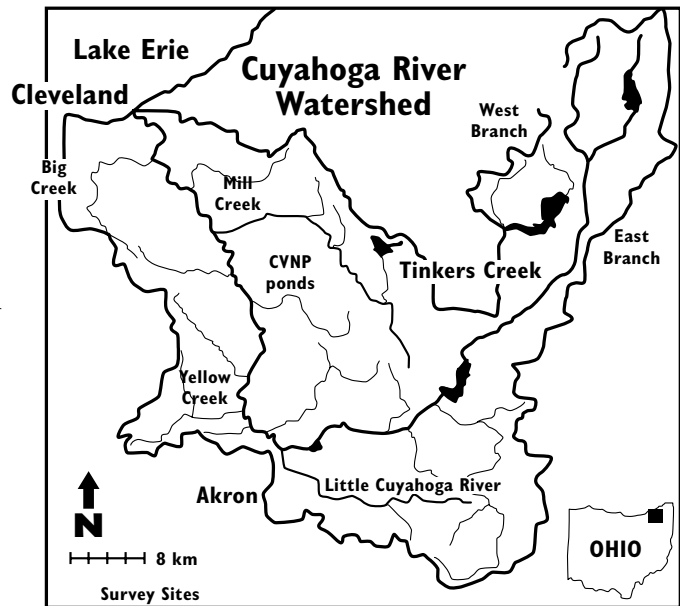


Figure 1. Map of the Cuyahoga River watershed, indicating major tributaries and the locations of 20 survey sites.

of deviation. The categories are Best of the Best (BOB), Best of the Worst (BOW), Worst of the Best (WOB), and Worst of the Worst (WOW). Three sites were chosen from each of the site categories. The additional eight sites were chosen along the stretch of the main channel of the Cuyahoga River.

At each of the 20 sites chosen for the study, a 100-m transect was located through the center of the stream, using a measuring tape that could adjust to the contours of the stream. Within the 100-m transect, a thorough survey of aquatic macrophytes was conducted. In a 10-m long rectangular subplot, we counted the number of aquatic macrophyte species, the number of shoots (by species), and the percent cover (by species). The width of the subplot was equal to the width of the stream at each end.

To sample submerged aquatic vegetation in deep and turbid water, a benthic grab sampler was used. It was important to identify as accurately as possible all aquatic macrophytes in order to exclude those that occupy banks and are partially or fully submerged following storm events. Areas of streams covered by water 85% of the time or greater were considered in-stream (Thiebaut *et al.*, 2002). In accessing

the stream and river sites, a wading technique that is standard for sampling in shallow bodies of water was used (Capers, 2000). Swamps and backwaters were avoided because of the tendency for large changes in species composition and abundance.

Every 10 m within each transect, various measurements were taken to quantify physical stream characteristics, including stream depth, stream width, and canopy coverage. Dissolved oxygen and pH levels were taken using digital meters. Water samples (500 ml) were also tested for orthophosphate (PO<sub>4</sub>), nitrate (NO<sub>3</sub>) and ammonia (NH<sub>4</sub>) concentrations. A Qualitative Habitat Evaluation Index (QHEI) worksheet was scored at each site.

## Results and Discussion

Aquatic macrophytes were found at seven of the 20 sites. An overall total of 11 species were found — two floating, two submerged, and six emergent aquatic macrophytes, and one aquatic bryophyte (moss) (Table 1). Ten of the 11 species surveyed were native, and one species was an invasive, *Potamogeton crispus* (curly pondweed).

Shoot abundance was quantified for each species at each site. *Elodea canadensis*, *Iris versicolor*, and *Pontederia cordata* exhibited the greatest shoot abundance. The most common species found

was *E. canadensis*. It is a submerged macrophyte that often occurs in large assemblages. One of the assemblages surveyed contained more than 1,600 shoots. PO<sub>4</sub>, NO<sub>3</sub>, and NH<sub>4</sub> concentrations differed among site types (Figure 2).

One introduced invasive species, *P. crispus*, was found in the Cuyahoga River. It is perennial and readily identifiable by its curly, flattened leaves (Stuckey, 1979). Leaves are generally submerged and broadly linear to oblong. *Potamogeton crispus* is found in lakes, ponds, rivers, and streams. Its range includes all of the continental United States except Maine and South Carolina (Waterway Experiment Station, 2004). Interestingly, *P. crispus* reaches maximum growth during the early part of the year and forms turions to survive the harsh summer months (Sastroutomo, 1981). Thus, *P. crispus* does not directly compete with many native aquatic plants growing later in the season.

*Potamogeton crispus*, native to Eurasia, was most likely introduced into the United States by the middle of the 1800s. *Potamogeton crispus* was initially confined to the northeastern United States, but by 1930 it had spread to the Great Lakes region. The species has apparently reached its current range as a result of waterfowl migration, deliberate planting in wildlife areas, and shipment of fishes and eggs to hatcheries. *Potamogeton crispus* has been placed on invasive

Common Name		Scientific Name
Canadian waterweed	s	<i>Elodea canadensis</i> Michx.
Harlequin blueflag	e	<i>Iris versicolor</i> L.
Fontinalis moss		<i>Fontinalis sphagnifolia</i> (C. Müll.) Wijk & Marg.
American bur-reed	e	<i>Sparganium americanum</i> Nutt.
Broadleaf arrowhead	e	<i>Sagittaria latifolia</i> Willd.
American water plantain	e	<i>Alisma subcordatum</i> Raf
Green arrow arum	e	<i>Peltandra virginica</i> L. Schott & Endl.
American white waterlily	f	<i>Nymphaea odorata</i> Aiton
Curly pondweed	s	<i>Potamogeton crispus</i> L.
Pickerelweed	e	<i>Pontederia cordata</i> L.
Common duckweed	f	<i>Lemna minor</i> L.

s = submerged; f = floating; e = emergent

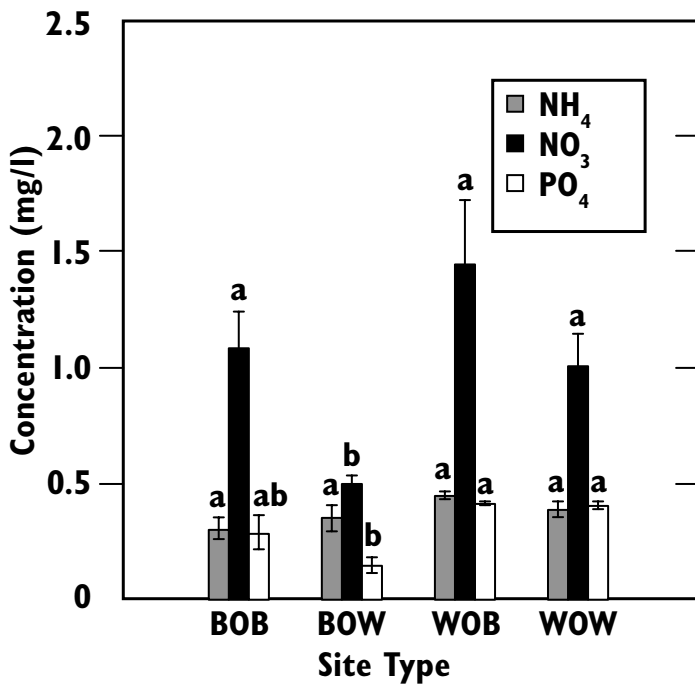


Figure 2. Nitrate (NO<sub>3</sub>), ammonia (NH<sub>4</sub>), and phosphate (PO<sub>4</sub>) concentrations in four site types. Different letters above the bars show significant differences in concentrations of a particular nutrient among site types. BOB = Best of Best, WOB = Worst of Best, BOW = Best of Worst, WOW = Worst of Worst.

species lists in California, Tennessee, and Wisconsin (Southeast Exotic Pest Plant Council, 1996; Hoffman and Kearns, Eds., 1997; California Exotic Pest Plant Council, 1999).

Although it is found in 43 other states, it has not spread significantly and has not evoked response from those state natural resource departments. It is important to note that *P. crispus* was discovered in small quantities at two sites in the Cuyahoga River. This indicates that widespread invasion has not yet occurred. Opportunities exist to control populations before *P. crispus* causes significant ecological and economic damage.

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# Combining On-Site Research, Monitoring, and Management Practices: A Case Study of Amur Honeysuckle and Garlic Mustard in an Ohio Woodland Restoration

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## Abstract

Removal of the invasive Amur honeysuckle shrub (*Lonicera maackii*) from eastern deciduous woods is often followed by expansion of garlic mustard (*Alliaria petiolata*) at affected sites. We developed a four-year restoration plan for both species to maximize effectiveness of management resources in view of the life histories and community dynamics of the species. Honeysuckle was controlled by spraying the foliage with 1% glyphosate on autumn days above 10°C (50°F) following a cold period when all other species have senesced and there was no risk to spring ephemerals. To minimize soil disruption, we only removed honeysuckle bushes to plant a native shrub.

Following honeysuckle eradication, management of garlic mustard invasion requires minimizing disturbance of the understory and preventing seed-set. Overwintering rosette mortality was 100% following honeysuckle spraying, which allowed us to focus efforts on reproductive plants in the second year following removal. Cutting the second-year plants to six inches as flowers began to fade prevented seeds from maturing. Using a four-year plan, native herbaceous diversity increased two-fold, and density increased three-fold in plots where honeysuckle was removed.

In year one, honeysuckle is eradicated with fall foliar glyphosate application. In year two, native shrubs are transplanted into some of the spaces formerly occupied by honeysuckle. In year three, mature garlic mustard plants are cut to six inches above ground after flowers begin to fade, and additional native shrubs are introduced. During year four, the re-emergent honeysuckle seedlings are mature enough to spray efficiently, and spot removal of second-year garlic mustard is completed. Utilizing management resources efficiently and effectively during the first four years of restoration promotes the return of native herbs and ultimately reduces management requirements in subsequent years.

## Introduction

A recent challenge in restoring eastern deciduous woodlands is removal of the invasive Amur honeysuckle (*Lonicera maackii*) shrub and management of the biennial garlic mustard (*Alliaria petiolata*), which often flourishes following honeysuckle removal. Our four-year restoration plan includes eradication of Amur honeysuckle and garlic mustard — two common invasive non-native species — from our 30-acre woodland and old fields. Honeysuckle exploits canopy gaps left by disturbance (7) and decreases herb cover and tree seedling establishment (10). Honeysuckle out-competes

native species for light by breaking dormancy earlier, thus shading spring ephemerals and other herbs, and also by senescing later than the native flora. Honeysuckle is also suspected of root competition by depleting moisture and nutrients (10).

Garlic mustard is a biennial herb with a 33-month life cycle that has the capability to invade mature second-growth forests (11) by spreading exclusively by seed (5). Seed production averages 200 per plant (12), but can be as great as 7,000 (13), and seeds are dispersed within two meters (5).

## Land History

Mount St. John (MSJ) is a 140-acre property in the urban-rural transition zone east of Dayton, Ohio (Greene County), under the stewardship of the Marianist Environmental Education Center (MEEC). Traditionally an oak-hickory woodland community with open pockets of wet prairie, MSJ was subjected to tree clearing and fragmentation, cultivation, and domestic animal foraging beginning in the 1910s and ending in the 1960s when farming ceased and the land went fallow.

During the 1960s, when Amur honeysuckle was spreading and found abundantly in Ohio pastures and woodlands (4), the invasive shrub quickly established in the light gaps and disturbed soil of the MSJ woods. Garlic mustard and many other invasive non-natives soon followed, the frequency increasing with disturbance. By the late 1980s, bush honeysuckle was present throughout and successfully reproducing in more than half of the 30-acre woodland and old fields.

The native herb-layer showed a reduction in species richness and cover (10) from the more than 30 species typical of a historic oak-hickory woodland (9). This prompted Dr. Donald Geiger, plant physiologist and restorationist, to initiate a bush honeysuckle control regime and to found the MEEC to implement restoration plans and monitor re-establishment of the native flora.

## Honeysuckle Management

We have tested various methods of bush honeysuckle eradication available to Ohio land managers. Initially, MEEC managers sawed mature bushes off near ground level and treated the stump with a 20% solution of glyphosate (Roundup©) to prevent re-emergence. This method minimized soil disturbance, which is often a precursor for colonization by other non-native invasive species. However, it was labor-intensive, and managers began experimenting with a foliar spray application of glyphosate.

Because bush honeysuckle continues photosynthesis and senesces later in the season than indigenous species, fall applications can be made with minimal danger to desirable native species. A 1% application covering 75% of the plant, including all major branches, is sufficient to cause death (6). Honeysuckle spraying is safest after several days of cold temperatures or a frost heavy enough to induce dormancy in native species (6). Honeysuckle is most susceptible to treatment when above 10°C (50°F), the minimum temperature for glyphosate uptake. Garlic mustard is the non-target species that shows the highest death rate.

Because bush honeysuckle breaks dormancy earlier than native species, spring also presents an opportunity for foliar application; however, this is not recommended where spring ephemerals would be impacted. We have treated one acre of land that was invaded by honeysuckle with 80 gal of 1% glyphosate in four hours. To minimize soil disruption, we only remove honeysuckle bushes when planting a native shrub (including dogwoods, hazelnuts, and roses) from our shrub nursery, which is another shield against re-invasion.

## Monitoring and Research Plots

To monitor reestablishment of the native plant community, managers began honeysuckle control at the woodland edges and moved inward each year. Once honeysuckle was removed, we observed a flush of garlic mustard as well as the emergence of several native herbs. To assess the plant community dynamics

following honeysuckle removal, successive treatment plots (of approximately 500 m<sup>2</sup>) were established for monitoring. Plot 1 was a control plot, in which honeysuckle was not removed. In Plot 2, honeysuckle was removed in the fall of 2001, and it represents the first growing season following treatment. In Plot 3, honeysuckle was removed in the fall of 2000, and it represents the second growing season post-treatment. To assess garlic mustard and other herbaceous layer species (woody and herbaceous) density and cover, quadrats (1m<sup>2</sup>, n = 18) were randomly placed within each plot. Garlic mustard presence was reported by life history stage, first year (seedling, basal rosette) and second year (flowering plant).

## Results and Discussion

### Native Species

Native herbaceous species diversity was twice the control (Figure 1) and density three times the control (Figure 2) one year following honeysuckle removal. Prevalent new native species were summer-flowering herbs, such as white snakeroot (*Eupatorium rugosum*) and enchanter's nightshade (*Circaea quadrisulcata*). Other native herbs have increased substantially — e.g., clearweed (*Pilea pumila*) was seven-fold more plentiful two years after honeysuckle removal.

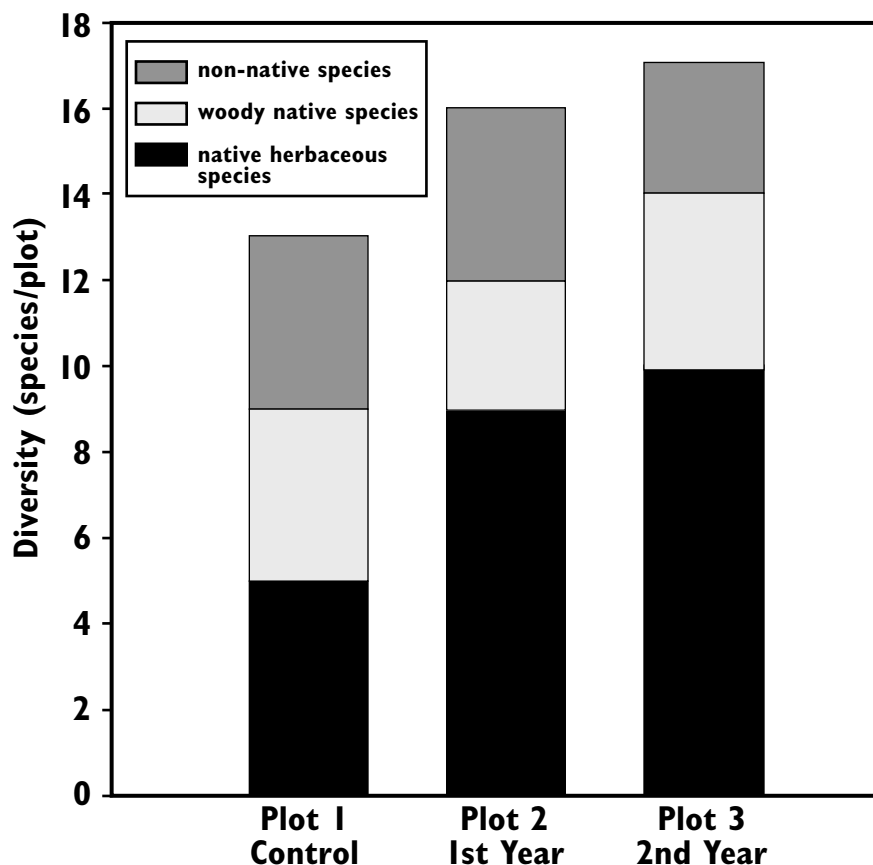


Figure 1. Woodland herbaceous layer species diversity in spring 2002, bush honeysuckle removal plots. Plot 1 – no treatment. Plot 2 – first growing season following treatment. Plot 3 – second growing season following treatment. Mean + SEM (error bars), n = 18. Within the first growing season following honeysuckle treatment, herbaceous layer native species re-established. Following honeysuckle removal, native herbaceous species (Plots 2 and 3) comprised more than 50% of total species diversity. Native woody species show signs of recovery after two years.

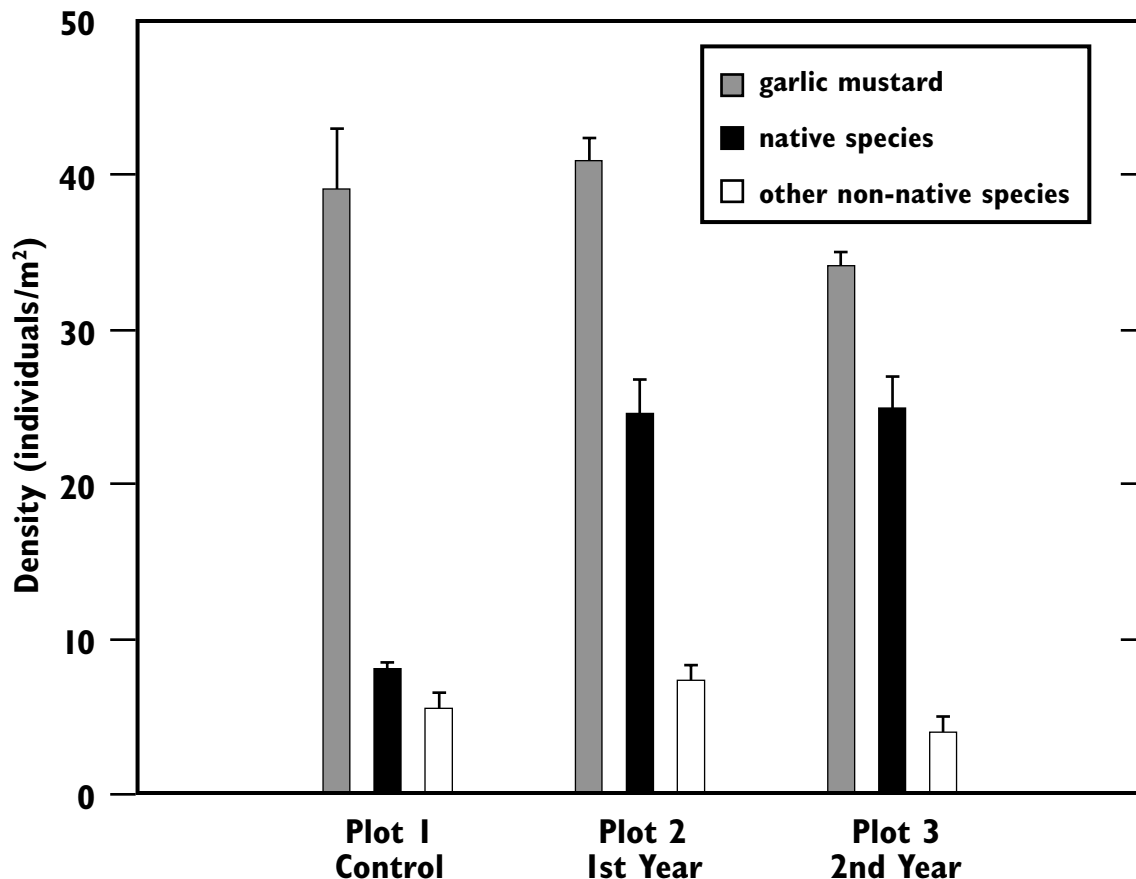


Figure 2. Herbaceous layer density in spring 2002, bush honeysuckle removal plots. Plots and sampling as in Figure 1. Density of native herbaceous plants was three-fold greater than the control, one year following honeysuckle removal.

Adjacent areas in which honeysuckle was removed 10 years ago now have populations of spring ephemerals such as violet (*Viola* spp.) and cut-leaved toothwort (*Dentaria lancinata*) which could serve as a seed source for our research plots (8). After two years, only two native trees were present — slippery elm (*Ulmus rubra*) and wild black cherry (*Prunus serotina*). Native tree establishment may take longer but shows promise, as plots treated to remove honeysuckle since 1995 now show 15 species in the understory (1).

### Honeysuckle

Honeysuckle seedling density was reduced following treatment of mature plants (5.0/m<sup>2</sup> in first-year Plot 2 and 3.3/m<sup>2</sup> in second-year Plot 3, compared with 8.2/m<sup>2</sup> in control). To prevent re-establishment, spot treatment with

1% glyphosate should be repeated on young honeysuckle growth within three to five years, prior to reaching maturity.

### Garlic Mustard

The age demographics of the garlic mustard population (Figure 3) shows the effects of non-target spraying and directs our control strategy. Achieving 100% mortality of rosettes in the first year post-treatment prevents seed rain for this year and reduces the longevity of management. Focusing on the reproductive (two-year-old) plants in the second year after honeysuckle removal is a key time to intervene and prevent replenishment of the seed bank. Control of first-year rosettes is an inefficient use of management resources, as only two to four percent of seedlings typically reach reproductive maturity (5).

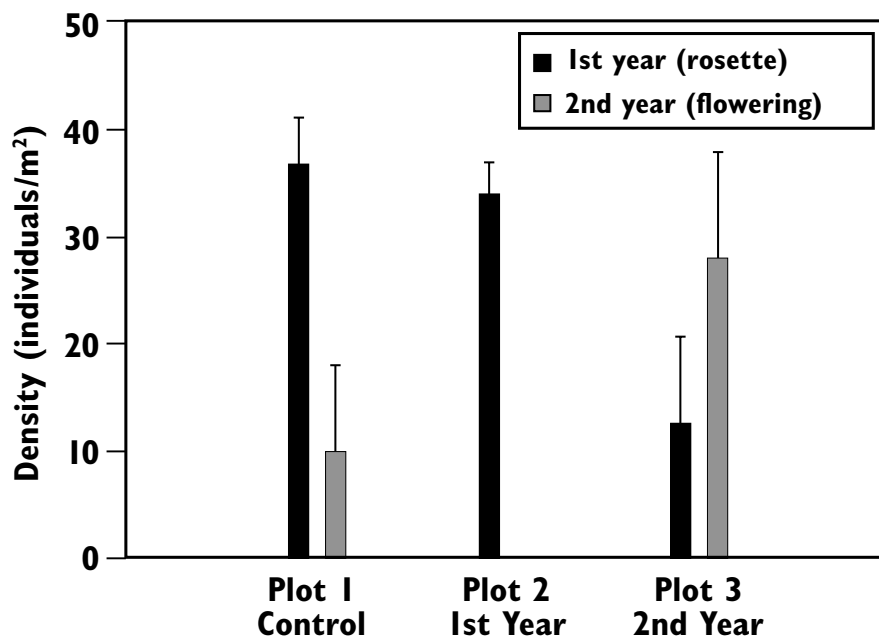


Figure 3. Biennial garlic mustard by age class in spring 2002, bush honeysuckle removal plots. Plots and sampling as in Figure 1. No mature garlic mustard was found one year post-removal (Plot 1), showing a 100% mortality rate of first-year rosettes through non-target spraying.

Since garlic mustard spreads by seed, all potential additions to the seed bank must be excluded. We do not recommend using the common labor-intensive approach to garlic mustard control of pulling second-year plants at flowering. The potentially negative consequences of pulling include risks of re-invasion through the soil disturbance and possibilities of seed maturation on removed plants if removal occurs too late in the season.

MEEC managers are experimenting with carefully timed weed sawing in the dense patches that are common at two years post-removal. Second-year plants are cut to six-inches high when flowers begin to fade, and seeds begin to mature. Accurate timing of the cut prevents production of a second group of flowers — a potential problem if plants are cut instead of pulled — and prevents seeds from falling and maturing on the forest floor (12).

In our first test of timing, we cut second-year plants during the third week of May 2003. Seeds were dried for eight months, and no viable seeds were found when tested using tetrazolium (14). Since no viable seeds were introduced into

the seed bank and no negative impacts were observed on the native herbaceous layer, we are pursuing this approach.

## Summary

Honeysuckle seedlings from seed rain must be controlled every three to five years to prevent maturity of secondary growth. Four years of treatment is also necessary to manage any garlic mustard seed rain and to deplete its seed bank (2). Our ecological research monitoring of the woodland herbaceous layer following honeysuckle removal indicates increases in native species density and diversity.

Utilizing management resources efficiently and effectively during the first four years of restoration promotes the return of native herbs and ultimately reduces the intensity of management required in the long-term. Our recommended strategy, showing timing, application, and monitoring for managing both honeysuckle and garlic mustard, is shown in Table 1.

**Table 1. Recommended management strategies for honeysuckle and garlic mustard over four years.**

Year/Season	Honeysuckle	Garlic Mustard
<b>Year 1 Summer</b>		
Year of First Herbicide Fall Treatment	<ul style="list-style-type: none"> <li>• Measure density of mature shrubs and seedlings for baseline data.</li> <li>• Apply 1% foliar glyphosate.</li> </ul>	<ul style="list-style-type: none"> <li>• Monitor density of first-year rosettes and mature plants for baseline data.</li> <li>• Mature plants have set seed, but first-year rosettes will be killed by glyphosate.</li> </ul>
<b>Year 2 Spring</b>		
First Year After Honeysuckle Removal  Fall	<ul style="list-style-type: none"> <li>• Transplant native shrubs into spaces occupied by dead honeysuckle. If you do not plan to introduce native shrubs, leave the honeysuckle standing so as not to disturb the soil.</li> <li>• No control recommended for first-year honeysuckle seedlings.</li> <li>• Spot spraying of honeysuckle not killed by previous fall glyphosate application.</li> </ul>	<ul style="list-style-type: none"> <li>• Second-year plants should be sparse, if present. Spot removal by cutting.</li> <li>• No control recommended for first-year rosettes.</li> </ul>
<b>Year 3 Spring</b>		
Second Year After Honeysuckle Removal	<ul style="list-style-type: none"> <li>• Continue to introduce native shrubs as resources permit.</li> <li>• No management recommended for honeysuckle seedlings.</li> </ul>	<ul style="list-style-type: none"> <li>• Few first-year rosettes.</li> <li>• Cut dense growth of second-year plants to six inches after flowers begin to fade.</li> </ul>
<b>Year 4 Spring and Future</b>		
Fall	<ul style="list-style-type: none"> <li>• Transplant native shrubs as necessary.</li> <li>• Fall foliar 1% glyphosate application to young honeysuckle as needed.</li> </ul>	<ul style="list-style-type: none"> <li>• Spot removal of second-year plants in spring.</li> </ul>

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# Do Invasives Use Roadsides as Corridors or as Habitat in the Wayne National Forest?

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Roads figure prominently in discussions of biological invasions. Casual observation shows that roadsides are full of nonnative species, and reports of roadside infestations are common in the published literature (Benniger-Truax, 1992; Gelbard and Parendes and Jones, 2000; Tikka *et al.*, 2001; Belnap, 2003). The linear structure of roads suggests a corridor function. Perhaps nonnative species use roads as avenues of invasion, an impression supported by observations of range expansion as measured by roadside populations (*e.g.*, Braun, 1921; Brothers, 1992; Matlack, 2002).

The idea is intuitively reasonable. Roadsides provide continuous strips of habitat, potentially allowing an invading species to spread without crossing sections of nonnative habitat. Dispersal may be facilitated along roads by the movement of traffic. Plant propagules do occasionally adhere to cars (Wace, 1977; Schmidt, 1989), and nonnative species are sometimes sucked along in the eddies behind trains (Kent, 1960; Mack, 1986), suggesting a similar function with cars.

Conduit function cannot be assumed, however. Conceptual models show that even modest irregularities in a corridor can interfere with plant movement along the corridor (Soule and Gilpin, 1991). Field studies in non-road situations show that species of some dispersal modes have difficulty crossing gaps in habitat (Dzwonko, 1993; Matlack, 1994). It is possible that the abundance of nonnative species along roads simply reflects the suitability of roadside habitat for weedy species, and that roads actually serve little conduit function. Distinguishing

between habitat and conduit functions is critical in understanding how a particular invasion can be managed, so assessing their relative contributions in a real situation is an important research issue.

As in most studies of biological invasions, the investigator does not have the luxury of measuring roadside invasion as it happens. He or she is faced with interpreting a long-term dynamic process from snapshot data.

We are presently applying this approach to *Rosa multiflora* (multiflora rose), an invasive shrub with animal-dispersed fruits, in the Wayne National Forest. We use the degree of aggregation of stems along a stretch of road and within adjacent forest and the shape of the edge of the distribution as indicators of invasion progress. We begin by asking if roadsides have been invaded differently from surrounding land. We quantify distributions and examine the dispersal process in terms of removal of fruits.

## Methods

To document distributions, 100-meter belt transects two-meters wide were established parallel to unpaved forest roads and in forest interior. The two-meter width was measured from the edge of regular road maintenance. Comparing landscape position, transects were sampled along valley and ridge roads and nearby forest interior. All transects were placed at least 50 m from any road, trail, or other obvious anthropogenic disturbance. Sampling was conducted in each transect by recording

*R. multiflora* stem number, area coverage of *R. multiflora*, and crown canopy cover in consecutive 2 x 2m quadrats.

Dispersal of *R. multiflora* was monitored from roadsides and interior habitats. During the first week of October 2003, six “bouquets” composed of *R. multiflora* stems bearing fruit and foliage were placed in roadside and forest interior sites at least 50m from a road (Note: *R. multiflora* was already present at these sites). Fruit removal from these “bouquets” was recorded biweekly for six months.

Percent cover of *R. multiflora* was regressed against canopy openness to determine if canopy openness supports greater *R. multiflora* growth. An autocorrelation analysis of the transect data was done to determine the spatial extent of *R. multiflora* within each transect. Autocorrelation is a spatial analysis tool that indicates the degree and the spatial scale at which the presence of one variable accounts for the presence of another (Cliff and Ord, 1973). For example, a positive autocorrelation value of 1.0 at a distance of 6m would indicate that the presence of *R. multiflora* in any given quadrat is completely positively correlated with the presence of another *R. multiflora* 6m away.

## Results

*Rosa multiflora* was found in 35% of the road quadrats and 20% of interior quadrats. *Rosa multiflora* showed a higher percent cover in areas with lower canopy cover. We infer that roadside is superior habitat for this species, and that habitat quality is defined in terms of light availability. *Rosa multiflora* stems are more strongly autocorrelated at fine scale along roads than interior habitats, which is consistent with spread along roads from individual colonization events. Within the forest interior, there is weaker autocorrelation at fine scale than along roads, but *R. multiflora* remains weakly autocorrelated throughout the transect (Figure 1). Such distributions are consistent with colonization of roadsides by lateral spread from germination sites, whereas the more-isolated, less-strongly autocorrelated forest stems suggest colonization in many separate germination events.

Fruit disappeared from bouquets in both roadside and forest interior habitats in distinct removal events — removal was not a gradual process. After one month, more interior “bouquets” retained greater than 20% of their fruit than roadside bouquets. We conclude that roadside plants enjoy better dispersal service than conspecifics away from roads.

## Discussion

*Rosa multiflora* is clearly invading roadside habitats within the Wayne National forest. In addition to providing habitat for *R. multiflora*, autocorrelation and seed removal results suggest that roadsides serve a corridor function for its spread. The most effective management will address both habitat and corridor function. From the habitat perspective, invasion might be slowed by allowing greater shading along roads; open verges should be avoided. A more shaded road may also forestall spread by reducing dispersal effectiveness. In the most critical habitat areas, road construction should be avoided entirely. If roads need to be constructed, narrow roads with closed canopies providing less suitable habitat would be preferable to roads with open-canopy habitats.

The project continues. We are applying these methods to two other species (*Microstegium vimineum* and *Tussilago farfara*) — surely species of different life histories experience the roadside habitat differently. We will also consider the wave-front shape of the spreading vegetation patches using epidemiology theory. Ultimately, however, a better answer will be produced by long-term monitoring, allowing us to view the invasion in real time.

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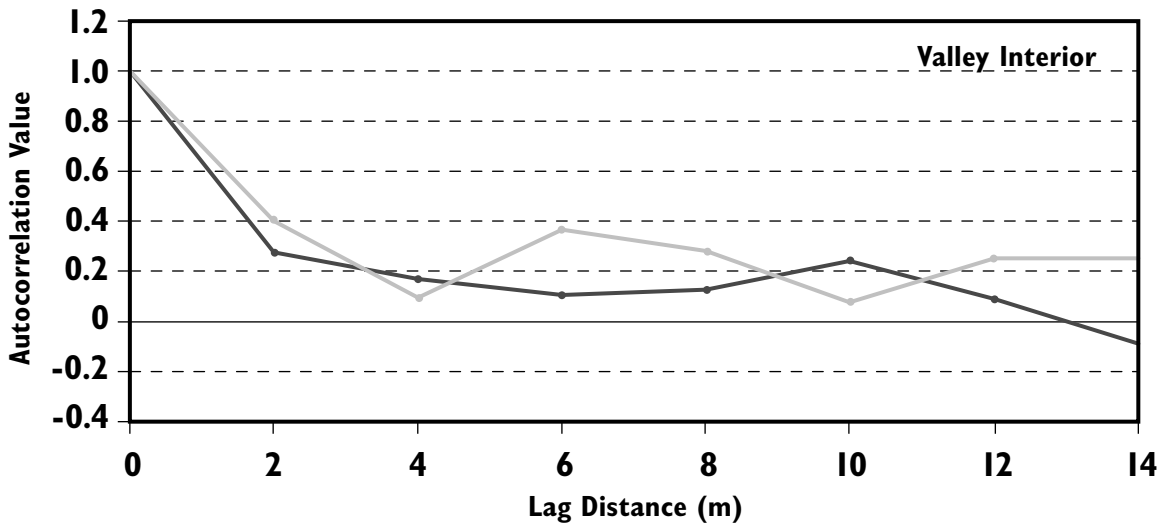
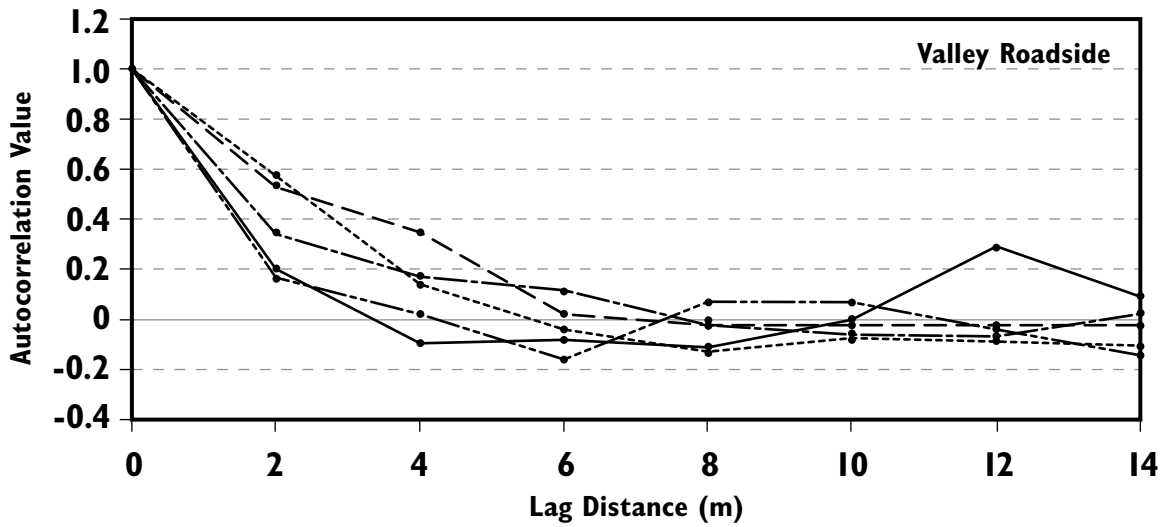


Figure 1. Autocorrelation of *Rosa multiflora* along valley transects in roadside and interior habitats. *Rosa multiflora* has stronger autocorrelation at fine scale in roadside habitats than interior habitats.

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# Testing Predictions of the Evolution of Increased Competitive Ability Hypothesis in Garlic Mustard (*Alliaria petiolata*): Chemical Defenses and Growth in European and North American Populations

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## Introduction

Garlic mustard [*Alliaria petiolata* (Bieb.) Cavara and Grande; Brassicaceae] is a European biennial herb, first recorded on Long Island, New York, in the 1860s, that is expanding rapidly in northeastern and Midwestern forests in the United States and in southern Canada. Garlic mustard flourishes in moist woodlands with moderate exposure to light, but it can grow in a diversity of other habitats. It is found in natural areas, woodlots, and along edges of agricultural fields and lawns throughout North America.

Several life history traits likely contribute to the invasiveness of this species. It is highly inbreeding and can produce numerous seeds. It exhibits remarkable morphological plasticity to local environmental conditions. It can exude allelopathic chemicals (glucosinolates and their hydrolysis products) that can reduce seed germination and growth of some species and affect mycorrhizal potential of soils. Garlic mustard has been shown to out-compete some ecologically and commercially important hardwoods in short-term experiments, and its presence in natural areas is associated with reduced native herb abundance and diversity.

Garlic mustard can also negatively impact salamander populations that rely on litter-

dwelling animals for food, and it can endanger populations of the rare butterfly *Pieris virginiensis* by serving as an oviposition site for adults on which larvae cannot survive. Because of its known or potential negative impacts in natural and agricultural ecosystems, garlic mustard is an important target for chemical and biological control efforts.

The Evolution of Increased Competitive Ability (EICA) hypothesis predicts that invasive plants in novel habitats, lacking substantial pressure by natural enemies, will evolve reduced expression of costly, unneeded chemical defenses to the benefit of growth and reproduction. We tested predictions of this hypothesis in garlic mustard, a European native that lacks substantial specialist herbivory in North America, where it is also largely resistant to generalist herbivores (Figure 1).

## Methods

We grew plants in the greenhouse from field-collected seeds of four North American garlic mustard populations from Ohio and Pennsylvania and seven European populations from the United Kingdom and the Netherlands. Plants were grown for 35 days, at which time length and width of the third true leaf were taken, and half of the plants were treated with a

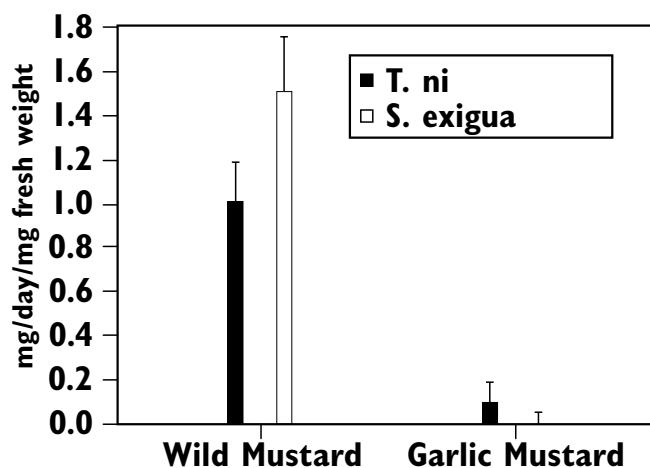


Figure 1. Relative growth rate of seven-day-old *Trichoplusia ni* and *Spodoptera exigua* on leaves of wild mustard and garlic mustard. N = 6-10.

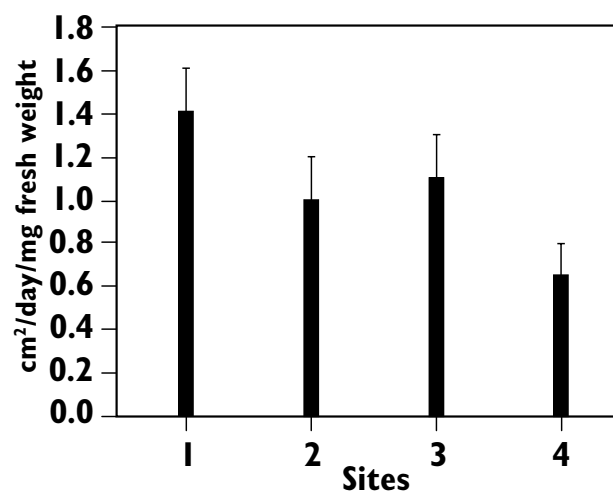


Figure 2. Relative consumption rate of *Trichoplusia ni* on garlic mustard leaves from different sites in the field. N = 9.

foliar spray of jasmonic acid (JA). Jasmonic acid is a wound-related hormone involved in the induction of several chemical defenses associated with insect and pathogen resistance. Exogenous treatment of this hormone can induce defenses in a manner similar to herbivore attack, without the confounding effects of leaf damage.

Four days later, samples from the fourth true leaves were harvested for analysis of specific leaf weight and several constitutive and JA-inducible defense proteins and secondary metabolites that range from general resistance factors to defenses unique to garlic mustard. Levels of some of these defenses have been shown to vary among populations in the field, which may explain variation in herbivore resistance among naturalized populations (Figure 2). Data were analyzed with mixed model ANOVA with continent, population within continent, and JA treatment as main effects.

Glucosinolates, secondary compounds characteristic of the mustard family, are involved in numerous species interactions including specialist herbivore attraction, generalist herbivore resistance, and interactions with soil fungi. Total glucosinolates (of which sinigrin is a major component in garlic mustard) were assessed using the glucose release method. Activity of the phenolic oxidizing enzyme, peroxidase, was assessed in soluble protein extracts using a spectrophotometric assay with

guaiacol as a substrate. Trypsin inhibitors, capable of competitively inhibiting digestive serine proteases of animals, were assessed in soluble protein extracts using a radial diffusion assay through a trypsin-containing agar. Unique secondary compounds of garlic mustard that have been shown to impart resistance to specialist herbivory include the cyanoglucoside, alliarinoside, and the flavone glycoside, isovitexin-6''-O-B-D-glucopyranoside. Levels of these compounds in water-soluble fractions of ethanol extracts were analyzed by HPLC.

## Results

Total glucosinolate content differed significantly among populations within continents (Figure 3A). In addition, the response of populations to JA treatment by continent was marginally significant, with North American populations tending to be more inducible by JA than European populations (Figure 3). No variation among continents in peroxidase activity was found, although variation was found among populations within continents (Figure 3B). Although not significant, an interesting pattern was present in the peroxidase response of populations to JA. Two North American populations displayed higher peroxidase activities after JA treatment, and two populations displayed lower peroxidase activities. Six of seven European populations displayed lower

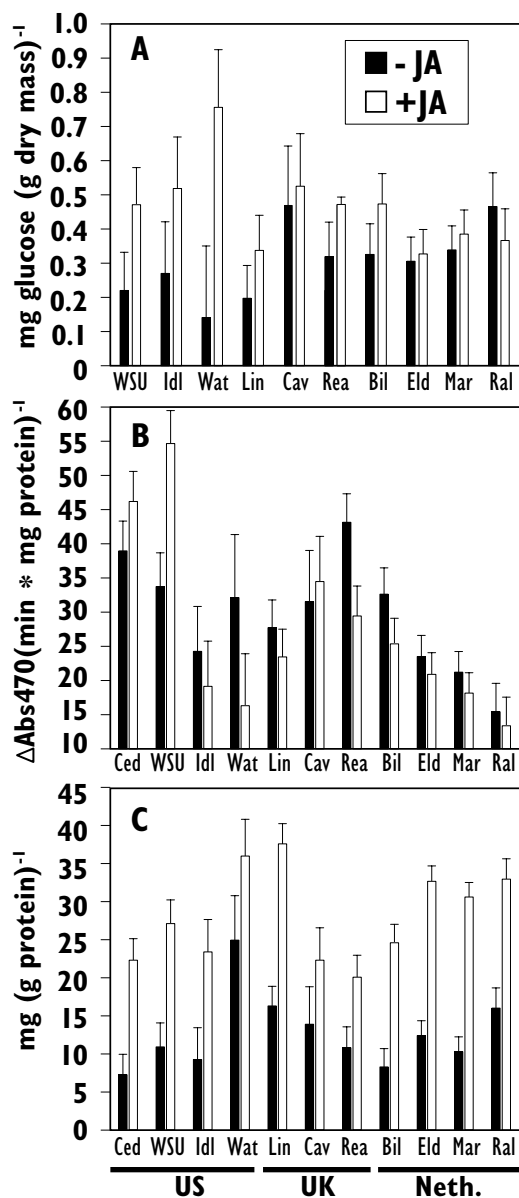


Figure 3. Glucosinolate (A), peroxidase (B), and trypsin (C) inhibitor levels in third true leaves of garlic mustard from the United States, U.K., and the Netherlands. N = 5-10.

peroxidase levels after JA treatment. Garlic mustard expressed substantial activity of trypsin inhibitor (Figure 3C). There was significant variation in trypsin inhibitor levels among the populations within each continent, but no trends could be significantly attributed to their continental origin (Figure 3C). JA significantly increased trypsin inhibitor expression, but there was no significant variation among populations in their response to JA.

A representative HPLC chromatogram of alliarinoside and isovitexin-6''-O-B-D-glucopyranoside is shown in Figure 4. North American populations had more variable amounts of alliarinoside (Figure 5A) and isovitexin-6''-O-B-D-glucopyranoside (Figure 5B) than European populations, and generally expressed higher amounts of isovitexin-6''-O-B-D-glucopyranoside. JA did not consistently induce higher expression of either compound. Due to low sample sizes, levels of these two compounds were not statistically analyzed.

Length (Figure 6A) and width (Figure 6B) of the third true leaf, measured prior to JA treatment, significantly varied among populations within continents, but did not vary with continental origin. However, specific leaf weight of the fourth true leaf varied by continent, and among populations within each continent (Figure 6C). In particular, North American populations had higher specific leaf weight than European populations.

## Discussion

Our results provide mixed support for predictions of the Evolution of Increased Competitive Ability hypothesis in garlic mustard. Leaf growth traits, such as higher

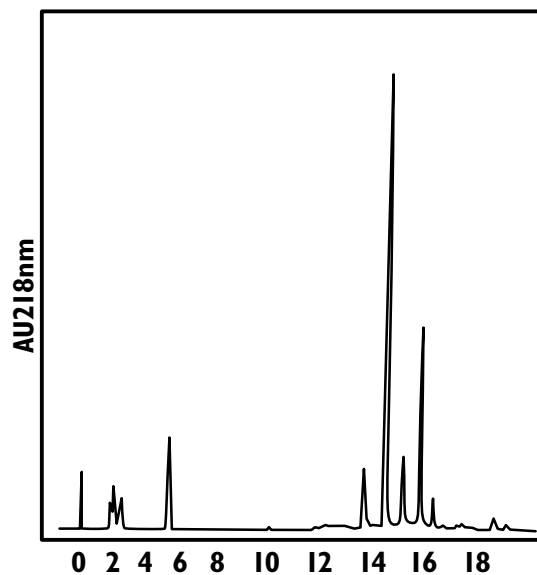


Figure 4. Representative HPLC trace of flavonoids isolated from garlic mustard leaves. Peak 1 = alliarinoside, Peak 2 = isovitexin-6''-O-B-D-glucopyranoside.

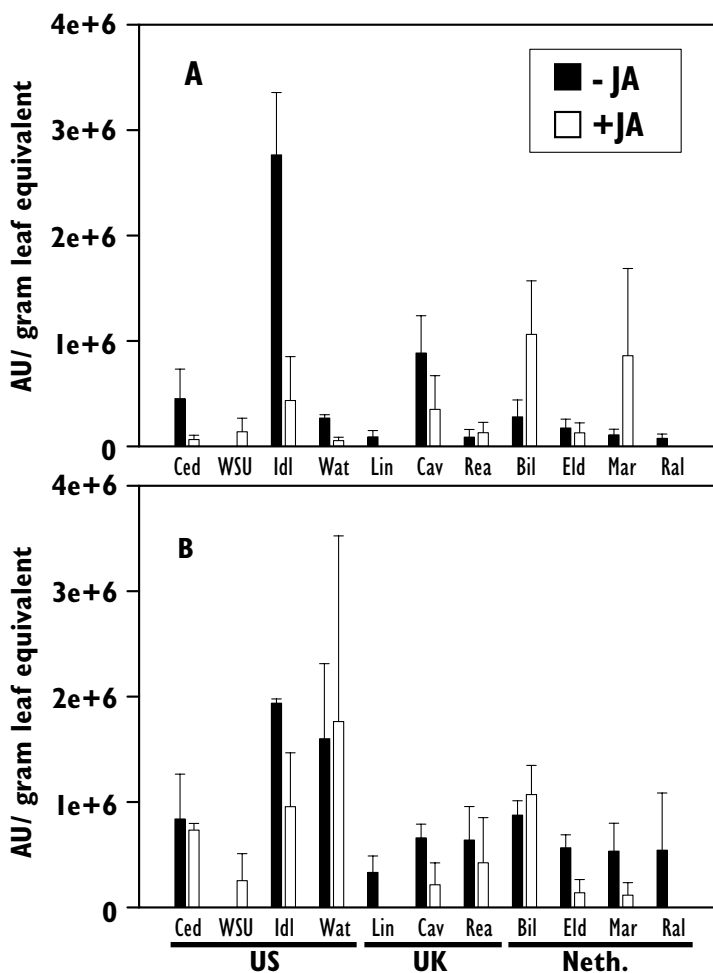


Figure 5. Alliarinoside (A) and isovitexin-6''-O-B-D-glycopyranoside (B) levels in third true leaves of garlic mustard from the United States, U.K., and the Netherlands. N = 5-10.

specific leaf weight, were suggestive of increased productivity in North American populations as predicted, but this must be verified with longer-term studies.

In contrast to predictions, no evidence of reduced expression of chemical defenses was found in North American populations relative to European populations. In fact, greater inducibility of glucosinolates by JA and tendencies for peroxidase activity to be higher in North American populations suggest that the opposite may be true for some defenses. Invasive garlic mustard populations may both grow fast and defend well, despite the tradeoff typically posited between these traits.

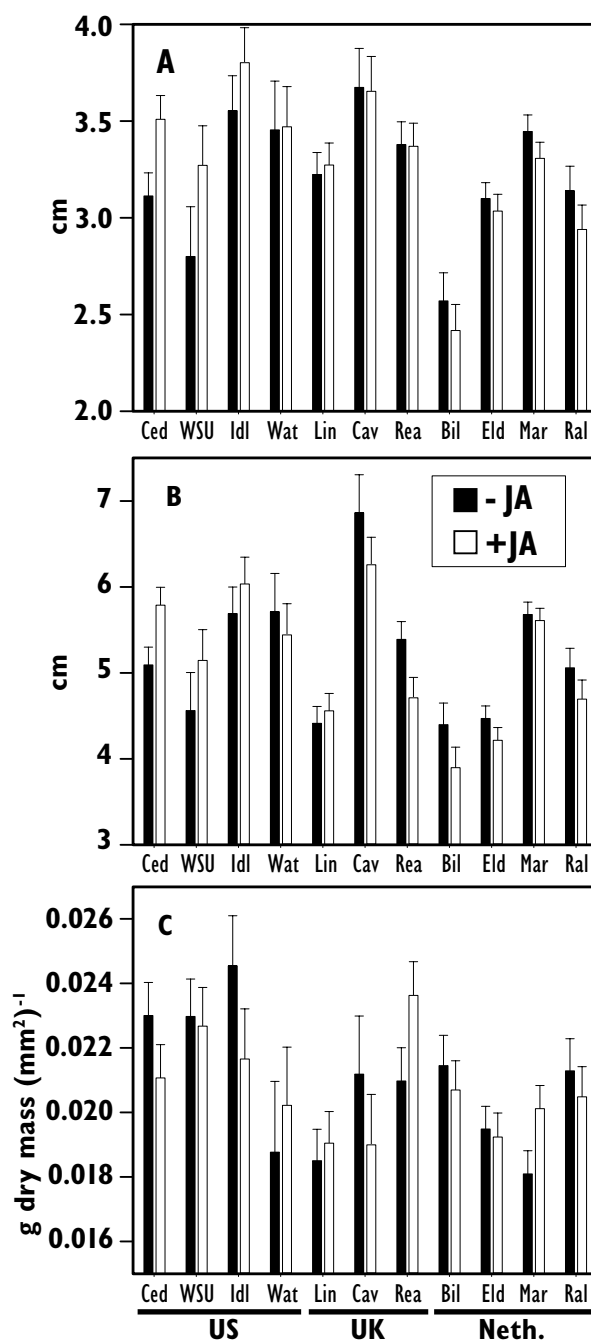


Figure 6. Leaf length (A) and leaf width (B) of third true leaves, and specific leaf weight (C) of fourth true leaves of garlic mustard from the United States, U.K., and the Netherlands. N = 5-10.

Future experiments will include a greater biogeographical representation of garlic mustard. In addition, chemical defenses will be assessed more thoroughly throughout the life cycle and quantitatively related to herbivore resistance and seed production as determined in laboratory bioassays and field studies.

# Impact of the Invasive Species *Lonicera maackii* on Individual Plants and Plant Community Structure

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## Introduction

Control of the invasive shrub *Lonicera maackii*, commonly known as bush honeysuckle, poses a serious problem for managers of nature reserves across the Midwestern United States. *Lonicera maackii* is an invader of forest and open environments. It grows best in high light areas, including canopy gaps, forest edges, and disturbed forests (Nyboer, 1992; Hutchinson and Vankat, 1997; Luken, 1998). It is unlikely to be found in old-growth forests even when the forest is adjacent to an invaded area (Hutchinson and Vankat, 1998).

Management techniques for control of *L. maackii* vary according to site characteristics of the invaded region. Cut shrubs readily resprout unless the entire root mass is also removed (Nyboer, 1992). Shrubs are less resilient in forested areas without canopy gaps, so a cutting regime can be sufficient in lower light areas. However, repeated cuttings are necessary to permanently remove the species (Luken and Mattimiro, 1991). Open areas require stump application of herbicides after cutting (Luken and Mattimiro, 1991). Since *L. maackii* is difficult to permanently eradicate, attempts to do so should be limited to places where it is least likely to regenerate. Target sites could include recently invaded environments, young shrub environments, and mature forests without canopy gaps (Luken, 1993; Deering and Vankat, 1999).

*Lonicera maackii* causes problems associated with most invasive plants. It competes with native plants for resources, especially light

(Nyboer, 1992; Collier *et al.*, 2002). This shrub grows taller than native herb species and has the advantage of being one of the first plants to leaf out in the spring and one of the last to lose its leaves in the fall (Luken and Thieret, 1995; Collier *et al.*, 2002). *Lonicera maackii* has been shown to reduce richness, diversity, and abundance of native species above ground and in the seedbank (Hutchinson and Vankat, 1997; Hartman and McCarthy, 2001; Collier *et al.*, 2002). Tree seedlings are especially uncommon under *L. maackii* thickets (Luken *et al.*, 1997), indicating that forests may not regenerate in its presence.

Even after *L. maackii* is removed, many species do not grow back and those that do are often those common to disturbed sites, annuals, or other invasive species (Luken, 1997; Luken *et al.*, 1997; Collier *et al.*, 2002). These factors indicate that *L. maackii* may slow succession and possess allelopathic properties (Nyboer, 1992; Luken *et al.*, 1997; Gorchov and Trisel, 2003).

The primary goal of this ongoing study is to examine the effects of *L. maackii* on individual transplanted plants and plant community structure. We are also interested in determining if effects remain after *L. maackii* has been removed, therefore impacting succession, and identifying the mechanisms involved in the shrub's invasion (namely, whether it is allelopathic). It is hoped that results will aid in control of this shrub and restoration of areas where it has been removed.

This paper focuses on initial results of an ongoing experiment that examines the survival, growth, and fecundity of three herbaceous

species transplanted to sites where *L. maackii* is present or absent, or has been recently removed, or historically removed.

## Methods

### Historical Sites Investigating Long-Term Impacts of *L. maackii*.

All of the *in situ* portions of this experiment are being conducted in Sugarcreek MetroPark. In April 1996, 10 30-m x 30-m plots in Sugarcreek were cleared of honeysuckle (DiSalvo, 1997), using a 20-m x 20-m plot within that area for study. An adjacent 20-m x 20-m plot where honeysuckle was present was chosen as the control. Within each large plot, 20 randomly selected 1-m x 1-m plots were sampled from June to August of 1996 and May of 1997 for species present and percent cover of each species. Nine of these 10 sites will be resampled in spring and late summer 2003-2004. These data will be used to determine if *L. maackii* has long-term effects on succession after its removal.

### Current Study of Impacts of *L. maackii* on Transplanted Herbs

Of the 10 aforementioned sites, two were chosen for further study (Sites A and B). Each of these two sites now consists of four adjacent 20-m x 20-m plots — one plot labeled P (Present) where *L. maackii* is present (also the control for the previous study); the second labeled HR (Historical Removal) where all *L. maackii* was removed in April 1996; the third labeled NR (New Removal), which was selected for removal of additional *L. maackii* plants; and the fourth plot labeled A (Absent) where no *L. maackii* plants naturally exist with the exception of a few small seedlings.

Five points were randomly selected within each plot. At each point, a 2-m x 2-m fenced plot was randomly planted with four plants each of *Impatiens capensis*, *Alliaria petiolata*, and *Asarum canadense*. Each plant will be monitored for its survival, growth, and fecundity over the course of the following two growing seasons. The sites will also be monitored for light availability, soil saturation, and pH. These measurements will

be used to find out how *L. maackii* may impact native species, as well as to determine possible confounding differences among plots. The results of this experiment are the focus of this paper.

### Allelopathy Greenhouse Experiments.

The effects of *L. maackii* root and leaf extracts on the germination of *I. capensis*, *A. petiolata*, *L. maackii*, and *Arabidopsis thaliana* grown in Petri dishes were tested. *Arabidopsis thaliana* was also grown from seed in the Wright State University greenhouse in soils from sites where *L. maackii* was absent and sites where *L. maackii* was present. Six different treatments were applied to each soil type — nutrient application, root extract, leaf extract, root extract + nutrient application, leaf extract + nutrient application, and control (water only). All plants will be monitored throughout the experiment for growth and survival. Results of these experiments have not yet been evaluated.

## Results

Light varied marginally by site, and significantly by treatment and the interaction of site and treatment (Figure 1). At Site A, light levels were lowest in the *L. maackii* Present treatment and highest in the New Removal treatment. At Site B, light levels were highest in the Historical Removal treatment. Soil moisture and pH were relatively similar among all plots.

Seed weights for *I. capensis* tended to be lowest

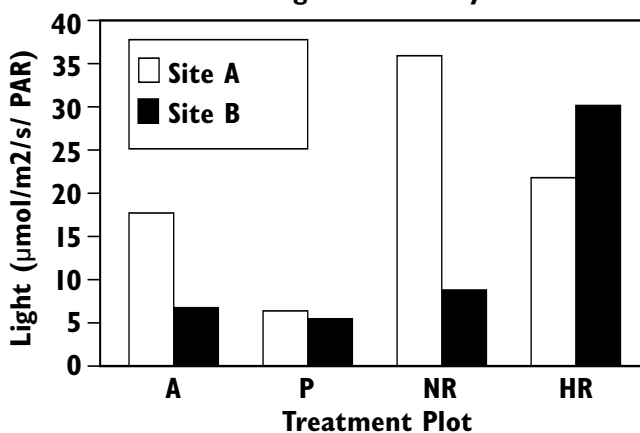


Figure 1. Average light availability for the five points within each treatment plot varied by site and treatment.

in the *L. maackii* Present treatment and greatest in the Historical Removal treatment (Figure 2A). The number of reproductive plants was always greatest in the Historical Removal treatment. Average seed weight per plot was correlated with light availability (Figure 2B).

The proportion of *I. capensis* plants surviving was also correlated with light in both July and August. Treatment effects for survival of *I. capensis* varied by site. Survival was lowest in the Present treatment and highest in the New Removal treatment for Site A (Figure 2C). At Site B, survival was much lower in the New Removal and Present treatments and highest in the Historical Removal treatment (Figure 2D).

was positively correlated with light availability. Although no significant treatment effects were found, survival tended to be lowest in the *L. maackii* Present treatment at both sites (Figure 3A). There were no significant effects of treatment on survival of *A. canadense*, although effects approached significance. Survival tended to be lowest in the *L. maackii* Present treatments (Figure 3B).

Effects of the treatments on survival of *A. petiolata* and *A. canadense* will be assessed at the end of the 2004 growing season. Effects on reproduction of *A. petiolata* will also be assessed at this time. Results of the 2003-2004 study period are incomplete and therefore have not

The proportion of surviving *A. petiolata* plants

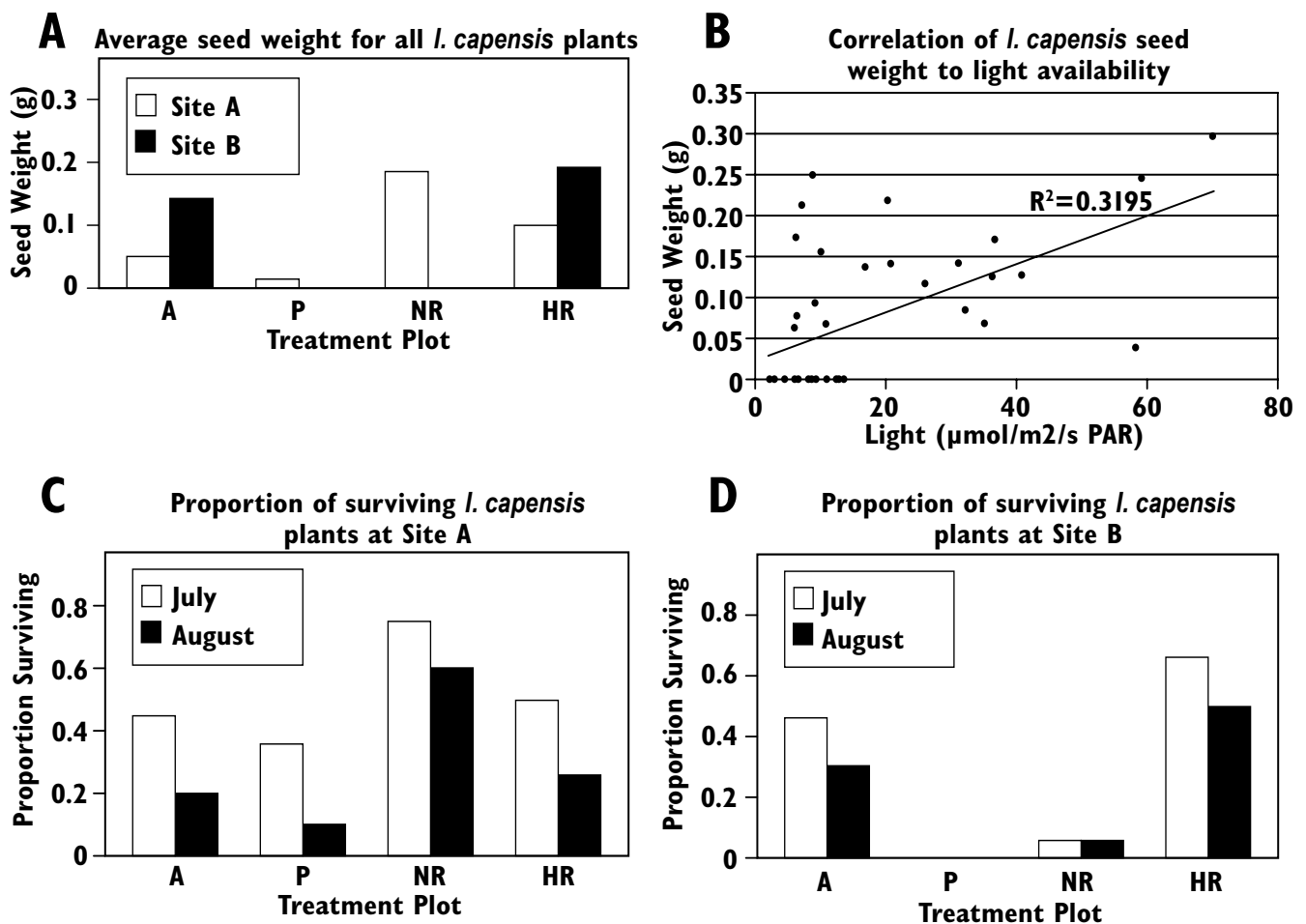


Figure 2. (A) Seed weights for *Impatiens capensis* tended to be lowest in the *Lonicera maackii* Present treatment and highest in the Historical Removal treatment. (B) Average seed weight of *I. capensis* plants was correlated with light availability in the plot (C) and (D). The proportion of *I. capensis* plants surviving varied by site and treatment.

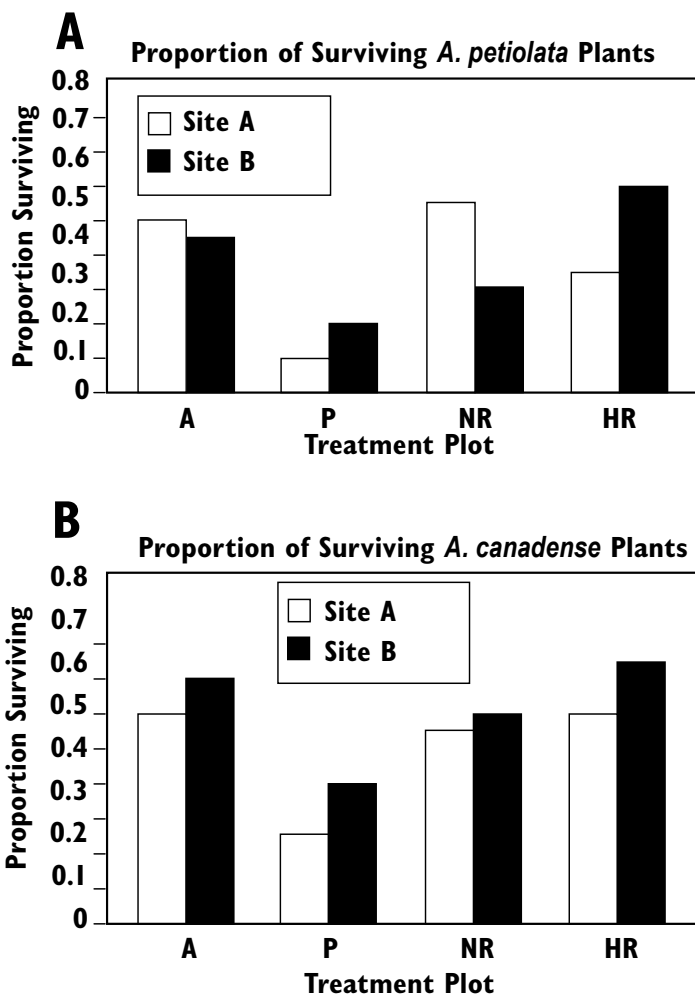


Figure 3. The survival of (A) *Alliaria petiolata* and (B) *Asarum canadense* plants tended to be lowest in plots where *Lonicera maackii* was present.

been analyzed. Experiments to examine the potential allelopathic effects of *L. maackii* are underway but are also incomplete.

## Discussion

Light appears to be the most influential factor on the survival and reproduction of *I. capensis*. The presence of *L. maackii* decreased light availability, and therefore the survival and reproduction of *I. capensis* also decreased. Seed weight for *I. capensis* plants was highest overall in the Historical Removal treatment, indicating a positive response to *L. maackii* removal.

This response was also seen in the New Removal treatment at Site A, where light levels were high. However, due to the removal of single shrubs rather than the clearing of the entire plot, light

levels in the New Removal treatment were not elevated at Site B. This is probably the reason for the poor performance of *I. capensis* at this site.

Though the results are not significant, it appeared that the population of *A. canadense* plants decreased in the presence of *L. maackii*. This further supports the theory that *L. maackii* has a negative impact on native herbs. Survival of *A. petiolata* was lowest in the presence of *L. maackii*. This is probably due to the absence of light in these plots. The fact that *A. petiolata* survival was correlated with increasing light availability indicates that the absence or removal of *L. maackii* and the shade it provides allows easier invasion by another invasive species.

Some complications were experienced. Some sites have been disturbed by storm or other damage. It is difficult to evaluate if decreased survival, growth, and reproduction are due to herbivores, trampling, and transplant stress rather than actual treatment effects. There may also be a competition factor in the Absent treatment, since these plots already have an herbaceous understory that may compete with the transplanted species. It is hoped that these and other incidental factors will not confound the results of the experiment.

At this point in the study, light availability as affected by *L. maackii* appears to be the most significant factor influencing survival, growth, and fecundity of the herbaceous plants tested. For this reason, *L. maackii* growth should be suppressed to increase light availability. The results of these experiments may indicate the best time to transplant new plants to a site after *L. maackii* is removed if this option is chosen.

New information about the mechanisms facilitating the spread of *L. maackii* may also assist managers in preventing its invasion and spread. Even after *L. maackii* is removed, sites should be monitored for regrowth of *L. maackii* and invasion by other exotic species such as *A. petiolata*. This species is suppressed by *L. maackii* and is known to invade sites where *L. maackii* has been removed.

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# Assessing Herbicidal Damage in Amur Honeysuckle, *Lonicera maackii*, Stem Tissue

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## Abstract

Microscopic examination of structure, cellular viability, and metabolic activity revealed that glyphosate lethally damages the phloem and vascular cambium tissues within stems of Amur honeysuckle. Combined with results from glyphosate uptake and translocation measurements, the data suggest that Amur honeysuckle plants are killed early and late in the growing season, when the plants are just coming out of or preparing for winter dormancy.

Shoots sprayed with glyphosate were analyzed and compared with non-treated plants to assess structural damage, tissue metabolism, and cell viability. Tissue-specific histochemical stains, such as aniline blue and phloroglucinol, high-resolution scanning-electron microscopy, and fluorescent microscopy made it possible to determine the location and amount of structural damage and to differentiate metabolically active, living stem tissue from stems killed by glyphosate. Herbicide-induced losses in metabolic activity and tissue viability corresponded with deterioration of structural organization in treated stem sections. In addition, to measure the amount of herbicide absorbed by the plant, tracer amounts of [<sup>14</sup>C]glyphosate were applied as droplets to leaf tissue and measured for amount of uptake through the cuticle and transport to the stem.

## Introduction

Over the past several decades, the invasive woody shrub, Amur honeysuckle (*Lonicera maackii*), has been infesting the forests of much

of the eastern United States (Luken and Thieret, 1996). In Ohio, the Department of Natural Resources considers Amur honeysuckle one of the top noxious, invasive plant species in the state.

Non-invaded temperate forests in Ohio are composed of a diverse herbaceous layer, a moderate to thick canopy layer, and minimal shrub and sapling cover (Gordon, 1969). However, when introduced into pristine environments, Amur honeysuckle out-competes herbaceous plants and emerging saplings (Collier and Vankat, 2002; Gorchov and Trisel, 2003), decreasing the biodiversity and dynamics of the system (Vitousek *et al.*, 1997; Wilcove *et al.*, 1998). In the absence of biological controls, curtailing the spread and removal of this species is laborious and time-consuming.

When properly used, herbicides, such as glyphosate (N-phosphonomethyl glycine), have proved to be effective when applied to the plant as a foliar spray (Conover and Geiger, 1999), through stem injection (Franz and Keiffer, 2000), or directly to a cut stump (Conover and Geiger, 1999). For unknown reasons, control effectiveness depends on seasonality. To date, a detailed mechanism illustrating stem-tissue death in honeysuckle is not known. Winter tissue dormancy and seasonal changes in metabolism are important aspects of the life cycle of all temperate woody plant species that may provide potential signals by which to control honeysuckle.

Glyphosate is a non-selective, broad spectrum herbicide that inhibits 5-enolpyruvylshikimate 3-phosphate synthase (EPSP synthase), which interrupts the shikimic acid pathway (Steinrücken and Amrhein, 1980) in plants. Blockage of the pathway, which involves 60% or more of a plant's dry weight (Jensen, 1986), disrupts a number of essential processes including chlorophyll (Kitchen *et al.*, 1981), protein, and nucleic acid synthesis (Foley *et al.*, 1983), growth, photosynthesis, and carbon metabolism (Fuchs *et al.*, 2002).

Glyphosate works most effectively in metabolically active tissues (Franz *et al.*, 1997). To kill the plant, these tissues must be active sinks at the time of herbicide application. However, since woody plants go through a series of seasonal metabolic and developmental changes (Dickson and Nelson, 1982), herbicide susceptibility varies with seasonal shoot development. The most effective time to apply glyphosate to woody plants is later in the growing season, before leaf senescence (Wendel and Kochenderfer, 1982) when plants are storing carbohydrates in anticipation of winter dormancy.

For effective control, it is also important to understand the process of shoot development of a shrub as it occurs throughout the seasons. In spring and early summer, organic nutrients from photosynthesis are translocated to the developing leaves; in late summer and early fall, transport patterns shift as carbohydrates accumulate in the xylem rays and phloem of stems and roots (Scarascia-Mugnozza *et al.*, 1999). These tissues are sites of seasonal starch storage and mobilization (Sauter and Neumann, 1994; Witt and Sauter, 1994).

The specific focus of this study is to understand how glyphosate affects honeysuckle stem tissue. It is hypothesized that glyphosate applied to the plant as a foliage spray late in the growing season lethally damages tissues within the stem and disrupts the plant's ability to overwinter or initiate sap flow and bud-out the following spring.

## Materials and Methods

### Sampling Site

Field research was conducted on three- to five-year-old stands of invasive Amur honeysuckle plants in an oak-hickory-maple forest located in Beaver Creek, Ohio. Management of invasive species and restoration efforts have been ongoing at this site since 1986. This site contains areas that were never invaded, areas where the honeysuckle was removed, and areas that are highly infested with honeysuckle. Where non-invaded or managed for honeysuckle shrubs, the site consists of a temperate forest composed of a canopy layer containing an open shrub layer and a diverse herbaceous layer. Where invaded with honeysuckle, the area consists of a canopy layer covering a dense shrub monoculture with little herbaceous ground cover (Figure 1).

### Tissue Collection and Sectioning

First- or second-year stem samples were collected in the field from mature non-treated plants and those treated with foliage sprays of glyphosate (1.3% Roundup Pro Dry). Collected stem segments were cross sectioned with the aid of a modified rotary microtome to a thickness between 60 to 80  $\mu\text{m}$  and quickly placed into 50mM HEPES buffer (pH 7.4) for live tissue analysis or fixed for histochemistry or scanning electron microscopy (SEM) in a solution containing either formalin, acetic acid and ethanol (FAA), or 4% glutaraldehyde, respectively (Ruzin, 1999).

For analysis of cell viability in honeysuckle stems, the fluorescent dye 6-carboxyfluorescein diacetate (CFDA) was employed. Stem sections were incubated in a buffered solution (50mM HEPES, pH 7.4) containing CFDA for five minutes, washed in buffer, and viewed under fluorescent microscopy using a Nikon FITC filter cube (B-2 E/C FITC) with an excitation wavelength of 465-495nm and a narrow band barrier filter wavelength of 515-555nm.

### [<sup>14</sup>C]Glyphosate Uptake

To measure the amount of herbicide taken up by the plant at different times throughout the



Figure 1. Research site of Amur honeysuckle project at Mt. St. John in Beavercreek, Ohio. The photographs show a comparison of (A) a restored forest and (B) one that has been invaded by honeysuckle. In this picture, note the density and early leafing out of the shrub layer (arrow).

year, tracer amounts of [ $^{14}\text{C}$ ]glyphosate were applied to leaf tissue and analyzed for uptake and translocation following a procedure by Geiger *et al.* (1999). To determine the amount of activity of radioisotope within the plant after 24 hours, leaf and stem tissues were separated, oven dried, and [ $^{14}\text{C}$ ]glyphosate extracted from the tissue through multiple washes in 5% EtOH. Washes were combined and scintillation counted for activity. Extraction was completed when scintillation counts were less than three times background levels.

## Results

The presence of starch and chlorophyll in primary xylem, xylem ray cells, and phloem tissues indicated these as metabolically active tissues capable of photosynthesis and as storage sites for carbohydrates. Comparison of glyphosate-treated tissue one month after application with non-treated tissue illustrates the primary location of damage to be within the phloem band. Compared to the non-treated stem, glyphosate-treated stems showed a loss of structure, integrity, and organization of cells within the phloem band (Figure 2).

In non-treated honeysuckle sections, CFDA taken up by the cells was able to show that the primary xylem, xylem ray cells, and phloem tissue accounted for the location of living tissue

within the stem. On the other hand, glyphosate-treated tissue showed structural deformation of the phloem band, illustrating that these tissues had been killed (Figure 3).

Studies measuring [ $^{14}\text{C}$ ]glyphosate uptake showed a pattern of seasonality where the herbicide is taken up and translocated more effectively. Throughout the season, radioactive glyphosate applied to the leaf was taken up most effectively in early spring and then again in late summer and early fall. During these months, nearly 25% of radioactive glyphosate applied to the leaf was absorbed. During the summer, the vast majority of glyphosate was unable to penetrate the cuticle. It is likely that seasonal differences in assimilation, translocation, and allocation of carbon and nutrients factor into herbicide-induced plant death.

## Discussion

In contrast to contact herbicides that act locally, glyphosate acts both at the point of application and in actively growing tissues to which it is translocated (Devine, 1989; Schulz *et al.*, 1990). Woody plants with seasonal patterns of carbohydrate allocation and annual dormancy (Witt and Sauter, 1994) are more susceptible to herbicides at certain times of the year. Presumably, seasonal effectiveness needs to take advantage of the metabolic mechanisms

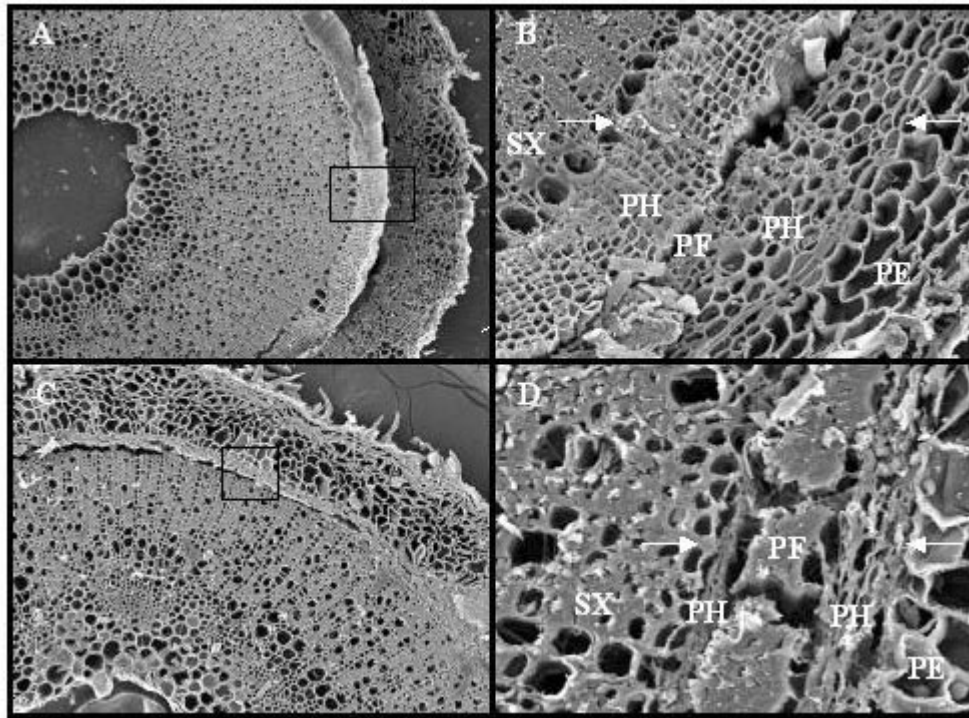
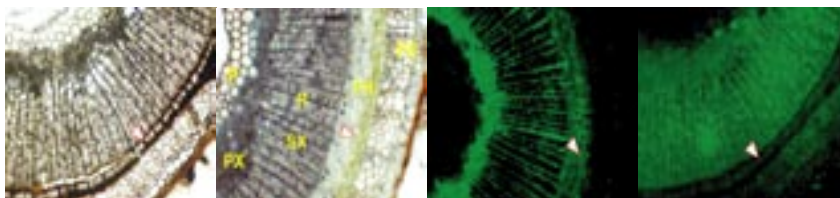


Figure 2. Scanning electron micrographs depicting non-treated and glyphosate-treated honeysuckle stem cross sections. Images show overviews of (A) non-treated [90x magnification] and (C) glyphosate-treated stems [120x magnification] with corresponding close-ups of the xylem-phloem band interface in a (B) non-treated [400x magnification] and (D) glyphosate-treated stem [800x magnification]. The arrows represent the phloem band. Note the cellular distortion and reduced band size of the phloem in the stem treated with glyphosate. Sections were fixed with glutaraldehyde, dehydrated through an ethanol series, critically dried, and gold sputter coated.



A B C D

Figure 3. Amur honeysuckle stem fresh sections depicting stem tissue anatomy and corresponding viability analysis under visible light or fluorescent microscopy. Images depict (A) a non-treated and (B) a glyphosate-treated light micrograph under visible light and (C) a non-treated and (D) a glyphosate-treated CFDA-stained micrograph under fluorescent light. P = Pith, PX = Primary Xylem, SX = Secondary Xylem, R = Xylem Ray cells, PH = Phloem, PE = Periderm. The arrow represents the phloem band. Note the lack of this band in stems treated with glyphosate and compare with scanning electron micrographs in Figure 2 [images taken at 100x magnification].

by which temperate woody plants prepare for winter dormancy.

Control increases when glyphosate is translocated together with sucrose to metabolically active areas within the stem. Herbicide effectiveness based on seasonality and shoot development is due to the method by which temperate woody plants metabolically prepare for winter dormancy. By annually renewing the functional xylem and phloem, the main function of the vascular cambium is to ensure the perennial life of trees (Plomion *et al.*, 2001). Since the vascular cambium is damaged in plants treated with glyphosate, stems that are injured later in the season, after bud-set, become incapable of over-wintering successfully or re-sprouting new bud growth the following year. Interference with protein and carbohydrate storage may disrupt the plant's ability to adapt to cold, thereby reducing its ability to survive over winter.

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# Ecological Restoration of Irwin Prairie State Nature Preserve: Control of Glossy Buckthorn in a Unique Oak-Openings Habitat

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## Introduction

Irwin Prairie State Nature Preserve, located in Spencer Township, Lucas County, lies in the Oak Openings region of northwestern Ohio. One of Ohio's 127 state nature preserves, Irwin Prairie contains the largest preserved area of the rare twigrush-wiregrass wet prairie community (Schneider and Cochrane, 1997). Known only in northwestern Ohio, this community, dominated by *Cladium mariscoides* and *Carex lasiocarpa* (Table 1), is considered globally imperiled by NatureServe, formerly the Association for Biodiversity Information (Faber-Langendoen, 2001).

Irwin Prairie is a remnant of a once-larger wet sedge meadow that extended approximately seven-miles long and one-mile wide. The increase of human activity in the area, including urban sprawl, hydrologic modification, and fire suppression, has led to the conversion of sedge meadows to impervious surfaces and cultural vegetation, as well as shrub- and tree-dominated communities. Successional processes have been accelerated at Irwin Prairie by the invasion of a non-native shrub, glossy buckthorn (*Rhamnus frangula*). In 2001, the Ohio Division of Natural Areas and Preserves undertook a large-scale ecological restoration effort to restore native prairie in areas where *R. frangula* had invaded. These efforts are critical to ensure the future viability of the twigrush-wiregrass wet prairie community.

## Methods

*Rhamnus frangula* has been managed at Irwin Prairie since the 1980s using prescribed burning and cut-stump herbicide treatments. However, these efforts alone were not enough to control invasives at the site. The Division began large-scale restoration efforts in 2001. The existing *R. frangula* clumps were mapped using GPS units and ArcView GIS software (Figure 1). After management areas were identified, three separate techniques were implemented:

- High-concentration, low-volume foliar spray.
- Low-concentration, high-volume foliar spray.
- Mowing and spraying.

### Method 1

Using backpack sprayers, Division staff treated stands of *R. frangula* during the growing season with a 5 to 10% solution of the broadleaf herbicide triclopyr (amine salt preparation) and a surfactant. After the herbicide took effect, the standing dead *R. frangula* was mowed. Resprouts and seedlings were sprayed after they reached one to two feet in height.

### Method 2

In 2002, the Division contracted NOVCO, a private vegetation control company, to spray a dense area of *R. frangula* occupying nearly four acres of prairie (Figure 1). In June, the contractor

<b>Scientific Name</b>	<b>Microhabitat</b>	<b>Frequency in Microhabitat</b>	<b>Habit</b>	<b>State Status</b>
<i>Physocarpus opulifolius</i>	Moderately wet	Common	Shrub	-
<i>Ilex verticillata</i>	Moderately wet	Occasional	Shrub	-
<i>Salix petiolaris</i>	Moderately wet	Occasional	Shrub	Endangered
<i>Carex lasiocarpa</i>	Moderately wet	Dominant	Sedge	Potentially threatened
<i>Cladium mariscoides</i>	Moderately wet	Dominant	Sedge	-
<i>Hypericum majus</i>	Moderately wet	Frequent	Forb	Potentially threatened
<i>Carex stricta</i>	Moderately wet	Occasional	Sedge	-
<i>Euthamia remota</i>	Moderately wet	Occasional	Forb	Threatened
<i>Hypericum kalmianum</i>	Moderately wet	Occasional	Forb	Threatened
<i>Lythrum alatum</i>	Moderately wet	Occasional	Forb	-
<i>Solidago riddellii</i>	Moderately wet	Frequent	Forb	-
<i>Gentianopsis crinita</i>	Moderately wet	Rare	Forb	Potentially threatened
<i>Calamagrostis canadensis</i>	Moderately wet	Sub-dominant	Grass	-
<i>Calamagrostis inexplansa</i>	Moderately wet	Sub-dominant	Grass	-
<i>Rhamnus frangula</i>	Moderately wet	Locally dominant	Shrub	-
<i>Proserpinaca palustris</i>	Wet	Frequent	Forb	-
<i>Carex sartwelli</i>	Wet	Locally dominant	Sedge	-
<i>Iris versicolor</i>	Wet	Occasional	Forb	-
<i>Carex atherodes</i>	Wet	Rare	Sedge	Potentially threatened

applied a 0.75% solution of triclopyr (amine salt preparation) and a surfactant. The *R. frangula* was allowed to stand for one year and surviving stems were retreated in 2003. The standing dead *R. frangula* is currently being cut down, and resprouts and seedlings will be treated.

### Method 3

The third method involved mowing large stands of *R. frangula* and treating re-sprouts with a 5 to 10% solution of triclopyr (amine salt preparation) and a surfactant. This method is being used along a wall of *R. frangula* that is invading the prairie (Figure 1). This area of dense *R. frangula* has been difficult to penetrate with backpack sprayers alone.

By establishing permanent transects, each management technique will be assessed for its effectiveness in eliminating *R. frangula*. Of particular interest are the plant species that colonize the management areas.

### Results

The preliminary results of our management efforts at Irwin Prairie have been very encouraging. *Rhamnus frangula* clumps treated using Method 1 in 2001 are now reverting to prairie. Although the treated areas contain transitional plant communities, rhizomatous sedges and perennial grasses have become established (Figure 2).



Figure 1. Aerial view of the 27.8-acre management unit of Irwin Prairie. Within this unit, *Rhamnus frangula* has invaded more than 13 acres.

In the NOVCO managed areas treated in 2002, approximately 80 to 90% of the *R. frangula* died. Immediately following spraying, the area was found to be relatively species poor. Transects have been set up to study the re-colonization of the area. It is predicted that sedges will quickly colonize the area after removal of the standing dead stems.

Method 3 has not yet been systematically evaluated since herbicide application occurred in 2003; however, the *R. frangula* canopy has been removed, opening the area to sedge and grass colonization.

## Discussion

Many factors have guided the Division's management strategy at Irwin Prairie. Because *R. frangula* is clumped and grasses and sedges dominate the community, foliar application of broadleaf herbicide has caused very little damage to non-target species. In cases where small *R. frangula* clumps are scattered throughout an area, staff can efficiently use backpack sprayers.

The Division contracted NOVCO to treat large areas that would have been difficult to reach with these sprayers. This turned out to be very cost effective because of NOVCO's specialized expertise and equipment. NOVCO may be used again to deal with large stands of *R. frangula*

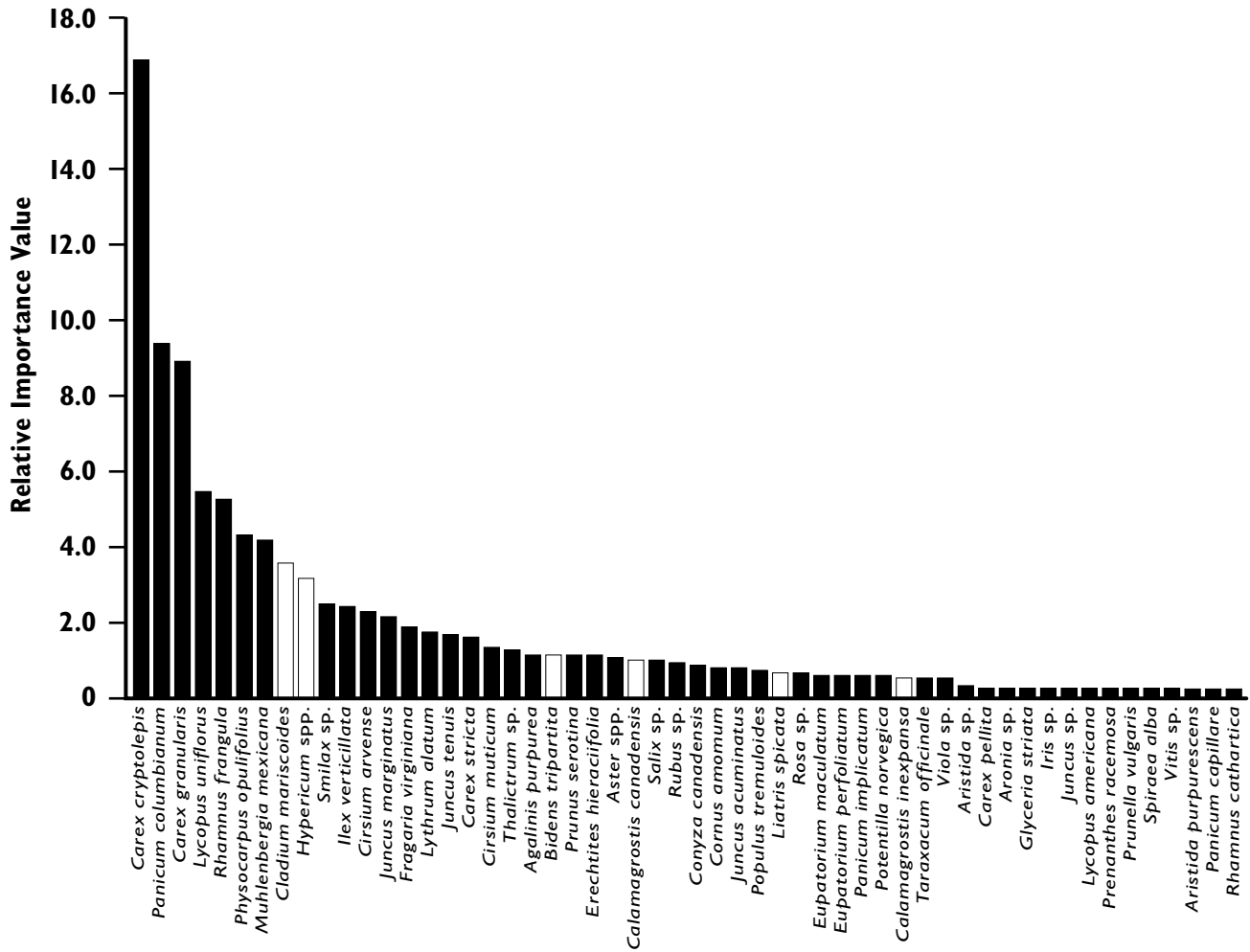


Figure 2. Plant community composition of a management area two years after initial herbicide treatment.

in place of using Method 3 in which clumps are mowed and re-sprouts treated. Planting sedges and grasses in the managed areas is not necessary, since individual stems of desired sedges can often be located within *R. frangula* clumps, where they persist in a suppressed state. Many of the sedges are rhizomatous and are able to spread into cleared areas.

The importance of using GPS and GIS has been critical in our efforts. With these technologies, we are able to track the fate of individual clumps, as well as the wall of *R. frangula*. GPS and GIS have also been important tools for estimating acreage. These methods are being applied throughout the preserve.

The Division's strategy at Irwin Prairie is designed to ensure the long-term persistence of the twigrush-wiregrass community. After large

stands of *R. frangula* have been eliminated, the restored prairie community can be maintained by burning, mowing, and occasional herbicide application. As with any invasive-species management program, a strong commitment is necessary to achieve success. The Ohio Division of Natural Areas and Preserves is dedicated to continuing these efforts.

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# The Effect of Interplant Variation on Emergence Patterns of *Ambrosia trifida* Populations

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## Abstract

*Ambrosia trifida* (giant ragweed) is a summer annual that can reduce species diversity in disturbed and successional habitats. *Ambrosia trifida*'s success is partly attributed to seedling emergence, which occurs intermittently during the growing season. *Ambrosia trifida* diaspores exhibit a high degree of size variation among individual plants. Diaspore size is known to affect emergence phenology of other species.

This experiment examined the influence of interplant variation on emergence phenology of two giant ragweed populations. Diaspores from 25 giant ragweed individuals (*i.e.*, 25 half-sib families) from each population were planted at a uniform depth in the autumn of 2002. In spring of 2003, emergence was monitored on a regular basis. Diaspore dimensions were determined with image analysis software, and relationships between diaspore dimensions and emergence were examined.

We identified two forms of emergence phenology among half-sib families — synchronous and continuous. Negative relationships between diaspore dimensions and days to 95% emergence were detected in one population. Definitive conclusions concerning diaspore size and emergence phenology require additional experiments. Nonetheless, results of this experiment suggest that unique emergence behaviors exist among half-sib families.

## Introduction

*Ambrosia trifida* is a summer annual that colonizes disturbed sites and persists in successional communities. When present, *A. trifida* suppresses and eliminates many plant species from the community (Abul-Fatih and Bazzaz, 1979). *Ambrosia trifida*'s success is partly attributed to seedling emergence, which occurs intermittently during the growing season (Hartzler, 2003). Intermittent emergence is problematic for weed management since late-emerging seedlings can evade weed-control practices.

Within a population, dissimilar emergence behaviors can be a consequence of:

- Differences among progeny from individual plants (intraplant variation) or
- Differences among progeny from different individual plants (interplant variation) (Andersson and Milberg, 1998).

*Ambrosia trifida* diaspores (single-seeded woody dispersal units) exhibit a high degree of size variation among individual plants (Sako *et al.*, 2001). *Ambrosia trifida* diaspore dimensions range from 3 to 14 mm long and 2 to 10 mm wide. In many species, polymorphic diaspores display different germination behaviors (Guterman, 2000). In particular, diaspore size often influences germination (Leishman *et al.*, 2000).

In this experiment, we hypothesized that the progeny of different *A. trifida* individual plants exhibit unique emergence behaviors. Furthermore, this investigation examined

relationships between *A. trifida* diaspore size and emergence phenology.

## Materials and Methods

### Plant Material and Experimental Design

On November 5, 2002, diaspores from 25 individuals (half-sib families) were harvested from two central Ohio populations (Old Field = OF; Railroad Embankment = RR). The study was conducted on the Columbus campus of The Ohio State University at a site with no history of *A. trifida* colonization. On November 18 to 20, fiberglass screen baskets were incorporated into the soil. Within each basket, 10 diaspores from an individual were planted 3.0 cm deep. Half-sib families were arranged in a completely randomized design with four replicates.

### Emergence Analysis

In spring 2003, the number of emerged seedlings was recorded at three- to five-day intervals. Dates of first and last emergence were plotted for each half-sib family, and days to 95% emergence were determined with linear regression. Following the conclusion of the 2003 emergence season, diaspores of non-emergent half-sib families were collected and placed on moist substrate at representative winter temperatures (5°C) for six weeks (cold stratification). Following cold stratification, diaspore germination was determined at 20°C, 24-hr light.

### Diaspore Dimension Analysis

Digital images and image analysis software were used to determine average diaspore dimensions (Sako *et al.*, 2001), including length, width, and area. The effects of diaspore dimensions on days to 95% emergence were evaluated by regression analysis.

## Results

Emergence occurred from March 26 to June 3, 2003. Two patterns of emergence were identified among half-sib families — synchronous and continuous (Figure 1). Dates of initial and final emergence varied among half-sib families. Four half-sib families of the OF population failed to

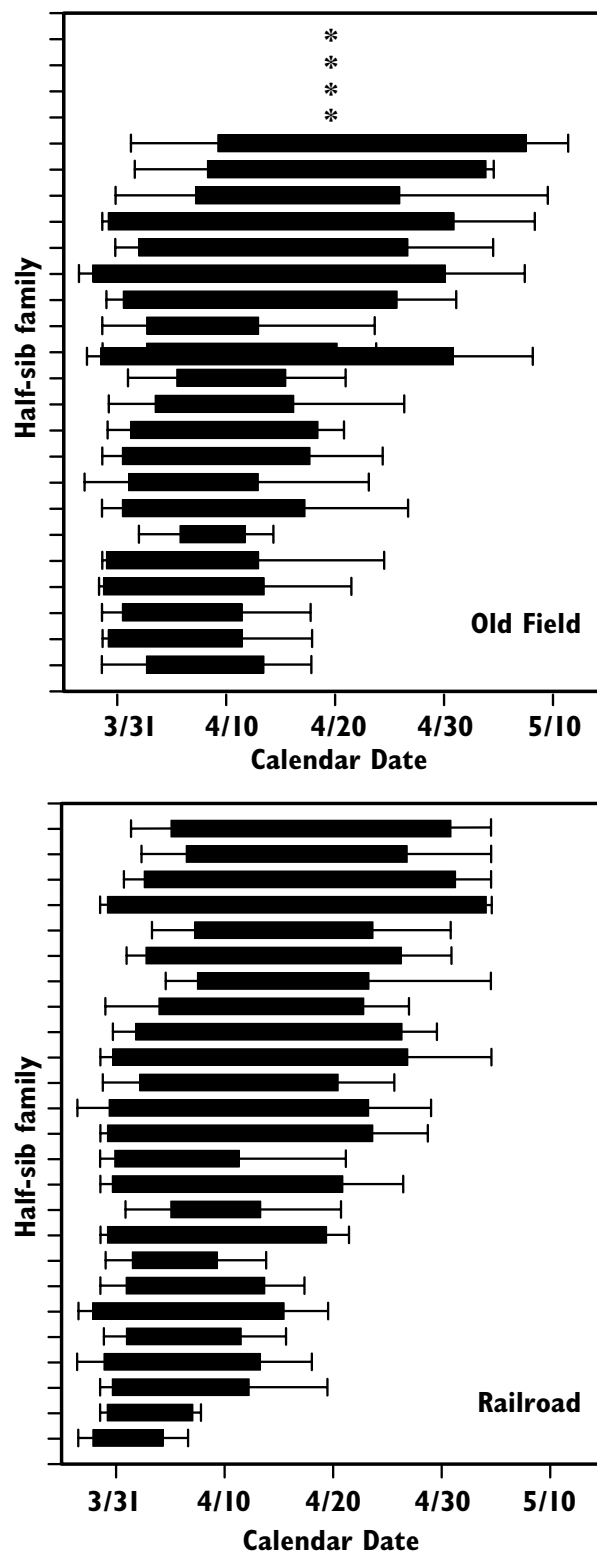


Figure 1. Emergence phenology of half-sib families collected from two *Ambrosia trifida* populations (OF, RR). Solid bars represent average emergence season; error bars are the standard error associated with date of initial emergence and date of final emergence ( $n = 4$ ). \* Indicates no emergence.

emerge. Diaspores of non-emergent half-sib families germinated following cold stratification. In the RR population, diaspore length and diaspore area were negatively correlated with days to 95% emergence (Table 1).

*Ambrosia trifida's* success in multiple environments is partly attributed to its emergence phenology (Hartzler, 2003). In successional habitats, *A. trifida* seedlings that emerge early are more competitive than seedlings that emerge late (Abul-Fatih and Bazzaz, 1979). However, in habitats characterized by disturbance, delayed seedling emergence is advantageous (Abul-Fatih and Bazzaz, 1979). Distinct emergence patterns were detected among half-sib families. This suggests that some individual *A. trifida* plants produce offspring that are better suited to particular environments.

Natural selection is strong during the seedling establishment stage of a plant's life cycle (Leishman *et al.*, 2000). Presumably, natural selection influences *A. trifida* emergence phenology. However, in order for natural selection to occur, traits that confer a competitive advantage must be heritable. While germination and emergence of many species is genetically influenced (Baskin and Baskin, 1998; Foley and Fennimore, 1998), the heritability of *A. trifida* emergence phenology is unknown at this time.

Diaspore (seed) size influences emergence phenology of many species (Gutterman, 2000; Leishman *et al.*, 2000). Results concerning the relationship of *A. trifida* diaspore dimension and emergence are inconclusive. In the RR population, days to 95% emergence were negatively correlated with diaspore length

and area, which suggests that larger diaspores emerged at a faster rate than smaller diaspores. However, a relationship between diaspore size and emergence was not detected in the OF population.

Knowledge of biological mechanisms that contribute to *A. trifida's* success can lead to more effective and efficient control measures. Results of this experiment indicate that interplant variation contributes to emergence phenology variation. Plants that produce progeny that exhibit continuous emergence are more problematic than plants that produce synchronous emergent offspring. Therefore, future research efforts should work towards investigating mechanisms by which some plants generate continuous emergent progeny.

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**Table 1. Regression analysis of diaspore size effects on days to 95% emergence for two *Ambrosia trifida* populations (OF, RR). Non-emergent half-sib families were excluded from regression analysis.**

	RR Population			OF Population		
	r	r <sup>2</sup>	p-value	r	r <sup>2</sup>	p-value
Area	-0.5997	0.3597	0.0019	-0.1949	0.0380	0.4236
Length	-0.6693	0.4479	0.0003	-0.1892	0.0358	0.4379
Width	-0.2375	0.0564	0.2638	-0.2585	0.0668	0.2852

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# Successional Management in Restored Old-Field Wetlands

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## Abstract

Exotic plants are known to have invaded various ecosystems throughout the United States. Wetlands, in particular, have a number of invasive exotic plants known to affect both floral and faunal communities, as well as ecosystem function. Loss of species diversity and ecosystem function are concerns shared by managers of both natural wetland preserves and constructed treatment wetlands alike.

In northeastern Ohio, exotic species such as purple loosestrife (*Lythrum salicaria*), reed canary grass (*Phalaris arundinacea*), and common reed grass (*Phragmites australis*) are of particular concern. Six created wetland cells on formerly drained fields are being used to test different management strategies for the control of invasive plant species and to determine what management strategies provide the best ecological services while still maintaining a high plant species diversity.

Our objectives are to see not only which management strategy best reduces invasive and exotic plant species establishment in this part of Ohio, but also to determine which strategy can provide the most cost-effective means of preserving diversity as well as restoring ecological function. Three treatments are replicated — two planted wetland cells being actively managed for invasive and exotic species; two planted cells being allowed to vegetate without continued management; and two cells being left as unplanted controls. The project was initiated in the fall of 2003 with the creation,

seeding, and planting of the experimental cells. The collection of field data began in early spring of 2004.

## Introduction

Many studies have shown that anthropogenic activities in and around wetlands have resulted in the increased size and occurrence of monotypic stands of both exotic and native invasive species, especially *Phragmites australis*, *Phalaris arundinacea*, and *Typha* (cattail) spp. These shifts in community composition have been linked to the increased sediment (Werner and Zedler, 2002) and nutrient (Woo and Zedler, 2002) loads associated with stormwater runoff — both from urban and agricultural (Galatowitsch *et al.*, 2000; Owen, 1999) settings.

Differences in morphology and life history characteristics of many invasive species have been shown to provide them with selective advantages over many native species when present in areas of hydrologic modification (Wetzel and van der Valk, 1998). These dense stands may hinder the establishment of other native species, thereby limiting biodiversity (Marks *et al.*, 1994). As a result, many anthropogenically affected (Ailstock *et al.*, 2001) and unplanted wetland restorations (Moore *et al.*, 1999; Mulhouse and Galatowitsch, 2003) display high abundances of invasive and exotic species and lower than expected native-species richness and diversity (Galatowitsch and van der Valk, 1996).

Despite mitigation and restoration efforts, recent studies have shown a disproportionate amount of restored and mitigated wetlands to be neither achieving, nor approaching, functional and community structures likened to that of many natural reference wetlands (Fionnessey, 1997; Zedler and Callaway, 1999).

We initiated a project in 2003 to determine which management strategy reduces invasive and exotic plant species establishment in this part of Ohio. In addition, we hope to determine which strategy can provide economical means of preserving diversity while restoring ecological function.

The George Jones Memorial Farm (Figure 1) in Oberlin, Ohio, has provided us with a unique opportunity to study the effects of three restoration management strategies on both long- and short-term changes in wetland community compositions, invasibility, ecological function, and wetland soil development. Before restoration, the farm was the site of intensive drainage and agricultural practices for more than 50 years. This report describes the design of the experimental system.

## Methods

The experimental wetland cells are located on Oberlin College's George Jones Memorial Farm in northeastern Ohio (Figure 2). Cells are approximately one-half acre each in size (115' x 255'). All cells in the study have near identical water levels, basin size, and shape. Three management regimes are duplicated in two cells each. Since the cells are adjacent to each other on the same site, their drainage history, initial soil properties, and catchment areas are very similar.

The first five years of the study will focus on the development of the macrophyte communities of each cell, with an emphasis on the occurrence of native and exotic invasive species (*Phragmites australis*, *Phalaris arundinacea*, *Typha latifolia*, and *Lythrum salicaria*). Initial plots and transects for vegetative sampling are identical in each cell and have been permanently set with rebar to facilitate standard assessment of plant community development over time.

The species richness and diversity of amphibian and macroinvertebrate communities will also be compared among management treatments using techniques described by the Ohio EPA's Wetland Ecology Unit and in Micacchion *et al.* (2000). Seed bank samples provide baseline data for each cell, while seasonal soil cores from various elevations document changes in organic matter accumulation and soil development.

## Short-Term Goals

- To document plant community changes and invasive species establishment and to compare these results with local reference wetlands.
- To compare amphibian and macroinvertebrate populations and communities.
- To document differences in organic matter accumulation and soil development among treatments.

## Long-Term Goals

- To determine which treatment is most cost-effective with regard to resulting species diversity and ecosystem functionality.
- To determine when and if each treatment will become functionally and floristically similar to that of an existing reference wetland.
- To formulate a step-by-step protocol, with cost estimates, for farmers interested in pursuing future wetland restorations in old-field or low agricultural productivity settings.

In future studies planned by Oberlin faculty, identical nutrient and sediment loads will be pulsed through each wetland cell during storm events to simulate nutrient and sediment inputs that would be received in an agricultural treatment setting. Shifts in plant community composition and differences in ecological functions that may result will continue to be observed. This will be done through continued floral, faunal, and soil surveys and by measuring

# Conservation Reserve Enhancement Program Wetland/Meadow Restoration Area

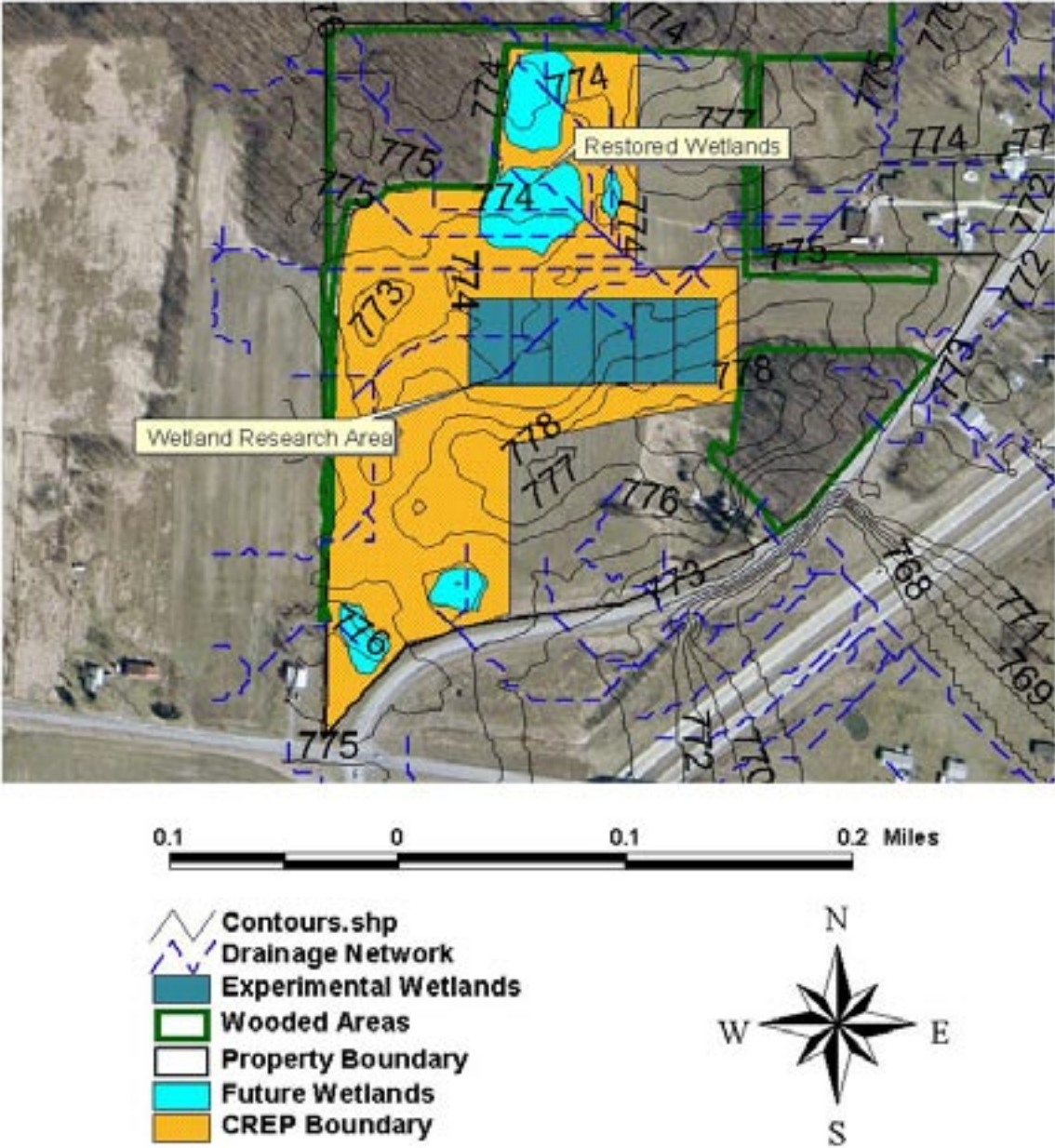


Figure 1. An aerial view of the study site outside of Oberlin, Ohio, adjacent to U.S. 20. The six experimental wetland cells, their relative size, shape, and locations on the property are depicted by the rectangles (map by Brad Masi).



Figure 2. A photograph from the north bank of the wetland cells facing southeast. The protruding piping in each cell is connected to control boxes that will be used to maintain equal water levels in each wetland. This summer will mark the first growing and sampling season of the study.

water-quality parameters at the inflow and outflow of each cell.

Data will be analyzed with ANOVA using SAS software (Copyright © 1999-2001, Version 8e) to determine significant differences among management treatments. Fisher's post-hoc LSD or Tukey tests will be applied to significant ANOVA tests.

In a more comprehensive assessment, a Partial Canonical Correspondence Analysis may be used to determine the amount of variance associated with physical (*e.g.*, soil, elevation), temporal, and treatment variables.

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# Herbicide-Resistant Weeds in Ohio

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## Introduction

The use of carbon-based herbicides began in the 1940s with the introduction of 2,4-D. Today, many herbicides with many different sites-of-action are available to farmers and land managers. In 1952, the first report of a herbicide-resistant weed biotype in the world was made in Canada in *Daucus carota* (wild carrot) with 2,4-D, a synthetic auxin herbicide. The first case of herbicide resistance in the United States occurred in the state of Hawaii in 1957 with 2,4-D in *Commelina diffusa* (spreading dayflower).

Herbicide-resistant weeds first appeared in Ohio in the late 1970s with atrazine, a photosystem II inhibitor herbicide, in *Chenopodium album* (common lambsquarters), but was not confirmed at the time. The first known confirmation of herbicide-resistant weeds in Ohio was in 1993 in *D. carota* with 2,4-D. Since that time, numerous greenhouse and field studies have been conducted at The Ohio State University to confirm the presence of herbicide-resistant weed biotypes in Ohio.

## Methods

The general method used by Ohio State to confirm herbicide resistance under greenhouse conditions was to compare the response of a sensitive wild-type biotype to that of the biotype suspected of being herbicide-resistant for each herbicide treatment. Seeds used in this research were collected primarily from fields where herbicide resistance was suspected due to poor herbicide activity. In a primary screening to

determine herbicide resistance, plant response was compared for rates equivalent to the field-use rate and up to four times this rate. For a more detailed characterization of resistance, a logarithmic scale of rates ranging from 1/1000 or 1/100 the field-use rate to 100 or 1,000 times that rate was used in dose response studies. Response was evaluated through visual assessment of plant injury and measurements of plant biomass approximately 14 to 24 days after treatment.

## Results

A number of field and greenhouse studies between 1997 and 2003 confirmed resistance to acetolactate-synthase-inhibiting (ALS) herbicides in a number of common weed species. Field studies conducted in 1997 in Madison County confirmed the presence of resistance to imazethapyr in *Amaranthus tuberculatus/rudis* (common/tall waterhemp).

In greenhouse studies in 1998 using seeds of *A. powellii* (Powell amaranth) collected in 1997, resistance to soil-applied cloransulam and imazaquin and foliar-applied chlorimuron, imazethapyr, primisulfuron, and thifensulfuron was confirmed in one population in Hancock County. An additional population of this same species from Hancock County was confirmed ALS-resistant a few years later, and dose response studies showed 809-fold resistance to imazamox and greater than 7,940-fold resistance to thifensulfuron.

Two populations of *Ambrosia artemisiifolia* (common ragweed) collected in 1998 from Defiance and Clark Counties were confirmed

resistant to foliar-applied chlorimuron, cloransulam, and imazamox. In subsequent screenings in 1999 through 2002 with seeds of *A. artemisiifolia* collected from suspect fields primarily in the northwestern quarter of Ohio, approximately 75 of 113 populations were confirmed ALS-resistant. Dose response studies showed >1,100-fold resistance to imazamox, >1,500-fold resistance to chlorimuron, and >12,000 fold resistance to cloransulam.

A population of *Ambrosia trifida* (giant ragweed), collected in 1998 in Union County, was confirmed resistant to a foliar application of cloransulam in early 1999. In subsequent screenings from 1999 to 2001 with seeds of *A. trifida* collected from suspect fields primarily in the western half of Ohio, approximately 23 of 74 populations were confirmed ALS-resistant. Dose response studies showed 24-fold resistance to imazamox and greater than 1,000-fold resistance to chlorimuron and cloransulam.

Screenings of *Conyza canadensis* (maretail) seeds collected in 1999 confirmed resistance to foliar-applied chlorimuron and cloransulam in nine populations from six counties. In subsequent screenings with seeds of *C. canadensis* collected in 2000 through 2003, approximately 60 of 97 populations from 10 counties were confirmed ALS-resistant. Dose response studies showed 32 to 168-fold resistance to cloransulam and 34 to 934-fold resistance to chlorimuron.

A population of *Xanthium strumarium* (common cocklebur) collected in 1999 in Miami County was confirmed resistant to a foliar application of chlorimuron and cloransulam. This was the only population of this species confirmed ALS-resistant out of 10 populations from various areas of western Ohio.

*Sorghum bicolor* (shattercane) seeds collected from a field in Fairfield County in 2000 were confirmed resistant to foliar-applied imazethapyr, nicosulfuron, and primisulfuron. A dose response study showed greater than 151,976-fold resistance to nicosulfuron, greater than 373,938-fold resistance to imazethapyr, and greater than 1,000,000-fold resistance to primisulfuron.

*Amaranthus hybridus* (smooth pigweed) seeds collected in 2001 from a field in Madison County were confirmed resistant to flumetsulam and thifensulfuron, but not imazamox. One out of six *C. album* populations collected in 2001 and 2002 was resistant to thifensulfuron but not to imazamox; the resistant population was from Putnam County.

In screening for resistance to sites-of-action other than ALS inhibition, a *D. carota* population from Williams County collected in 1998 was confirmed to be resistant to 2,4-D. *Chenopodium album* seeds collected from a population in Fairfield County in 2000 was confirmed resistant to atrazine. Another population collected in 2001 from Darke County, but not screened until late 2003, was also triazine-resistant.

Resistance to glyphosate in *C. canadensis* was confirmed in 10 of 13 populations collected from Brown, Clermont, Clinton, and Highland Counties in 2002. Seeds of 35 additional populations of *C. canadensis* were collected in 2003, and greenhouse screening confirmed glyphosate resistance in 60 percent, representing 13 counties in southwestern Ohio.

Since 2001, a total of 96 populations of *C. canadensis* have been tested with glyphosate, and out of that, 31 have been confirmed glyphosate-resistant. Dose response studies showed 33- to 39-fold resistance to glyphosate. One of these populations in Montgomery County exhibited resistance to multiple sites-of-action, glyphosate, and ALS inhibitors. The distribution of confirmed herbicide-resistant weeds in Ohio is shown in Figure 1, with the exception of *Kochia scoparia* (Kochia), for which herbicide resistance is only suspected.

## Discussion

Much is known about the distribution of herbicide-resistant weeds in Ohio. For the most part, herbicide resistance occurring in agricultural fields has had little effect on non-farm land managers to date. This is largely because few ALS- and photosystem II-inhibiting herbicides have been used by non-farm land managers.

# Location of Herbicide Resistant Weeds in Ohio by County 1995 to 2003

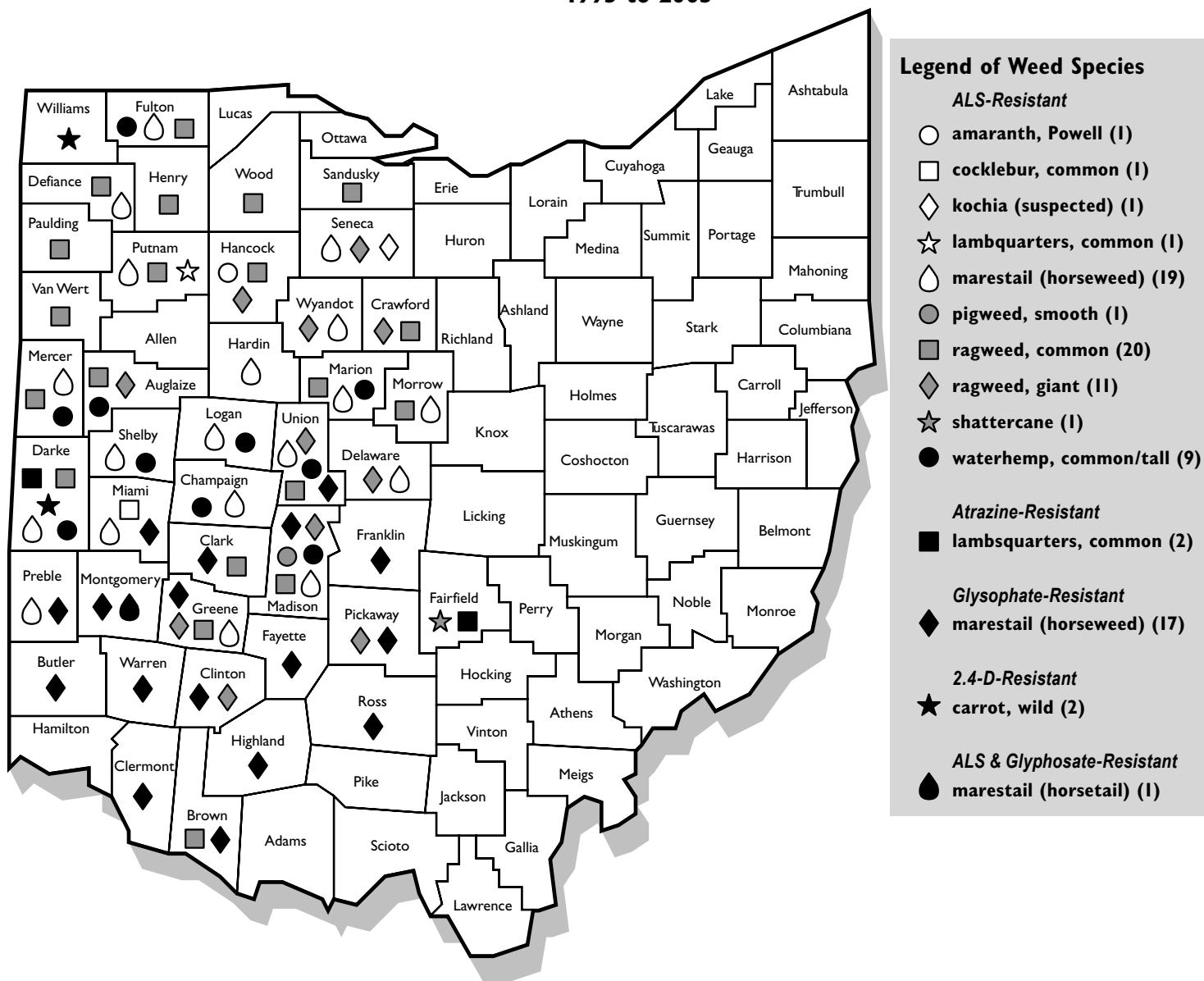


Figure 1. The distribution of confirmed herbicide-resistant weeds in Ohio, with the exception of *K. scoparia*, for which herbicide resistance is only suspected.

However, the development of glyphosate-resistant *C. canadensis* in agricultural fields and the long-distance wind dispersal of *C. canadensis* seeds may be of greater concern to many non-farm land managers, who often rely heavily on glyphosate. As herbicide-resistant weeds become

more prevalent in Ohio and the frequency of rural-urban interfaces increases, herbicide-resistant weeds may become more like invasive species and require specific management in many areas.

# Allelopathy as a Mechanism for Resisting Invasion: The Case of the Florida Scrub

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## Abstract

Recent papers linking the success of certain invasive plants to allelopathy have led to the proposal that allelopathy may be an important mechanism in the success of exotic plant invasions. This claim is reviewed in light of work done on allelopathic mechanisms in the Florida sand pine scrub. This community contains a number of perennial shrubs (e.g., *Polygonella myriophylla*, *Conradina canescens*, *Ceratiola ericoides*, *Chrysoma pauciflosculosa*, and *Calamintha ashei*) for which there is evidence of allelopathic interference toward invasive grasses of the adjacent sand hill community. Scrub vegetation is vulnerable to fire, and grasses would provide fuel for fires if they became established.

Field and laboratory chemical studies with the woody shrub *Polygonella myriophylla* have supported a role for gallic acid and hydroquinone. Recent work indicates that non-microbial and microbial oxidation is important in activating and in degrading these allelochemicals. Studies with other scrub perennials also point to the importance of environmental and microbial degradation processes in activating phytotoxins.

Environmental stress factors such as nutrient limitation have been implicated in contributing to the toxicity of scrub allelochemicals. While more work remains to be done, a combined approach coupling laboratory and field studies has helped us to understand better the apparent

mechanisms that keep grasses out of the Florida scrub. Furthermore, these studies suggest that the role of allelopathy as a mechanism in plant invasions is more complex than has been appreciated. While in some cases allelopathy may allow exotic invaders to succeed, the Florida scrub provides a counter-example in which allelopathy appears to play a primary role in preventing invasion.

## Introduction

### *The Florida Scrub*

The Florida scrub occurs on well-drained, sandy soils along Florida's central ridge and coastal dunes. Scrub sites contain essentially no herbaceous understory (Figure 1). Younger scrubs are dominated by widely spaced perennial shrubs, including the ubiquitous Florida rosemary (*Ceratiola ericoides* Michx.) and other locally abundant shrubs including *Chrysoma pauciflosculosa* (Michx.) Greene and *Polygonella myriophylla* (Small) Horton (Figure 1). Scrub communities form striking boundaries, or ecotones, where they border roads or abandoned fields (Figure 1).

### *The Allelopathy-Fire Cycle Hypothesis*

Richardson and Williamson (1988) proposed that chemical interference by fire-sensitive scrub perennials prevents the invasion of the scrub by grasses and herbs, both from the neighboring sandhill and along roadsides. These grasses



Figure 1. Patches of *Polygonella myriophylla* (left) dominate the edge of a scrub near Sun Ray, Florida. This scrub borders an abandoned citrus field that has been invaded by bahiagrass (*Paspalum notatum*), and other ruderal species. The bare zone is approximately 1 m wide. (Original figure appeared in Weidenhamer and Romeo, 1989; used with permission of Plenum Publishing Corp.)

would otherwise provide fuel for fires that would kill scrub plants.

### *Evidence for Allelopathy*

Over the past two decades, a team of chemists and ecologists has investigated the hypothesis that allelopathic mechanisms prevent invasion of the scrub by grasses and herbs that would fuel fires in this fire-sensitive community. This work has been the subject of a number of detailed reviews (Fischer, 1994; Weidenhamer, 1996). Among the findings:

- Bioassays and field observations show strong evidence of allelopathy.

Weidenhamer and Romeo (1989) found that both germination and biomass of bahiagrass

was reduced in soil from beneath *Polygonella myriophylla* compared to adjacent bare zones.

- Suspected phytotoxins and activation mechanisms have been identified.

For several scrub plants, environmental processes increase the toxicity of allelochemicals produced by the plant. *Polygonella myriophylla* produces large quantities of hydroquinone and gallic acid glycosides. Recent work (Weidenhamer and Romeo, 2004) shows that microorganisms in scrub soil readily convert arbutin, a glycoside of hydroquinone, to hydroquinone and benzoquinone.

- Environmental factors may intensify allelopathic effects.

Scrub soils are almost 100% sand, and available nutrients are low. Hydrocinnamic acid, a breakdown product of the compound ceratiolin found in the leaf washes of *Ceratiola ericoides*, is more toxic to the sand-hill grass *Schizachyrium scoparium* in low-N and low-K treatments (Williamson *et al.*, 1992).

## Discussion

Recent papers linking the success of certain invasive plants to allelopathy have led to the proposal that allelopathy may be an important mechanism in the success of exotic plant invasions (Callaway and Aschehoug, 2000; Bais *et al.*, 2003; Hierro and Callaway, 2003). Hierro and Callaway (2003) argue allelopathy should be considered as a hypothesis for the success of exotic invasive weeds, because invaders often establish in near-monoculture in what was once a diverse community. Furthermore, they assert that allelopathy is likely to be more important in new communities that are “naive” to the allelopathic chemicals produced by the invaders. Hierro and Callaway (2003) also note the potential for nutrient limitation to intensify allelopathic effects and predict that “the invasibility of plant communities should increase as resource availability decreases.”

Studies of allelopathic mechanisms in the Florida scrub community provide a strong rebuttal of this prediction. The scrub environment, as noted previously, has a number of aspects that may intensify allelopathic effects, including nutrient limitation, periodic moisture stress, and high temperatures. However, these factors do not appear to make the scrub more vulnerable to invasion. To the contrary, they appear to play a role in enhancing the effectiveness of allelochemicals from scrub species and thereby contributing to the allelopathic exclusion of grasses and herbs that might otherwise invade the scrub.

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# Abstracts

# Winter Application of Glyphosate for Garlic Mustard Control

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## Abstract

Current chemical control applications for garlic mustard (*Alliaria petiolata*), an exotic woodland biennial, are limited to warm days during fall and spring. Our goal was to determine if glyphosate could effectively control garlic mustard when applied to rosettes during the winter when most native herbs are dormant. This would allow land managers to implement effective control programs while minimizing risks to native species. Specific objectives were to:

- Evaluate effectiveness of glyphosate for rosette control at temperatures below 10°C.
- Evaluate glyphosate impact on non-target plant species.
- Measure glyphosate impact on newly germinated garlic mustard seedlings.

Experiments were conducted during the fall and winter of 2000-2001 and 2001-2002 in two study sites — Wooster Memorial Park and King Farm Woods — located near Wooster, Ohio. Plots (1.5 x 3.0 m) were established within dense stands of garlic mustard rosettes.

Treatments involved spraying a solution of glyphosate (Roundup Ultra) (1% v:v) and ammonium sulfate (19.25g/L) at three times spanning the cold months — late autumn: 11/14/00, 12/21/01; mid-winter: 2/13/01, 2/12/02; late winter: 3/20/01, 3/16/02 — plus an unsprayed control, with four replicates of

each treatment. The mixture was applied at ca. 325L/ha.

Treatment days were chosen during 2000-2001 for rain-free conditions and during 2001-2002 for near-freezing temperatures. Survival of targeted rosettes was monitored the following springs.

In 2000-2001, glyphosate applied at air temperatures of 1.0°C (late autumn), 5.6°C (mid-winter) and 12.8°C (late winter) resulted in 100%, 87%, and 94% mortality of targeted rosettes, respectively, during the primary bolting period, compared with only 12% mortality of control plants. In 2001-2002, similar results were obtained at air temperatures below freezing. Glyphosate applied at -4.2°C (late autumn), -4.0°C (mid-winter), and -0.8°C (late winter) resulted in 84%, 97%, and 94% mortality of targeted rosettes, respectively, compared with 41% mortality of control plants.

Few plants (average of 0 to 0.3 plants/m<sup>2</sup>) survived to bolt following the 2000-2001 spray treatments, compared to 49.3 plants/m<sup>2</sup> surviving in the control. Bolting plants in the spray treatments were extremely stunted. Similar results were obtained in 2001-2002, except for the mid-winter spray (5.2 plants/m<sup>2</sup> survived to bolt), where light snow cover may have inhibited or diluted the effectiveness of the spray treatment.

Non-target species density (all species combined) for both study periods was lower in control plots than in treated plots in mid-April and -May.

However, differences disappeared by mid-June. The higher initial densities of spring flora in treated plots may reflect the release of native species from competition by invasive species. Late winter spraying had a small incidental impact on garlic mustard seedlings. However, by summer, density-dependent mortality erased any differences. Delaying treatment of the current garlic mustard generation until seedlings emerge may increase risks to native species without impacting the new generation.

In summary, garlic mustard rosettes can be controlled effectively by glyphosate applied

when temperatures are below freezing. Fall and winter glyphosate application can release native species from competition with garlic mustard. Late winter applications may kill garlic mustard seedlings, but density-dependent mortality negates these differences.

These results demonstrate that glyphosate (label rates) can be used during winter at temperatures far below the label-recommended minimum temperature (50°C) to effectively control garlic mustard, while minimizing impacts on non-target plant species, thereby increasing forest restoration success.

# Methods for Garlic Mustard Seed Prevention and Destruction

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## Abstract

A common control strategy for garlic mustard (*Alliaria petiolata*), a profusely seeding European biennial invading North American forests, involves pulling bolting plants before seeds are shed. Harvested plants are either left on-site, or bagged and taken off-site. However, garlic mustard plants can form viable seeds even when harvested before fruits are mature, so both methods of disposal pose risks for continued invasion.

We conducted studies to develop strategies that prevent input of seeds into the seedbank from harvested garlic mustard. We evaluated the ability of bolting plants to form viable seeds when pulled at different flowering stages, and when roots or inflorescences were separated from stems (seed prevention). We also evaluated the seed destruction potential of different materials used to bag plants pulled at an advanced fruiting stage (seed destruction). All experiments were carried out in 2001 in a small forest in Wayne County, Ohio.

Seed prevention treatments were a factorial combination of four flowering stages at plant harvest ( $\leq 5$  or  $> 5$  flowers on May 4, post-flowering on May 21 and 30), and three types of stem separation (removal of roots or inflorescence, neither). Ten plants were used per treatment, with four replicates. Treated plants were spread in a single layer on the litter in 1 m<sup>2</sup>-plots, and viable seed formation evaluated indirectly with biweekly seedling counts in spring 2002.

Seedlings (thus viable seeds) were produced for all flowering stages of plant harvest. The two early stages resulted in far lower seedling frequency (31% and 19% of plots) and number (0.3 seedlings/m<sup>2</sup>) than the two late stages (88% and 100% of plots, 16 and 19 seedlings/m<sup>2</sup>, respectively). Removing the inflorescence or roots from stems did not affect seedling production, suggesting that root and stem resources are not necessary for seed maturation in pulled plants.

Seed destruction treatments involved bagging plants with well-developed fruits (harvested June 10) in one of four bag types (double-layer paper feed, woven-mesh plastic feed, black plastic garbage, no bag), and leaving on-site in 1 m<sup>2</sup>-plots. Approximately 500 bolting plants were used per treatment, with four replicates. Seeds were sampled monthly through February 2002 to measure weight and viability (Tetrazolium test). Plots were monitored for seedlings in spring.

All seeds produced by plants bagged in plastic lost viability after two months. After eight months, seed viability differed only slightly among plants bagged with paper (73%) or mesh (70%), or unbagged (90%). Seed weight (3.3 mg initial) after eight months was lowest for plants bagged in plastic (0.5 mg), and did not differ among plants bagged in paper (1.0 mg) or mesh (1.2 mg), or unbagged (1.1 mg).

By mid-April, hundreds of seedlings were present around unbagged plants and those bagged in paper (bags mostly decomposed), and

mesh bags were inflated with growing seedlings. No seedlings were present in or out of plastic bags.

In summary, pulled bolting garlic mustard can produce viable seeds, even when harvested at early stages of flowering. However, the risk is much greater when plants are pulled at advanced stages of fruit development. Removing the inflorescence or roots from pulled stems has no impact on seed production.

Bagging pulled plants in heavy-duty, black plastic quickly destroys seeds, whereas decomposable or porous materials provide only a minor seed destruction benefit. However,

bagging plus the off-site disposal required for non-decomposable bags is more resource-intensive than on-site disposal.

Using a "sacrifice area" (an area heavily infested and with low conservation value) for on-site disposal, in combination with pulling at early flowering stages, could minimize the hazards of seed production and spread. The risks of both strategies should be weighed.

Ultimately, a comprehensive control strategy for garlic mustard would include targeting of first-year rosettes as well as second-year bolting plants.

# Dendroecological Analysis of the Effects of an Invasive Shrub, Amur Honeysuckle (*Lonicera maackii*), on Forest Overstory Tree Growth

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## Abstract

Amur honeysuckle (*Lonicera maackii*) is a nonindigenous shrub that has invaded many hardwood forests, particularly in glaciated southwestern Ohio. The presence of Amur honeysuckle has been linked to a number of changes in forest understories, including reductions in herb diversity, woody seedling diversity and survival, and abundance of seedbank propagules. Most studies examining the community- or ecosystem-level effects of an invasive species focus on the stratum dominated by that species. The goal of this study was to examine the potential impact of understory invasion by Amur honeysuckle on overstory growth patterns.

We sampled 16 sites throughout southwestern Ohio. The oldest honeysuckle shrubs were aged within each stand to determine time of invasion. Increment cores were then collected from trees ( $n = 192$  total) within heavily infested areas of each stand. Tree cores were cross-dated to check for missing or double rings, and then

standardized to correct for differences in stand productivity. A 20-year window was established around each stand invasion date.

Radial growth patterns and tree growth rates were compared for a 10-year period before and after date of invasion and analyzed using paired t-tests. We found that mean radial tree growth was reduced by  $0.89 \text{ mm} \pm 0.06 \text{ SE}$  ( $P = 0.02$ ) following honeysuckle invasion, and the radial growth rate of canopy trees was depressed by  $0.15 \text{ mm/year} \pm 0.01 \text{ SE}$  ( $P < 0.001$ ). Thus, understory invasion by Amur honeysuckle is negatively impacting overstory tree growth and productivity. Honeysuckle must be competing for limited resources needed for tree growth.

We are unaware of any previous study that has documented this sort of influence. The resulting implications could be profound regarding successional dynamics, tree carbon storage, and forest stability. Considerably more study is warranted, especially how tree growth would respond following honeysuckle removal.

# The Effects of Forest Floor Disturbance on Garlic Mustard (*Alliaria petiolata*) Density and Cover

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## Abstract

The invasive biennial herb *Alliaria petiolata* (garlic mustard) is considered a threat to native forest herbs in the eastern United States. However, the factors that contribute to its invasion and subsequent spread have not been thoroughly explored. We assessed the effects of forest-floor disturbance on *A. petiolata* density and cover in an old-growth and second-growth forest stand in Hueston Woods State Nature Preserve, Preble County, Ohio.

Disturbance was assessed by visually estimating percent bare ground in 25 1- x 1-m plots in each stand in October 2002, and by point frame sampling in May 2003. The effect of each measure of bare ground on log-transformed *A. petiolata* rosette density and cover data taken in May 2003 in each stand was tested by linear regression, and the effect on the presence v. absence of adult *A. petiolata* in May 2003 was tested by logistic regression.

In the old-growth stand, neither October 2002 nor May 2003 measures of bare ground had a significant effect on *A. petiolata* rosette density or cover. However, presence of *A. petiolata* was significantly dependent on the October 2002 bare ground measure ( $\chi^2 = 4.74$ ,  $df = 1$ ,  $P = 0.029$ ); plots with adult garlic mustard had more bare ground than those without. In the second-growth stand, neither measure of bare ground had an effect on *A. petiolata* rosette density or cover or on adult *A. petiolata* presence.

Leaf litter disturbance may promote *A. petiolata* invasion in the old-growth stand by creating litter-free sites with high light availability during summer months, enhancing growth and survival of *A. petiolata*. In both sites, effects of forest floor disturbance may be overwhelmed by early summer precipitation. June precipitation was strongly correlated with October *A. petiolata* rosette density across the years (2001-2003). Understanding the factors that promote *A. petiolata* invasion can lead to efficient, cost-effective control strategies.

# Chemical and Physical Methods to Break Seed Dormancy in *Alliaria petiolata*

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## Abstract

*Alliaria petiolata* (Bieb) Cavara and Grande (garlic mustard) is an invasive biennial that reproduces solely by seeds produced in late spring and early summer by second-year rosettes. The seeds are dormant at maturity and require a period of cold stratification (approximately 104 days), with temperatures fluctuating around freezing, for germination to occur. This requirement increases the preparation time for conducting greenhouse and laboratory studies.

Our goal was to identify a fast and easy method to break dormancy in *A. petiolata* seeds. Seeds of *A. petiolata* were germinated following pre-treatments (immersion in 3% H<sub>2</sub>O<sub>2</sub> for 12, 24, or 48 hours; immersion in concentrated H<sub>2</sub>SO<sub>4</sub> for one or five minutes; mechanical abrasion in an

electric seed scarifier for one or three seconds; nicking seed coat with a razor blade; untreated control), with and without gibberellic acid (GA<sub>3</sub>) in the germination substrate, under two temperature regimens (20/10°C and 15/6°C) for 35 days.

Control seeds did not germinate regardless of substrate or temperature. Scarified seeds, except in the H<sub>2</sub>O<sub>2</sub> treatments, required GA<sub>3</sub> for germination. Cumulative germination percentages were greatest for the mechanically abraded (usually > 73%) and acid treated (> 66%) seeds. The best germination responses for most treatments occurred in the 15/6°C temperature cycle. Results suggest that mechanical abrasion and acid immersion are valuable techniques for effecting rapid germination in *Alliaria petiolata*.

# Woody Ornamental Plants as Invasive Species: A Study of the Spread of *Pyrus calleryana* From Cultivation

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## Abstract

*Pyrus calleryana* Dcne. (Callery pear; Rosaceae) is a commonly planted ornamental pear tree, cultivars of which include 'Bradford' and 'Aristocrat.' Callery pear is presently one of the most popular and widely planted ornamental tree species in the United States. Originally thought by some to be a sterile cultivar, 'Bradford' pear was frequently used by the horticultural industry, though it is not as widely used now due to its susceptibility to wind and ice damage.

With the introduction of additional cultivars of Callery pear, cross-pollination among cultivars resulted in heavy fruit set, with often hundreds or thousands of fruits forming on a single tree. Since these fruits are popular with birds, seeds of the species have been broadcast from cultivation, and many populations of Callery pear have been appearing in disturbed areas. This spread is being noted with increasing frequency in literature reports, but the actual extent to which *P. calleryana* has escaped has not previously been compiled.

Herbarium specimens of the species were examined from 30 herbaria to determine the extent to which it has spread. Field-work was also conducted in several states in the Midwestern and Southern United States — Alabama, Illinois, Indiana, Kentucky, Missouri, Mississippi, North Carolina, Ohio, Tennessee, Wisconsin — to see if additional populations could be located.

Escaped populations of Callery pear have been documented for 152 counties or parishes in 25 states and the District of Columbia. Many of these populations consist of relatively young individuals just reaching sexual maturity, while others consist of mature reproductive trees along with younger individuals of many different ages.

In addition, some individuals exhibit morphology indicating hybridization of *P. calleryana* with *P. betulifolia* and perhaps also with *P. bretschneideri*. It is possible, given the well-known ability of many *Pyrus* species to interbreed, that hybrids with other species may be discovered as well.

It is now known unequivocally that Callery pear has escaped and is reproducing extensively in the wild. In some states, such as Pennsylvania and Arkansas, dense thorny thickets of the species have been spreading very rapidly. While it has not yet been documented to invade undisturbed forests, time will tell whether it has the potential to do so.

In Arkansas, Callery pear has become problematic in relatively undisturbed pine savannah areas. *Pyrus calleryana* has the potential to become a seriously invasive plant throughout much of the lower 48 United States, especially in USDA Zones 4-8, in the more moist areas of the Midwest and South, mainly in marginal habitats and disturbed areas.

# Apple-of-Peru (*Nicandra physalodes*): A New Invasive Weed Threatening Ohio Agro-Ecosystems

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## Abstract

Apple-of-Peru (*Nicandra physalodes*), also known as Shoofly, was originally reported as an occasional adventive species in Ohio in the late 1800s. During the summer of 2002, this species was discovered in farm fields and along tree lines in Sandusky County, Ohio. Most infestations seem to be concentrated in Seneca and Sandusky Counties. Infestations have also been reported in North Carolina, Virginia, Tennessee, and Georgia in peanut, tomato, and soybean fields. Apple-of-Peru is a serious weed problem in Asia, Australia, east and southern Africa, and South America, and is one of the worst weeds in soybeans in Brazil.

Apple-of-Peru is a member of the Nightshade (Solanaceae) family and has a seed anatomy similar to that of eggplants. Seeds have a relatively thick seed coat, consisting of an outer and inner layer, and are innately dormant at maturity. Apple-of-Peru is an annual, reproducing only by seed. Seed germination occurs in late spring and continues throughout the summer if moisture is available. Flowering occurs 15 to 22 days after emergence, when plants have 6 to 15 leaves and are 15 to 53 cm high.

Leaves are arrowhead-shaped and pointed at the tip, with irregularly toothed margins. Flowers are trumpet-shaped and lavender in color (occasionally white). Fruits are borne singly and consist of a berry covered by a papery, bladder-like casing, resembling that of smooth- and clammy groundcherry. Each berry may contain

400 to 550 seeds. A mature plant is capable of producing thousands of seeds and may grow 1.8 to 2.0 m tall.

Our initial objective was to further our understanding of Apple-of-Peru biology relating to seed germination, seedling response to herbicides, extent of Apple-of-Peru distribution in Ohio, aggressiveness in open environments and with a crop, and ability to expand once introduced into a field.

Freshly harvested seeds from local Apple-of-Peru plants did not germinate except when soaked in sodium hypochlorite (household bleach) or concentrated sulfuric acid as a pre-treatment to break dormancy, or when potassium nitrate was used as a wetting agent in the Petri dish. Soaking seeds in sodium hypochlorite for 2 minutes resulted in 98% germination, whereas those immersed in concentrated sulfuric acid had 25 to 45% germination. Using 0.2% potassium nitrate as a wetting solution resulted in 25 to 40% germination.

A series of pre-emergence and post-emergence herbicide applications were done under controlled conditions in 2002 and 2003 to evaluate Apple-of-Peru response. Members of the triazine group of herbicides resulted in acceptable levels of control. However, most herbicides had poor to fair control of Apple-of-Peru. Unsatisfactory levels of control using pre-emergence applications of other herbicides were observed under laboratory testing in both 2002 and 2003. Field studies have confirmed these observations.

Surveys were conducted in 2003 throughout Seneca and Sandusky Counties to determine the extent of Apple-of-Peru's distribution. Infested fields were mapped using a GPS. The infested area totaled about 809 ha (2,000 acres) in a 8-km (5-mile) radius around Fremont.

An Apple-of-Peru and soybean competition study was initiated in 2003 in Wooster. A simulated average population of 1,452 Apple-of-Peru plants per 0.4 ha (1 acre) germinating at the same time with soybeans resulted in yield reduction of 1.2 T/ha (8.43 bushels per acre).

Initial findings from these studies suggest that Apple-of-Peru poses a serious threat due to tolerance to many commonly used herbicides and competitiveness with crops. Its ability to produce dormant seeds in large quantities suggests the potential to quickly build up a long-lasting seedbank. In addition, we have confirmed that Apple-of-Peru can serve as an alternate host for cucumber mosaic virus, which can cause significant yield reductions in a wide variety of vegetable crops including peppers, cucumbers, tomatoes, melons, squash, and onions.

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