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THE IMPACTS OF IMAZAPIC ON GARLIC MUSTARD AND NON-TARGET FOREST FLOOR VEGETATION IN CENTRAL KENTUCKY'S HARDWOOD FORESTS

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THE IMPACTS OF IMAZAPIC ON GARLIC MUSTARD AND NON-TARGET FOREST FLOOR VEGETATION IN CENTRAL KENTUCKY'S HARDWOOD FORESTS

THESIS

A thesis submitted in partial fulfillment of the
requirements for the degree of Master of Science in Forest and Natural Resource Sciences in the
College of Agriculture, Food and Environment
at the University of Kentucky

By

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Lexington, Kentucky

Director: Dr. John J. Cox, Associate Professor of Wildlife and Conservation Biology

Lexington, Kentucky

2021

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ABSTRACT OF THESIS

THE IMPACTS OF IMAZAPIC ON GARLIC MUSTARD AND NON-TARGET FOREST FLOOR VEGETATION IN CENTRAL KENTUCKY'S HARDWOOD FORESTS

Alliaria petiolata is an invasive biennial herb that poses a substantial threat to various ecosystems across the United States. Imazapic herbicide can control *A. petiolata* infestations, but there is limited peer-reviewed data on impacts of pre-emergent imazapic spraying to forest floor communities. This research examined the impacts of pre-emergent imazapic (0.84 kg/ha) with Pentra-Bark® surfactant on ground cover of *A. petiolata* and the spring perennials *Claytonia virginica* and *Erigenia bulbosa*. Experimental populations in randomized blocks within two forest stands in central Kentucky received the following treatments at 0.84 kg ai/ha: imazapic with Pentra-Bark®, glyphosate with Pentra-Bark®, Pentra-Bark® alone, and a control with no herbicide. Imazapic treatments significantly reduced ground cover of all tested species, while other herbicidal treatments led to no significant ground cover responses. Imazapic treatments did not always eliminate these species from experimental units, although some *C. virginica* and *E. bulbosa* individuals exhibited superficial injury. These findings suggest imazapic (0.84 kg/ha) with Pentra-Bark® surfactant is highly effective against *A. petiolata*, but may also harm some non-target forest floor plants. Additional research is required to determine impacts at other application rates to these and other non-target forest floor plant species.

KEYWORDS: *Alliaria petiolata*, garlic mustard, exotic invasive species, herbicide, forest floor, non-target impacts

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DEDICATION

To Dr. Thomas G. Barnes for his mentorship during 2012-2014 prior to his passing, and to my father and sister for their support and understanding as I worked towards my personal and professional goals.

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1.1 Introduction

The United States (US) has a myriad of terrestrial ecosystems. The continental U.S. itself is comprised of several classes of grasslands, scrublands, forests, wetlands, steppes, and other ecosystems spread across varying terrain and climatic regions (Comer et al. 2003; Sayre et al. 2009). Natural ecosystem processes such as soil nutrient cycling, water cycling, and pollination provide various resources that benefit human well-being (Fisher et al. 2009). Species in their native ranges play various ecological roles influencing these processes, and the loss of valuable ecological communities poses long- and short-term risks to both ecosystem function and human societies. The key to preserving our forest ecosystems is in balancing our needs to protect desirable forest communities and to satisfy our societal demands for forest resources. Many of the short- and long-term risks, however, are subtle and sometimes difficult to assess with our current ecological knowledge.

Understanding species diversity and the ecological niches species occupy in an ecosystem is vital to sustainable ecosystem management. Significant declines in species diversity may compromise multiple ecosystem functions and services, thereby impacting the provisioning of ecosystem functions (Lefcheck et al. 2015; Delgado-Baquerizo et al. 2016). One of the greatest threats to biodiversity, alongside habitat destruction, is exotic, invasive species (Pimentel et al. 2005). Within the U.S., almost half of the federally listed threatened and endangered species are at risk primarily due to competition with or predation by exotic invaders. Exotic invasive species can alter ecosystem functions such as nutrient cycling and hydrology, reducing habitat suitability for native species and increasing potential for other biological invasions (Didham et al. 2005). Most exotic invasive plants within the U.S. were originally introduced for food, fiber, or ornamental needs. Pimentel et al. (2005) estimated that the U.S. lost $120 billion per year to exotic invasive species, and $34.6 billion of these annual losses were due to exotic invasive plants. These conservative estimates reflect impacts to agriculture, forestry, and public health sectors, as well as the costs of invasive species management programs, and do not account for indirect economic costs driven by factors such as declines in biodiversity, ecosystem services, and aesthetic value.

Temperate forest floor plants comprise the vast majority of all plant species in a forest stand, provide wildlife cover and forage (Gilliam 2007; Weng et al. 2017) and influence future forest stand composition by exerting competitive pressure on tree seedling germination success. The spatial distribution and density of understory species helps determine the relative success of different species of tree seedlings through competition, soil nutrient/water cycling, impacts to fungal association formation, and various other biotic and abiotic factors (Shannon et al. 2014; Weng et al. 2017). Interactions between herbaceous layer cover density and soil nitrogen cycling may also influence canopy foliage density, thereby influencing deciduous tree leaf litter accumulation rates in the organic soil layer (Elliot et al. 2015).

Invasive understory plant species have various disruptive impacts on overall forest health and composition. Some research in North America points towards increased vulnerability to biological invasion in forests high in soil calcium and net nitrogen mineralization (Howard et al. 2004; Gilbert and Lechowicz 2005). An important characteristic among many successful invasive plants is their ability to alter soil nutrient cycling dynamics, such as nitrogen mineralization, to favor their continued proliferation (Ehrenfeld 2003; Meisner et al. 2012). Invasive shrubs such as *Lonicera maackii* and *Ligustrum vulgare* decrease arbuscular mycorrhizal fungi (AMF) availability in soils, which may compromise water and phosphorous uptake in AMF-dependent competitor plant species during times of water- and phosphorus-limitation (Smith and Read 2008; Shannon et al. 2014). Vectors for invasive plant species spread often lead to multiple species invasions, resulting in a suite of invasive species establishing in new regions (Moser et al. 2009). This means that land managers pursuing invasive species control must correctly identify and limit vectors responsible for invasive species spread. Restricting invasive plant spread becomes more complex if invasive plants can successfully establish within a multitude of disturbed and undisturbed ecosystems.

1.2 Garlic Mustard Life History Traits

Alliaria petiolata, or garlic mustard, is an exotic, biennial herb in the Brassicaceae family native to Europe and western and central Asia that was introduced to North America in the eighteenth century as a culinary herb (Durka et al. 2005; Rodgers et al. 2008). *A. petiolata* has since spread throughout various disturbed and undisturbed ecosystems in North America. *A. petiolata* germinates in spring, forming sexually immature rosettes during the first year that grow close to the ground and remain green year-round. Overwintering rosettes later bolt during the following spring, producing white cruciform flowers borne in a raceme that eventually give rise to green siliques after pollination. Second-year plants die some time after depositing seeds. Various intrinsic traits have facilitated *A. petiolata* establishment and persistence in North American forests, including pollination strategies, seed dispersal, allelopathy, and herbivory deterrence.

Generalist pollinator traits and a long-lived seed bank are major contributors to *A. petiolata* reproductive success in various ecosystems. Cruden et al. (1996) discovered that small- to medium-sized solitary bees (*Apidae*; *Andrenidae*; *Halictidae*) and syrphid (*Syrphidae*) flies serve as cross-pollinators. If outside pollinators are absent, then *A. petiolata* plants will attempt self-pollination. *A. petiolata* seeds require a period of cold stratification before they can germinate, after which germination usually begins around mid-February to early March (Roberts and Boddrell 1983; Baskin and Baskin 1992). This allows *A. petiolata* to emerge earlier than many other surrounding plants. Each second-year plant can produce hundreds of seeds, allowing solitary individuals to establish a population (Cruden et al. 1996). Some seeds may remain dormant for over ten years, but most seeds germinate after one or two seasons of overwintering (Cruden et al. 1996; Redwood et al. 2018). These seeds can continue maturing on uprooted second-year plants, and should be bagged and removed during invasive plant control efforts (Solis 1998).

Some plant species exhibit allelopathy, the ability to produce and emit secondary metabolites that alter reproduction, growth, and/or survival in other plants. Plants in the order Brassicales, such as *A. petiolata*, produce a set of various sulfur-containing compounds, glucosinolates, that hydrolyze to form phytotoxic byproducts. Vaughn and Berhow (1999) extracted several glucoside and glucosinolate metabolites within the leaves, stems, and roots of *A. petiolata*. The metabolites extracted from some of these leaves were applied to wheat (*Triticum aestivum*) and garden cress (*Lepidium sativum*) seedings to assess the efficacy of garlic mustard phytotoxins on germination rates. Analyzing extract from the remaining leaf collection revealed allyl isothiocyanate (20.4%), benzyl isothiocyanate (35.6%), and 2,3-epithiopropylnitrile (8.1%) to be metabolic by-products of sinigrin and glucotropaeolin breakdown. The extract constituents were found to slow down or completely halt germination within *L. sativum* and *T. aestivum*. While allelopathy in *A. petiolata* reduces competition from surrounding vegetation, North American populations may exhibit less allelopathic potential than their European conspecifics. Prati and Bossdorf (2004) noted that seed germination in rough avens (*Geum laciniatum*), a North American forest perennial, was inhibited by both North American and European *A. petiolata* allelochemicals. When these effects were compared to those on wood avens (*Geum urbanum*), a European forest perennial, only European *A. petiolata* allelochemicals inhibited *G. urbanum* seed germination. This may be explained by a decrease in glucosinolate production within North American *A. petiolata* populations over many generations, likely due to the relative lack of natural enemies such as insect herbivores in North America as compared to Europe (Szentesi 1991; Lankau et al. 2009). This likely led to lower selection pressure in the North American range that otherwise would have favored maintaining chemical expression traits in the face of ample insect herbivores in Europe. Despite this, genetically similar North American *A. petiolata* populations may still exhibit considerable plasticity in glucosinolate production, peroxidase activity, and trypsin (Cipollini 2002). This plasticity may limit the adaptive responses of generalist and some specialist insect herbivores to chemical defense expression in North American *A. petiolata* populations.

Riper et al. (2010) demonstrated a negative relationship between native plant species diversity in *A. petiolata*-infested forests and *A. petiolata* cover, and Meekins and

McCarthy (1999) found chestnut oak (*Quercus montana*) seedlings grown in pots with *A. petiolata* had lower biomass than when grown in monocultures. Even after *A. petiolata* removal, residual allelochemicals in the soil may prevent plants from re-establishing (Hochstedler et al. 2007). Additional observed impacts on forest ecosystems include inhibition of ectomycorrhizal fungi growth within surrounding soil (Wolfe et al. 2008; Cantor et al. 2011), which can impact root structure in tree species such as eastern white pine (*Pinus strobus*) by decreasing soil nutrient absorption. Arbuscular mycorrhizal colonization in tree species such as northern red oak (*Quercus rubra*), sugar maple (*Acer saccharum*), red maple (*Acer rubrum*), and white ash (*Fraxinus americana*) also decline with increasing *A. petiolata* ground cover, suggesting this invasive could impact long-term seedling recruitment rates for some hardwood trees (Stinson et al. 2006; Castellano and Gorchov 2012).

Very few North American herbivores are capable of controlling *A. petiolata* populations, which has contributed to the spread of this species throughout North America (Rodgers et al. 2008). Some members of the Brassicaceae family produce cyanide compounds in leaves, and cyanide levels in *A. petiolata* leaves can reach up to 100 ppm fresh weight (Cipollini and Gruner 2007). This amount is considered toxic to many vertebrates, although cyanide production alone may not defend against all forms of herbivory (Eisler 1991; Gleadow and Woodrow 2002). Cipollini and Gruner (2007) found that green peach aphids (*Myzus persicae*) feeding on *A. petiolata* rosette leaves reduced cyanide levels in these leaves, suggesting that *M. persicae* feeding either removed cyanide or inhibited cyanide synthesis in leaves.

The relationship between *A. petiolata* seed predation and *A. petiolata* seed bank longevity necessitates additional research. Invasive earthworms have been observed in the litter layers of Minnesota forests, indicated by litter composed of recently fallen leaves and branches and previous information on invasive earthworm activity in Minnesota forests (Riper et al. 2010). These invaded sites had low litter layer thicknesses ranging from 0.1 to 2.4 cm. Bartuszevige et al. (2007) noted that *A. petiolata* establishment was significantly higher at sites where the leaf litter layer was absent than at sites with intact leaf litter; however, this could also work against *A. petiolata* in sites with abundant invertebrate seed predators. Cassin and Kotanen (2016) found that the earthworm *Lumbricus terrestris* can significantly reduce the seed bank of *A. petiolata* populations.

Certain environmental conditions affect the degree of competition between *A. petiolata* and native plants and the performance of *A. petiolata* in different ecological niches. Meekins and McCarthy (2000) discovered that *A. petiolata* rosettes and flowering plants reacted to the combined conditions of low population density, high light availability, and ample soil nutrients by producing more leaves and increasing overall biomass. In conditions of high *A. petiolata* population density and low light availability however, rosettes and flowering plants exhibited increased leaf chlorophyll content. Under low light, Meekins and McCarthy (2000) also found that flowering plants also allocated more biomass towards roots, while rosettes demonstrated increased shoot biomass. This study suggested that light conditions play a crucial role in influencing relative abundances of rosettes and flowering plants within an *A. petiolata* population. This was supported by Riper et al.'s (2010) findings on *A. petiolata* cover in Minnesota's hardwood forests. Second-year *A. petiolata* abundance was positively correlated with light availability and was associated with reduced cover of first-year *A. petiolata* rosette cover. This also suggests two-point cycling within *A. petiolata* populations, where first- and second-year life stage individuals may alternate site dominance each year.

1.3 Garlic Mustard Management

1.3.1 *Fire*

A. petiolata's deleterious ecological impacts have led to the application of many standard invasive plant species control methods, each with varying success. Prescribed burning is often used to remove ground-layer vegetation and encourage native species growth and species diversity. Within *A. petiolata*-infested areas, however, prescribed burning impacts on *A. petiolata* population density vary according to timing and intensity. Burns prior to seed germination may encourage post-burn populations to form denser cover in areas where forest floor vegetation is removed. Luken and Shea (2000) found that burned and unburned plots after three consecutive annual burns in Kentucky shared similar long-term changes in *A. petiolata* population abundance. Burning had no significant effect on overall *A. petiolata* abundance, but burned plots had greater *A. petiolata* flowering plant and lower seedling densities in the summers following burns. Bowles et al. (2007) found that forest floor plant communities in burned plots showed a 97% reduction in shrub and small sapling abundance after 17 years of annual dormant-season burning in Illinois. These areas also exhibited increased *A. petiolata* abundance, likely due to *A. petiolata* recolonization from adjacent unburned forest stands and after burns removed other forest floor plant cover in burned plots. This contrasts with previous research by Nuzzo et al. (1996) which reported that periodic burning suppressed *A. petiolata* populations, albeit without completely removing them. For these reasons, habitat management plans involving burning would have to include herbicide and/or hand-pulling to ensure *A. petiolata* does not reestablish after a burn.

1.3.2 *Manual Removal*

Hand-pulling can help control *A. petiolata* if conducted before individuals flower and disperse seed; however, it is more feasible for second-year individuals given the difficulty of removing young first-year rosettes, and the fact that any remaining root crowns in the soil can continue to leach allelopathic compounds (Drayton and Primack 1999; Herold et al. 2011). Control of *A. petiolata* with hand-pulling is especially challenging given the large seed bank associated with most *A. petiolata* populations. Drayton and Primack (1999) tested the effects of hand-pulling on *A. petiolata* populations in eastern Massachusetts. The majority of un-pulled populations showed steep population growth, with 97% of these populations demonstrating a growth rate greater than 100%. In contrast, only 25% of hand-pulled populations exhibited steep growth, while 30% maintained population size stability and the remainder either declined or went extinct. The 25% of the hand-pulled populations in this study exhibiting growth and the continued existence of hand-pulled populations both indicate the importance of *A. petiolata*'s prolific seed production in maintaining population sizes. A 3-year old population can still survive even if 95% of flowering individuals are completely removed from an area.

Herold et al. (2011) assessed the effects of three years of removal of second-year *A. petiolata* plants on percent vegetation cover of first-year *A. petiolata* and native herbaceous plants in upland and lowland Illinois forests. They found that first-year rosette cover was greater in control than in hand-pulling treatment plots, which suggested that removal of second-year plants led to a decrease in seed input into the ecosystem. Nevertheless, Herold et al. (2011) found that rosette populations showed increased density, caused by increased seed germination due to soil disturbance. Collectively, these findings suggest hand-pulling requires consistent removal of pre-flowering plants before they produce viable seeds for several years before *A. petiolata* populations undergo significant decline. Hand-pulling is therefore more feasible for either smaller *A. petiolata* infestations or for mop-up efforts after other control methods.

1.3.3 *Biocontrol*

A. petiolata has at least 69 insect herbivores in Europe that are largely absent in North America (Szentesi 1991). Evans and Landis (2007) found herbivores within forests in Michigan's Lower Peninsula removed or damaged almost 3% leaf area on average per *A. petiolata* individual, while Riper et al. (2010) noted insect herbivores within Minnesota hardwood forests removed only 2% leaf area on average per individual. Davis et al. (2006) conducted a study on four European weevil species noted for feeding on *A. petiolata* in their native range: *Ceutorhynchus constrictus*, *C. alliariae*, *C. roberti*, and *C. scrobicollis*. These weevil species feed specifically on *A. petiolata* and a few other plants, and were easy to obtain as research specimens. All weevil species significantly affected fecundity in *A. petiolata* populations by either decreasing or stimulating seed production. Matrix model simulations of C. *alliariae* and *C. roberti* as single herbivores suggested that these species could stimulate *A. petiolata* seed production. *C. scrobicollis* appeared to be the only weevil capable of reducing survival rates of both seeding and rosette individuals to subsequent life stages. Dual-species combinations of herbivores showed *A. petiolata* populations decreased in number for all scenarios, where herbivores had the greatest negative impacts on seed and rosette survivorships except for the highest values for seed survival and fecundity (99% and 600 seeds/plant, respectively).

The potential of *C. scrobicollis* as an effective biocontrol agent eventually led to the Centre for Agriculture and Bioscience International successfully petitioning for its release as a biocontrol agent against *A. petiolata* within the Canadian province of Ontario in 2018 (CABI 2020). Other North American nations, however, have not yet approved release of *C. scrobicollis* for *A. petiolata* management. One pressing concern for biocontrol programs is the simultaneous release of other species accompanying a biocontrol species from its native range. *Perilitus consuetor*, an insect endoparasitoid found in adult and larval *C. scrobicollis* hosts in Europe, is one example. Katovich et al. (2020) suggested it may be possible to rear *C. scrobicollis* populations on caged *A. petiolata* plants for at least one generation to allow for separation of potentially parasitized *C. scrobicollis* adults from eggs before new larvae emerge. This may minimize *P. consuetor* transmission risk to future *C. scrobicollis* larvae.

North American *A. petiolata* populations do experience some fungal infections, despite the presence of antifungal allelochemicals. The amount of research on North American fungal biocontrol candidate species, however, is sparse in comparison to studies on insect candidates. Chen (1996) attempted to identify fungal pathogens endemic to Illinois that had successfully infected *A. petiolata* populations. Among these species were *Alternaria* spp., *Fusarium oxysporum, Fusarium solani, Phoma* spp., *and Scerotinia sclertiorum*. Pathogenicity testing revealed that *F. solani* caused severe disease in *A. petiolata* greenhouse individuals, causing root and basal stem rot and killing 75% percent of *A. petiolata* after three weeks. Field trials with *F. solani*, however, did not lead to significant death rates or stop seed production within infected *A. petiolata* populations. Other research suggests using a powdery mildew fungus (*Erysiphe crusiferarum*) to control *A. petiolata* (Ciola and Cipollini 2011). Ciola and Cipollini (2011) assessed geographic areas of southeastern Ohio for powdery mildew fungal infection rates in *A. petiolata*, and which species of wild and cultivated Brassicaceous plants could become hosts for the fungus. The researchers observed a positive correlation between the number of *A. petiolata* infections and the proximity of each site to the Dayton, OH metropolitan area. Native Brassicaceous plants developed moderate to mild infection in greenhouse conditions, but phenologically escaped from infections in the field. *A. petiolata* populations, however, exhibit considerable variation in resistance to *E. crusiferarum* infections across both North American and European ranges (Cipollini et al. 2020).

1.3.4 *Herbicides*

Herbicide products such as glyphosate have frequently been used to treat garlic mustard. Glyphosate is a broad-spectrum herbicide, effective in killing or injuring a wide variety of plants via inhibition of EPSP synthase, an enzyme that catalyzes the formation of aromatic amino acids tyrosine, tryptophan, and phenylalanine, which are crucial to plant growth (Duke and Powles 2008). Glyphosate binds strongly to soil particles and is readily broken down by soil microbes (Sprankle et al. 1975; Haney et al. 2000). This limits glyphosate to post-emergent applications, since glyphosate can only be reliably absorbed if the target plant is metabolically active and above ground. Frey et al. (2007) assessed whether cold-weather application (<10ºC) of 1% (v/v) glyphosate solution application could significantly curb *A. petiolata* rosette survival. They conducted two trials of three glyphosate applications: November 14-March 20, and December 21-March 16. Results from the first trial showed 87-100% mortality within treated plots and 12% within nontreated plots. The second trial resulted in 84-94% mortality in treated plots, as opposed to 41% for nontreated plots.

Long-term effects of continued dormant-season glyphosate spraying show modest alterations to native forest floor plant communities (Hochstedler et al. 2007). Hochstedler et al. (2007) analyzed the effects of spraying *A. petiolata* with glyphosate on native forest floor plant communities in old-growth and second-growth forest stands in Ohio. Plots at each stand were sprayed every November of 2000-2004, and plant cover was evaluated during May and June of each following year. The study revealed that although community composition differed each year between both stands, neither species richness nor diversity were significantly impacted by dormant-season spraying. Furthermore, while *A. petiolata* flowering plants were wiped out after every treatment, rosettes in each stand persisted.

Dormant-season glyphosate experiments suggested that native plant species densities were higher in sprayed areas during the spring in the first year following fall application (Frey et al. 2007; Hochstedler et al. 2007). Frey et al. (2007) found non-target plant species density was still higher in treated than non-treated areas during the second spring season, but new *A. petiolata* seedlings tended to emerge in almost all cases. Furthermore, new *A. petiolata* seedlings that emerged the spring following treatment in autumn and winter displayed no response to glyphosate applied during these latter seasons (Frey et al. 2007). Habitat managers delaying treatment of *A. petiolata* until seedling emergence in spring face increased risk of harming native plant species without significant reduction of newly-germinated garlic mustard seedlings.

Continued glyphosate usage, however, carries the risk of creating glyphosate-resistant *A. petiolata* individuals (Nandula et al. 2005). Resistance has been documented in several species of weeds in agricultural fields, such as common ragweed (*Ambrosia artemisiifolia*), asthmaweed (*Conyza bonariensis*), Canadian horseweed (*Conyza canadensis*), and Indian goosegrass (*Eleusine indica*). These plants have been exposed to glyphosate at varying application rates and timings and are no longer inhibited by this herbicide. In addition, several glyphosate-resistant plant lineages have emerged in naturally occurring populations of field bindweed (*Convolvulus arvensis*) and birdsfoot trefoil (*Lotus corniculatus*) without glyphosate use. Species such as tropical spiderwort (*Commelina benghalensis*), Asiatic dayflower (*Commelina communis*), Chinese foldwing (*Dicliptera chinensis*), lambsquarters (*Chenopodium album*), and velvetleaf (*Abutilon theophrasti*) have also become difficult to control with glyphosate applications. The development of this resistance within the past two decades requires agricultural and habitat management specialists to find new ways to control these weeds.

Among other herbicides used for *A. petiolata* control are 2,4-dichlorophenoxyacetic acid (2,4-D) and triclopyr, two systemic growth regulator herbicides that kill by mimicking auxin plant growth hormones and causing uncontrolled plant cell division. 2,4-D alone offers limited post-emergent control of *A. petiolata*, but can be paired with triclopyr for more effective control (DiTomaso et al. 2013). 2,4-D selectively kills dicots through pre- or post-emergent application, and has been applied via salt, ester, or acid formulations on numerous weeds within various settings in turf management, forestry, agriculture, and aquatic habitats (Peterson et al. 2016). Examples of susceptible weed species include: blue mudplantain (*Heteranthera limosa*); dayflower

(*Eclipta prostrata*); flatspine bur ragweed (*Ambrosia acanthicarpa*); dandelion (*Taraxacum officinale*); broadleaf plantain (*Plantago major*); creeping woodsorrel (*Oxalis corniculata*); and Eurasian watermilfoil (*Myriophyllum spicatum*) (Elmore 1996; Scott et al. 2013; Schardt and Netherland 2020). Despite its successful history as an herbicide, its continued use in multiple settings has contributed to the development of 2,4-D resistant plants. As of 2021, 2,4-D-resistance has been documented worldwide in at least 47 plant species (Heap 2021).

Among other general concerns for the use of 2,4-D include environmental persistence and toxicology. Wilson et al. (1997) found half-life duration in soils across 35 sites in the United States ranged 1.7-13.1 days, with less than 5% of applied 2,4-D moving below 6 inches in the soil. 2,4-D breakdown in soil occurs primarily due to microbial activity, and 2,4-D dissipation rates appear directly correlated with soil moisture content (Wilson et al. 1997; Peterson et al. 2016). Despite these findings, the acidic carboxyl group and generally low soil adsorption increase 2,4-D mobility in aqueous systems (Islam et al. 2017). Low organic content and/or low clay content can contribute to faster and deeper chemical infiltration through soil strata, and 2,4-D is known to easily enter runoff from treated sites.

Triclopyr selectively targets both broadleaf herbs and woody plants. It can be marketed in acid, salt, or ester forms, and is often used in woodlands, rights of way, pastures, and agricultural fields (NPIC 2002). This herbicide has been used to control weeds such as Eurasian watermilfoil (*Myriophyllum spicatum*), tree of heaven (*Ailanthus altissima*), alligatorweed (*Alternanthera philoxeroides*), Virginia creeper (*Parthenocissus quinquefolia*), and common hawthorn (*Crataegus monogyna*) (UK 2021). There is little documentation on triclopyr resistant-species other than *Soliva sessilis*, a turf weed originally from South America (Harrington et al. 2001; Heap 2021).

Microbial breakdown serves as the primary means of triclopyr degradation in soils, although triclopyr's soil half-life generally varies between 8-46 days (NPIC 2002; Strid et al. 2018). Triclopyr is fairly mobile in soils, and thus is likely to enter runoff or groundwater. A major concern with triclopyr use is the formation of 3,5,6-trichloro-2-pyridinol (TCP), a toxic byproduct of microbial degradation that is mobile and more persistent that triclopyr in soils. Triclopyr can rapidly degrade via photolysis in aqueous solution within a few days, although remains far longer in groundwater in the absence of sunlight (Woodburn et al. 1993). This breakdown rate, however, is unlikely to prevent runoff from reaching nearby aboveground terrestrial and aquatic habitats before complete chemical degradation.

One proposed alternative to glyphosate is bentazon, a post-emergence herbicide that selectively controls broadleaf weeds and sedges. Bentazon kills target plants that cannot metabolize it by interrupting photosynthesis. A study in a northern Illinois mesic forest showed late-fall bentazon application was less effective than glyphosate in reducing *A. petiolata* cover, but also less detrimental to non-target vegetation (Nuzzo 1996). Bentazon binds weakly to soil particles, however, allowing the herbicide to easily enter groundwater. This limits its effectiveness as a pre-emergence herbicide, and poses a considerable risk for groundwater contamination.

Imazapic is also an important herbicide used to control invasive plant species. Imazapic suppresses target plants by inhibiting the formation of acetolactate synthase (ALS), an enzyme responsible for catalyzing the formation of branched-chain amino acids leucine, isoleucine, and valine. This mode of action was first implemented within chlorosulfuron in 1982 (Saari et al. 1994; Tranel and Wright 2002). Imazapic targets several plants except for certain perennial grasses and forbs, making it valuable for grassland, rangeland, and shrubland rehabilitation (Bangsund et al. 1999; Beran et al. 1999; Bahm and Barnes 2011). Many studies looking at the role of imazapic in invasive species control have focused on rangeland and grassland habitats in the central and western US.

Downy brome (*Bromus tectorum*) is an invasive plant species of the western U.S. that, like *A. petiolata*, is sustained by large seed banks. Applying imazapic during fall reduces cover and provides residual control of *B. tectorum* for two months (Morris et al. 2009). Spraying imazapic in grassland and rangeland areas for *B. tectorum* control, however, negatively impacts overall plant biomass and height in several perennial forage grasses such as big bluegrass (*Poa secunda*), smooth brome (*Bromus inermis*), orchardgrass (*Dactylis glomerata*), crested wheatgrass (*Agropyron cristatum*), western wheatgrass (*Pascopyrum smithii*), hard fescue (*Festuca brevipila*), ‘Regar’ meadow brome (*Bromus biebersteinii*), bluebunch wheatgrass (*Pseudoroegneria spicata*), and intermediate wheatgrass (*Thinopyrum intermedium*) (Shinn and Thill 2004).

Leafy spurge (*Euphorbia esula*) is an invasive perennial herb sharing some similar life history traits as *A. petiolata*, including allelopathy, deep root production, and prolific seeding. *E. esula* has spread across much of the northern U.S. in prairies and open fields (Bangsund et al. 1999). Markle and Lym (2001) conducted a study to determine the effects of various adjuvants with imazapic on *E. esula* and many native warm- and cool-season grasses. They compared imazapic against picloram plus 2,4-D, a dual-herbicide combination typically used to eliminate *E. esula*. They found that spraying imazapic with methylated seed oil adjuvants both reduced *E. esula* by an average of 72% twelve months after treatment and was more effective than imazapic without adjuvants and picloram with 2,4-D, which only reduced *E. esula* by 33% and 40% respectively. The methylated seed oil adjuvant proved more effective than when compared to ionic, organosilicone, and silicone adjuvants. Imazapic applications to cool-season grasses without the tested adjuvants only reduced crested wheat-grass (*Agropyron cristatum*) and smooth brome (*Bromus inermis*) biomass. On the other hand, applications of imazapic with any adjuvant reduced biomass in all cool-season grasses and warm-season grasses.

When deciding to use imazapic for exotic invasive plant species control, it is important to consider whether any plant species exhibit resistance to this herbicide. Herbicide resistance typically occurs when prolonged use of a single herbicide within an area selects for herbicide-resistant plant variants. It is estimated that at least 22 monocots and 48 dicots had developed resistance to imazapic by 2002 (Tranel and Wright 2002). Prostko et al. (2009) investigated the geographical distribution of and levels of resistance in Palmer amaranth (*Amaranthus palmeri*), an annual flowering plant native to southern North America, within the U.S. state of Georgia. This study showed that *A. palmeri* has spread throughout Georgia, and some sub-populations could resist up to 1,400 g ai/ha of imazapic. Wind-distributed pollen from *A. palmeri* has spread genetic resistance to imazapic throughout Georgia and other areas of southern North America.

Prostko et al. (2009) discussed that traditional breeding methods have been used to create imadizolinone-resistant plant cultivars for landscaping. Their field study examined the tolerance of three imadizolinone-resistant sunflower cultivars (Dekalb 880CL, Mycogen 8H419CL, and Mycogen 8N386CL) to postemergent imazapic applications.

Resistance in these cultivars arose from continued use of imazapic for landscaping efforts, and imazapic's impacts on above-ground biomass, height, and seed-head production in these cultivars were negligible. While this study was primarily focused on consequences for landscaping efforts, it raises the question of whether the emergence of imazapic resistance in non-target vegetation could have impacts on how habitat managers apply imazapic for invasive species control in natural areas. There is scant research on imazapic resistance in *A. petiolata*, but managers may have to take extra care to remove surviving *A. petiolata* individuals before they deposit seed. Chemical control should complement rather than wholly replace non-chemical management for *A. petiolata*.

Another consideration is environmental persistence, and although imazapic's half-life is usually around 120 days in soil, this can vary depending on setting. Aerobic microbial activity plays a crucial role in imazapic degradation, and degradation rates increase with higher temperature, soil pH, and soil moisture (Su et al. 2019). Imazapic's low soil adsorption at near-neutral pH and high solubility in water may make it more likely to infiltrate into groundwater in near-neutral soil pH conditions (Aichele and Penner 2005; Martini et al. 2011; Christiansen et al. 2015). This herbicide can degrade within a few days via photolysis in aqueous solution (Harir et al. 2007), but has been observed lasting longer than two years in some groundwater supplies (Refatti et al. 2017; da Costa Marinho et al. 2019). This is an especially important consideration for land managers using imazapic close to bottomland areas or watersheds.

A. petiolata remains a top invasive plant species in many of Kentucky's forest communities, including many protected areas (Bossdorf 2004). Plateau®, an imazapic formulation manufactured by BASF, can be applied at 0.28-0.42 kg/ha (4-6 oz/acre) for either preemergent or postemergent control, but there was insufficient peer-reviewed research on non-target impacts of pre-emergent imazapic to forest floor vegetation prior to 2014. I also wanted to evaluate the suitability of Pentra-Bark®, a nonionic organosilicone surfactant, for pre-emergent herbicide use and expand public knowledge on potential phytotoxicity of Pentra-Bark®. Continued glyphosate use to control invasive plants may result in glyphosate-resistant variants of *A. petiolata* and other invasive plants over time. Strategies for managing herbicide-resistant invasive plants generally involve integrating multiple or alternating herbicidal and/or non-herbicidal (e.g. manual removal, biocontrol, prescribed burning) techniques over time as part of an integrated pest management approach (Heap 2013; Clay 2021). Herein, I examine the efficacy of imazapic with Pentra-Bark® surfactant in controlling *A. petiolata* populations, and characterize its effects on native plants in the Bluegrass Region of Kentucky. My findings should help inform land managers in evaluating the risks and benefits of using imazapic as a chemical alternative to glyphosate for *A. petiolata* control within and around forests of this region with possible application to other ecosystems.

1.4 Research Objectives

Objective 1: Compare the efficacy of two herbicide-surfactant combinations (imazapic-Pentra-Bark®, glyphosate-Pentra-Bark®) for controlling pre-emergent *Alliaria petiolata* in central Kentucky forests.

- H_0 : Herbicide treatments will have no effect on *A. petiolata* abundance
- H_a : One or more herbicide treatments will reduce *A. petiolata* abundance

Objective 2: Compare the effects of two herbicide-surfactant combinations (imazapic-Pentra-Bark®, glyphosphate-Pentra-Bark®) on two non-target forest floor plant species (*Claytonia virginica* and *Erigenia bulbosa*) in central Kentucky forests.

- H_0 : There was no difference in the abundance of either species after herbicide application.
- H_a : One or both herbicide treatments resulted in reduced abundance for at least one non-target species.

1.5 Methods

I conducted research at two study sites in central Kentucky: Curtis Gates Lloyd Wildlife Management Area (Grant Co., KY) and Raven Run Nature Sanctuary (Fayette Co., KY) (Figure 1.1). Research blocks at the Curtis Gates Lloyd Wildlife Management Area (WMA) site served to assess herbicide treatment effects on *Alliaria petiolata* ground cover, while blocks at the Raven Run Nature Sanctuary site served to assess *Claytonia virginica* and *Erigenia bulbosa* ground cover responses. I originally planned to utilize a third site at “Canoe Creek” (Garrard Co., KY) for assessing ground cover responses of *Acer saccharum*, *Acer nigrum*, and *Cardamine concatenata*. These species were also present in blocks at one or both other sites, and I planned to combine blocks from “Canoe

Creek" and the other sites for comparing treatment effects on ground cover. Post-hoc power testing revealed low experimental power for these species within individual or multiple site combinations, however, and I therefore chose to omit the aforementioned species from statistical reporting. These sites were characterized by varying degrees of *A. petiolata* infestation and were opportunistically used primarily because of either public access or the desire of public land managers of these areas to participate in the study. These sites are located within the Interior Low Plateaus, a region largely underlain with calcareous rock (Fenneman 1938; Jones 2005) and dominated by oak (*Quercus* sp.)-hickory (*Carya* spp.)-ash (*Fraxinus* sp.) forests as described by Jones (2005).

The Curtis Gates Lloyd Wildlife Management Area (WMA) is situated off US 25 about 1 mi (1.6 km) south of Crittenden, KY and is managed by the Kentucky Department of Fish and Wildlife Resources. Curtis Gates Lloyd WMA is located within the Outer Bluegrass physiographic region (Fenneman 1938). Research blocks were located within a mature forest stand characterized by major tree species such as *Acer saccharum*, *Carya* spp., *Fraxinus* spp, *Juglans nigra*, *Liriodendron tulipifera*, *Quercus alba* and *Quercus rubra* (Sewell and Smitson 2008). Soils were characterized by Eden flaggy silty clay, with 20-30% slopes around the research zones and extreme erosion (NRCS 2016). The area was used for agriculture before becoming public land, but now serves a variety of recreational uses.

Raven Run Nature Sanctuary is located south and slightly east of Lexington, KY, and is partially bordered to the east by the Kentucky River. This property is managed by the Lexington-Fayette Urban County Government Division of Parks and Recreation. It is an amalgam of old agricultural fields and both early successional and mature tree forest stands. Raven Run Nature Sanctuary sits within the Inner Bluegrass physiographic region described by Fenneman (1938). Non-target species research blocks were located in a forest stand with primarily Fairmount very rocky silty clay loam soil and comprised of tree species such as, but not limited to, *Acer saccharum*, *Acer nigrum*, *Aesculus glabra*, *Carya* spp., *Fraxinus americana, Quercus muhlenbergia, Quercus rubra*, *Quercus shumardii*, and *Ulmus* spp. These species are similar to those previously reported by Campbell et al. (1995) for mature forest stands on mesic areas of this property. Raven Run Nature Sanctuary today serves to protect natural land aesthetic and historic value, and restricts visitor use to pre-approved events and hiking along foot trails.

1.5.1 Pre-Emergent Herbicide Impacts on *A. petiolata*

Objective 1: Compare the efficacy of two herbicide-surfactant combinations (imazapic-Pentra-Bark® and glyphosate-Pentra-Bark®) for controlling pre-emergent *Alliaria petiolata* in central Kentucky forests.

I tested the effects of herbicide treatments on pre-emergent (first-year) *A. petiolata* by establishing treatment blocks (n=3) at Curtis Gates Lloyd WMA. I originally intended to analyze impacts of the aforementioned treatments to second-year *A. petiolata* plants, but second-year individuals were not consistently present throughout blocks in post-treatment assessments. Blocks were 8m^2 and contained four 2m^2 treatment plots assigned using a randomized block design. My primary goal for block placement was to include *A. petiolata* throughout each plot of each block. Blocks were kept at least 1m away from each other, but distances between any one block and the closest other block on any site were up to 15m apart depending on occurrence of *A. petiolata*. Per manufacturer's recommendation, blocks were established at least 15.24m (50ft) away from terrestrial bodies of water to reduce the chance of herbicide runoff into these areas. By creating blocks with adjacent plots, I hoped to focus on differences between treatments in each block and minimize confounding environmental factors associated with spatial gradients. The plot size and block quantities were established during initial research agreements on experimental unit size and replication with Raven Run Nature Sanctuary staff, and both the staff and I wished to minimize non-target damage in areas critical to forest management. The same experimental unit size and replication was then applied to other sites as well to ensure similar study design at each site.

Herbicide treatments occurred in late winter 2014 and included 20 mL/L imazapic (Plateau®) with 20 mL/L Pentra-Bark®; 20 mL/L glyphosate (Mad Dog® Plus) with Pentra-Bark® (20 mL/L), Pentra-Bark® (20 mL/L), and a control (Tables 1.1 and 1.2). Blocks at each site were treated once prior to *A. petiolata* rosette emergence using a wand boom connected to a CO_2 pressurized backpack sprayer. An application rate of 0.84 kg ai/ha (12 oz ai/acre) was used for each treatment (Table 1.2). I treated Curtis Gates Lloyd WMA blocks on March 1, 2014 (Table 1.1) . Plateau® manufacturer label instructions recommend 0.28-0.42 kg/ha (4-6 oz/acre) for pre-emergent *A. petiolata* control, but there was little peer-reviewed information on pre-emergent impacts of imazapic or Pentra-Bark® to forest floor vegetation at the time of this study. I wished to establish a baseline application rate for analyzing pre-emergent impacts of imazapic with Pentra-Bark® surfactant to forest floor vegetation, and therefore sprayed at the maximum annual limit of Plateau® herbicide to analyze impacts on *A. petiolata* ground cover at this application rate.

Post-treatment counts of *A. petiolata* rosettes were conducted in $8m^2$ blocks at Curtis Gates Lloyd WMA to quantify the effects of herbicide treatments. Sampling was conducted using a $1m^2$ quadrat frame. Although each treatment plot was $2m^2$, sampling was conducted within a $1m^2$ quadrat at the center of each plot and away from plot edges where the risk of cross-contamination would be highest. I visited Curtis Gates Lloyd WMA once every two weeks for the first three months, and then once every month thereafter until the end of 2014 for post-treatment ground cover assessments. I visually estimated ground cover for each and every identified species values by counting individual plants and assigning each values based on individual plant canopy area that I converted to percent ground cover. I did not engage in physical removal or disturbance of organic litter in the blocks to avoid disturbing the soil and/or accidentally killing forest floor plants, both of which could facilitate competitive release of conspecifics or other plant species and impact observed relative species abundances in subsequent site visits. This approach led to difficulty in estimating percent ground cover of newly-germinated *A. petiolata* rosettes, as not all first-year rosettes were initially visible above the leaf litter layer after germination. I therefore only analyzed and reported treatment effects on ground cover observed during May 16, 2014, the date when I recorded the highest *A. petiolata* ground cover values at Curtis Gates Lloyd WMA (Tables 1.3 and 1.4).

Herbicides may not immediately eradicate target plants, but can still injure plant tissue in various ways. I therefore visually assessed *A. petiolata* populations for easily-identifiable aboveground injury symptoms that loosely indicate herbicidal activity, but did not perform statistical modelling of these observations. Variable forest floor lighting conditions sometimes made it difficult to determine injury severity and the ratio of injured to uninjured plants in each plot, and inadequate sample size precluded statistical analysis. I thus only recorded whether or not any injured *A. petiolata* individuals were present in each treatment plot. Leaf tissue chlorosis, necrosis and malformation within imazapic-treated plants in other studies informed and confirmed my criteria for evaluating imazapic injury (Brosnan et al. 2012; Grichar et al. 2012; Grey et al. 2017). Although glyphosate typically deactivates when binding to soil particles, I still evaluated populations for leaf chlorosis as seen in and confirmed by other studies (Felix et al. 2011; Huang et al. 2015). These studies also evaluated stunting/biomass changes due to glyphosate and imazapic, but nondestructive sampling prevented me from comparing biomass among treatment populations. Information on Pentra-Bark® phytotoxicity without pesticide additives is scarce, so I evaluated Pentra-Bark®-treated populations for any easily recognizable plant discoloration, wilting, and/or tissue desiccation symptoms.

I conducted all statistical tests within R software, version 4.1.0, using RStudio (R Core Team, 2021; RStudio Team, 2021). I assumed herbicide treatments were independent of each other, and omitted blocks when I suspected herbicide cross-contamination based on the presence of injured plant groups or bare ground extending from the edge of one experimental plot partway (≤50% plot surface area) into another adjacent plot. *A. petiolata* percent ground cover data was arcsine-transformed for all statistical analyses. Prior to one-way ANOVA, I assessed *A. petiolata* populations with Shapiro-Wilks normality testing by running *shapiro.test* from the "stats" package in RStudio. After this, I analyzed treatment populations with Levene's test for homoscedasticity by executing *leveneTEST* from the "car" R package, version 3.0.10 (Fox and Weisberg 2018). Shapiro-Wilks normality tests did not report significant departures from normality (Table 1.3), and Levene's test (Table 1.4) did not find heteroscedasticity among populations. I therefore proceeded with one-way ANOVA using the *aov* formula from the "stats" R package to detect differences in arcsine-transformed ground cover between treatment populations. I then employed the *TukeyHSD* formula from the same R package to perform Tukey's Honestly Significant Difference (HSD) tests for post-hoc analysis. I conducted a post-hoc power analysis for ANOVA by running *power.anova.test* in the "stats" package of R, and calculated effect size similarly to Cohen's (1988) guidelines for ω^2 as follows:

$$\omega^2 = \frac{SS_b - (df_b \times MS_w)}{SS_{total} + MS_w} \qquad \text{(eqn. 0.1)}$$

In this formula, SS_b represents the sum of squares between subjects, df_b is degrees of freedom between subjects, MS_w is mean square within groups, and SS_{total} is the total sum of squares.

1.5.2 Pre-Emergent Herbicide Impacts on Non-Target Species

Objective 2: Compare the effects of two herbicide-surfactant combinations (imazapic-Pentra-Bark®, glyphosphate-Pentra-Bark®) on two non-target forest floor plant species (*Claytonia virginica* and *Erigenia bulbosa*) in central Kentucky forests.

I chose *C. virginica* and *E. bulbosa* due to their regular appearance in experimental blocks (n=4) and high experimental power (≥0.8). *C. virginica* is a perennial herb within the Portulaceae family with opposite, cauline leaves and a raceme inflorescence with pale pink or white flower petals. It blooms during March-May in Kentucky, and is typically found in lawn and mesic forest habitats across this state (Jones 2005). *E. virginica* is a perennial herb within the Apiaceae family possessing alternate, compound leaves with highly dissected segments. It produces an inflorescence consisting of compound umbels, blooms during March-April in Kentucky, and is typically encountered in mesic woods across the state (Jones 2005). Block placement priority at Raven Run Nature Sanctuary was initially given to patches of *C. virginica* and *Delphinium tricorne* large enough to span each block, as I believed these native perennials would co-occur with various other native forest floor plants. This meant distances between any one block and the closest other block could vary from 1m-15m. *D. tricorne*, however, was omitted from statistical analysis once post-hoc power analysis revealed experimental power was below 0.80. The experimental setup is almost identical to that for the previous objective using a randomized complete block design with the same herbicide treatments. The null and alternative hypotheses for this objective were:

- H_0 : There was no difference in the abundance of either species after herbicide application.
- H_a : One or both herbicide treatments resulted in reduced abundance for at least one non-target species.

I applied the aforementioned herbicides on March 8, 2014 at Raven Run Nature Sanctuary (see Table 1.1) and conducted post-treatment ground cover assessments every two weeks for the first three-month period and once every month thereafter until the end of 2014. These assessments consisted of percent ground cover estimations carried out within a sampling quadrat, in which I assigned individual plants of each and every identified species values based on individual plant canopy area that I converted to percent ground cover. I also recorded non-target plant injury as previously described to provide a loose indicator of herbicide activity, but did not use this data for hypothesis testing. I arcsine-transformed ground cover estimates from April 5, 2014 and assessed for normality among treatment populations (see Table 1.3) using *shapiro.test* in RStudio (R Core Team

2021; RStudio Team 2021). Shapiro-Wilk reported non-normality within imazapic-treated *C. virginica* and *E. bulbosa* populations, but I ignored these findings since the ranges of untransformed ground cover values never exceeded 1%. I then ran *leveneTest* in RStudio (Fox and Weisberg 2018) to evaluate populations for homoscedascity (see Table 1.4). These findings later informed my decision to employ a one-way ANOVA test to assess for significant treatment differences in ground cover observed on April 5, 2014 via *aov* in RStudio. I ran a post-hoc power analysis to ensure experimental power for both ANOVA tests was at least 0.80, and calculated effect size ω^2 according to equation 1.1. I finally employed post-hoc testing with Tukey's HSD via *TukeyHSD* in RStudio to evaluate individual treatment population differences.

1.6 Results

1.6.1 *Alliaria petiolata* (First-Year Rosettes)

A one-way ANOVA found significant differences (F[3,8]=8.24, p=0.008) in first-year *A. petiolata* responses to herbicide treatments at Curtis Gates Lloyd WMA (Table 1.5). Post hoc analysis with Tukey's HSD indicated ground cover of imazapic-treated populations was significantly lower than that of control (p=0.009), Pentra-Bark®- (p=0.02), and glyphosate-treated ($p<0.05$) populations (Figure 1.2 and Table 1.6). Tukey's HSD indicated no other significant differences between treatments in other pairwise comparisons. Post-hoc power analysis revealed that ANOVA testing had 0.94 power and a large effect size (ω^2=0.64) according to Cohen's (1988) guidelines.

Imazapic treatments did not completely eliminate *A. petiolata* from experimental plots by May 16, 2014, or nine weeks after spraying (Table 1.7). Although there were significant differences in treatment populations, a visual scan did not reveal any injury symptoms among control or herbicide-treated populations. This does not mean that injury is absent, and it is possible that aboveground visual appearance alone may not reliably indicate phytotoxicity. A subsequent ground cover assessment in December 18, 2014 revealed *A. petiolata* had survived in all but one imazapic-treated plot. I did not collect injury data during this date, however, and it is unknown if experimental populations survived to reproductive maturity in spring of the following year.

1.6.2 Non-Target Species

One-way ANOVA testing revealed significant differences among herbicide-treated *Claytonia virginica* (F[3,12]=13.21, $p<0.001$) and *Erigenia bulbosa* (F[3,12]=11.17, $p<0.001$) populations (Table 1.5). Post-hoc testing with Tukey's HSD found *C. virginica* ground cover for imazapic-treated populations was significantly lower at $p<0.05$ than those of Pentra-Bark®-treated ($p<0.05$), glyphosate-treated ($p=0.001$), and control ($p<0.001$) populations (Figure 1.3 and Table 1.8). Similarly, post-hoc testing on *E. bulbosa* found imazapic-treated populations had significantly lower values at $p<0.05$ than control ($p=0.004$), Pentra-Bark®- ($p=0.004$), and glyphosate-treated ($p=0.001$) populations (Figure 1.4 and Table 1.9). Tukey HSD test results reported no significant differences in other pairwise comparisons for either species. Power for *C. virginica* and *E. bulbosa* ANOVA tests were approximately 0.997 ($\omega^2=0.70$) and 0.99 ($\omega^2=0.66$) respectively, with both exhibiting large effect sizes.

C. virginica populations were present within each treatment plot of every block during the counting date, but ground cover of imazapic-treated plots was consistently 3-4% (Table 1.10). Injury was only evident within one imazapic-treated plot. Chlorosis was discernable within most *C. virginica* here, but more difficult to distinguish in two individuals. *E. bulbosa* was present in all but two imazapic-treated plots, where cover did not exceed 1%. Chlorosis was evident in all leaves, and some individuals also exhibited withering of leaf tissue.

1.7 Discussion

1.7.1 *Alliaria petiolata* (First-Year Rosettes)

Late winter treatment trials with imazapic and Pentra-Bark® surfactant led to significant reduction of first-year *A. petiolata* ground cover nine weeks after application. This was expected, given that the Plateau® manufacturer label recommends *A. petiolata* for pre- and post-emergent control at 0.28-0.42 kg/ha (4-6 oz/acre), and the U.S. Forest Service recommends this product for selective control (Miller et al. 2013). These recommendations, however, do not apply to forest sites except for managed conifer plantations. Insufficient samples and lack of visual detection of plant injury precluded statistical testing on injury data, however, and non-destructive sampling in this study prevented collection for biomass comparisons. Biomass changes should be evaluated in *A. petiolata* to understand overall herbicidal impacts to plant development. My sampling regimen focused on collecting ground cover data during times of greatest first-year *A. petiolata* abundance and within the first typical half-life period of imazapic (~120 days) to increase likelihood of detecting significant differences in ground cover between treated populations.

Glyphosate/Pentra-Bark®-treated and Pentra-Bark®-treated first-year *A. petiolata* populations showed no significant differences in ground cover between each other or control populations. Results with glyphosate soil applications were expected since glyphosate typically binds to soil particles and becomes ineffective when sprayed on the ground (Sprankle et al. 1975; Haney et al. 2000). The addition of Pentra-Bark® surfactant did not alter glyphosate's performance on *A. petiolata* germinating after treatment, and these findings on glyphosate's pre-emergent impacts are consistent with those reported by Frey et al. (2007). Pentra-Bark® alone also appeared to be ineffective at significantly reducing *A. petiolata* ground cover. Very little peer-reviewed information was available prior to this research on pre-emergent Pentra-Bark® impacts to exotic invasive herbaceous plants, and my findings provide limited data on *A. petiolata* responses to Pentra-Bark®.

Observations in December revealed *A. petiolata* survived in all but one imazapic-treated plot (Table 1.7). It is possible that first-year *A. petiolata* may have experienced additional herbicidal mortality during 2015, although I cannot account for individuals that may have survived to produce viable offspring. Intense intraspecific competition is a major factor leading to high mortality rates during the growing season within *A. petiolata* populations in North American habitats (Meekins and McCarthy 2000; Riper et al. 2010). It may be prudent to spray during fall or winter when population densities are lower than during spring or summer, thereby requiring less herbicide for ground cover reduction.

Assuming that Raven Run Nature Sanctuary treatment plots received their intended treatment amounts at 0.84 kg ai/ha, this may highlight a major consideration with using pre-emergent imazapic (Plateau®) with Pentra-Bark® surfactant for *A. petiolata* control in forests. Markle and Lym (2001) demonstrated that using imazapic with silicone-based and nonionic surfactants typically leads to reduced impacts to both target and non-target vegetation as compared to methylated seed oils in rangeland settings. Future studies could improve knowledge on non-target phytotoxicity within forest floor plant species by evaluating impacts of different adjuvants/surfactants.

1.7.2 Non-Target Species

Significant reductions in ground cover of imazapic-treated *Claytonia virginica*, and *Erigenia bulbosa* demonstrated imazapic's potential for non-target damage to forest floor perennials in Central Kentucky's hardwood forests. These species do not characterize forest floor flora within these forests by themselves but may serve as important bioindicator species to assess damage from late-winter imazapic spraying at other application rates in the future. It remains unclear exactly how changes in these species' relative abundances may impact overall forest floor community health at my sites, especially with lingering imazapic soil activity. Plateau® label warnings indicate imazapic applied in or around forest sites may injure several forbs and seedlings of desirable tree species. Various past research on southern U.S. pineland systems found herbicides generally reduced ground layer woody and herbaceous plant species richness, although these studies do not include data on imazapic (Litt et al. 2001). Imidazolinone herbicides used for grassland weed control have been known to harm some wildflower species while facilitating establishment of others (Beran et al. 1999). Once imazapic soil activity declines below injurious levels due to microbial degradation or leaching, it is possible for other plant species to colonize areas previously occupied by *C. virginica* and *E. bulbosa*. This could favor increased *A. petiolata* cover, given its early germination strategy and long-lived seed bank. (Cruden et al. 1996; Redwood et al. 2018).

Several other species were originally considered for analysis due to their abundance throughout research blocks: *Acer saccharum*; *Acer nigrum*; *Cardamine concatenata*;

Delphinium tricorne; *Laportea canadensis*; and *Podophyllum peltatum*. I only looked at seedling life stages for *A. saccharum* and *nigrum* and combined these together during counts due to difficulties in distinguishing between new seedlings and these species' tendencies to form hybrids (St. Hilaire et al. 2001; Grimm et al. 2007). Post-hoc power analyses for preliminary ANOVA tests on all these species revealed experimental power far below 0.80 for each. Although ANOVA testing failed to detect significant differences in all these species during their times of peak abundance, limited block quantities very likely precluded any ability to detect significant treatment differences. Statistically-justifiable research data from larger sample sizes is required to fully elucidate treatment impacts. I decided not to exceed use of four blocks for each objective per study site to limit spraying that might cause excessive non-target damage and conflict with landowners' forest management interests at my study sites. Future research on imazapic's impacts to specific plant species could utilize a greenhouse study design, which may allow for standardization of various soil and climatic factors influencing experimental unit population sizes. This design could forgo excessive trial spraying in forests that could severely compromise forest community health.

1.7.3 Limitations

One major limitation with this data is in interpreting overall impacts to *A. petiolata* across all life stages. I separated *A. petiolata* counts by life-stage, keeping counts of first-year rosettes separate from second-year individuals. The intent was to conduct analysis on both life stages, but second-year individuals during the treatment year were not consistently present throughout all plots. As such, I was unable to assess second-year *A. petiolata* population density responses to post-emergent chemical treatments. This limits my understanding of how declines in ground cover of different life stages of *A. petiolata* in response to chemical treatments may influence intraspecific competition, which is an important factor driving the predominance of first-year or second-year life stages of *A. petiolata* at natural sites (Riper et al. 2010).

Yet another complication in data analysis is the lack of data beyond the 2014 sampling period. Personal time constraints in 2015 prevented subsequent returns to the field to survey for *A. petiolata* individuals that may have survived from the previous year and produced siliques. This may be an important consideration if surviving *A. petiolata* individuals are imazapic-resistant and contribute their offspring to the seed bank (Tranel and Wright 2002). I am unable to recommend pre-emergent imazapic treatment for *A. petiolata* at this time without knowing how many *A. petiolata* rosettes from 2014 survived to become reproductively mature in 2015.

Not all non-target species within experimental units could be identified and incorporated for analysis due to difficulty in finding plant structures for keying or failure to complete field identifications within one day. Species identification for specimens within genera such as *Carex*, *Sanicula, Dicentra,* and sometimes *Viola* was left at the genus level (especially with non-destructive sampling restrictions across all sites). As such, I chose not to calculate species diversity or richness within my study sites. Furthermore, only *Acer saccharum/nigrum* seedlings, *Cardamine concatenata*, *Claytonia virginica*, *Delphinium tricorne*, *Erigenia bulbosa*, *Laportea canadensis*, and *Podophyllum peltatum* were commonly present throughout at least four blocks in any one site. Post-hoc power analysis revealed, however, that only ANOVA tests with *C. virginica* and *E. bulbosa* populations had the necessary experimental power to allow detection of significant herbicidal impacts. This led to species from one site, "Canoe Creek," being omitted from statistical analyses (Figure 1.1).

Apart from concerns over experimental power, it should be noted that other highly competitive invasive plants were present in and around research blocks at Raven Run Nature Sanctuary. *A. petiolata*, *Lonicera maackii*, and *Stellaria media* were among a few invasives spotted within and around some experimental plots in research blocks in early April 2014. Block placement procedures prioritized inclusion of ample desirable non-target vegetation, even areas with substantial non-invasive plant cover had some invasive shrubs and forbs. The presence of highly competitive invasive plants in research blocks may introduce confounding factors when analyzing treatment responses in plant ground cover. Non-destructive sampling precluded removal of invasives from research blocks, however herbicidal injury symptoms and significant ground cover reduction in *C. virginica* and *E. bulbosa* populations still suggested imazapic treatments had consistent impacts on these species.

One issue that can lead to inability to detect differences in treatment responses is loss of chemical via runoff or sprayer failure. Historical weather records from the U.S. National Oceanic and Atmospheric Administration revealed no precipitation 1 hour after treatment times at each site, and very little (≤1 cm) precipitation 48 hours after treatment (NOAA 2021). There also appears to have been no clearly identifiable source of physical disturbance that could facilitate translocation of contaminated topsoil or detritus from treatment plots shortly after treatment, although these factors are expected in field settings. I tested my backpack sprayer just prior to treatments, but it still malfunctioned a few times at Curtis Gates Lloyd WMA due to spray nozzle damage during operation. These sprayer problems did not stop experimental treatment applications, and replacement nozzles were available on-hand if needed. Nevertheless, sprayer nozzle issues may have reduced chemical delivery in some treatments. This may have reduced my ability to detect significant differences in *L. canadensis* and *P. peltatum*.

I can assume that imazapic at 0.84 kg/ha with Pentra-Bark® surfactant has the potential to seriously injure some forest floor plant species. As no species richness or diversity analyses were performed in light of issues with plant identification, however, it is difficult to assess the degree to which this may alter overall forest floor community composition. Furthermore, this study can only provide a baseline at 0.84 kg/ha for imazapic application in understanding non-target impacts to forest floor communities. Future studies would have to consider lower application rates to determine susceptibility within different species.

1.7.4 Forest Management Implications

Pre-emergent application of 0.84 kg/ha (12 oz/acre) imazapic with Pentra-Bark® surfactant is highly effective in reducing first-year *A. petiolata* ground cover, but impacts to *C. virginica* and *E. bulbosa* indicate imazapic may reduce ground cover of some non-target forest floor plants. Insufficient data on non-target impacts to other forest floor plants limits my understanding of the full extent of non-target damage to forest floor flora, and I currently cannot recommend late winter application of imazapic with Pentra-Bark® surfactant for *A. petiolata* control in forests. My observations at 0.84 kg ai/ha, however, provide a baseline to continue testing for impacts at other application rates with Pentra-Bark® surfactant, and indicate Pentra-Bark® may serve as an appropriate soil surfactant candidate for pre-emergent herbicide spraying. Depending on federal and/or state regulations, managers considering imazapic use in other ecological settings could consider post-emergent fall application over pre-emergent late winter application to allow more time for herbicide degradation prior to spring. This may also allow managers to limit the amount of herbicide sprayed to treat first-year *A. petiolata*, which typically experiences significant population declines during the growing season due to intense intraspecific competition (Riper et al. 2010).

Forest managers should consider other management strategies for *A. petiolata* in forests for now. Dormant-season glyphosate application for *A. petiolata* infestation likely poses fewer long-term risks to forest health than imazapic due to lack of soil activity (Sprankle et al. 1975; Frey et al. 2007). Triclopyr is an alternative to glyphosate for *A. petiolata* herbicide management, and can be used in spot treatments to minimize non-target plant damage (DiTomaso et al. 2013; Miller et al. 2013). Managers controlling infestations should physically remove *A. petiolata* that survive the first growing season before flowering, taking care to dispose of individuals offsite so seeds do not contribute to the seed bank (Solis 1998). Removing individuals prior to flowering may also minimize the chance that herbicide-resistant *A. petiolata* individuals will contribute herbicide-resistant offspring to the seed bank if there are survivors from any previous herbicide spraying. It remains more feasible for now to manage smaller infestations around areas with desirable vegetation than attempt full eradication of large-scale infestations. The active biocontrol program in Canada with *Ceutorhynchus scrobicollis* may present an opportunity to expand management options and minimize herbicide use for *A. petiolata* control within or around some forests in the US. Additional data on non-target impacts and *Perilitus consuetor* parasitoid activity in Canadian forests is required, however, to thoroughly evaluate potential risks to beneficial flora and fauna within various U.S. ecosystems.

Table 1.1 Experimental herbicide treatment and analysis times for *A. petiolata* and non-target vegetation research blocks, Bluegrass Region of Kentucky, 2014

Study Site*	**Research Block Purpose**	**Herbicide Treatment Date**	**Post-Treatment Analysis Dates**	**List of Blocks**
Curtis Gates Lloyd WMA	*A. petiolata* analysis	March 1, 2014	May 16, 2014	LN1, LN2, LN3
Raven Run Nature Sanctuary	Non-target	March 8, 2014	April 5, 2014	RN1, RN2, RN3, RN4

* Curtis Gates Lloyd WMA and Raven Run Nature Sanctuary are located in Crittenden, KY (Grant Co.) and Lexington, KY (Fayette Co.) respectively

Table 1.2 Experimental herbicide treatments applied in research blocks within the Bluegrass Region of Kentucky, 2014

Active Herbicide Ingredient	**Brand Used (Manufacturer)**	**Purpose/Mode of Action**	**Liquid v/v active ingredient (%)**	**Application Rate**
Ammonium salt of imazapic (±)-2-[4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1*H*-imidazol-2-yl]-5-methyl-3-pyridinecarboxylic acid	Plateau® (BASF Corporation)	Herbicide/amino acid inhibition	2	0.84 kg ai/ha (12 oz ai/acre)
Glyphosate, N-(phosphonomethyl) glycine, in the form of its isopropylamine salt	Mad Dog® Plus (Loveland Products, Inc.)	Herbicide/amino acid inhibition	2	0.84 kg ai/ha (12 oz ai/acre)
Alkylphenol ethoxylate, polysiloxane polyether copolymer, propylene glycol	Pentra-Bark® (Quest Products Corporation)	Nonionic organosilicone wetting agent	2	0.84 kg ai/ha (12 oz ai/acre)
control treatment (no active ingredient)	none	none	0	0

Table 1.3 Shapiro-Wilk normality test results (arcsine-transformed) for *A. petiolata*, *C. virginica*, and *E. bulbosa* ground cover, Bluegrass Region of Kentucky, 2014

Species	**Test Results**	**Control**	**Pentra-Bark®**	**Glyphosate**	**Imazapic**
A. petiolata	W	1.00	0.99	0.98	0.85
	p-value	0.93	0.77	0.73	0.24
C. virginica	W	0.78	1.00	0.82	0.73
	p-value	0.07	0.98	0.15	0.02*
E. bulbosa	W	0.82	0.85	0.92	0.73
	p-value	0.14	0.23	0.55	0.02*

* These p-values for imazapic-treated populations were ignored. Imazapic-treated *C. virginica* populations ranged only between 3-4% ground cover, and imazapic-treated *E. bulbosa* populations never exceeded 1% ground cover.

Table 1.4 Levene's test results for homogeneity of variance (arcsine-transformed) among herbicide-treated populations of normally-distributed plant species, Bluegrass Region of Kentucky, 2014

Species	**F-value**	**p-value**	**df (between groups)**	**df (within groups)**
A. petiolata	0.67	0.59	3	8
C. virginica	0.44	0.73	3	12
E. bulbosa	1.63	0.23	3	12

Table 1.5 One-way ANOVA test results for herbicide treatment effects on plant ground cover, Bluegrass Region of Kentucky, 2014

Species	**Total blocks (n)**	**Sum of squares (between groups)**	**Sum of squares (within groups)**	**Sum of squares (total)**	**df (between groups)**	**df (within groups)**	**Mean square (within groups)**	**p-value**	**F**	**F_{crit}**
A. petiolata	3	0.84	0.27	1.11	3	8	0.03	0.008*	8.24*	4.07
C. virginica	4	0.11	0.03	0.15	3	12	0.003	<0.001*	13.21*	3.49
E. bulbosa	4	0.15	0.05	0.20	3	12	0.004	<0.001*	11.17*	3.49

* ground cover differences significant at p<0.05 and given F values

Table 1.6 Tukey's HSD testing for significant differences in percent ground cover (arcsine-transformed) of herbicide-treated *Alliaria petiolata* populations

Pairwise Comparisons	**Mean Difference**	**95% Confidence Interval**		**p-value**
		Lower Bound	**Upper Bound**	
Glyphosate – Control	-0.07	-0.55	0.41	0.96
*Imazapic – Control	-0.68	-1.16	-0.20	0.009
Pentra-Bark® – Control	-0.19	-0.67	0.29	0.62
*Imazapic – Glyphosate	-0.61	-1.09	-0.12	0.02
Pentra-Bark® – Glyphosate	-0.12	-0.60	0.37	0.87
*Pentra-Bark® – Imazapic	0.49	0.008	0.97	<0.05

* findings significant at $p<0.05$, suggesting significant differences in ground cover between treatment populations

Table 1.7 *Alliaria petiolata* percent ground cover data collected during growing and dormant seasons in 2014, Curtis Gates Lloyd WMA, Crittenden, KY

***Research Block**	**Treatment Plot**	**Ground Cover (%)**	
		May 16, 2014	**December 18, 2014**
LN1	Control	62	1
	Pentra-Bark®	43	1
	Glyphosate	57	1
	Imazapic	1	0
LN2	Control	83	1
	Pentra-Bark®	47	2
	Glyphosate	33	1
	Imazapic	2	1
LN3	Control	35	2
	Pentra-Bark®	37	4
	Glyphosate	71	7
	Imazapic	16	4

Table 1.8 Tukey's HSD testing for significant differences in mean percent ground cover (arcsine-transformed) of herbicide-treated *Claytonia virginica* populations

Pairwise Comparisons	**Mean Difference**	**95% Confidence Interval**		**p-value**
		Lower Bound	**Upper Bound**	
Glyphosate – Control	-0.02	-0.13	0.09	0.95
*Imazapic – Control	-0.21	-0.33	-0.10	<0.001
Pentra-Bark® – Control	-0.10	-0.21	0.01	0.08
*Imazapic – Glyphosate	-0.19	-0.31	-0.08	0.001
Pentra-Bark® – Glyphosate	-0.08	-0.19	0.03	0.20
*Pentra-Bark® – Imazapic	0.11	0.001	0.23	<0.05

* findings significant at $p<0.05$, suggesting significant differences in ground cover between treatment populations

Table 1.9 Tukey's HSD testing for significant differences in mean percent ground cover (arcsine-transformed) of herbicide-treated *Erigenia bulbosa* populations

Pairwise Comparisons	**Mean Difference**	**95% Confidence Interval**		**p-value**
		Lower Bound	**Upper Bound**	
Glyphosate – Contol	0.03	-0.11	0.17	0.89
*Imazapic – Control	-0.21	-0.35	-0.07	0.004
Pentra-Bark® – Control	-0.002	-0.14	0.14	1.00
*Imazapic – Glyphosate	-0.24	-0.38	-0.10	0.001
Pentra-Bark® – Glyphosate	-0.04	-0.18	0.11	0.88
*Pentra-Bark® – Imazapic	0.21	0.07	0.35	0.004

* findings significant at $p<0.05$, suggesting significant differences in ground cover between treatment populations

Table 1.10 Percent ground cover of *Claytonia virginica* populations within experimental plots, Raven Run Nature Sanctuary, Lexington, KY, April 2014

Research Block	Treatment Plot	Ground Cover (%)
RN1	Control	19
	Pentra-Bark®	13
	Glyphosate	20
	Imazapic	3
RN2	Control	17
	Pentra-Bark®	5
	Glyphosate	11
	Imazapic	3
RN3	Control	9
	Pentra-Bark®	10
	Glyphosate	12
	Imazapic	4*
RN4	Control	17
	Pentra-Bark®	8
	Glyphosate	13
	Imazapic	4

* Indicates injury is present within at least one plant in this plot

Table 1.11 Percent ground cover of *Erigenia bulbosa* populations within experimental plots, Raven Run Nature Sanctuary, Lexington, KY, April 2014

Research Block	**Treatment Plot**	**Ground Cover (%)**
RN1	Control	8
	Pentra-Bark®	11
	Glyphosate	15
	Imazapic	1*
RN2	Control	9
	Pentra-Bark®	10
	Glyphosate	9
	Imazapic	1*
RN3	Control	5
	Pentra-Bark®	3
	Glyphosate	5
	Imazapic	0
RN4	Control	5
	Pentra-Bark®	4
	Glyphosate	6
	Imazapic	0

* Indicates injury is present within at least one plant in this plot

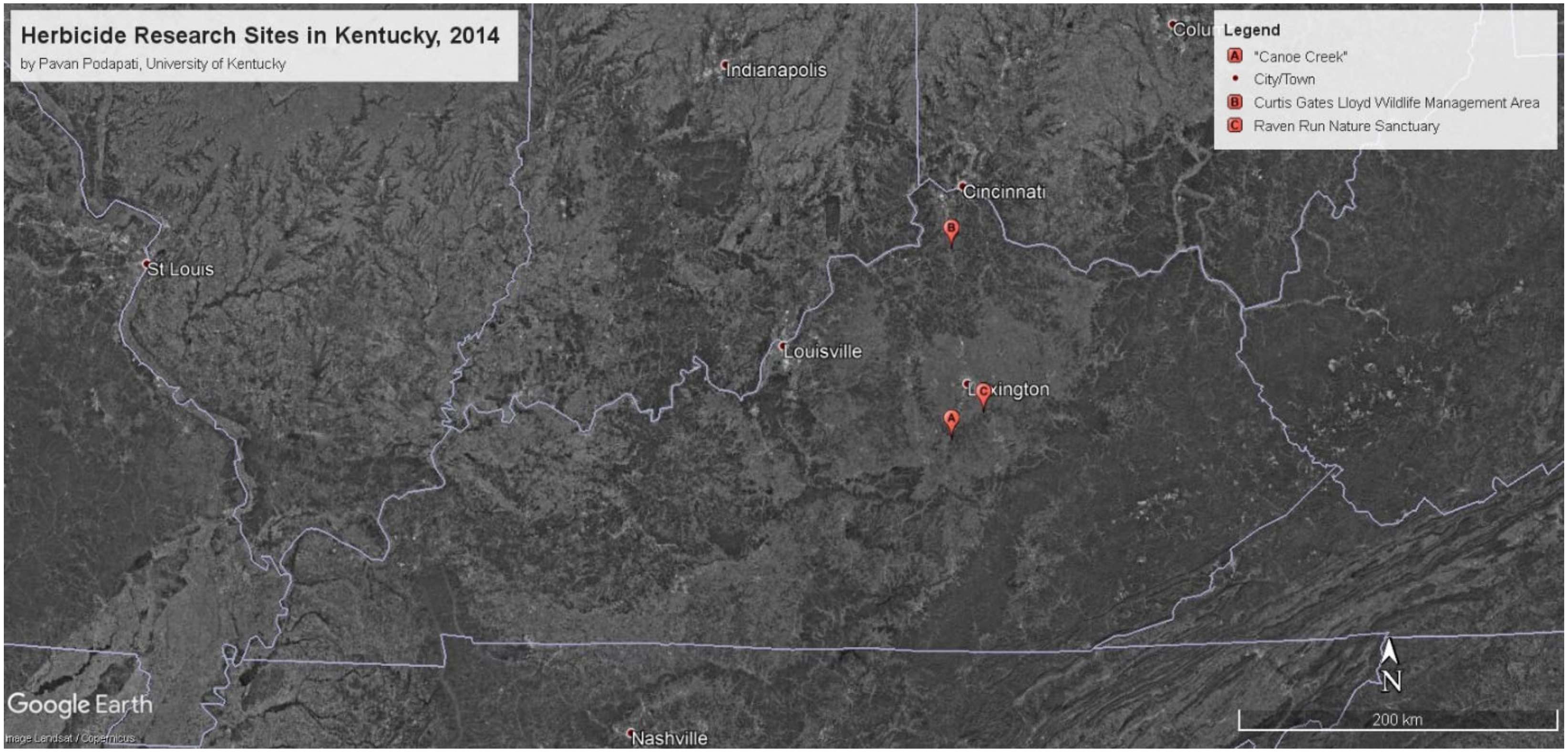

Figure 1.1 *Alliaria petiolata* and non-target plant study sites are marked by icons "B" and "C" respectively. Site "A" ("Canoe Creek") blocks were omitted from analysis, but I recommend future non-target plant research on additional non-target plants prevalent in blocks at this site (see "Discussion"). Landscape imagery dates to December 2013. Map data © 2013 Google.

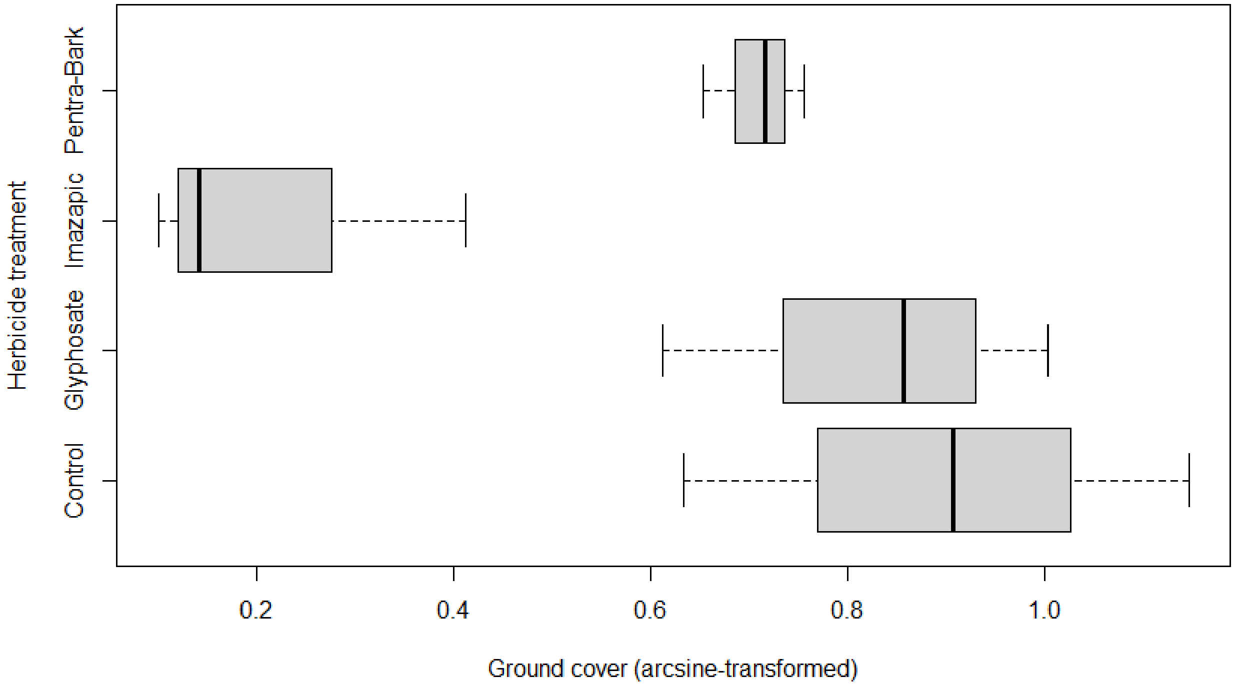

Figure 1.2 Percent ground cover (arcsine-transformed) distributions of *Alliaria petiolata* treatment populations at Curtis Gates Lloyd WMA, Crittenden, KY, May 2014.

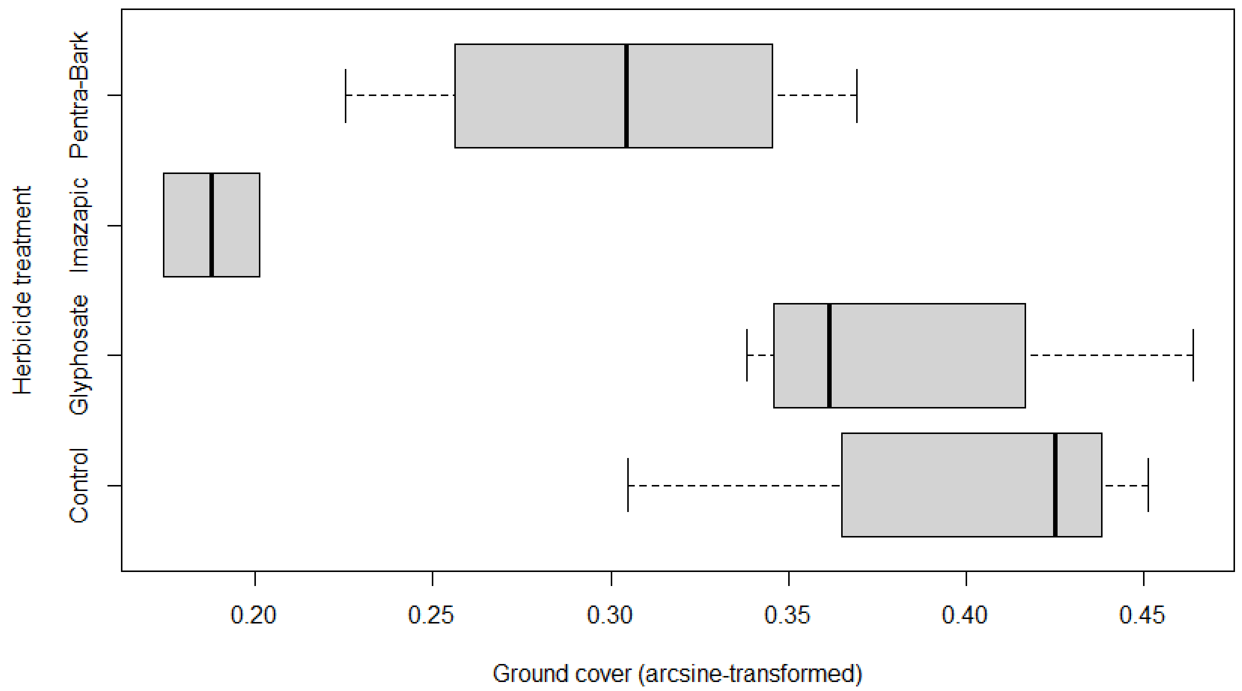

Figure 1.3 Percent ground cover (arcsine-transformed) distributions of *Claytonia virginica* treatment populations at Raven Run Nature Sanctuary, Lexington, KY, April 2014.

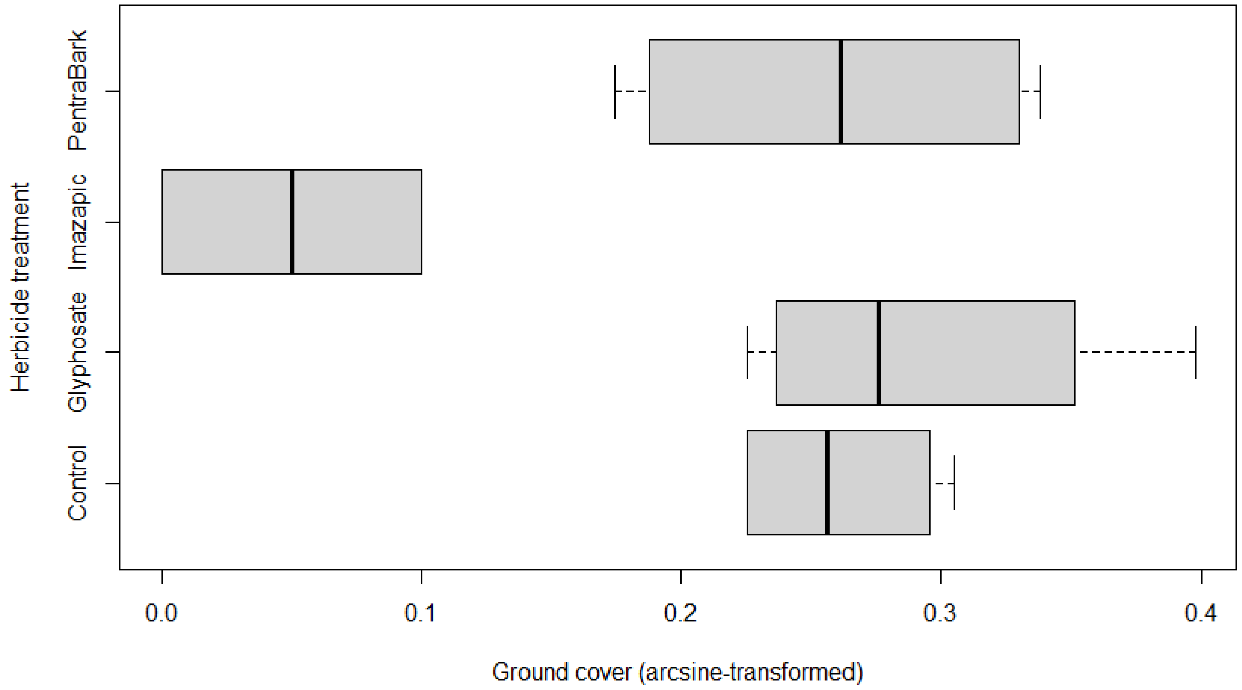

Figure 1.4 Percent ground cover (arcsine-transformed) distributions of *Erigenia bulbosa* treatment populations at Raven Run Nature Sanctuary, Lexington, KY, April 2014.

APPENDICES

APPENDIX A. SOIL SAMPLE AND OVERSTORY DENSITY MEASUREMENTS AT HERBICIDE TREATMENT SITES

I measured overstory density using a convex spherical densiometer, and submitted soil samples to the University of Kentucky Soil Testing Laboratories for routine soil analysis. Soil pH in the routine soil test used 1M KCl instead of water. For producer reports, the University of Kentucky Soil Testing Laboratories calculated soil-water pH using the following equation based on the analysis of 240 soil samples from other separate soil studies in March 2009:

$$\textit{soil-water pH} = 0.91 \times 1\ M\ KCl\ \textit{soil pH} + 1.34$$

Site	**Soil Sample Date**	**Overstory Density Estimate Date**	**Block Name**	**Soil Texture**	**Soil Sand (%)**	**Soil Silt (%)**	**Soil Clay (%)**	**1M KCl Soil pH**	**Calculated Soil-Water pH**	**Sikora II Buffer pH**
Raven Run Nature Sanctuary	6 Aug 2013	15 Aug 2013	RN1	Silt loam	15.96	63.52	20.52	4.71	5.63	6.44
		15 Aug 2013	RN2	Silt loam	15.96	63.52	20.52	4.71	5.63	6.44
		15 Aug 2013	RN3	Silt loam	15.96	63.52	20.52	4.71	5.63	6.44
		15 Aug 2013	RN4	Silt loam	15.96	63.52	20.52	4.71	5.63	6.44
Curtis Gates Lloyd WMA	28 Jul 2013	28 Jul 2013	LN1	Silt loam	6.54	79.21	14.25	4.15	5.12	6.41
		28 Jul 2013	LN2	Silt loam	6.54	79.21	14.25	4.15	5.12	6.41
		28 Jul 2013	LN3	Silt loam	6.54	79.21	14.25	4.15	5.12	6.41
		28 Jul 2013	LN4	Silt loam	6.54	79.21	14.25	4.15	5.12	6.41

Site	**Soil Sample Date**	**Overstory Density Estimate Date**	**Block Name**	**Soil P (kg/ha)**	**Soil K (kg/ha)**	**Soil Ca (kg/ha)**	**Soil Mg (kg/ha)**	**Soil Zn (kg/ha)**	**Overstory Density (%)**
Raven Run Nature Sanctuary	Aug 6, 2013	August 15, 2013	RN1	818	171	4682	208	1.5	88
		August 15, 2013	RN2	818	171	4682	208	1.5	94
		August 15, 2013	RN3	818	171	4682	208	1.5	93
		August 15, 2013	RN4	818	171	4682	208	1.5	94
Curtis Gates Lloyd WMA	July 28, 2013	July 28, 2013	LN1	146	251	1604	161	4.6	91
		July 28, 2013	LN2	146	251	1604	161	4.6	87
		July 28, 2013	LN3	146	251	1604	161	4.6	92
		July 28, 2013	LN4	146	251	1604	161	4.6	86

APPENDIX B. PERCENT GROUND COVER OF FOREST FLOOR PLANTS AT RAVEN RUN NATURE SANCTUARY

Species counts shown here are not comprehensive, as two species were omitted due to lack of identification. *Viola pubescens* and *V. sororia* were previously identified at this site, although other *Viola* spp. were not always distinguishable. Gray-shaded cells indicate presence of injured plants (April 5, 2014, Lexington, KY).

Block	**Treatment**	*Alliaria petiolata (1st-year)*	*Alliaria petiolata (2nd-year)*	*Acer nigrum/saccharum*	*Aesculus flava*	*Aesculus glabra*	*Allium tricoccum*	*Allium vineale*	*Bignonia capreolata*	*Cardamine concatenata*	*Cardamine douglassii*	*Claytonia virginica*	*Delphinium tricorne*	*Erigenia bulbosa*	*Erythronium americanum*	*Hydrophyllum macrophyllum*	*Lonicera maackii*	*Stellaria media*	*Stellaria pubera*	*Trillium sessile*	*Viburnum prunifolium*	*Viola* spp.
RN1	Pentra-Bark®	1	0	5	0	0	7	0	3	7	5	13	7	11	0	0	0	8	8	0	0	4
RN1	Imazapic	0	0	2	7	0	0	0	3	4	0	3	5	1	0	5	0	0	0	1	0	2
RN1	Glyphosate	1	2	3	0	2	1	0	3	7	4	20	13	15	0	0	0	0	0	3	0	3
RN1	Control	0	0	4	0	0	4	0	1	3	2	19	11	8	1	5	2	0	0	1	0	4
RN2	Pentra-Bark®	1	0	5	0	0	0	0	1	6	1	5	9	10	0	11	0	0	0	1	0	5
RN2	Imazapic	0	0	1	0	0	0	0	0	3	2	3	9	1	0	16	0	0	0	0	0	0
RN2	Control	1	0	2	0	0	0	1	0	10	3	17	5	9	4	26	0	0	0	1	0	0
RN2	Glyphosate	1	0	2	0	0	0	4	0	8	2	11	6	9	4	13	0	0	0	2	0	3

Block	**Treatment**	*Alliaria petiolata (1st-year)*	*Alliaria petiolata (2nd-year)*	*Acer nigrum/saccharum*	*Aesculus flava*	*Aesculus glabra*	*Allium tricoccum*	*Allium vineale*	*Bignonia capreolata*	*Cardamine concatenata*	*Cardamine douglassii*	*Claytonia virginica*	*Delphinium tricorne*	*Erigenia bulbosa*	*Erythronium americanum*	*Hydrophyllum macrophyllum*	*Lonicera maackii*	*Stellaria media*	*Stellaria pubera*	*Trillium sessile*	*Viburnum prunifolium*	*Viola*
RN3	Imazapic	1	0	0	0	0	0	0	0	0	0	4	1	0	0	13	0	4	2	0	0	3
RN3	Pentra-Bark®	1	0	2	0	0	0	0	0	1	3	10	5	3	0	13	1	9	13	0	6	3
RN3	Control	0	0	2	0	5	0	0	0	5	3	9	0	5	1	13	0	11	7	2	0	5
RN3	Glyphosate	1	0	1	4	0	0	0	0	0	0	12	0	5	2	0	0	8	11	0	0	0
RN4	Glyphosate	1	0	0	0	0	0	0	2	6	1	13	3	6	11	0	0	3	4	0	0	2
RN4	Control	1	3	0	0	0	0	0	3	0	2	17	1	5	12	0	0	1	3	2	0	2
RN4	Pentra-Bark®	0	0	1	0	0	0	0	0	8	1	8	1	4	15	0	0	0	0	1	0	0
RN4	Imazapic	1	0	1	0	0	0	0	0	0	0	4	1	0	15	4	0	0	0	3	0	0

APPENDIX C. PERCENT GROUND COVER OF FOREST FLOOR PLANTS AT CURTIS GATES LLOYD WMA

Species counts shown here are not comprehensive. *Valerianella* and *Viola* spp. identification was left at the genus level due to time restraints or sometimes indistinguishable individuals, although *Viola pubescens* and *V. sororia* were previously observed in preliminary surveys. Gray-shaded cells indicate presence of injured plants (May 16, 2014, Crittenden, KY).

Block	**Treatment**	*Alliaria petiolata (1st-year)*	*Acer nigrum/saccharum*	*Cardamine concatenata*	*Caulophyllum thalictroides*	*Erigenia bulbosa*	*Hydrophyllum macrophyllum*	*Laportea canadensis*	*Parthenocissus quinquefolia*	*Pilea pumila*	*Podophyllum peltatum*	*Polygonatum biflorum*	*Valerianella* spp.	*Viola* spp.
non1	Pentra-Bark®	43	0	1	0	1	0	83	0	0	5	0	0	0
non1	Control	62	0	0	0	0	0	91	0	0	19	0	0	0
non1	Glyphosate	57	0	0	0	0	0	57	0	0	0	0	0	0
non1	Imazapic	1	0	0	0	0	0	5	0	0	2	0	0	0
non2	Glyphosate	33	0	0	14	0	0	23	0	0	11	0	0	0
non2	Imazapic	2	2	1	1	0	0	26	0	0	11	1	0	0
non2	Pentra-Bark®	47	0	1	13	0	5	10	0	0	10	4	0	0
non2	Control	83	3	0	0	0	0	11	0	0	27	0	0	2
non3	Glyphosate	71	2	0	0	0	3	37	0	0	11	0	0	0
non3	Control	35	3	1	0	0	0	25	0	0	21	0	0	0
non3	Pentra-Bark®	37	0	0	0	0	0	23	0	0	21	0	0	0
non3	Imazapic	16	0	0	0	0	0	57	0	0	9	0	13	0

REFERENCES

Aichele, T.M., and D. Penner. 2005. Adsorption, desorption, and degradation of imidazolinones in soil. Weed Technology 19:154-159.

Bahm, M.A., and T.G. Barnes. 2011. Native grass and forb response to pre-emergent application of imazapic and imazapyr. Natural Areas Journal 31:75-79.

Baskin, J.M., and C.C. Baskin. 1992. Seed germination biology of the weedy biennial Alliaria petiolata. Natural Areas Journal 12:191-197

Bangsund, D.A., F.L. Leistritz., and J.A. Leitch. 1999. Assessing economic impacts of biological control of weeds: the case of leafy spurge in the northern Great Plains of the United States. Journal of Environmental Management 56:35-43.

Bartuszevige, A.M., R.L. Hrenko, and D.L. Gorchov. 2007. Effects of leaf litter on establishment, growth and survival of invasive plant seedlings in a deciduous forest. American Midland Naturalist 158:472–477.

Beran, D.D., R.E. Gaussoin, and R.A. Masters. 1999. Native wildflower establishment with imadizolinone herbicides. HortScience 34:283-286.

Bowles, M.L., K.A. Jacobs, and J.L. Mengler. 2007. Long-term changes in an oak forest's woody understory and herb layer with repeated burning. Journal of the Torrey Botanical Society 134:223-237.

Bossdorf, O., D. Prati, H. Auge, and S. Bernhard. 2004. Reduced competitive ability in an invasive plant. Ecology Letters 7:346-353.

Brosnan, J.T., G.K. Breeden, M.T. Elmore, A.J. Patton, and D.V. Weisenberger. 2012. Zoysiagrass seedhead suppression with imidazolinone herbicides. Weed Technology 26:708-713.

Burke, D.J. 2008. Effects of *Alliaria petiolata* (garlic mustard; Brassicaceae) on mycorrhizal colonization and community structure in three herbaceous plants in a mixed deciduous forest. American Journal of Botany 95:1416-1425.

[CABI] Centre for Agriculture and Bioscience International. 2020. Biological control of garlic mustard. Accessed 27 May 2020 from <https://www.cabi.org/projects/biological-control-of-garlic-mustard/>.

Campbell, J., D.G. Ruch, and W. Meijer. 1995. The flora and vegetation of Raven Run Nature Sanctuary, Fayette County, Kentucky. Pp. 139-184 *in* Proceedings of the Indiana Academy of Science. Indiana Academy of Science, Indianapolis, IN.

Cantor, A., A. Hale, J. Aaron, M.B. Traw, and S. Kalisz. 2011. Low allelochemical concentrations detected in garlic mustard-invaded forest soils inhibit fungal growth and AMF spore germination. Biological Invasions 13:1-11.

Cassin, C., and P. Kotanen. 2016. Invasive earthworms as seed predators of temperate forest plants. Biological Invasions 18:1567-1580.

Castellano, S.M., and D.L. Gorchov. 2012. Reduced ectomycorrhizae on oak near invasive garlic mustard. Northeastern Naturalist 19:1-24.

Chen, W. 1996. Use of endemic pathogens to control garlic mustard (*Alliaria petiolata*). Center for Biodiversity Technical Report 1996 (25). Illinois Natural History Survey, Champaign, IL.

Christiansen, A., A. Peterson, S.C. Anderson, R. Lass, M. Johnson, and A.M. Nienow. 2015. Analysis of the photodegradation of the imidazolinone herbicides imazamox, imazapic, imazaquin, and imazamethabenz-methyl in aqueous solution. Journal of Agricultural and Food Chemistry 63:10768-10777.

Ciola, V., and D. Cipollini. 2011. Distribution and host range of a powdery mildew fungus infecting garlic mustard, *Alliaria petiolata*, in southwestern Ohio. The American Midland Naturalist 166:40-52.

Cipollini, D. 2002. Variation in the expression of chemical defenses of *Alliaria petiolata* (Brassicacaeae) in the field and common garden. American Journal of Botany 89:1422-1430.

Cipollini, D., and B. Gruner. 2007. Cyanide in the chemical arsenal of garlic mustard, Alliaria petiolata. *Journal of Chemical Ecology, 33*(1), 85-94.

Cipollini, D., S. Davis, D. Lieurance, K. Cipollini, and V. Bahn. 2020. Biogeographic variation in resistance of the invasive plant, *Alliaria petiolata*, to a powdery mildew fungus and effect of resistance on competitive dynamics. Biological Invasions 22:1657-1668.

Clay, S.A. 2021. Near-term challenges for global agriculture: herbicide-resistant weeds. Agronomy Journal. doi: 10.1002/agj2.20749.

Cohen, J. 1988. Statistical power analysis for the behavioral sciences (2nd ed.). L. Erlbaum Associates, Hillsdale, NJ.

Comer, P., D. Faber-Langendoen, R. Evans, S. Gawler, C. Josse, G. Kittel, S. Menard, M. Pyne, M. Reid, K. Schulz, K. Snow, and J. Teague. 2003. Ecological Systems of the United States: A Working Classification of U.S. Terrestrial Systems. NatureServe, Arlington, VA.

Cox, C. 2003. Imazapic. Journal of Pesticide Reform 23:10-14.

Cruden, R.W., A.M. McClain, and G.P. Shrivastava. 1996. Pollination biology and breeding system of *Alliaria petiolata* (Brassicaceae). Bulletin of the Torrey Botanical Club 123:273-280.

Davis, A.S., D.A. Landis, V. Nuzzo, B. Blossey, E. Gerber, and H. Hariet. 2006. Demographic models inform selection of biocontrol agents for garlic mustard (*Alliaria petiolata*). Ecological Applications 16:2399-2410.

Delgado-Baquerizo, M., F.T. Maestre, P.B. Reich, T.C. Jeffries, J.J. Gaitan, D. Encinar, M. Berdugo, C.D. Campbell, and B.K. Singh. 2016. Microbial diversity drives multifunctionality in terrestrial ecosystems. Nature Communications 7. doi: 10.1038/ncomms10541.

Didham, R., J. Tylianakis, M. Hutchinson, R. Ewers, and N. Gemmell. 2005. Are invasive species the drivers of ecological change? Trends in Ecology & Evolution 20:470-474.

da Costa Marinho, M.I., A.I.G. Costa, N.M. Vieira, M.C.G. Paiva, F.C.L. de Freitas, and A.A. da Silva. 2019. Validation and application of a QuEChERS based method for estimation of half-life of imidazolinone herbicides in soils by LC-ESI-MS/MS. Ecotoxicology and Environmental Safety 167:212-217.

DiTomaso, J.M., G.B. Kyser, S.R. Oneto, R.G. Wilson, S.B. Orloff, L.W. Anderson, S.D. Wright, J.A. Roncoroni, T.L. Miller, T.S. Prather, C. Ransom, K.G. Beck, C. Duncan, K.A. Wilson, and J.J. Mann. 2013. Weed Control in Natural Areas in the Western United States. University of California Weed Research and Information Center, Davis, CA.

Drayton, B., and R.B. Primack. 1999. Experimental extinction of garlic mustard (*Alliaria petiolata*) populations: implications for weed science and conservation biology. Biological Invasions 1:159-167.

Duke, S.O., and S.B. Powles. 2008. Glyphosate: a once-in-a-century herbicide. Pest Management Science 64:319-325.

Durka, W., O. Bossdorf, D. Prati, and H. Auge. 2005. Molecular evidence for multiple introductions of garlic mustard (*Alliaria petiolata*, Brassicaceae) to North America. Molecular Ecology 14:1697-1706.

Ehrenfeld, J. 2003. Effects of exotic plant invasions on soil nutrient cycling processes. Ecosystems 6:503-523.

Elliott, K.J., J.M. Vose, J.D. Knoepp, B.D. Clinton, and B.D. Kloeppel. 2015. Functional role of the herbaceous layer in eastern deciduous forest ecosystems. Ecosystems 18**:**221–236

Elmore, C.L. 1996. Use of phenoxy herbicides in turfgrass in the United States. Pp. 103-125 *in* Biologic and Economic Assessment of Benefits from Use of Phenoxy Herbicides in the United States. l-PA-96, US Department of Agriculture, National Agricultural Pesticide Impact Assessment Program, Washington, DC.

Eisler, R. 1991. Cyanide hazards to fish, wildlife, and invertebrates: a synoptic review. Biological Report 85(1.23), US Fish and Wildlife Service, Patuxent Wildlife Research Center, Laurel, MD.

Evans, J.A. and D.A. Landis. 2007. Pre-release monitoring of *Alliaria petiolata* (garlic mustard) invasions and the impacts of extant natural enemies in southern Michigan forests. Biological Control 42:300–307.

Felix, J., R. Boydston, and I.C. Burke. 2011. Potato response to simulated glyphosate drift. Weed Technology 25:637-644.

Fenneman, N.M. 1938. Physiography of eastern United States. McGraw-Hill Book Co., New York, NY.

Fox, J., and S. Weisberg. 2018. An R Companion to Applied Regression. 3rd ed. Sage Publications, Thousand Oaks, CA.

Fisher, B., R.K. Turner, and P. Morling. 2009. Defining and classifying ecosystem services for decision making. Ecological Economics 68:643-653.

Frey, M.N., C.P. Herms, and J. Cardina. 2007. Cold weather application of glyphosate for garlic mustard (*Alliaria petiolata*) control. Weed Technology 21:656-660.

Gerber, E., G. Cortat, H.L. Hinz, B. Blossey, E. Katovich, and L. Skinner. 2009. Biology and host specificity of *Ceutorhynchus scrobicollis* (Curculionidae; Coleoptera), a root-crown mining weevil proposed as biological control agent against *Alliaria petiolata* in North America. Biocontrol Science and Technology 19:117-138.

Gilbert, B., and M.J. Lechowicz. 2005. Invasibility and abiotic gradients: the positive correlation between native and exotic plant diversity. Ecology 86:1848–1855.

Gilliam, F.S. 2007. The ecological significance of the herbaceous layer in temperate forest ecosystems. BioScience 57: 845-858.

Gleadow, R.M., and I.E. Woodrow. 2002. Constraints on effectiveness of cyanogenic glycosides in herbivore defense. Journal of Chemical Ecology 28:1301-1313.

Grey, T.L., E.P. Prostko, C.W. Bednarz, and J.W. Davis. 2017. Cotton (*Gossypium hirsutum*) response to simulated imazapic residues. Weed Technology 19:1045-1049.

Grichar, W.J., D.L. Jordan, and E.P. Prostko. 2012. Weed control and peanut (*Arachis hypogaea* L.) response to formulations of imazapic. Crop Protection 36:31-36.

Grimm, G.W., T. Denk, and V. Hemleben. 2007. Evolutionary history and systematics of *Acer* section *Acer* – a case study of low-level phylogenetics. Plant Systematics and Evolution 267:215-253.

Haney, R.L., S.A. Senseman, F.M. Hons, and D.A. Zuberer. 2000. Effect of glyphosate on soil microbial activity and biomass. Weed Science 48:89-93.

Harrington, K.C., A.J. Ward, and D.M. Wells. 2001. Herbicide resistance in black nightshade and Onehunga weed. New Zealand Plant Protection 54:152-156.

Harir, M., A. Gaspar, M. Frommberger, M. Lucio, M. El Azzouzi, D. Martens, A. Kettrup, and P. Schmitt-Kopplin. 2007. Photolysis pathway of imazapic in aqueous solution: ultrahigh resolution mass spectrometry analysis of intermediates. Journal of Agricultural and Food Chemistry 55:9936-9943.

Heap, I. 2013. Global perspective of herbicide-resistant weeds. Pest Management Science 70:1306-1315.

Heap, I. 2021. The International Survey of Herbicide Resistant Weeds. Accessed 31 March 2021 from <http://www.weedscience.org>.

Howard, T.G., J. Gurevitch, L. Hyatt, M. Carreiro, and M. Lerdau. 2004. Forest invasibility in communities in southeastern New York. Biological Invasions 6:393–410.

Herold, J., M.R. Anderson, J.T. Bauer, V. Borowicz, and R.C. Anderson. 2011. Comparison of the effect of early and late removal of second-year garlic mustard (*Alliaria petiolata*) on first-year plants and deciduous forest spring and summer dominant herbaceous groundlayer species in Central Illinois, USA. Ecological Restoration 29:225-233.

Hochstedler, W.W., B.S. Slaughter, D.L. Gorchov, L.P. Saunders, and M.H.H. Stevens. 2007. Forest floor plant community response to experimental control of the invasive biennial, *Alliaria petiolata* (garlic mustard). The Journal of the Torrey Botanical Society 134:155-165.

Huang, Y., K.N. Reddy, S.J. Thomson, and H. Yao. 2015. Assessment of soybean injury from glyphosate using airborne multispectral remote sensing. Pest Management Science 71:545-552.

Islam, F., J. Wang, M.A. Farooq, M.S.S. Khan, L. Xu, J. Zhu, M. Zhao, S. Muños, Q.X. Li, and W. Zhou. 2017. Potential impact of the herbicide 2,4-dichlorophenoxyacetic acid on human and ecosystems. Environment International 111:332-351.

Jones, R. 2005. Plant Life of Kentucky: An Illustrated Guide to the Vascular Flora. The University Press of Kentucky, Lexington, KY.

Katovich, E.J., R.L. Becker, E. Gerber, H.L. Hinz, and G. Cortat. 2020. Lessons learned: rearing the crown-boring weevil, *Ceutorhynchus scrobicollis* (Coleoptera: Curculionidae), in containment for biological control of garlic mustard (*Alliaria petiolata*). The Great Lakes Entomologist 52:78-93.

Lankau, R.A., V. Nuzzo, G. Spyreas, and A.S. Davis. 2009. Evolutionary limits ameliorate the negative impact of an invasive plant. Proceedings of the National Academy of Sciences of the United States of America 106:15362-15367.

Lefcheck, J.S., J.E.K. Byrnes, F. Isbell, L. Gamfeldt, J.N. Griffin, N. Eisenhauer, M.J.S. Hensel, A. Hector, B.J. Cardinale, and J.E. Duffy. 2015. Biodiversity enhances ecosystem multifunctionality across trophic levels and habitats. Nature Communications 6. doi: 10.1038/ncomms7936.

Litt, A.R., B.J. Herring, and L. Provencher. 2001. Herbicide effects on ground-layer vegetation in southern pinelands, USA: a review. Natural Areas Journal 21:177-188.

Luken, J.O., and M. Shea. 2000. Repeated prescribed burning at Dinsmore Woods State Nature Preserve (Kentucky, USA): responses of the understory community. Natural Areas Journal 20:150-158.

Markle, D.M., and R.G. Lym. 2001. Leafy spurge (*Euphorbia esula*) control and herbage production with imazapic. Weed Technology 15:474-480

Martini, L.F.D., L.A. Avila, K.M. Souto, G. Cassol, J.P. Refatti, E. Marchesan, and C.A.P. Barros. 2011. Imazethapyr + imazapic leaching in lowland soil as affected by rice irrigation management. Planta Daninha 29:185-193.

Meekins, J.F., and B.C. McCarthy. 1999. Competitive ability of *Alliaria petiolata* (garlic mustard, Brassicaceae), an invasive, nonindigenous forest herb. International Journal of Plant Sciences 160:743-752.

Meekins, J.F., and B.C. McCarthy. 2000. Responses of the biennial forest herb *Alliaria petiolata* to variation in population density, nutrient addition and light availability. Journal of Ecology 88:447-463.

Meisner, A., W. de Boer, J.H.C. Cornelissen, and W.H. van der Putten. 2012. Reciprocal effects of litter from exotic and congeneric native plant species via soil nutrients (litter effects of exotic plant species). PLoS ONE 7. doi: 10.1371/journal.pone.0031596.

Morris, C., T.A. Monaco, and C.W. Rigby. 2009. Variable impacts of imazapic rate on downy brome (*Bromus tectorum*) and seeded species in two rangeland communities. Invasive Plant Science and Management 2:110-119.

Moser, W.K., E.L. Barnard, R.F. Billings, S.J. Crocker, M.E. Dix, A.N. Gray, G.G. Ice, M. Kim, R. Reid, S.U. Rodman, and W.H. McWilliams. 2009. Impacts of nonnative invasive species on US forests and recommendations for policy and management. Journal of Forestry 107:320-327.

Miller, J.H., S.T. Manning, and S.F. Enloe. 2013. A management guide for invasive plants in southern forests. GTR-SRS-131, US Department of Agriculture, Forest Service, Asheville, NC.

Nandula, V.K., K.N. Reddy, S.O. Duke, and D.H. Poston. 2005. Glyphosate-resistant weeds: current status and future outlook. Outllooks on Pest Management 16:183-187.

[NOAA] National Oceanic and Atmospheric Administration. 2021. Record of climatological observations. National Centers for Environmental Information. Accessed 3 March 2021 from <https://www.ncdc.noaa.gov/cdo-web/datatools/lcd>.

[NPIC] National Pesticide Information Center. 2002. Triclopyr (Technical Fact Sheet). Oregon State University, Corvalis, OR.

[NRCS] Natural Resources Conservation Service. 2016. Web Soil Survey. Accessed 30 July 2016 from <https://websoilsurvey.sc.egov.usda.gov/≥.

Nuzzo, V.A. 1991. Experimental control of garlic mustard [*Alliaria petiolata* (Bieb.) Cavara & Grande] in northern Illinois using fire, herbicide, and cutting. Natural Areas Journal 11:158-167.

Nuzzo, V.A. 1996. Impact of dormant season herbicide treatment on the alien herb garlic mustard (*Alliaria petiolata* [Bieb.] Cavara and Grande) and groundlayer vegetation. Transactions of the Illinois State Academy of Science 89:25-36.

Nuzzo, V. 1999. Invasion pattern of the herb garlic mustard (*Alliaria petiolata*) in high quality forests. Biological Invasions 1:169-179.

Nuzzo, V.A., W. McClain. and T. Strole. 1996. Fire impact on groundlayer flora in a sand forest 1990-1994. The American Midland Naturalist 136:207-221.

Overbeck, W. 2014. "The Vascular Flora of Garrard County, Kentucky." Master’s thesis, *Online Theses and Dissertations* (228), http://encompass.eku.edu/etd/228

Owen, S.M., C.H. Sieg, and C.A. Gehring. 2011. Rehabilitating downy brome (*Bromus tectorum*)–invaded shrublands using imazapic and seeding with native shrubs. Invasive Plant Science and Management 4:223-233.

Pardini, E.A., J.M. Drake, J.M. Chase, and T.M. Knight. 2009. Complex population dynamics and control of the invasive biennial *Alliaria petiolata* (garlic mustard). Ecological Applications 19:387-397.

Peterson, M.A., S.A. McMaster, D.E. Riechers, J. Skelton, and P.W. Stahlman. 2016. 2,4-D past, present, and future: a review. Weed Technology 30:303-345.

Pimentel, D., R. Zuniga, and D. Morrison. 2005. Update on the environmental and economic costs associated with alien-invasive species in the United States. Ecological Economics 52:273-288.

Prati, D., and O. Bossdorf. 2004. Allelopathic inhibition of germination by *Alliaria petiolata* (Brassicaceae). American Journal of Botany 91:285-288.

Prostko, E.P., T.L. Grey, and J.W. Davis. 2009. Imidazolinone-resistant sunflower tolerance to imazapic. Weed Technology 23:188-190.

R Core Team. 2021. R: A language and environment for statistical computing (version 4.1.0). R Foundation for Statistical Computing, Vienna, Austria. Accessed 1 June 2021 from <https://www.R-project.org/>.

RStudio Team 2021. RStudio: Integrated Development Environment for R (version 1.4.1106). RStudio, PBC, Boston, MA. Accessed 1 June 2021 from <http://www.rstudio.com/>.

Redwood, M.E., G.R. Matlack, and C.D. Huebner. 2018. Seed longevity and dormancy state suggest management strategies for garlic mustard (*Alliaria petiolata*) and Japanese stiltgrass (*Microstegium vimineum*) in deciduous forest sites. Weed Science 66:190-198.

Refatti, J. P., De Avila, L. A., Noldin, J. A., Pacheco, I., & Pestana, R.R. (2017). Leaching and residual activity of imidazolinone herbicides in lowland soils. Ciência Rural 47. doi: 10.1590/0103-8478cr20151147.

Riper, L.C.V., R.L. Becker, and L.C. Skinner. 2010. Population biology of garlic mustard (*Alliaria petiolata*) in Minnesota hardwood forests. Invasive Plant Science and Management 3:48-59.

Roberts, H.A., and J.E. Boddrell. 1983. Seed survival and periodicity of seedling emergence in eight species of Cruciferae. Annals of Applied Biology 103:301-304.

Rodgers, V.L., K.A. Stinson, and A.C. Finzi. 2008. Ready or not, garlic mustard is moving in: *Alliaria petiolata* as a member of eastern North American forests. BioScience 58:426-436.

Saari, L.L., J.C. Cotterman, and D.C. Thill. 1994. Resistance to acetolactate synthase inhibiting herbicides. Pp. 83-139 *in* S.B. Bowles and J.A.M. Holtum, eds., Herbicide Resistance in Plants: Biology and Biochemistry. 2nd ed. CRC Press, Boca Raton, FL.

St. Hilaire, R., W.R. Graves, and R.L. Small. 2001. Variation in TaqI-digested DNA of sugar and black maples is independent of taxon and plant origin. HortScience 36:1327-1328.

Sayre, R., P. Comer, H. Warner, and J. Cress. 2009. A new map of standardized terrestrial ecosystems of the conterminous United States. Professional Paper 1768, US Department of the Interior, Geological Survey, Reston, VA.

Schardt, J., and M.D. Netherland. 2020. 3.7.1 Chemical control of aquatic weeds. Pp. 71-88 *in* L.A. Gettys, W.T. Haller, D.G. Petty, eds., Biology and Control of Aquatic Plants: A Best Management Practices Handbook. 3rd ed. Aquatic Ecosystem Restoration Foundation, Marietta, GA.

Scott, B., J. Norsworthy, T. Barber, and J. Hardke. 2013. Rice weed control. Pp. 51-61 *in* J.T. Hardke, ed., Arkansas Rice Production Handbook. MP192, University of Arkansas Division of Agriculture, Cooperative Extension Service, Little Rock, AR.

Sewell, A., and C. Smitson. 2008. Kentucky forest stewardship plan. Case Record No. 081-08-150, Kentucky Division of Forestry, Frankfort, KY.

Solis, K. 1998. Update: new results indicate flowering garlic mustard should be bagged and destroyed (Wisconsin). Restoration and Management Notes 16:223-224.

Sprankle, P., W.F. Meggit, and D. Penner. 1975. Adsorption, mobility, and microbial degradation of glyphosate in the soil. Weed Science 23:229-234.

Shannon, S.M., J.T. Bauer, W.E. Anderson, and H.L. Reynolds. 2014. Plant-soil feedbacks between invasive shrubs and native forest understory species lead to shifts in the abundance of mycorrhizal fungi. Plant & Soil 382:317-328.

Shinn, S.L., and D. Thill. 2004. Tolerance of several perennial grasses to imazapic. Weed Technology 18:60-65.

Smith, S.E., and D. Read. 2008. Mycorrhizas in ecological interactions. Pp. 573-610 *in* Mycorrhizal Symbiosis. 3rd ed. Academic Press, Oxford, UK.

Strid, A., W. Hanson, A. Cross, and J. Jenkins. 2018. Triclopyr general fact sheet. Oregon State University Extension Services, National Pesticide Information Center. Retrieved 1 April 2021 from <http://www.npic.orst.edu/factsheets/triclopyrgen.html>.

Stinson, K.A., S.A. Campbell, J.R. Powell, B.E. Wolfe, R.M. Callaway, G.C. Thelen, S.G. Hallett, D. Prati, and J.N. Klironomos. 2006. Invasive plant suppresses the growth of native tree seedlings by disrupting belowground mutualisms. PLoS Biology 4. doi: 10.1371/journal.pbio.0040140.

Su, W., H. Hao, M. Ding, R. Wu, H. Xu, F. Xue, C. Shen, L. Sun, and C. Lu. 2019. Adsorption and degradation of imazapic in soils under different environmental conditions. PLoS ONE 14. doi: 10.1371/journal.pone.0219462.

Szentesi, A. 1991. Controversial components of plant apparency in *Alliaria petiolata* Cavara & Grande (Cruciferae). Symposium Biologia, Hungarica 39:237-244.

Tranel, P.J., and T.R. Wright. 2002. Resistance of weeds to ALS-inhibiting herbicides: what have we learned? Weed Science 50:700-712.

[UK] University of Kentucky. 2021. Chem-Weed-List-T. Department of Horticulture. Retrieved 31 March 31 2021 from <https://www.uky.edu/Ag/Horticulture/masabni/xreflist/Chem-Weed%20Files/Chem-Weed-List-T/Chem-Weed-List-T.htm>.

Vaughn, S.F., and M.A. Berhow. 1999. Allelochemicals isolated from tissues of the invasive weed garlic mustard (*Alliaria petiolata*). Journal of Chemical Ecology 25:2495-2504.

Weng, C.-Y., K.-C. Yang, C.-F. Hsieh, C. Hsieh, and M.-H. Su. 2017. Local neighborhood communities in the understory play a critical role by affecting regeneration niches and subsequent community assembly in a montane cloud forest. Ecological Research 32:821-833.

Wilson, R.D., J. Geronimo, and J.A. Armbruster. 1997. 2,4-D dissipation in field soils after applications of 2,4-D dimethylamine salt and 2,4-D 2-ethylhexyl ester. Environmental Toxicology and Chemistry 16:1239-1246.

Wolfe, B.E., V.L. Rodgers, K.A. Stinson, and A. Pringle. 2008. The invasive plant *Alliaria petiolata* (garlic mustard) inhibits ectomycorrhizal fungi in its introduced range. *Journal of Ecology* 96:777-783.

Woodburn, K.B., F.R. Batzer, F.H. White, and M.R. Schultz. 1993. The aqueous photolysis of triclopyr. Environmental Toxicology and Chemistry 12: 43-55.

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