

THE ROLE OF BIOTIC INTERACTIONS IN BIOLOGICAL INVASIONS

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ABSTRACT

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Invasive species are one of the major drivers of biodiversity loss, and it is estimated that invasive species cost billions in damage per year, globally. Given the economic costs and the potential ecological consequences of invasive species, it is important to understand how introduced species become integrated into natural communities and the consequences of invasion over longer time scales. To better predict and prevent future invasions, we must identify the mechanisms driving a small proportion of introduced species to become invasive.

Biotic interactions, such as herbivores and competitors, are among the major drivers of plant community structure and population dynamics. Release from antagonistic biotic interactions during the process of introduction may drive the explosive population growth rates of invasive species when they are transported to new ranges. However, subsequent acquisition of novel biotic interactions in the introduced range could explain why so many of the plants introduced around the world fail to become invasive. The Enemy Release Hypothesis (ERH) is one of the leading hypotheses explaining the success of invasive species and states that species once controlled by antagonistic biotic interactions in their native range will be able to reach high abundances once released from this control.

My dissertation research takes an integrative approach to rigorously test the oft-cited Enemy Release Hypothesis. Using field experiments including over 50 plant species, and a meta-analysis of the published literature to test ERH across a wider range of environments and species, I address four main questions:

1. Do invasive, noninvasive exotic, and native species experience different amounts of damage from enemies in the introduced range?
2. Does enemy release result in increased performance for invasive species compared to native and noninvasive exotic species?
3. Is enemy release lost with increased residence time and geographic spread in the introduced range?
4. Does tolerance to enemy damage or competitive ability drive invasiveness?

I found no evidence suggesting enemy release is a general mechanism contributing to invasiveness. Invasive species received the most damage from enemies and were equally affected by the presence of antagonistic biotic interactions, compared to native and noninvasive exotic species. Invasive species were no more tolerant to enemy damage than were native or noninvasive exotic species. For both invasive and noninvasive introduced plants, damage and the performance effects of that damage, increased with longer residence times and larger areas of spread in the introduced range. Our results show that invasive and exotic species fail to escape enemies, particularly over longer temporal and larger spatial scales. Key differences between introduced species that become invasive and those that do not may be the formation of successful mutualisms in the introduced range, and release from competition compared to native and noninvasive exotic species in the introduced range.

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To my fiancé, Jim.
I can't wait to spend our lives together
on the shores of Lake Michigan.

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CHAPTER 1: INTRODUCTION

Background

Biotic interactions, such as herbivores and competitors, are major drivers of plant community structure and population dynamics (Crawley 1989, Lubchenco 1978, Louda 1982, Klironomos 2002, Fitzsimons and Miller 2010). When species are introduced into new ranges, they leave behind many biotic interactions from their native range, however they are immediately met with a new suite of species in the invaded range. These species may eventually become new competitors, herbivores, pathogens and mutualists for the invader and may be just as influential on performance as lost interactions.

Elton (1958) was the first to describe the two potential roles played by biotic interactions for introduced species: release from antagonistic interactions from the native range may play a role in the explosive population growth rates of invasive species, and acquisition of antagonists in the introduced range may explain why so many of the species introduced around the world fail to establish and spread. The enemy release hypothesis (ERH) posits that invasiveness is driven by escape from enemies that constrained performance in the native range of an introduced species, and predicts that invasive species are less damaged and controlled by antagonistic biotic interactions than native species or noninvasive exotics.

Therefore, escape may only be a temporary feature experienced during early phases of invasion, and the magnitude of enemy release could change over time. Enemy release may be ephemeral, and plants and animals with longer residence times and larger areas of spread in their introduced range may be more strongly controlled by biotic interactions (Hawkes 2007, Mitchell et al. 2010).

Previous studies testing the ERH have focused primarily on enemy damage levels, and few have taken the next step to see if damage drives the increased performance of invasive species compared to native and noninvasive exotic species. Further, identifying the mechanisms that differ between introduced species that become invasive and those that do not is crucial to understand invasiveness. In addition, the effects of enemy release may be dynamic over the course of invasion as enemies accumulate in the introduced range. My dissertation addresses these shortcomings in the ERH literature by focusing on four main questions: (1) Do invasive, noninvasive exotic, and native species experience different amounts of damage from enemies in the introduced range? (2) Does enemy release result in increased performance for invasive species compared to native and noninvasive exotic species? (3) Is enemy release lost with increased residence time and geographic spread in the introduced range? (4) Does tolerance to enemy damage or competitive ability drive invasiveness?

Organization of the Dissertation

Chapter 2

In collaboration with Jennifer A. Lau and Andrea E. Berardi, I used three years of data from 61 plant species planted into common gardens to determine whether invasive, noninvasive exotic, and native species experience differential damage from insect herbivores and mammalian browsers, and whether enemy release is lost with increased residence time and geographic spread in the introduced range. We found no evidence suggesting enemy release is a general mechanism contributing to invasiveness in this region. Invasive species received the most insect herbivory, compared to native and noninvasive exotic species, and damage increased with longer residence times and larger range sizes at three spatial scales. Our results show that invasive and

noninvasive exotic species fail to escape enemies, particularly over longer temporal and larger spatial scales.

Chapter 3

To test whether enemy release explains invasive success, and how the benefits of enemy escape may change with increasing residence time, I conducted a three-year field experiment, manipulating the presence of insect herbivores, mammalian browsers and fungal disease on 50 native, noninvasive exotic, and invasive species. Counter to predictions, I found that native, noninvasive exotic, and invasive species experienced similar fitness benefits from enemy removal. On average, invasive species were no more tolerant to enemy damage than native or noninvasive exotic species, though some invasive species showed evidence for increased performance when damaged. Additionally, I found that enemy release was lost over time and strongest for recently introduced species. Though ERH was not a mechanism shared by invaders in this system, it may operate for some introduced species and early in the invasion process.

Chapter 4

In collaboration with Daniel MacGuigan, I performed a greenhouse experiment to investigate whether increased tolerance to herbivore damage or competitive ability could contribute to invasive plant success. We investigated whether invasive plants are more competitive than native and noninvasive exotics, and whether they are less affected by competition or exert stronger competitive effects on native species. We found the effects of competition and herbivory to be additive; the presence of our competition treatment did not affect the outcome of our herbivory clipping treatment, and vice versa. We also found that

introduced species were equally affected by herbivory and competition, compared to native species, yet they exerted stronger competitive effects on the native grass, *Elymus canadensis*. Therefore, competitive effects on native competitors, potentially through allelopathy and novel weapons, may contribute to the success of introduced species.

Chapter 5

In collaboration with Jennifer A. Lau and Ines Ibáñez, I performed a meta-analysis on studies that experimentally tested effects of biotic interactions (competition, disease/parasitism, herbivory, mutualism, plant-soil feedbacks, predation) on native, invasive, and noninvasive exotic plant and animal performance. We included cross-continental studies that manipulated biotic interactions in both the native and introduced range of a species, and studies comparing introduced species to co-occurring natives in the introduced range. Our meta-analysis provides strong evidence that antagonistic biotic interactions do not generally differ between native, invasive, and noninvasive exotic species. Removal of antagonistic biotic interactions increased performance equally for native, invasive, and noninvasive exotic species, indicating that biotic resistance is occurring for introduced species and that release is not a general mechanism explaining invasive species' success. However, while competition reduced performance for native and noninvasive exotic species, invasive species were generally not affected. In general, native and invasive species were negatively affected by the experimental removal of mutualist partners, while noninvasive exotic species were not. Therefore, key differences between introduced species that become invasive and those that do not may be the formation of successful mutualisms in the introduced range, and release from competition compared to native and noninvasive exotic species in the introduced range.

CHAPTER 2: NO RELEASE FOR THE WICKED: ENEMY RELEASE IS DYNAMIC AND NOT ASSOCIATED WITH INVASIVENESS

Introduction

Most introduced species do not establish, and even fewer become invasive (Williamson and Fitter 1996). Although invasive species have fascinated scientists for decades (Thellung 1912, Darwin 1895), the causal mechanisms of invasiveness are still undetermined. Some of the earliest writers on invasiveness predicted that loss of enemies in the introduced range might drive the success of invasive species over natives (Thellung 1912, citations within Kowarik and Pyšek 2012). Today, the Enemy Release Hypothesis (ERH) is the predominant, and most extensively tested, mechanism addressing the success of invasive species, and posits that invasive species gain a competitive advantage in their introduced range by escaping enemies that constrained their growth in their native range (Elton 1958, Callaway and Aschehoug 2000, Keane and Crawley 2002). An extension of the ERH is that invasive species are expected to receive reduced damage from enemies compared to co-occurring native species in their introduced range. Enemy release may result in increased population densities, and could explain how invasive species overcome usual controls on population growth such as density dependence and life history tradeoffs (Blair and Wolfe 2004, Martin et al. 2010).

Species introduced into new ranges sometimes experience reduced enemy diversity and damage compared to their native ranges (Mitchell and Power 2003, Torchin et al. 2003, Liu and Stiling 2006), and this reduced damage may translate into increased performance (Maron and Vila 2001). For example, parallel experiments in the native and introduced ranges of *Cynoglossum officinale* found that reduced insect herbivory in the introduced range led to increased performance and population growth rates for this species (Williams et al. 2010, see

also DeWalt et al. 2004). Similarly, a review of 473 species found that plants were attacked by 24% fewer virus and 84% fewer pathogen species in their introduced range, compared to their native range, and those species with a lower diversity of pathogens were more invasive, supporting ERH (Mitchell and Power 2003).

However, release from enemies found in the native range does not mean complete release from all enemy pressures. While enemy release may play a role in the explosive population growth rate of invasive species, the acquisition of new enemies in the introduced range could explain why so many introduced species fail to become invasive (Carpenter and Cappuccino 2005, Hawkes 2007). Elton (1958) was the first to describe the dual roles played by enemies during biological invasions: an introduced species leaves behind many of its enemies, but is immediately met with a novel set of potential enemies in its introduced range. These new interactions could be just as important as those initially lost, limiting the establishment and geographic spread of an introduced species, preventing it from becoming invasive (Elton 1958, Maron and Vila 2001). Introduced species released from enemy pressures are likely to experience increased population growth and competitive ability and as a result become invasive, while introduced species that do not experience release should not (Keane and Crawley 2002).

In this paper, we will follow the conventions used in previous studies (Cappuccino and Carpenter 2005, Liu et al. 2007, Parker and Gilbert 2007, Jogesh et al. 2008) and consider the following patterns of enemy damage evidence for ERH: (1) if enemy release explains the success of invasive species in their introduced range, we expect invaders to receive reduced damage compared to the native species with which they now compete (invasives < natives) and (2) if enemy release explains the differential success between introduced species that become invasive and introduced species that fail to become invasive (i.e., noninvasive exotics), we expect

invasives to receive less damage than noninvasive exotics (invasives < noninvasive exotics).

These same patterns would hold for ERH studies looking at other responses to enemies, such as enemy effects on plant performance and survival.

Many previous studies on ERH do not differentiate between introduced invasive and noninvasive exotic species, allowing them to test only the first prediction. Given the dual roles enemies play in invasions, homogenizing invasive and noninvasive exotic species into one group could miss important information on the drivers of invasiveness, and thus provide only a conservative estimate for whether invasive species experience enemy release. Studies that do not differentiate between these two types of introduced species have found that introduced plants receive less (Agrawal et al. 2005), no difference (Agrawal and Kotanen 2003, Hawkes 2007, Chun et al. 2010), or more (Ashton and Lerdau 2008, Stricker and Stiling 2014) enemy damage compared to natives. These same patterns are found in studies looking at enemy abundance or the performance consequences of enemy damage (reviewed in Colautti et al. 2004). For example, seed pathogens and predators have similar effects on the fecundity of native and introduced species (Blaney and Kotanen 2001a, Blaney and Kotanen 2001b, Blaney and Kotanen 2002).

A recent systematic review of the ERH literature found that there is as much evidence for ERH as there is against it (Heger and Jeschke 2014), and studies that find support for ERH tend to include just one pair of native and introduced congeners, while large multi-species experiments tend to find no difference in enemy effects between native and introduced species (Colautti et al. 2004). A meta-analysis by Chun and collaborators (2010) found that introduced plants in general receive similar amounts of damage as native species and their performance was reduced to a greater degree than was natives'. This lack of evidence for ERH may be due to combining invasive and noninvasive exotic species in analyses.

Studies that partition introduced species into invasive and noninvasive exotics are more rare (Liu and Stiling 2006) and find mixed support for ERH as well (ex. Cappuccino and Carpenter 2005, Carpenter and Cappuccino 2005, Parker et al. 2006, Liu et al. 2007, Parker and Gilbert 2007, Jogesh et al. 2008, Dawson et al. 2014). In a study of native, noninvasive exotic, and invasive *Eugenia* species, native species did in fact receive higher damage levels than invasives, supporting ERH Prediction 1 (Liu et al. 2007; see also Dietz et al. 2004 and Liu and Stiling 2006). The same study found no support for ERH Prediction 2 – invasive and noninvasive exotic *Eugenia* species received similar amounts of damage. Other studies support ERH Prediction 2, finding that invasive species received less enemy damage than noninvasive exotic species, or that introduced species that are more invasive tend to receive less herbivore damage or disease (Mitchell and Power 2003; Cappuccino and Carpenter 2005; Carpenter and Cappuccino 2005). These studies reveal that the relationship between enemy pressures and invasiveness is complex and variable across species, study systems, and time (e.g., Agrawal and Kotanen 2003, Agrawal et al. 2005).

Dynamic Invasions

An extension of the ERH is that the effects of enemy release may be dynamic over the course of invasion as enemies accumulate in the introduced range. As an introduced species spends more time in its introduced range, expanding into new habitats and occupying a greater area, its likelihood of encountering an enemy that can attack it increases, potentially leading to increased damage with increased residence time and geographic spread. While enemy release may facilitate colonization and establishment during the early stages of an invasion, these benefits could be lost over time as introduced species acquire enemies (Elton 1958, Mitchell et

al. 2006, Mitchell et al. 2010).

Species can accumulate enemies in their introduced range in three ways: (1) as invaders expand their range, they increase their probability of encountering an enemy in the introduced range that can attack them, (2) new introductions may bring enemies from the species' native range from which they had previously escaped, and (3) evolutionary changes or plasticity in native enemies or the introduced species may result in enemies being able to exploit an introduced species as a novel resource (Goßner et al. 2009). Therefore, the magnitude of enemy release is predicted to decrease over time and with range expansion into new habitats (Hawkes 2007, Mitchell et al. 2010).

Studies on both crops and undomesticated species find that introduced species accumulate enemies over time and with increasing geographic spread in the introduced range (Strong et al. 1977, Hawkes 2007). In a study of 124 plant species introduced to North America from Europe, Mitchell and collaborators (2010) found that pathogen richness increased with species' time since introduction and geographic range. Other work has found no relation between time since introduction and damage from enemies (Carpenter and Cappuccino 2005).

Despite the widespread ecological and evolutionary processes that vary over the course of an invasion, only 40% of recent invasion literature mentions the residence times of species (Strayer et al. 2006), and even fewer factor this into their experimental design. If enemy release is dynamic, it could explain some of the contradictory findings in previous studies comparing enemy attack on noninvasive exotic, invasive, and native species (Colautti et al. 2004).

Understanding whether invasive species acquire enemies over time and with range expansion will help to predict the long-term effects of biological invasions (Mitchell et al. 2006, Strayer et

al. 2006). These distinctions underscore the need for multi-species experiments to test the generality and persistence of enemy release.

Here, we address this need by testing the dynamic nature of enemy release, while differentiating between damage received by invasive and noninvasive introduced species. We conducted multi-year field experiments using 61 plant species from multiple families, three provenances (status = native, noninvasive exotic, invasive), and with a variety of introduction dates and areas of geographic spread. The objectives of our study are to test the major predictions of the ERH (listed above), and determine how damage from insect herbivores and mammalian browsers changes over the course of invasion. We address two questions: (1) Do invasive, noninvasive exotic, and native species experience different amounts of damage from enemies? (2) Is enemy release lost with increased residence time and geographic spread in the introduced range? We predict that if ERH contributes to invasiveness, invasive plants should receive less damage from insect herbivores and mammalian browsers, compared to native and noninvasive exotic plants. Further, if introduced species lose the benefits of enemy release over time and with increased geographic spread in the introduced range, we predict that noninvasive exotic and invasive plants with earlier introduction dates and larger regional distributions will experience increased insect herbivory and browsing damage.

Methods

Study Species

We planted 61 plant species into an old field community in Michigan, near the W.K. Kellogg Biological Station (Latitude: 42°24' N, Longitude: 85°23' W) (Table A1). Species were categorized as native, noninvasive exotic, or invasive (n = 25, 25, and 11 species respectively).

We defined native species as those naturally occurring in Michigan, prior to widespread European settlement. Invasive and noninvasive exotic species were both introduced to Michigan from outside the U.S., either accidentally or intentionally by humans, according to herbarium and historical records (Reznicek et al. 2011). While noninvasive exotic species assimilated into the native community with little effect, invasive plants aggressively colonized natural areas, threatening biodiversity and human interests.

Invasiveness for this study was determined by inclusion on one or more of the following local invasive species lists, as of June 2014 (Table A1): (1) Michigan Natural Features Inventory (Borland 2009), (2) Listed by Czarapata (2005) as “major invader of natural areas” and not categorized as needing disturbance to establish, (3) Wild Type Plants (<http://www.wildtypeplants.com/invasive.html>), and (4) the Michigan Seed Law (Act 329 of 1965) ([http://www.legislature.mi.gov/\(S\(4zhwk1by1svk1hgs1ooxf03f\)\)/documents/mcl/pdf/mcl-act-329-of-1965.pdf](http://www.legislature.mi.gov/(S(4zhwk1by1svk1hgs1ooxf03f))/documents/mcl/pdf/mcl-act-329-of-1965.pdf)). Inclusion on these lists means the species have been categorized as invasive within the Midwestern United States based on reports from land managers, inclusion on government invasive species lists, or published documentation of their effects on native plant and animal communities. Final decisions on status were made in consult with local land managers. We acknowledge that the classification of 'invasive' is not an absolute; it can depend on many biotic and abiotic factors [i.e., depends on context]. For invasive species found on only one list, we conducted a second analysis of our data listing them as noninvasive exotic; this analysis did not alter the main findings of the paper so we only report the analysis with them listed as invasive.

We chose species based on the following criteria: First, to test for the generalizability of the ERH, we used a species mix that represented a wide range of phylogenetic diversity,

residence times (number of years in Michigan), and geographic spread (number of counties occupied) (Ahern et al. 2010). Second, we included only herbaceous species to control for life form. Third, we used species already reported in herbarium records for Kalamazoo County (Reznicek et al. 2011) and commonly found in old field or grassland habitats to ensure that experimental plants grew in conditions similar to where they typically occur and also to make certain that we did not introduce species into parts of Michigan where they were not previously found. Finally, we preferentially chose species for which we could obtain seeds from nearby populations, either from personal field collections or orders from local growers, although some species were obtained from a broader geographic region (Table A1).

Experimental Design

We planted two common garden field experiments: the first running from June through November 2011 and the second from May 2012 through September 2014. For Experiment 1 in 2011, we germinated seedlings of 30 species from six plant families (13 native, 11 noninvasive exotic, and 6 invasive) (Table A1) in greenhouses at the W.K. Kellogg Biological Station. We then transplanted two to three replicate seedlings of each species into randomly assigned locations within a 10 x 10 planting grid located within each of nine field plots (N = 540 seedlings). These nine field plots represented the control plots of a large manipulative field experiment. Field plots were 2m x 2m in size, with 2m separating each plot. Species were planted within a grid of 100 cells within each plot, and were separated from the nearest experimental seedling by 20 cm. From October 11 to November 3, we measured damage from insect herbivores as the proportion of leaf area removed on 10 leaves per plant, selected as every third leaf starting at the top of the plant, and damage from mammalian browsers as the

proportion of aboveground vegetation removed by browsing damage, calculated as the proportion of stems with browsing damage for all plant families except the Poaceae. For the Poaceae, browsing was calculated as the proportion of tillers with browsing damage. If the individual was fully browsed down to the soil surface, we recorded this as 100% browsed. Author Schultheis collected all damage data to ensure estimates of aboveground vegetation removal were consistent.

In 2012 we established Experiment 2, which included 50 species from three plant families (20 native, 20 noninvasive exotic, and 10 invasive) (Table A1). We transplanted two replicate seedlings of every species into randomly assigned locations within each of five field plots (N = 500 seedlings). These five field plots represent the control plots of a large common garden experiment manipulating insect herbivores, mammalian browsers, and disease to study their fitness effects on native, noninvasive exotic, and invasive plants. Plots were 2m x 2m in size, with 2m separating each of the 40 plots. Within each plot, species were located within a grid of 100 cells and were separated from the nearest experimental seedling by 20 cm. From September 10 to October 4 in 2012 and August 26 to September 12 in 2013, we estimated insect herbivore damage and mammalian browsing damage as in Experiment 1. Annuals were harvested at the end of the 2012 growing season and are not present in the 2013 census. In Experiment 2 we focused on the plant families Asteraceae, Fabaceae, and Poaceae, which represent three of the four plant families with the most invasive species in Michigan (Ahern et al. 2010). Additionally, these families vary widely in chemical and structural traits, which could play a large role in herbivore defense strategies (Agrawal 2007 and citations within).

Both experiments were planted in the same old field in Hickory Corners, MI. Old field habitats are common in the area and are formed when abandoned agricultural areas convert back

to unmanaged land. These communities consist of a wide diversity of both native and introduced plant and animal species. Based on field observations and trapping experiments, the dominant mammalian browsers in this community were *Peromyscus maniculatus bairdii*, *Tamias striatus*, *Spermophilus tridecemlineatus*, *Sylvilagus floridanus*, and *Odocoileus virginianus* (P. Howell, unpublished data). These mammals are native to the area (Baker 1983), with *O. virginianus* existing at a moderate to high density of ~30 individuals per square mile (MDNR 2010). Detailed sampling of the insect community was performed in nearby prairie habitats (Robertson et al. 2011), however we were unable to find records that identified insect herbivores down to species, precluding our ability to assign them native or introduced status. It is likely that the community consists of a mix of native and introduced insect herbivores, which differ in their effects on introduced plants (Parker et al. 2006).

Residence Time and Spread Data

To study the dynamic nature of enemy release, we determined the residence time and geographic spread for our invasive and noninvasive exotic species. We defined spread as the number of counties occupied by a species, according to presence in herbarium records. We defined residence time as the number of years a species has occurred in Michigan, and calculated it as the year from the first herbarium specimen or historical record of introduction, subtracted from 2014. The method of using herbarium records to define spread and residence time of species is well established (citations within Ahern et al. 2010), but not without bias, including differential accessibility of field sites and variable sampling efforts over time. Therefore, residence time may actually indicate dates when an introduced species became apparent and occurred at high enough densities to be sampled, especially for species not intentionally

introduced and for which we have no historical record of introduction.

We collected Michigan spread data from a published dataset, constructed from herbarium and historical records compiled in the Michigan Flora (Reznicek et al. 2011) and updated with recent herbarium records from the University of Michigan Herbarium (Ann Arbor, MI) (Ahern et al. 2010). Spread data at a regional and broader geographic scale was collected from the USDA PLANTS Database (<http://plants.usda.gov/>), which records county level occurrence data for plant species (accessed January 2015). The USDA assigns county level occurrence by the presence of herbarium records and the scientific literature, similar to our dataset for Michigan. From these datasets we recorded (a) the number of counties invaded in Michigan (local scale), (b) the number of counties invaded in the five nearest states surrounding our study site (Michigan, Wisconsin, Illinois, Indiana, and Ohio) (regional scale), and (c) the number of counties invaded in the U.S. (broad geographic scale).

Phylogenetic Reconstruction

To control for phylogenetic non-independence in our study, we accounted for phylogenetic relatedness in all ANOVA analyses. Nucleotide sequences for matK, rbcL, and ITS were retrieved from NCBI Genbank for each species (accessed February 2015) (Table A1). If a species had no accession for a gene, a sequence from a closely related taxon was chosen if available. Gene sequences were aligned using the MUSCLE algorithm in Geneious v. 6.1.8 (Kearse et al. 2012). The ends of sequences were trimmed from each gene, and the three genes were concatenated using phyutility (Smith and Dunn 2008). We determined the optimal model of molecular evolution for the alignment using the Akaike Information Criterion (AIC), Bayesian Information Criterion (BIC), and Performance Based Selection (DT) using jModelTest2 v. 2.1.7

(Darriba et al. 2012). All three methods selected the General Time Reversible model, with rate heterogeneity including invariable sites and the rate of evolution at other sites as a gamma distribution (GTR + I + Γ), as the optimal model. Maximum likelihood (ML) analysis with 100 bootstrap replicates was implemented with the high performance computing version of RAxML v. 8.1.17 (Stamatakis 2014). We included a partition file for ML analysis to account for gene regions in the concatenated alignment. To assess phylogenetic structure for insect herbivory and mammalian browsing across the study species, we calculated Blomberg's K (Blomberg et al. 2003) separately for each year of the study, following methods found in Swenson (2014) and using the phytools package in R (v. 0.4-21, Revell, 2012). Blomberg's K is a measure of whether a trait shows more or less phylogenetic divergence than expected by a null model of Brownian Motion.

Statistical Analysis

We performed all analyses in R (v. 3.0.2, R Core Team 2015). Due to shared ancestry, traits in related species cannot always be viewed as being independent. We therefore incorporated comparative methods with linear models to determine whether invasive, noninvasive exotic, or native species differ in herbivore damage. We performed phylogenetic generalized least squares (PGLS) with Brownian Motion and Ornstein-Uhlenbeck (OU) models of trait evolution (Garland et al. 1993, Martins and Hansen 1997), with subsequent AIC model selection. PGLS was implemented by incorporating the constructed phylogeny into the covariance structure using the ape package (v. 3.1-4, Paradis 2012), after which the linear models were fit using the gls function in the nlme package in R (v. 3.1-119, Pinheiro et al. 2015). Proportion of leaf area removed and proportion of stems (or tillers) browsed were included as

separate response variables, and plant status (native, noninvasive exotic, or invasive) was included as a fixed predictor variable. Because species is our unit of replication for questions on status, we averaged individuals within a species within a year. Analyses on herbivory and browsing were conducted on within-year species averages; separate analyses were run for each year of data because species composition varied. To determine whether there is a relationship between the damage a species received from insect herbivores and that received from mammalian browsers, we performed a regression using the `lm` function in R. Species were excluded from some analyses due to high mortality in the field (Table A1, grayed out boxes), likely due to limited rainfall and water availability at the time of planting, competition from the background community, and enemy damage. Post-hoc tests were used to evaluate differences between treatment combinations when the main effect of status was significant ($P \leq 0.05$), and were implemented with a Holm multiple comparisons correction using the `phylANOVA` function in the `phytools` package in R. An additional analysis, including plant Family in our models in place of phylogenetic structure, can be found in Appendix B.

To determine whether enemy damage changes with increased residence time or spread, we performed non-linear ANCOVAs using the `glm` function in R (v. 3.1.1, R Core Team 2015). We included a logit link transformation in the generalized linear model to accommodate the nonlinear associations with county spread and time variables (Bolker 2008). We did not incorporate phylogeny into these models. Current phylogenetic methods that incorporate nonlinear relationships (such as independent contrasts) can reduce statistical power, and ignoring nonlinearity can affect biological inferences (Quader et al. 2004). Additionally, alternative techniques such as PGLS assume linear relationships and could not be used for our data.

Only invasive and noninvasive exotic species were included in spread and residence time

analyses. In our dataset, an introduced species' range size is a function of its residence time in the introduced range, meaning that generally when given more time, an introduced species will continue to expand its range (Ahern et al. 2010). Because of the high degree of correlation between time and spread ($r = 0.70$, $P < 0.001$), and between our different measures of spread (MI and 5 state spread: $r = 0.86$, $P < 0.001$; MI and U.S. spread: $r = 0.72$, $P < 0.001$), these predictor variables could not be tested simultaneously in one ANCOVA model (Underwood 1997, Miller and Chapman 2001). Therefore, when discussing how residence time and spread relate to herbivory and browsing damage, we cannot differentiate between their effects, though we can still explore the relationships between these variables and enemy damage (Miller and Chapman 2001). Here, we explore the effects of these variables separately, testing their individual influences in different models (Underwood 1997), and then discuss their effects making clear that either residence time or spread could be driving the observed patterns. To test whether enemy damage increases with residence time, proportion of leaf area removed and proportion of stems (or tillers) browsed were included as response variables; plant status, residence time, and their interaction were included as fixed factors. We tested the same model for each of our spread measures, substituting spread for time as a predictor variable. Model fit and hypothesis testing were conducted using likelihood ratio tests, and significance was assessed from the χ^2 distribution. Post-hoc contrasts were used to evaluate whether the slopes for invasive or noninvasive exotic species were significantly different from zero when a status by time, or status by spread, interaction was significant ($P \leq 0.05$).

Results

In our system, we found no evidence that invasive species receive reduced enemy

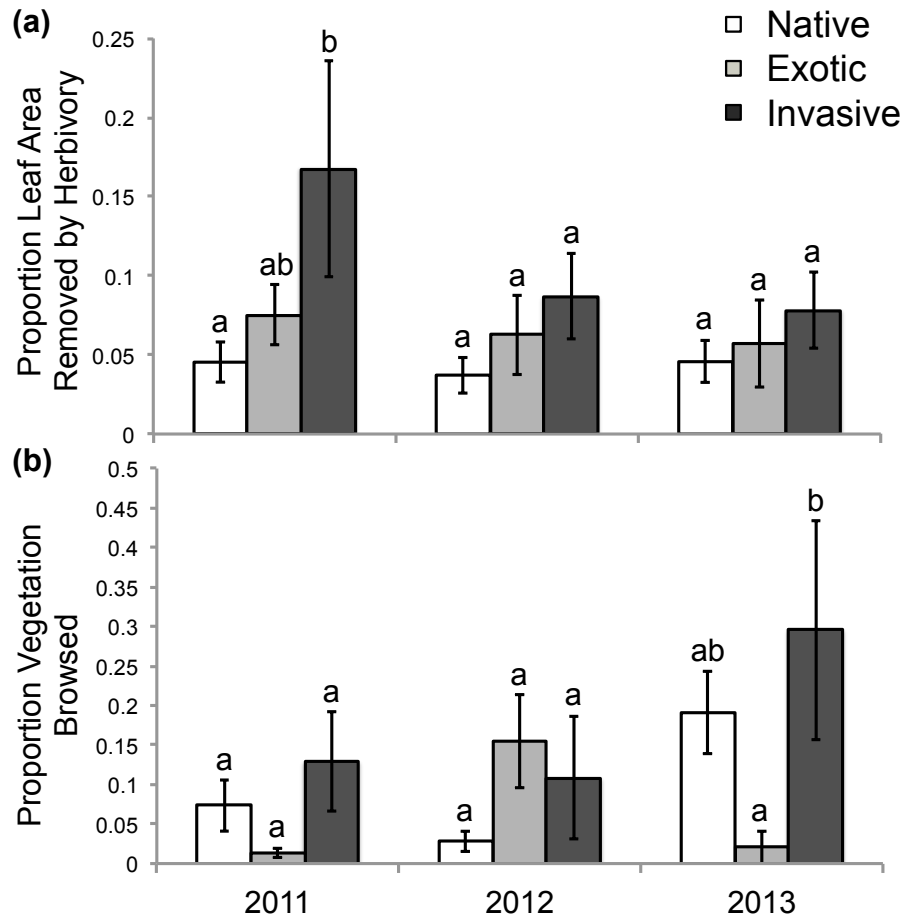


Figure 1: Three years of (a) insect herbivore and (b) mammal browser damage data on native (white bars), noninvasive exotic (gray bars), and invasive (black bars) plants. Bars indicate mean $\pm$ SE. Means with the same letter are not significantly different ($P \leq 0.05$) based on post-hoc contrasts.

damage, compared to native and noninvasive exotic species. Enemy damage from insect herbivores and mammalian browsers tended to be higher on invasive species, compared to native and noninvasive exotic species (Fig. 1, Table A2). In 2011, invasive species received significantly more damage from insect herbivores than natives, and noninvasive exotics received intermediate amounts of damage (Fig. 1a; 2011: $t_{2,27} = 2.20$, $P = 0.04$). Though not significant, this trend remained consistent through 2012 and 2013 (Fig. 1a; 2012: $t_{2,43} = 0.24$, $P = 0.81$; 2013: $t_{2,31} = 1.20$, $P = 0.24$). Notably, out of the top 10 species with the most insect herbivore damage,

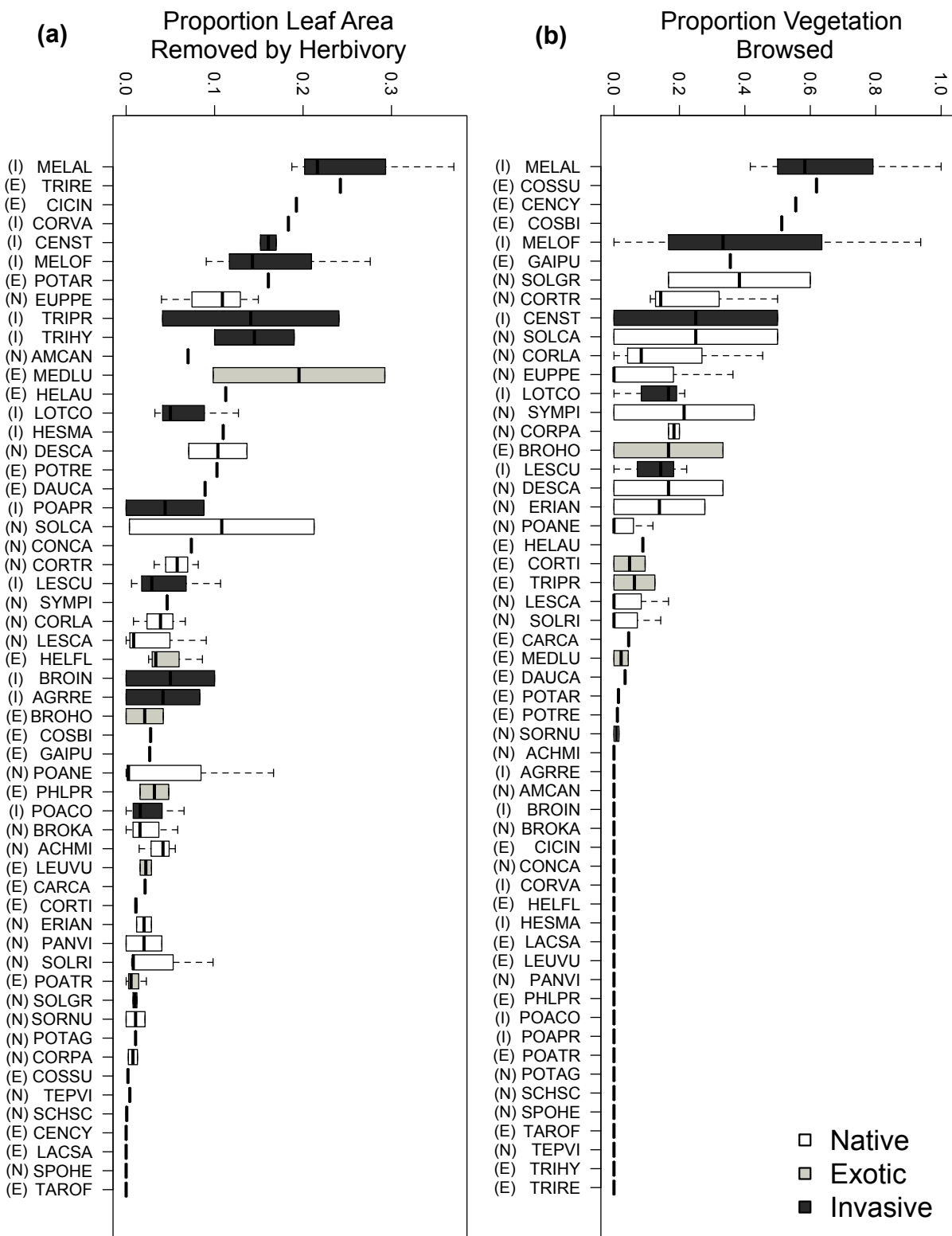


Figure 2: Boxplots of the median (black line), first and third quartiles (box), maximum, and minimum for (a) insect herbivory and (b) mammalian browsing for each species. Native species (N) are listed in white, noninvasive exotic species (E) in gray, and invasive species (I) in black. Species with one year of data, or the same amount of damage in all years, have boxplots only showing the median without quartiles. Species are organized in descending order by mean.

six were invasive and three are noninvasive exotic. Only one of the top 10 species with the most insect damage was native (Fig. 2).

Browsing was variable across years, but again we find no evidence consistent with the ERH (Fig. 1b, Table A2). In all years, native, noninvasive exotic, and invasive species did not differ significantly in browsing, but our post hoc contrasts revealed differences between noninvasive exotic and invasive species in 2013. In 2011 and 2013, invasives and natives generally received the most browsing damage (Fig. 1b; 2011: $t_{2,27} = -0.16$, $P = 0.56$; 2013: $t_{2,31} = 0.28$, $P = 0.78$). In 2012, invasive and noninvasive exotic species tended to receive more browsing damage than natives (2012: $t_{2,43} = 0.96$, $P = 0.34$). Of the 10 species with the most browsing damage, three were invasive, four were noninvasive exotic, and three were native. About half of species received little or no damage from mammalian browsers (Fig. 2). There was no relationship between the damage a species received from insect herbivores and that received from mammalian browsers ($R^2 = 0.01$, $F_{1,31} = 0.31$, $P = 0.58$).

The phylogeny created for PGLS analyses was well resolved with high bootstrap support at the nodes and expected grouping by genus and family (Fig. A1). All of our values for Blomberg's K were less than 1 (Table A2), indicating that leaf damage and stem browsing in close relatives were more divergent than expected across the phylogeny (Blomberg et al. 2003) and provides additional evidence that the lack of control for phylogeny in our ANCOVA analyses described below likely does not bias results.

Enemy damage was dynamic, depending on residence time and areas of spread in the introduced range (Fig. 3 & 4, Table A3), and these patterns were consistent across multiple geographic scales. With increased residence time, noninvasive exotic species experienced increased insect herbivore damage (Fig. 3a: $\chi^2 = 2.57$, $P = 0.02$), but this pattern was not

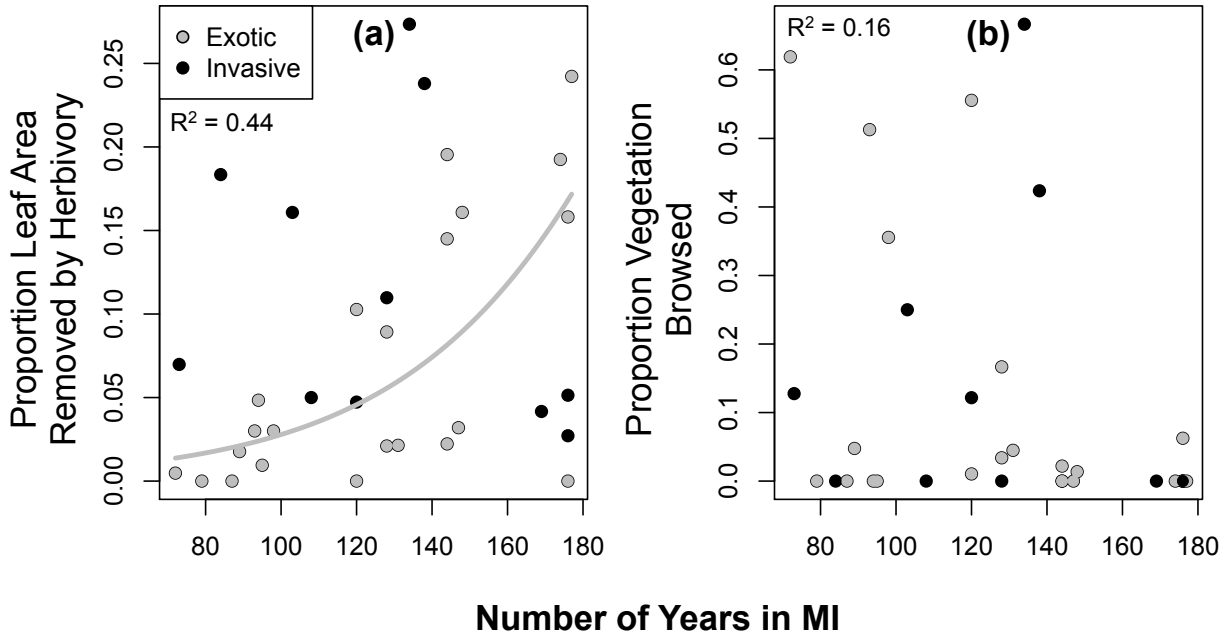


Figure 3: Insect herbivore (a) and mammalian browser (b) damage on noninvasive exotic (gray points) and invasive (black points) plants with increasing residence time in MI. Analysis was performed on species averages across the three study years; each point represents one species. The gray regression line indicates insect herbivory increases with residence time for non-invasive exotic species, but not invasive species.

observed for invasive species (Fig. 3a; status x time interaction: $\chi^2 = 1.76$, $P < 0.001$). Introduced species with longer residence times tended to experience less mammalian browsing, although this trend was marginally non-significant (Fig. 3b; $\chi^2 = 9.82$, $P = 0.06$). With increasing area occupied in Michigan (spread), noninvasive exotic species experienced greater insect herbivory (Fig. 4a; $\chi^2 = 2.04$, $P < 0.001$), and both invasive and noninvasive exotics experienced greater insect herbivory with increasing area occupied at larger spatial scales. (Fig. 4b-c; five states: $\chi^2 = 1.74$, $P < 0.001$; U.S.: $\chi^2 = 2.16$, $P = 0.001$). Noninvasive exotic species experienced reduced mammalian browsing with increasing spread at all three scales, and at larger scales invasive species experienced increased mammalian browsing with increasing spread (Fig. 4 d-f, status x county interaction at all three scales; Michigan: $\chi^2 = 7.83$, $P = 0.009$; five states: $\chi^2 = 5.43$, $P <$

0.001; U.S.: $\chi^2 = 5.86$, $P < 0.001$).

Discussion

We found no evidence consistent with ERH contributing to invasiveness in this system.

We detected few significant differences in damage between native, invasive, and noninvasive

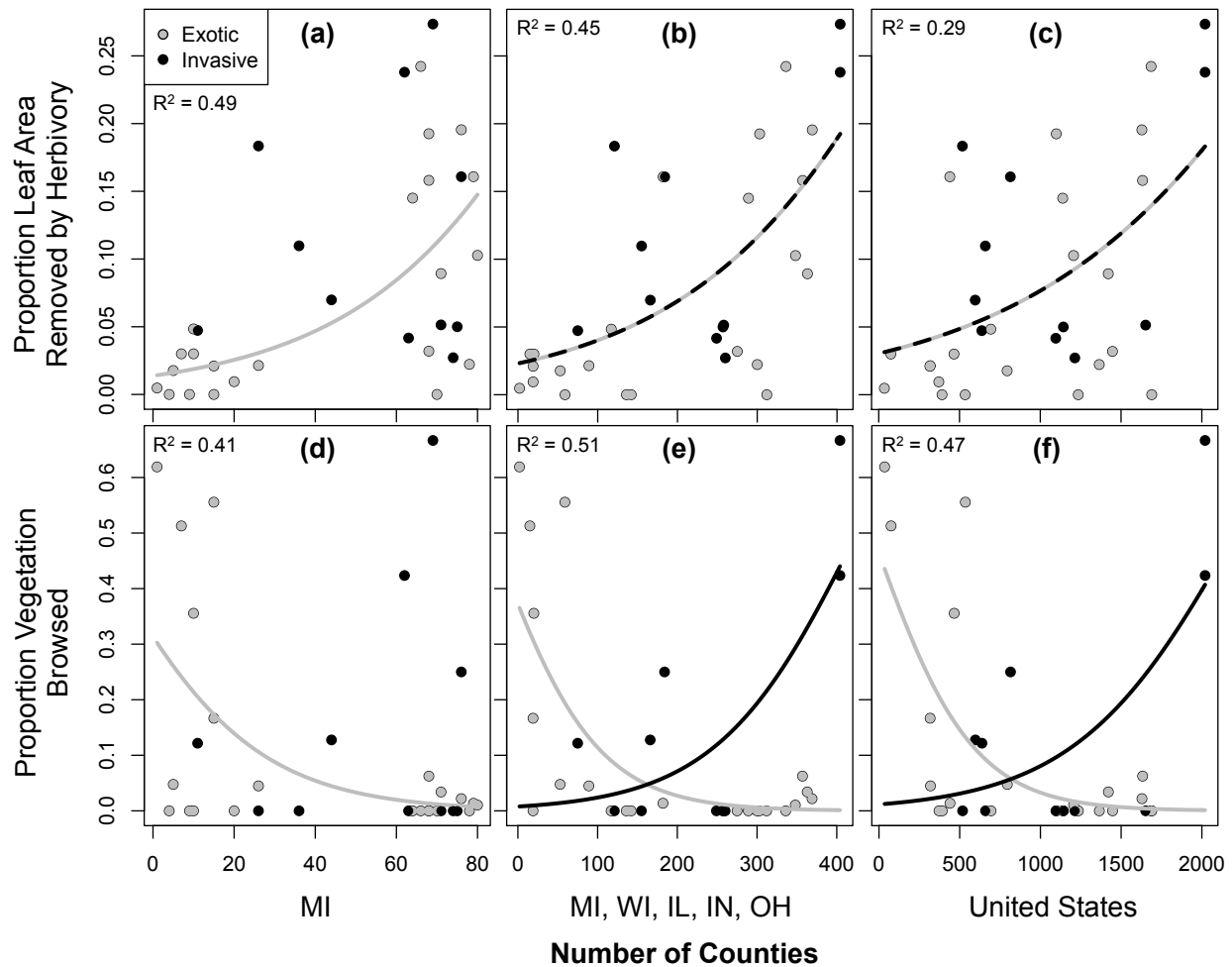


Figure 4: Insect herbivore (a-c) and mammal browser (d-f) damage on noninvasive exotic (gray points) and invasive (black points) plants with increasing spread. Spread measures for counties within MI (a & d), spread within MI, WI, IL, IN, OH (b & e), and spread within the U.S. (c & f). Analysis was performed on species averages across the three study years; each point represents one species. Regression lines show significant relationships ($P \leq 0.05$). A dashed black and gray regression line indicates insect herbivory increases with spread, but no difference between non-invasive exotic and invasive species. Black and gray lines indicate patterns only significant for invasive and non-invasive exotic species, respectively.

exotic species, although invasives tended to receive more damage from insect herbivores than did native or noninvasive exotic plant species across all study years (Fig. 1a). Browsing damage did not differ based on status, however native and invasive plants tended to get more browsing damage than did noninvasive exotics, not supporting either ERH prediction (Fig. 1b). Our results are consistent with other experiments using the common garden approach. For example, in a study of 12 temperate vine species, native and invasive vines experienced more foliar damage from insect and mammal herbivores than noninvasive exotics, not supporting ERH predictions (Ashton and Lerdau 2008). Similarly, invasive *Eugenia uniflora* sustained more insect herbivore damage than congeneric native and noninvasive exotic species in a common garden experiment, also not supporting ERH Predictions 1 and 2 (Stricker and Stiling 2014). In a study using 18 clover species, introduced and native species experienced similar amounts of disease, and the most invasive introduced species experienced the most disease (Parker and Gilbert 2007).

Further, we found that invasions are dynamic and enemy release from insect herbivores is lost over time for noninvasive exotic species, and with increasing spread at all scales for both invasive and noninvasive introduced species (Fig. 3a & 4a-c). Our herbivory results on the dynamic nature of invasions are consistent with other studies that have found that enemy release is lost with increased residence time (Siemann et al. 2006, Hawkes 2007, Diez et al. 2010; but see Carpenter and Cappuccino. 2005) and spread (Mitchell and Power 2003, Diez et al. 2010) in the introduced range. While there was no relationship between residence time and browsing (Fig. 3b), we found that the most widespread noninvasive exotic species actually received the least amount of browsing, contrary to our predictions (Fig. 4d-f). Because we are not able to determine the direction of causality, browsers may in fact be driving the pattern in spread, acting as a filter and determining which species can spread furthest in the landscape. In contrast,

invasive species with the largest ranges experienced higher amounts of browsing damage (Fig. 4e-f), indicating that these two types of introduced plants might interact differently with mammalian browsers, although this result should be interpreted cautiously given that two species in a single genus (*Melilotus officinalis* and *M. albus*) drive the observed patterns for invasives.

Due to the tight correlation between time and spread, we are unable to determine which variable is driving the patterns observed. Additionally, because we are unable to manipulate these two variables, other unmeasured correlated variables could be acting in our system. Previous analyses on our Michigan dataset of residence time and spread have found that the average introduced species will be present in 50% of counties after 160 years, with only the most invasive species spreading more quickly (Ahern et al. 2010). Given sufficient time, 10-20% of introduced plants will be listed as invasive, indicating that invasiveness may be a function of residence time in the introduced range (Ahern et al. 2010).

In our study, we find consistent patterns across all three years of data collection, despite slight variations in experimental species composition (Table A1). Enemy pressures can vary greatly across years and growing seasons (Agrawal and Kotanen 2003, Agrawal et al. 2005, Parker and Gilbert 2007). For example, in the first year of a common garden experiment, Agrawal and Kotanen (2003) found that introduced plants experienced more herbivory than did natives, similar to the results of our own study. They collected a second year of data on the same common garden and found that introduced plants now received less herbivory, supporting the ERH (Agrawal et al. 2005). They hypothesized that variable herbivore communities could drive these yearly differences, as well as ontogenetic changes in study plants and a potentially delayed response of the herbivore community to the establishment of their experiment. Thus, in their system, time periods where native species receive high amounts of enemy attack, but introduced

species receive little damage, may provide an opportunity window for introduced plants to dominate the system. In our experiment, no opportunity windows were apparent; the consistency of our results across three years provides strong support against ERH and suggests that enemy release windows, where invasive species experience reduced damage for a particular growing season, may be relatively infrequent in this system.

Several mechanisms could explain higher enemy damage to invasives than noninvasive exotics or natives. First, fast growing species tend to allocate less to defense, resulting in higher amounts of herbivore damage than slow growing species (Cebrian and Duarte 1994, Endara and Coley 2011), and invasive species may have faster growth rates than noninvasive exotic and native plant species (van Kleunen et al. 2010). Second, a lack of a shared coevolutionary history between introduced species and enemies in their introduced ranges could lead to higher amounts of damage because introduced species may lack defenses against these enemies, unlike native plants with coevolved defenses (increased susceptibility hypothesis; Hokkanen and Pimentel 1989; Colautti et al. 2004, Verhoeven et al. 2009). Consistent with this hypothesis, herbivore feeding trials on aquatic (Parker and Hay 2005, Morrison and Hay 2011) and terrestrial (Parker and Hay 2005) plants have shown that native herbivores preferentially consume introduced plants over natives, and the defensive chemistry of invasive plants serves as no more of a deterrent to herbivores than does the defensive chemistry of natives (Lind and Parker 2010). Third, invasive species may have higher local population densities than native or noninvasive exotic species (e.g. Herrera et al. 2011), which could potentially increase the abundance of enemies feeding on these species or make invasive plants more apparent to insect herbivores and mammalian browsers (Feeny 1976). This final hypothesis is unlikely in our system. In our common gardens, all species were planted at equal densities, and although some experimental

species also naturally occurred at our experimental site, we found that, herbivore ($r = 0.19$, $P = 0.42$) and browsing ($r = 0.003$, $P = 0.99$) damage were not correlated with species abundance at our site (percent cover estimated at 1% intervals for each species based on visual observation of 100 20x20 cm cells nested within 2x2 m experimental plots).

The question remains, if invasive plants tend to receive the most enemy damage, how is it that they are still invasive? There now exist over two dozen hypotheses attempting to explain invasiveness (Catford et al. 2009), and it is clear that no single hypothesis can explain the diversity of invasion strategies employed by today's invaders (Gurevitch et al. 2011, Lau and Schultheis 2015). Invasiveness could be driven, not by enemy release, but instead by performance and defense strategy traits of invasive species. In this study, we have identified differences in enemy damage between native, invasive, and noninvasive exotic species, and in future studies we will determine whether this damage translates into different effects on performance. Invasive species may lie on one end of a tradeoff between an individual's ability to resist damage and ability to maintain performance when damaged (i.e., tolerance) (Strauss and Agrawal 1999). Though invasive species in our system received the most insect herbivore damage, if they are also more tolerant, then their performance (growth, survival, fecundity) may be less affected by this damage compared to noninvasive exotics and natives. Contradictory to this hypothesis, a meta-analysis found introduced species to be *less* tolerant to damage (Chun et al. 2010). In a different meta-analysis, Parker and colleagues (2006) classified introduced species along a spectrum of invasiveness and determined that herbivores had similar effects on the performance of both noninvasive exotic and invasive species.

Alternatively, release from enemies not tested in our system, such as disease or belowground enemies, could contribute to invasion success. Mitchell and Power (2003)

demonstrated that plant species that experienced release from fungal and viral pathogens were more widely invasive than those that did not. Similarly, invasive plants experienced lesser effects from belowground enemies than rare, native plants (Klironomos 2002). In contrast, a meta-analysis by Levine and collaborators (2004) found herbivores (as well as competition and diversity in the native community) provided resistance to invasion, while fungal pathogens did not (Levine et al. 2004), indicating that some enemies may contribute more to ERH than others (Levine et al. 2004).

Though we find that ERH, mediated through aboveground herbivores, was not a common pattern across the species tested in our study location, some invasive and noninvasive exotic species did receive low amounts of enemy damage (Fig. 2), indicating that the success of these species may be driven by enemy release. During at least one study year, the invasive Poaceae species *Bromus inermis* (2012), *Poa compressa* (2013), and *Poa pratensis* (2013) experienced no damage from either insect herbivores or mammalian browsers. These species could be candidates for further study to assess whether enemy release contributes to increased fitness over native competitors.

Conclusion

The ERH remains among the most popular hypotheses explaining the successes and failures in introduced species, despite mixed support. A review on the ERH found that 36% of studies support it, while 43% do not (Heger and Jeschke 2014, see also Colautti et al. 2004). Meta-analyses and reviews on enemy richness and damage for introduced and native species find results both for (Liu and Stiling 2006, Hawkes 2007) and against the ERH (Chun et al. 2010).

Our study helps to identify some of the sources of variation in previous Enemy Release

Hypothesis studies, namely distinguishing between invasive and noninvasive exotic species and considering the dynamic interplay between an introduced species and their enemies over decadal timescales. Our findings indicate that invasive species generally receive more damage from enemies, compared to native and noninvasive exotic species, not supporting key predictions arising from the ERH. Therefore, we conclude that enemy release is not a general mechanism associated with invasiveness in our system, although enemy release could apply to specific cases of invasion and early on in the invasion process.

CHAPTER 3: PERFORMANCE CONSEQUENCES OF ENEMY RELEASE DEPEND ON INVASION AGE BUT DO NOT EXPLAIN INVASIVENESS

Introduction

The enemy release hypothesis (ERH) is among the most commonly invoked hypotheses to explain invasiveness, and states that the success of invasive species in their introduced range is driven by the loss of enemies, such as herbivores and disease, that constrain their performance in their native range (Elton 1958, Callaway and Aschehoug 2000, Maron and Vila 2001, Keane and Crawley 2002). According to the ERH, invaders should experience reduced enemy damage and as a result, fewer negative fitness effects of enemies in their introduced range compared to their native range. Additionally, the ERH predicts that invasive species will receive reduced damage, and less negative effects on performance from enemies compared to co-occurring native and noninvasive exotic species in their introduced range.

To date, most ERH studies have focused on measuring damage, and many studies have compared enemy damage on introduced and co-occurring native species in the introduced range (Agrawal and Kotanen 2003, Colautti et al. 2004, Agrawal et al. 2005, Chun et al. 2010). Few have explored whether damage differs for invasive and noninvasive exotic species (Mitchell and Power 2003, Parker et al. 2006, Liu et al. 2007, Parker and Gilbert 2007, Ashton and Lerdau 2008, Schultheis et al. 2015), which could reveal important differences between successful invaders and introduced species that do not become invasive. These observational studies of enemy damage have provided limited support for ERH, with only a few studies finding that invasive species receive less damage from enemies than natives or noninvasive exotics (Maron and Vila 2001, Colautti et al. 2004, Liu and Stiling 2006), and a number of studies and meta-analyses finding that invaders receive equal or even more damage (Parker et al. 2006, Ashton

and Lerdaun 2008, Zou et al. 2008, Chun et al. 2010, Morrison and Hay 2011, Dawson et al. 2014, Schultheis et al. 2015).

Three hypotheses may explain how invaders are able to receive high amounts of damage while still performing better than native and noninvasive exotic species. First, many ERH studies manipulate only a single enemy or examine a particular type of enemy damage, and release from multiple enemies may be necessary for invasive success. Second, invasive species may be more tolerant to enemy damage, maintaining performance and fitness despite receiving high levels of damage. Third, the fitness effects of enemies in the introduced range may increase over time as introduced species form novel biotic interactions with members of their new community, and ERH may only contribute to success early in the invasion process.

Community Complexity and Enemy Release

In native systems, plant performance is typically influenced by a multitude of biotic factors (Harper 1977, Louda 1982, Crawley 1989), and the effect of a particular biotic interaction often depends on a species' community context (Wootton 1994, Agrawal et al. 2007). However, ERH studies manipulating only one biotic interaction are unable to identify interactive and synergistic effects between antagonists. Perhaps, as in native systems, the removal of multiple antagonists will have additive or synergistic effects, and enemy release will only promote invasiveness when more than one antagonist is escaped. For example, only when competitors and herbivores were present was the performance of invaders *Triadica sebifera* and *Cirsium vulgare* reduced (Huang et al. 2012, Suwa and Louda 2012); escape from either competitors or herbivores alone would not have been sufficient for release, but escape from both antagonists could drive invasiveness.

Tolerance

Differences in damage between species may not directly translate into differences in performance. Tolerance, or a plant's ability to regrow and reproduce after damage, differs greatly between species (Marquis 1992, Strauss and Agrawal 1999). Therefore, tests of the ERH must go beyond observations of enemy damage and quantify performance effects of damage by manipulating the presence of enemies in the field (Maron and Vila 2001, Keane and Crawley 2002). Given the number of studies suggesting that invasive species receive as much or even more damage than native and noninvasive exotics, tolerance to, rather than release from, enemy damage may explain invasiveness.

Invasive species tend to be larger, grow more quickly, and produce denser stands compared to competing species in their introduced range and conspecific populations in their native ranges (Crawley 1987, Hinz and Schwarzlaender 2004, van Kleunen et al. 2010, Dawson et al. 2014; but see Thebaud and Simberloff 2001, Daehler 2003). Larger, more locally abundant species often lose more leaf tissue to herbivory and disease (Feeny 1976, Rhoades and Cates 1976, Paker et al. 2015), potentially due to increased detection by herbivores and increased density promoting disease spread (Anderson and May 1979, Burdon and Chilvers 1982). Additionally, invasive plants frequently have lower construction costs for tissues, and cheap tissues may be less defended against damage (Daehler 2003). Therefore, invaders may lie on one end of a tradeoff spectrum between defending against damage and tolerating damage once it occurs (Strauss and Agrawal 1999).

Dynamic Enemy Release

The lack of generalizability observed in ERH studies may be due to the fact that the traits and causal processes driving invader success early during invasion differ from those acting later (Dietz and Edwards 2006, Theoharides and Dukes 2007). For example, factors determining establishment success might differ from those controlling spread in the landscape (Richardson et al. 2000, Kolar and Lodge 2001, Heger and Trepl 2003, Levine et al. 2004, Kempel et al. 2013). In a study of 48 introduced plant species, propagule pressure contributed to species' establishment, but after three years of growth, traits related to interactions with insect herbivores and plant competitors became more important determinants of success (Kempel et al. 2013). Additionally, the probability of encountering new antagonists could increase with longer residence times; for example, native enemies of closely related species may evolve to use invaders as a host or food source, or enemies from the invader's native range could be introduced (Goßner et al. 2009, Mitchell et al. 2010, Flory and Clay 2013). Initial loss of enemies can be overshadowed by gain of new enemies, and the diversity of herbivores on introduced plants can be equal to that of natives (Maron and Vila 2001). ERH therefore may only be important and operate during the initial phases of invasion, and may be lost over time and with increasing spread in the introduced range (Elton 1958, Mitchell et al. 2006, Mitchell et al. 2010). Dynamic ERH predicts that introduced species with longer residence times in their new range will be more negatively affected by biotic interactions than species with more recent dates of introduction (Hawkes 2007, Mitchell et al. 2010).

There is now mounting evidence for the ephemeral nature of ERH (Hawkes 2007, Hayes and Barry 2008, Flory and Clay 2013). For example, in a study of 36 invasive and noninvasive exotic species, Schultheis and collaborators (2015) found that noninvasive exotic species

experienced increased insect herbivory with increasing residence times, while invasive species did not. In a study of 124 plant species, pathogen richness was six times higher on introduced plants that had been in their introduced range 400 years, compared to more recently introduced plants (Mitchell et al. 2010). However, these studies do not determine whether enemy accumulation translates to effects on performance (Flory and Clay 2013). Several studies of belowground enemies and plant-soil feedbacks (PSF) have demonstrated increasingly negative fitness effects of the soil community on introduced plants with time. The invasive plant, *Heracleum mantegazzianum*, accumulated belowground enemies and experienced reduced survival, biomass, and competitive ability when grown in soil collected from sites invaded longer ago compared to newly invaded sites (Dostál et al. 2013). In New Zealand, species with longer residence times experienced more negative PSF (Diez et al. 2010). However, to the best of my knowledge, no study has demonstrated whether the accumulation of aboveground enemies translates into performance differences over time for introduced species.

Here, I test the ERH by manipulating the presence of insect herbivores, mammalian browsers, and fungal disease on 20 native, 10 invasive, and 20 noninvasive exotic plant species in Michigan. Previously in this system, I found no evidence for reduced damage from insect herbivores or mammalian browsers on invasive species compared to native and noninvasive exotics (Schultheis et al. 2015), leading me to hypothesize that release from multiple antagonists or tolerance may play an important role in invasiveness. Additionally, enemy damage was dynamic; insect herbivory increased for noninvasive exotic species with longer residence times, but did not increase for invasives (Schultheis et al. 2015). Therefore, the performance effects of enemies may also increase for introduced species with longer residence times in the introduced range.

If ERH determines invasion success, I expect that invaders will experience fewer fitness benefits from the removal of enemies compared to native and noninvasive exotic species that are still controlled by enemies, either because invaders experience reduced damage or because invaders are highly tolerant. In addition, I predict that the removal of enemies will reduce the reproductive and growth advantages commonly observed for invasive species, relative to native species. Further, I predict that ERH will be ephemeral and lost for invasive and noninvasive exotic species with longer residence times in Michigan.

Methods

Experimental Design

To determine how antagonistic biotic interactions affect the performance of native, noninvasive exotic, and invasive plants, I conducted an enemy exclusion experiment in an old field community in southwest Michigan, near the W.K. Kellogg Biological Station (Latitude: 42°24' N, Longitude: 85°23' W). I constructed 40 2x2m field plots, with a 2m buffer between each plot, in which I experimentally manipulated the presence and absence of mammalian browsers, insect herbivores, and fungal disease in a 2x2x2 factorial design (n=5 plots per treatment). To exclude mammalian browsers I constructed four-foot deer fencing around the perimeter of treated plots and buried 0.64cm grid hardware cloth to a depth of 0.2 meters with a bent 3cm lip facing outwards to re-direct digging mammals away from the plot interior (Munger and Brown 1981). Hardware cloth extended 0.6m above the soil surface and was secured to deer fencing. To bury hardware cloth, I trenched the perimeter of each plot and backfilled once fencing was in place; to control for effects of trenching, all plots without fencing were also trenched and backfilled. To exclude insect herbivores and fungal pathogens I sprayed

experimental plots with insecticide or fungicide (Merit 75 WP at 0.031g/L and Heritage at 0.062g/L, respectively). Treatment applications used approximately 8L of liquid, and control plots were sprayed with an equal volume of water. I applied treatments biweekly throughout the growing season.

I germinated experimental seedlings in greenhouses at the W.K. Kellogg Biological Station, beginning in April 2012. From 14-22 May, 2012 I planted seedlings into the existing background community present in the field. Within experimental field plots, species locations were randomized in a 10x10 grid with 20cm between each seedling. Each plot contained two seedlings of 50 experimental species (N=4,000 seedlings), representing three plant families (Asteraceae, Fabaceae, Poaceae) and three provenances (status = native [n=20], noninvasive exotic [n=20], invasive exotic [n=10]) (Table C1).

Species used in this experiment naturally occur in Kalamazoo County and grow in similar conditions to those present at the field site. When possible, I collected seeds from nearby populations or used local seed sources (Table C1). Native status was assigned if a species occurred in Michigan prior to widespread European settlement. Invasive and noninvasive exotic species were introduced from outside the U.S., according to herbarium and historical records (Reznicek et al. 2011), but noninvasive exotic plants assimilate into the native community with little effect, while invasive plants invade natural areas and threaten biodiversity. Invasiveness of introduced species was determined for this study by inclusion on local invasive species lists and in consultation with local land managers (Schultheis et al. 2015). In addition, I focused on three plant families (Asteraceae, Fabaceae, Poaceae) that vary widely in chemical, structural, and growth traits, potentially playing a large role in enemy tolerance and resistance strategies (Agrawal 2007), and represent three of the four plant families with the most invasive species in

Michigan (Ahern et al. 2010).

At the end of each growing season I estimated enemy damage on the 4,000 experimental seedlings. I calculated damage from insect herbivores as the proportion of leaf area missing on 10 leaves per plant, selected as every third leaf starting at the top of the plant. I recorded leaves that were totally removed as 100% herbivory when I could observe the petiole was intact. Because it remains constant over the age of a leaf, percent leaf area missing serves as a reliable estimate of herbivory (Lowman and Heatwole 1992). To estimate disease incidence, I recorded the proportion of necrotic and chlorotic leaf tissue. I measured damage from mammalian browsers as the proportion of stems or tillers with evidence of browsing damage. If browsing resulted in complete removal of a focal plant, I recorded the individual as 100% browsed.

In addition, I measured performance metrics for each species, including survival, flower (or inflorescence) number, reproductive biomass, and vegetative biomass. An individual was recorded as alive in a particular year if aboveground biomass was present at any time during the growing season. Reproduction for the Asteraceae was estimated as the number of composite flower heads, and for Poaceae as the number of spikelets. Reproduction for all species was calculated as the sum of buds, flowers, and seed heads produced by an individual throughout the growing season. To estimate reproductive I collected all buds, flowers, and seed heads at the end of each growing season. To assess vegetative biomass, I harvested annuals at the end of the 2012, biennials in 2013, and perennials in 2014. I dried reproductive and vegetative biomass at 70°C for 72 hours before weighing.

To assess whether enemy release is dynamic and lost over time, I assigned invasive and noninvasive exotic species a residence time in Michigan based on a published dataset constructed from herbarium and historical records (Ahern et al. 2010). I define residence time as

the number of years a species has occurred in Michigan, and calculated it for each species as the year a species was first present in records, subtracted from 2014 (Schultheis et al. 2015). Using herbarium records to define residence time in a species' introduced range is a well-established method, particularly when other resources, such as historical introduction documentation, are not available (Ahern et al. 2010). However, this method is potentially biased due to differential accessibility of field sites, inconsistent sampling efforts over time, and introduced species becoming more apparent over time as they increase in number in their introduced range. Therefore, herbarium records may underestimate residence time, especially for introduced species with a long lag phase before they became abundant.

Statistical Analysis

Damage: I performed all analyses in R (v. 3.0.2, R Core Team 2015). To determine whether enemy removal treatments were effective at reducing enemy damage, I tested the effects of enemy exclusion on enemy damage with mixed model ANOVA using the lmer function in the lme4 package in R (v. 1.1-7, Bates et al. 2015). To test the efficacy of the fencing treatment, mammalian browsing was included as the response variable and fencing treatment as the fixed predictor variable. To test the efficacy of the insecticide treatment, the model included insect herbivory as the response variable and insecticide treatment as the fixed predictor variable. I was unable to run a model testing the efficacy of the fungicide treatment because no visible infection was detected on experimental plants throughout the experiment. Experimental plot is the unit of replication for questions on treatment effectiveness, so I included plot nested within treatment as a random factor in these models. P-values for mixed models were obtained using the lmerTest package in R (v. 2.0-20, Kuznetsova et al. 2015).

Performance: To determine whether enemies significantly altered the performance of experimental species, I tested the effects of enemy exclusion on plant performance with mixed model ANOVA. Status (native, noninvasive exotic, invasive), plant family (Asteraceae, Fabaceae, Poaceae), plant type (annual, biennial, perennial), fencing treatment (fenced, control), insecticide treatment (insecticide spray, control), fungicide treatment (fungicide spray, control), were included as fixed predictor variables. Species nested within status, plot nested within enemy treatment, and the species x enemy treatment interaction were included as random variables. Because of the potential for many interactions between predictor variables in the experiment, I lacked sufficient power to include all interaction terms in the models. I addressed this power issue using two methods. (1) First, I ran a series of models where I included all possible two and three way interactions between enemy removal treatments, which allowed me to identify any interactive or synergistic treatment effects. However, due to power issues, these models could not contain all interactions between treatments and status, or all necessary random terms to eliminate pseudoreplication from models. (2) Because these initial models revealed that only fencing significantly affected performance and enemy removal treatments did not interact, I conducted a second set of analyses including the fencing treatment only. These models allowed me to include all interactions between plant family, status, type, and fencing treatment, and include all necessary random terms. Both analyses yielded consistent results, so here I present only the second set of models.

I conducted separate analyses for each performance metric [survival, vegetative biomass (g), flower number, reproductive biomass (g)]. I calculated an individual plant's flower number and reproductive biomass by summing data across all years of the experiment. I only collected vegetative biomass once for each species (2012 for annuals, 2013 biennials, 2014 perennials) and

all years were analyzed together. To test for the effects of treatments on survival, and to explore how survival differed across years, I analyzed survival for each year of the experiment separately. For survival models using 2014 data, I did not include a plant perenniality (type) term because only perennials remained in the experiment.

For lmer models, I determined significance of fixed and random effects using the lmerTest package in R, and for random effects, I used chi-squared tests using the rand function. Flower number (Poisson distribution) and survival (binomial distribution) were analyzed using general linear mixed models with the glmer function in the lme4 package in R. For these glmer models, I determined significance for fixed and random effects using chi-squared tests based on log-likelihood ratio tests. Reproductive and vegetative biomass data were log transformed to improve normality and analyzed using linear mixed models and the lmer function in the lme4 package in R. When I found a significant main or interactive effect of status, plant family, plant type, or fencing treatment ($P \leq 0.05$), I conducted post hoc Tukey tests using the multcomp package in R (v. 1.4-0, Hothorn et al. 2015). I removed species perenniality from final models as it did not contribute to predictive ability and was never significant. Non-significant interaction terms were removed from final models to increase power for testing main effects (Crawley 2007).

Tolerance: To determine if invasive species are more tolerant of enemy damage than native or noninvasive exotic species, I tested the effects of enemy damage on plant performance with mixed model ANCOVA. To test whether invasive species are more tolerant than natives or noninvasive exotics, I included performance (vegetative biomass, reproductive biomass, flower number) as response variables. Plant status (native, noninvasive exotic, invasive), plant family (Asteraceae, Fabaceae, Poaceae), damage (proportion of leaves with insect herbivory or

proportion of stems/tillers browsed), and all interactions were included as fixed factors. Because tolerance can be measured as the slope of the regression of plant performance on damage (McNaughton 1983, Strauss and Agrawal 1999), a significant interaction between plant status and damage would indicate that native, noninvasive exotic, and invasive species differ in tolerance. Species nested within status and damage x species interactions were included as random factors. If the damage x species random term was significant, then I concluded that species differed in their tolerance to damage.

I conducted separate analyses for tolerance to insect herbivores and mammalian browsers. Only data from individuals for which I had performance and damage data could be used for tolerance analysis; species that died before I could estimate damage were therefore not included, possibly leading me to overestimate tolerance if these individuals died due to high levels of enemy damage. Enemy damage and biomass data were natural log transformed to improve normality. When the main effect of damage or the damage by status interaction was significant ($P \leq 0.05$), I used post-hoc contrasts to evaluate whether the slopes for native, invasive, or noninvasive exotic species were significantly different from zero.

Time: Species in the experiment spanned a range of residence times from 72 to 176 years. Based on preliminary data exploration, I grouped species into two categories for analysis – those that had been in Michigan for less than 120 years, or those that had been in Michigan ≥ 120 years. I was unable to treat time as a continuous variable in models due to insufficient replication for the invasive status ($n = 10$). To determine whether mammalian browser or insect herbivore removal treatments affected plant performance differently based on species' residence time in Michigan, I tested the effects of fencing, insecticide, and residence time on plant performance with mixed model ANOVAs. Response variables included vegetative biomass (g), reproductive

biomass (g), and flower number. Status (native, noninvasive exotic, invasive), insecticide (sprayed, control), fencing (fenced, control), residence time (< 120 years, ≥ 120 years), and all interactions were included as fixed predictor variables. Species nested within status, plot nested within insecticide treatment, and plot nested within fencing treatment were included as random variables. Biomass response variables were natural log transformed to improve normality. Because the insecticide treatment did not influence species' performance, all non-significant ($p > 0.05$) interaction terms with insecticide were dropped from final models.

Results

Damage

Fencing and insecticide treatments effectively reduced damage from mammalian browsers and insect herbivores in all years of study. Mammalian browsing was reduced by 97.4%, 99.0%, and 97.0% in 2012, 2013, and 2014 respectively (mean $\pm$ SE branches browsed. 2012: control $11.7 \pm 0.9\%$, fenced $0.3 \pm 0.1\%$ [$F_{1,27} = 65.3$, $p < 0.001$; 2013: control $19.9 \pm 1.6\%$, fenced $0.2 \pm 0.1\%$: $F_{1,32} = 102.7$, $p < 0.001$; 2014: control $29.9 \pm 3.0\%$, fenced $0.9 \pm 0.6\%$ [$F_{1,34} = 65.3$, $p < 0.001$]). Insect herbivory was reduced by 42.9%, 74.6%, and 56.5% in 2012, 2013, and 2014 respectively (mean $\pm$ SE % leaf area removed. 2012: control $5.6 \pm 0.4\%$, insecticide $3.2 \pm 0.3\%$ [$F_{1,2214} = 27.0$, $p < 0.001$], 2013: control $6.7 \pm 0.5\%$, insecticide $1.7 \pm 0.2\%$ [$F_{1,44} = 61.6$, $p < 0.001$]; 2014: control $6.9 \pm 0.9\%$, insecticide $3.0 \pm 0.5\%$ [$F_{1,44} = 9.2$, $p = 0.004$]).

Performance

Survival: In 2012 plants survived significantly better in fenced plots for all plant statuses (Fig. 5a; $F_{1,11} = 40.38$, $P = 0.01$). The effects of the mammalian browser removal on survival did

Table 1: Results from mixed model analysis of variance (ANOVA) testing the fixed effects of plant status (native, noninvasive exotic, invasive), family (Asteraceae, Fabaceae, Poaceae), and mammalian browser removal (fencing treatment, control) on plant performance. Biomass and floral biomass were log transformed to fit normality assumptions. Binomial (survival) and count (flower number) data were analyzed using general linearized mixed models; all other data were analyzed using linear mixed models. Chi square statistics based on log-likelihood ratio tests are presented for random factors and general linearized models. Statistically significant ($P \leq 0.05$) effects are in bold.

Model Terms	Vegetative Biomass (g)				Reproductive Biomass (g)				Flower Number			
	df	F	χ^2	P	df	F	χ^2	P	df	F	χ^2	P
status	2, 44	5.48		0.008	2, 37	13.56		<0.001	4, 14	2.98	14.8	0.005
family	2, 43	4.18		0.02	2, 40	0.24		0.79	4, 14	13.02	32.5	<0.001
fencing	1, 119	0.76		0.39	1, 164	0.33		0.57	3, 14	1.15	12.1	0.007
status x fencing	2, 91	1.48		0.23	2, 29	3.58		0.04	2, 16	1.22	2.2	0.33
status x family	4, 42	3.39		0.02	4, 38	3.87		0.01	4, 20	0.30	1.2	0.88
family x fencing	2, 41	5.60		0.007	2, 83	0.42		0.66	2, 20	8.85	12.3	0.002
status x family x fencing	4, 36	3.36		0.02	1, 229	0.40		0.53	2, 23	0.84	2.4	0.496
Random Effects												
(species)status			22.8	<0.001			13.2	0.001			17.2	<0.001
(plot)fencing			23.3	<0.001			7.8	0.02			23.8	<0.001
species x fencing			1.5	0.20			2.0	0.16			14.3	<0.001

Model Terms	Survival 2012				Survival 2013				Survival 2014			
	df	F	χ^2	P	df	F	χ^2	P	df	F	χ^2	P
status	2, 11	2.06	4.2	0.12	2, 11	2.67	0.1	0.74	2, 11	2.82	4.4	0.11
family	2, 11	0.65	1.3	0.52	2, 11	0.51	1.0	0.61	2, 11	11.04	18.3	<0.001
fencing	1, 11	40.38	6.3	0.01	1, 11	0.11	4.3	0.12	1, 11	2.06	1.8	0.18
Random Effects												
(species)status			73.1	<0.001			11.0	0.004			27.4	<0.001
(plot)fencing			53.1	<0.001			12.8	0.002			5.0	0.08
species x fencing			7.8	0.005			4.2	0.04			0.1	0.77

not differ based on status, family, or plant type (Table 1). In 2013, removal of mammalian browsers did not significantly increase survival, and survival did not depend on plant status or family (Table 1). In 2014, removal of mammalian browsers did not significantly increase survival (fencing: $F_{1,11} = 0.11$, $P = 0.12$), and survival was higher in Asteraceae and Fabaceae compared to Poaceae (family: $F_{2,11} = 11.04$, $P < 0.001$).

Vegetative Biomass: Fencing treatments differentially affected native, noninvasive exotic and invasive species, but these effects depended on plant family (status x fencing x family; $F_{4,36} = 3.36$, $P = 0.02$). Fencing treatments did not affect Asteraceae biomass for any status (Fig. 6a), but significantly increased invasive Fabaceae biomass and decreased native Poaceae biomass. Additionally, averaged across all treatments, biomass for invasive Fabaceae was significantly

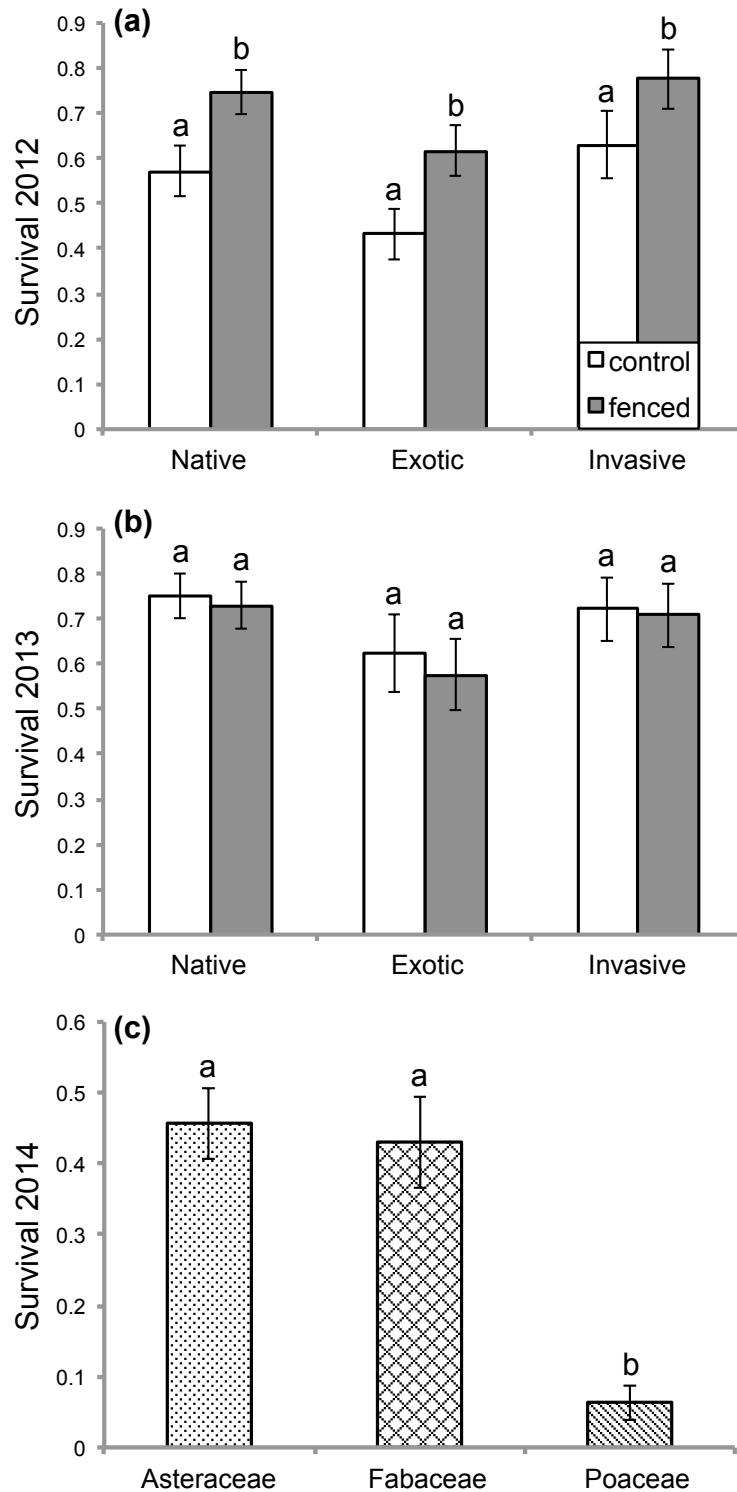


Figure 5: Three years of survival data for native, noninvasive exotic, and invasive plants in control (white bars) and fenced (gray bars) plots. Bars indicate mean $\pm$ SE. Status and family means with the same letter are not significantly different ($P \leq 0.05$) based on post-hoc contrasts.

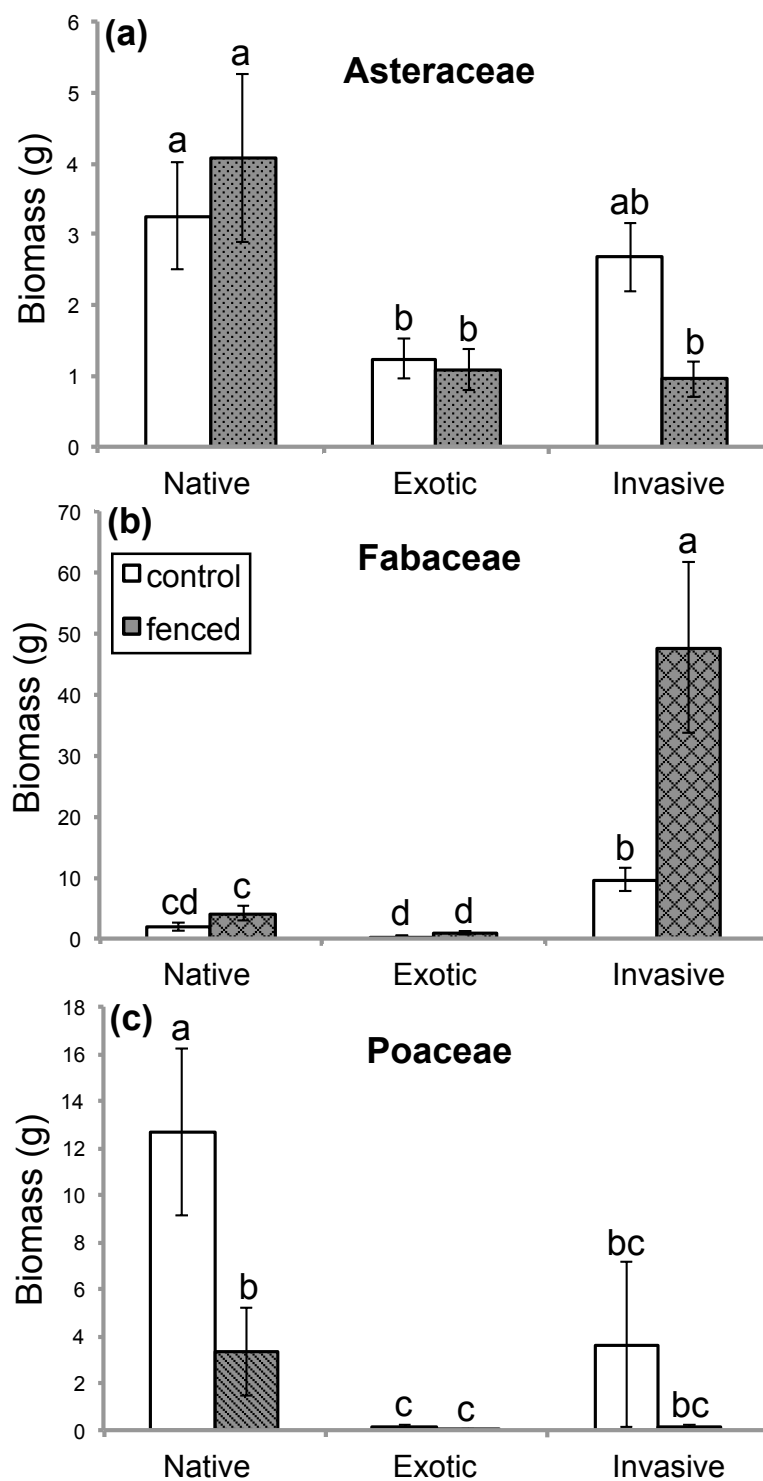


Figure 6: Biomass data for native, noninvasive exotic, and invasive plants in control (white bars) and fenced (gray bars) plots, divided by plant family. Bars indicate mean $\pm$ SE. Within family, means with the same letter are not significantly different ($P \leq 0.05$) based on post-hoc contrasts.

higher than that of natives or noninvasive exotics (Fig. 6b). Vegetative biomass was unaffected by insecticide and fungicide treatments (Table 1).

Flower Number and Reproductive Biomass: Flower number increased when mammalian browsers were removed, and was highest for the Fabaceae in fenced plots (family x fencing interaction: $F_{2,20} = 8.85$, $P = 0.002$). Plants in the Fabaceae produced by far the most flowers, while species in the Poaceae produced the least (family: $F_{4,14} = 13.02$, $P < 0.001$). Invasive species produced more flowers than did noninvasive exotic and native plants (status: $F_{4,14} = 2.98$, $P = 0.005$), however, though not statistically significant (status x family x fencing interaction: $F_{2,23} = 0.84$, $P = 0.50$), this pattern is driven by the Fabaceae family (Fig. 7b).

Reproductive biomass differed between native, noninvasive exotic, and invasive species (status: $F_{2,37} = 13.56$, $P < 0.001$), but this relationship depended on plant family and browser removal (Table 1; status x fencing interaction: $F_{2,29} = 3.58$, $P = 0.04$; status x family interaction: $F_{4,38} = 3.87$, $P = 0.01$). Invasive plants produced significantly more floral biomass when fenced, while floral biomass for native and noninvasive exotic species was unaffected (Fig. 7d-f). In the Asteraceae and Poaceae, native species had the highest floral biomass; in the Fabaceae invasive species had the highest floral biomass (Fig. 7d-f).

Tolerance

Species differed in their ability to tolerate insect herbivory and regrow vegetative biomass and produce flowers after damage. Tolerance to insect herbivory depended on species' status (status x herbivory interaction; vegetative biomass: $F_{2,375} = 3.95$, $P = 0.02$; flower number: $F_{2,11} = 5.46$, $P = 0.005$) (Table 2). Native species were able to, on average, compensate for insect herbivore damage in their regrowth of vegetative biomass and flowers, while noninvasive exotics

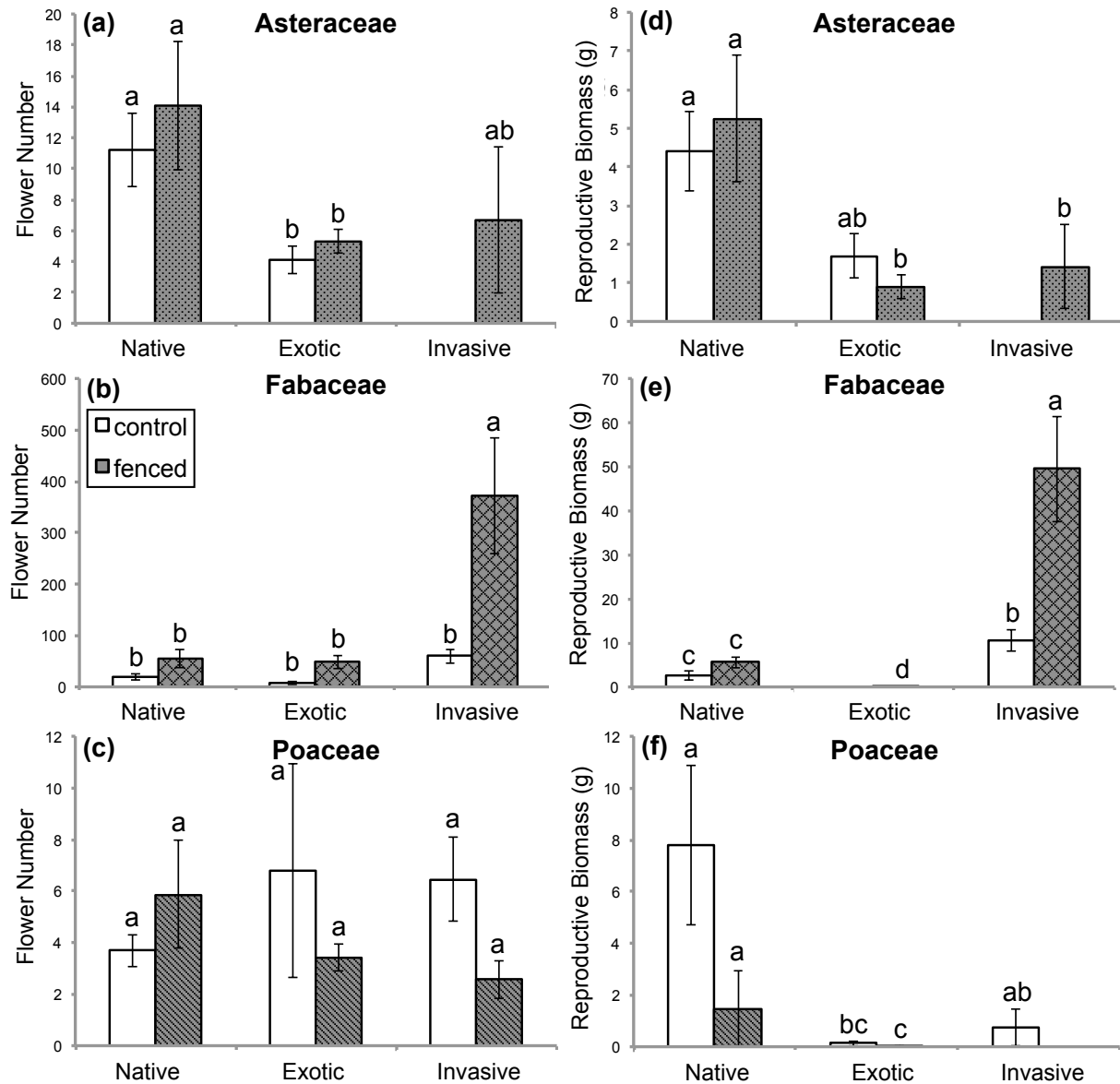


Figure 7: Flower number (a-c) and reproductive biomass (d-f) data for native, noninvasive exotic, and invasive plants in control (white bars) and fenced (gray bars) plots, divided by plant family. Bars indicate mean $\pm$ SE. Within family, means with the same letter are not significantly different ($P \leq 0.05$) based on post-hoc contrasts.

undercompensated and produced less biomass when damaged (Fig. 8a). Invasive species undercompensated for herbivory and produced fewer flowers when damaged, but fully compensated in terms of vegetative regrowth (Fig. 8c). Species identity contributed to tolerance above and beyond the variation explained by status for vegetative biomass regrowth and flower

Table 2: Results from tolerance mixed model analysis of variance (ANOVA) testing the fixed effects of plant status (native, noninvasive exotic, invasive) and enemy damage (insect herbivory and mammalian browsing) on plant performance (vegetative biomass, reproductive biomass, and flower number). Vegetative and reproductive biomass and damage data were log transformed to fit normality assumptions. Statistically significant ($P \leq 0.05$) effects are in bold.

Source	Vegetative Biomass (g)				Reproductive Biomass (g)				Flower Number			
	df	F	χ^2	P	df	F	χ^2	P	df	F	χ^2	P
<i>(a) Insect Herbivory Mixed Model ANCOVA</i>												
status	2, 42	5.14		0.01	2, 40	0.12		0.89	2, 11	5.64	2.9	0.09
family	2, 40	4.92		0.01	2, 33	5.49		0.009	2, 11	8.70	15.2	<0.001
herbivory	1, 428	6.67		0.01	1, 96	0.10		0.75	1, 9	3.78	3.4	0.06
status x herbivory	2, 375	3.95		0.02	2, 154	1.19		0.31	2, 11	5.46	10.6	0.005
status x family	4, 42	3.61		0.01	4, 34	1.30		0.29	4, 15	0.45	1.7	0.78
family x herbivory	2, 368	1.84		0.16	2, 154	0.05		0.95	2, 17	0.11	0.2	0.89
status x family x herbivory	4, 345	2.17		0.07	3, 194	0.58		0.63	4, 21	0.38	1.5	0.83
Random Effects												
(species)status			187.1	<0.001			70.0	<0.001			61.6	<0.001
species x herbivory			5.0	0.03			0.0	0.80			85413	<0.001
<i>(b) Mammal Browser Mixed Model ANCOVA</i>												
status	2, 80	4.11		0.01	2, 59	0.11		0.90	2, 9	7.22	3.6	0.16
family	2, 115	0.98		0.38	2, 126	0.09		0.92	2, 9	11.87	20.0	<0.001
browsing	1, 1106	2.46		0.12	1, 430	0.02		0.90	1, 9	8.33	6.5	0.01
status x browsing	2, 1105	2.63		0.07	2, 405	2.80		0.06	2, 11	0.93	1.9	0.39
status x family	4, 90	0.99		0.40	4, 47	1.77		0.15	4, 15	0.59	2.2	0.71
family x browsing	2, 1105	6.33		0.002	2, 422	0.43		0.65	2, 17	1.44	2.9	0.24
status x family x browsing	3, 1105	6.91		<0.001	2, 405	3.89		0.02	2, 19	0.38	0.8	0.67
Random Effects												
(species)status			67.0	<0.001			17.3	<0.001			5.5	0.06
species x browsing			0.0	1.00			0.0	0.90			10111	<0.001

production after damage (vegetative biomass: $\chi^2 = 5.0$, $P = 0.03$; flower number: $\chi^2 = 85413$, $P < 0.001$). The ability to maintain reproductive biomass when damaged did not vary based on species identity ($\chi^2 = 0.0$, $P = 0.80$) or plant status (Fig. 8a; $F_{2,154} = 1.19$, $P = 0.31$).

Tolerance to mammalian browsing, or the ability to maintain vegetative and reproductive biomass when browsed, depended on status but these effects varied across families (damage x status x family interaction; vegetative biomass: $F_{3,1105} = 6.91$, $P < 0.001$; reproductive biomass: $F_{2,405} = 3.89$, $P = 0.02$) (Table 2). In the Asteraceae, native and invasive species overcompensated and produced more vegetative and/or reproductive biomass (invasives only) when browsed, while exotic species compensated for browsing and maintained similar biomass levels (Fig. 8d). . In the Fabaceae, invasive species undercompensated and produced less vegetative and

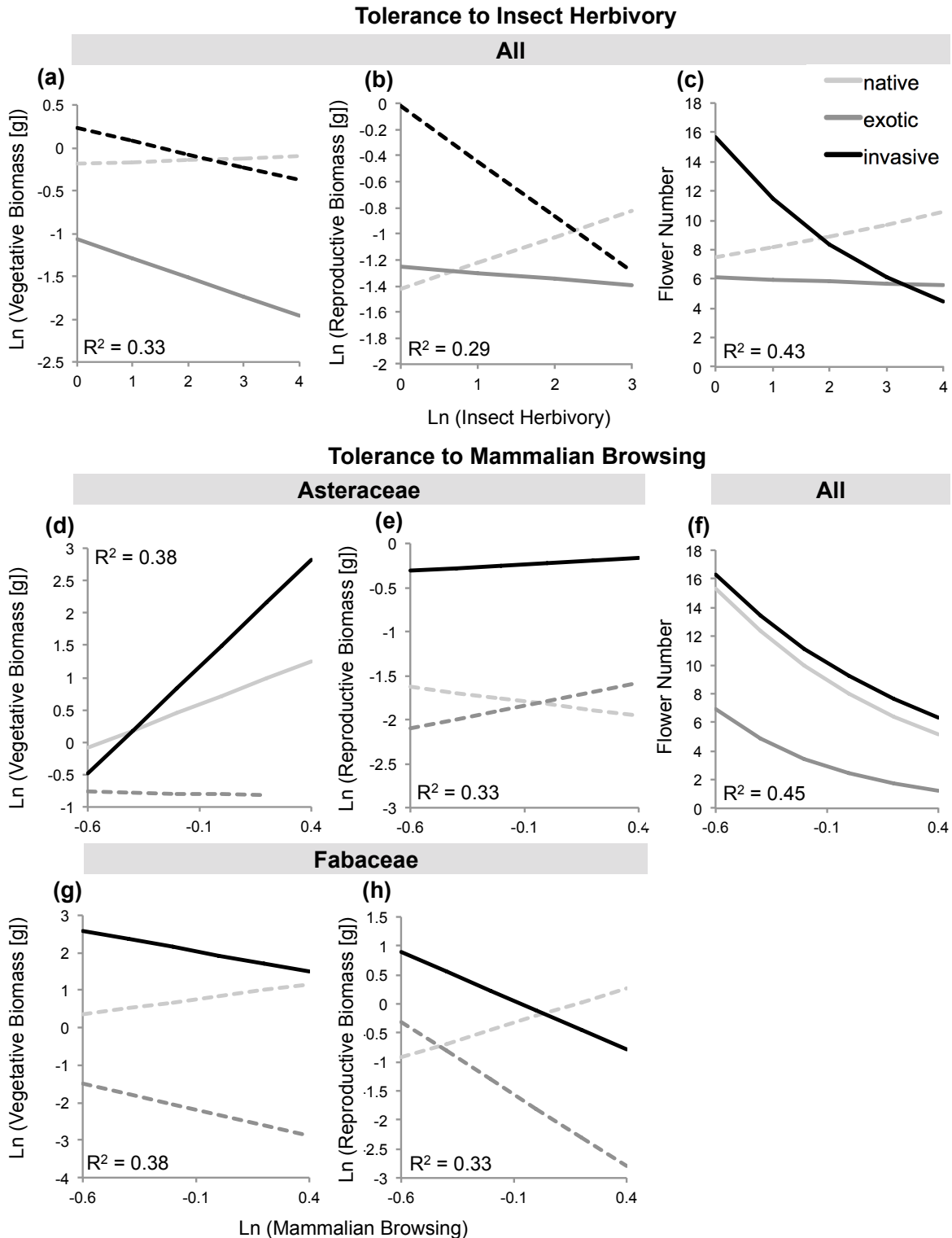


Figure 8: The effects of plant status and family on tolerance to insect herbivory (a-c) and mammalian browsing (d-h) in the field. Negative slopes indicate undercompensation and that the biomass and flower number of damaged plants is less than that of undamaged plants (undercompensation). Positive slopes indicate increased mass and flower number due to damage (overcompensation), and values of zero indicate compensation and no net change in growth rate. Solid regression lines show slopes that are significantly different from zero ($P \leq 0.05$), and dashed lines represent slopes that are not significantly different from zero.

reproductive biomass when browsed. Native and exotic species were able to compensate for mammalian browsing and produce similar levels of vegetative and reproductive biomass when browsed (Fig. 8g). In the Poaceae, most individuals that survived until biomass harvest did not receive any browsing damage and there was insufficient variation to calculate slopes using regression. For flower number, species differed in their ability to tolerate mammalian browsing ($\chi^2 = 10111$, $P < 0.001$), but this relationship did not depend on status ($F_{2,11} = 0.93$, $P = 0.39$) (Table 2). Native, invasive, and noninvasive exotic species all produced fewer flowers when browsed (Fig. 8f).

Time

Invasive species with longer residence times benefited more from mammalian browser removal than invasives more recently introduced (Fig. 9, Table 3). Invasives with residence times in Michigan equal to or longer than 120 years grew significantly larger and tended to produce more flowers when fenced (status x fencing x time; vegetative biomass: $F_{1,510} = 16.88$, $P < 0.001$; flower number: $F_{3,15} = 8.47$, $P = 0.04$). Species with residence times less than 120 years did not benefit from mammalian browser removal. Fencing effects on reproductive biomass did not differ based on status or residence time (status x fencing x time: $F_{1,284} = 0.01$, $P = 0.91$). The insecticide treatment did not affect species performance for any status or residence time (Table 3).

Discussion

I found no evidence that enemy release is a general mechanism contributing to invasiveness in this system. Invasive species were affected by enemy removal treatments,

Table 3: Results from mixed model analysis of variance (ANOVA) testing the fixed effects of plant status (noninvasive exotic, invasive), insecticide treatment (sprayed, control), fencing treatment (fenced, control), and time on plant performance. Vegetative and reproductive biomass data were log transformed to fit normality assumptions. Chi square statistics based on log-likelihood ratio tests are presented for random factors. Statistically significant ($P \leq 0.05$) effects are in bold.

Model Terms	Vegetative Biomass (g)				Reproductive Biomass (g)				Flower Number			
	df	F	χ^2	P	df	F	χ^2	P	df	F	χ^2	P
status	1, 22	6.26		0.02	1, 28	1.84		0.19	1, 9	1.95	1.94	0.16
time	1, 22	0.13		0.73	1, 28	1.77		0.19	1, 9	0.79	1.135	0.29
insecticide	1, 37	0.00		0.96	1, 283	1.81		0.18	1, 9	2.49	2.306	0.13
fencing	1, 41	2.63		0.11	1, 284	10.35		0.001	1, 9	42.33	6.513	0.01
status x fencing	1, 504	2.52		0.11	1, 283	2.46		0.12	1, 12	52.74	21.79	<0.001
status x time	1, 22	1.57		0.22	1, 28	0.13		0.72	1, 12	0.31	0.2	0.70
time x fencing	1, 500	9.90		0.002	1, 283	0.93		0.34	1, 12	441.40	390.6	<0.001
status x time x fencing	1, 510	16.88		<0.001	1, 284	0.01		0.91	3, 15	8.47	8.5	0.04
Random Effects												
(species)status			275.6	<0.001			141.5	<0.001			23300	<0.001
(plot)insecticide			0	1.00			0	1.00			0	1.00
(plot)fencing			9.9	0.007			0.1	1.00			0	1.00

similarly to native and noninvasive exotic species, and their performance was affected to the same degree by insect herbivores, mammalian browsers, and fungal disease. These findings are consistent with the few other studies that have experimentally manipulated enemy presence, which also found invasive species to be equally affected by biotic interactions (Parker et al. 2006, Heard and Sax 2012, Stricker and Stiling. 2012, Dawson et al. 2014). A meta-analysis of studies that manipulated biotic interactions on native, invasive, and noninvasive exotic species found that, in general, species benefitted from the removal of enemies, however this effect did not differ between native, noninvasive exotic and invasive species (Chapter 5).

Community Complexity and Enemy Release

Removal of mammalian browsers was the only treatment to significantly affect performance of experimental plants, and I found no significant interactions between insect herbivore, mammalian browser, or fungal disease removal treatments. These results suggest that escape from multiple enemies also is unlikely to explain invasiveness in this system. These

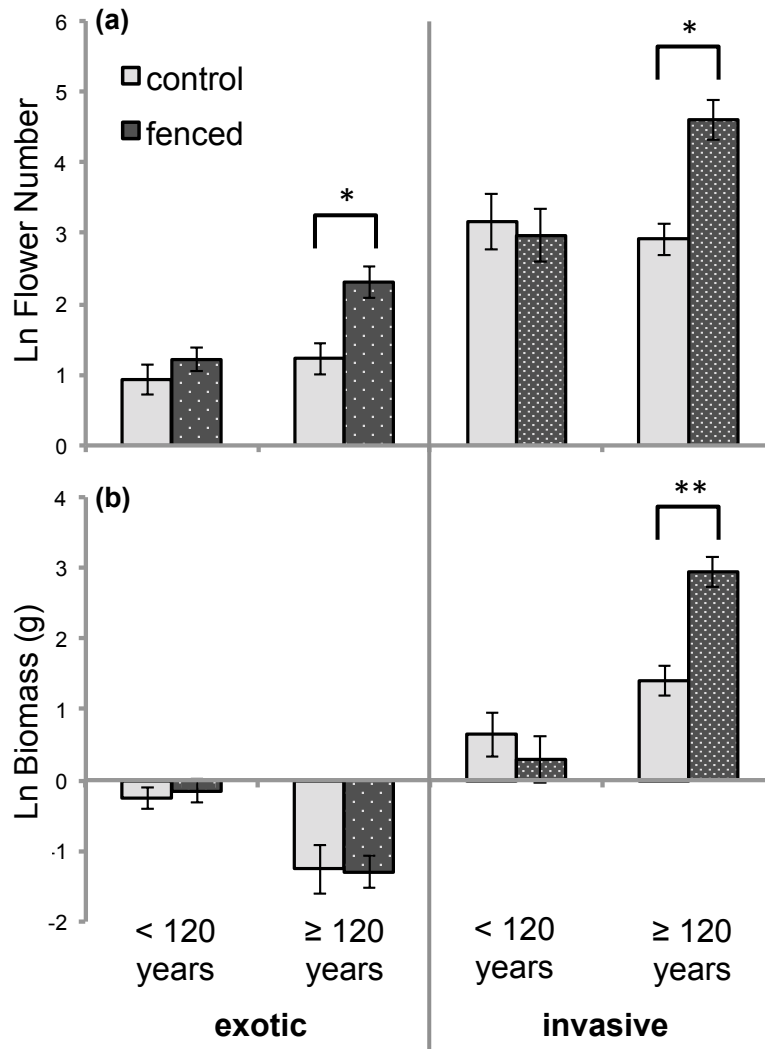


Figure 9: Natural log flower number (a) and natural log reproductive biomass (b) data for noninvasive exotic and invasive plants in control (light gray bars) and fenced (dark gray bars) plots, divided by species residence time.

findings are consistent with other studies that have manipulated multiple biotic interactions on native and introduced species. Prior to this study, 23 studies manipulated more than one biotic interaction on native and introduced species, and of those, nine found interactive effects of their treatments (Chapter 5). Only one previous study factorially manipulated three biotic interactions (competition, soil microbial community, and arbuscular mycorrhizal fungi) on invasive *Centaurea stoebe* and native *Ammophila breviligulata* and found no interactive effects between

treatments (Emery and Rudgers 2012). This study is the first to factorially manipulate three biotic interactions for a diverse set of native, noninvasive exotic, and invasive species, but also finds that escape from multiple enemies does not explain invasiveness.

Despite the fact that enemies had similar effects across plant status, invaders on average had higher biomass than native and noninvasive exotic species. This pattern was primarily driven by invasive Fabaceae, which were the largest and highest performing species in the experiment. Invasiveness in the Fabaceae may occur despite strong effects from enemies and instead, may be related to performance traits. Consistent with this hypothesis, a meta-analysis comparing the traits of invasive and exotic species found that invasiveness in plants is positively associated with performance-related traits, such as growth rate, size, and flower and seed number (van Kleunen et al. 2010).

Interestingly, invasives tended to have higher biomass, reproductive biomass, and flower numbers, but only for those individuals that were less damaged by herbivores and browsers. Across years and growing seasons, enemy abundance and damage can fluctuate greatly (Agrawal and Kotanen 2003, Agrawal et al. 2005, Parker and Gilbert 2007), and invasive species may be better able to exploit opportunity windows during periods with low enemy damage, while performing similarly to native and noninvasive exotics when enemy damage is high. During the first year of their study, Agrawal and Kotanen (2003) found that introduced plants were more damage by herbivores than were natives, similar to our own system (Schultheis et al. 2015). However, during the second year of their study, they found that introduced plants were less damaged, supporting the ERH (Agrawal et al. 2005). Therefore, invaders in their system may be able to dominate during these periods of low enemy damage. However, in this system, invasive species consistently received more damage from insect herbivores and mammalian browsers

across years (Schultheis et al. 2015), though enemy fluctuations may occur over longer timescales, providing opportunity windows in some years.

Tolerance

Because invasive species in this system tended to experience higher insect herbivore and mammalian browsing damage than did native and noninvasive exotic species, I hypothesized that tolerance could play a role in invasive success (Schultheis et al. 2015). However, in this study I found that invasive species were generally no more tolerant to insect herbivory or mammalian browsing than were native and noninvasive exotic species. Invasive and native species compensated equally for insect herbivore damage, although reduced tolerance of noninvasive exotics may explain their lack of invasion success. In general, the findings presented here are similar to those of a recent review of studies that measured both damage and performance on native and invasive plants, which found no evidence that invasive plants were more tolerant than natives (Chun et al. 2010). Additionally, Ashton and Lerdau (2008) found no difference in tolerance among native, noninvasive exotic, and invasive species under field levels of insect herbivory and mammalian browser damage.

Interestingly, invasive Asteraceae overcompensated for mammalian browsing and were more tolerant than native Asteraceae. I included 13 noninvasive exotic Asteraceae species in the experiment (Table C1), however there was only one invasive Asteraceae, *Centaurea stoebe*. Though these results for invasive species cannot be extrapolated beyond this one species, the data indicate that tolerance could play an important role in the success of this invader and for noninvasive exotics in general. In contrast, invasive Fabaceae undercompensated for browsing while native species fully compensated; therefore, increased tolerance cannot explain

invasiveness in this family. However, tolerance measures that do not take into account the full lifespan of a plant should be interpreted with caution, as they do not represent lifetime performance (Stowe et al. 2000). For annuals and biennials in this experiment, tolerance measures represent lifetime values, however perennials may have survived if not harvested. Compensation could be due to reallocation of belowground biomass to aboveground tissues, which could result in lower lifetime performance even though we observe no decrease in performance in one growing season. For example, while certain *Ipomopsis aggregata* genotypes exhibit overcompensation over their lifetime after mammalian browsing (Paige and Whitham 1987, Paige 1992), this species represents the extreme end of a continuum of known plant responses to antagonistic interactions (Maschinski and Whitham 1989).

Dynamic Invasions

While enemy release was not operating generally for invaders, I found evidence that release is dynamic and experienced only by invasives with shorter residence times in their introduced range. Both invasive and noninvasive exotic species with residence times equal to or longer than 120 years tended to produce more flowers and vegetative biomass when fenced, while species with residence times shorter than 120 years on average did not. These results indicate that both exotic and invasive species that have been in their introduced range for less than 120 years experienced minimal effects from mammalian browsers, supporting the ERH.

Enemy release could be lost over time in this system due to accumulation of both native and introduced enemies on introduced plants, which switch from species found in the resident community. Both disease and herbivore pressure can be driven local population abundances and phylogenetic relatedness to the resident community (Agrawal and Kotanen 2003, Parker and

Gilbert 2007, Dostál et al. 2013, Parker et al. 2015). Compared to species that grow in less dense populations, invasive species growing in dense populations are expected to accumulate pathogens at a faster rate (Bever 1994, Mitchell et al. 2010). Pathogens and herbivores are more likely able to infect close relatives of their hosts (Gilbert and Webb 2007, Goßner et al. 2009, Pearse and Hipp 2009, Hill and Kotanen 2010). Additionally, plants with longer residence times have greater geographic spread (Ahern et al. 2010), raising their encounter rates with novel enemies as they enter new habitats and come into contact with more enemy species (Goßner et al. 2009, Flory and Clay 2013).

Treatment Effects on Background Community

Given that this experiment was conducted in an existing old field community, enemy removal treatments may have had unintended effects on the resident community, contributing to the observed patterns. For example, fungicide increased the amount of thatch present in experimental plots by 8.5% ($F_{1,28} = 4.8$, $p = 0.04$), potentially by reducing the number of fungal decomposers in the community (Appendix D). Accumulation of thatch from grass leaf tissue can alter abiotic conditions in a habitat, for example reducing light availability, inhibiting nitrogen fixation and CO₂ uptake in the soil, and decreasing soil temperatures (Knapp and Seastedt 1986). Similarly, fencing increased the standing stock of the background community by 18.6% ($F_{1,28} = 6.38$, $p = 0.02$), potentially increasing the competitive environment for experimental seedlings (Appendix D). Removal of mammalian browsers also would have removed the disturbance effect from trampling by ungulates, which potentially favors introduced species (Vavra et al. 2007). At this field site, *Odocoileus virginianus* (white tailed deer) occurred at a moderate to high density of ~30 individuals per square mile (MDNR 2010). Therefore, removal of deer and other

mammals may have harmed species that are adapted to ungulate feeding and trampling, while benefitting those that are more palatable to these browsers (Augustine and McNaughton 1998, Vavra et al. 2007). Native grasses, which have many adaptations decreasing vulnerability to mammalian browsing, such as the presence of silica in tissues and rhizomatous growth form, had reduced biomass in fenced plots where ungulates were excluded, potentially because of increased competition in these treatments. These non-target effects on background community may have negated any direct effects of enemy removal on plant performance, and could have altered the strength or direction of treatments on experimental plants.

Conclusion

Due to accidental and purposeful transport of species into new regions, today introduced species are present in most communities across the globe (Lonsdale 1999). Introduced plants make up 34% of the flora in Michigan, and an average of 55.5 new species establish in Michigan each decade (Ahern et al. 2010). Loss of antagonistic biotic interactions during the introduction process is hypothesized to drive the population growth and success of invasive species. In this study, I manipulated three classes of enemies to study their effects on multiple native, invasive, and noninvasive exotic species' performance. I did not find evidence that ERH was a general mechanism explaining the success of invaders in this system. However, tolerance and competitive traits may explain the invasive success of some taxa (e.g., Asteraceae and Fabaceae respectively). Though ERH was not generally supported, I found evidence that ERH is dynamic and potentially lost with increasing time in the introduced range. Thus, while ERH may not be a universal mechanism behind the success of all invaders, it may still be important for some species at certain points in the invasion process (Heger and Jeschke 2014).

CHAPTER 4: COMPETITIVE ABILITY, NOT TOLERANCE, MAY EXPLAIN SUCCESS OF INTRODUCED PLANTS OVER NATIVES

Introduction

Invasive species are one of the greatest threats to biodiversity (Vilà et al. 2011, Powell et al. 2011, Powell et al. 2013), on par with habitat destruction and climate change (Sala et al. 2000, Tylianakis et al. 2008). While invasive species are rarely competitively dominant or major components in their native systems, in novel communities they often have larger populations, grow more densely, have higher fitness, and are able to outcompete natives (Hinz et al. 2004, Vilà et al. 2011, but see Firn et al. 2011). Biologists have struggled to identify the underlying mechanisms driving invasiveness and the effects of invaders on native communities.

One feature that is shared by all introduced species is that, during the process of introduction, they disassociate from many biotic interactions from their native range while simultaneously forming new biotic interactions in their introduced range (Hallett 2006, Mitchell et al. 2006). Novel biotic interactions could influence the performance of introduced species and promote invasiveness. For example, release from antagonists has been hypothesized to play a role in the prolific success of some of the most invasive species (Thellung 1912, Elton 1958). Additionally, evolutionary naïve native species may be more susceptible to novel competitive mechanisms, leading to decreased performance of native populations and loss of community diversity (Callaway and Aschehoug 2000).

Enemy Release and Tolerance

Enemies, such as insect herbivores, mammalian browsers, competitors, disease, and predators, may all regulate the population dynamics and performance of native and introduced

plants (Harper 1977, Louda 1982, Crawley 1989, Levine et al. 2004, Chapter 5). Therefore, loss of key enemies from the native range may explain the increased performance experienced by invasive species in their introduced ranges (Enemy Release Hypothesis [ERH]; Elton 1958, Callaway and Aschehoug 2000, Maron and Vila 2001, Keane and Crawley 2002). However, comparisons between native and introduced range populations and between native and invasive species in introduced communities find that invaders are not consistently less damaged by enemies (Chun et al. 2010, Dostál et al. 2013), and often times are more damaged (Colautti et al. 2004, Torchin and Mitchell 2004, Agrawal et al. 2005, Carpenter and Cappuccino 2005, Morrison and Hay 2011, Dawson et al. 2014, Schultheis et al. 2015). Therefore, the ability to maintain performance when damaged may play an important role in invasiveness. Invasive species may not be those released from enemy damage, but instead those that are better able to tolerate high levels of damage in their introduced ranges (Maron and Vila 2001, Keane and Crawley 2002).

Plants defend against enemies in two ways – resistance and tolerance (Marquis 1992, Stowe et al. 2000). Resistance traits reduce the amount of enemy damage sustained, while tolerance traits allow the plant to maintain performance once damaged (Strauss and Agrawal 1999, Stowe et al. 2000). Plant architecture and resource allocation patterns both contribute to tolerance; for example, individuals that store more resources belowground may be more tolerant to aboveground damage (Hochwender et al. 2000). Additionally, plants with a greater number of meristems can be more tolerant to herbivory; for instance, mammalian browsing can release from dormancy secondary meristems when the primary meristem is damaged in grass species (Olson and Richards 1988). Tolerance can also vary based on traits related to performance under different abiotic conditions, for example tolerance to antagonistic soil microbes was correlated

with ability to maintain performance under low-light conditions in 21 tropical tree species (McCarthy-Neumann and Kobe 2008).

Competitive Ability of Invasive Species

The mechanisms responsible for invasive species establishment and effects on the native community are rarely identified (Levine et al. 2003). However, a review of the studies that identified mechanisms found that most invaders had strong negative effects on native community members through competition for resources like light and water and through allelopathy (Levine et al. 2003). Thus, successful invaders may be those plants that are competitively superior in their new communities (Crawley 1987, Hinz and Schwarzlaender 2004, van Kleunen et al. 2010, Dawson et al. 2014), utilizing resources more efficiently and growing larger and more densely in their introduced range (Hinz et al. 2004). Additionally, invaders may compete through mechanisms novel to the community, such as allelochemicals not previously present, leading to competitive dominance of naïve native neighbors (Callaway and Aschehoug 2000).

Both herbivory and competition contribute to biotic resistance of the native community to invasion (Levine et al. 2004), yet few studies have explored the effects of competition and herbivory on invaders simultaneously (Heard and Sax 2013), while many have studied them independently (Chun et al. 2010, Levine et al. 2004, Chapter 5). Release from enemies may increase competitive ability, by making more resources available for competitive traits, or over longer timescales as invasive species evolve to reallocate resources from defensive to competitive traits (Blossey and Nötzold 1995). The simultaneous manipulation of both herbivory and competition not only tests the two major hypothesis addressing invasive species success and

effect on native species, but could reveal non-additive or synergistic effects that cannot be observed when both are studied in isolation.

Here, we test whether invasive species are more tolerant to herbivory or are more competitive compared to native and noninvasive exotic species. Using a manipulative greenhouse experiment we ask the following questions: (1) Do invasive plant species have higher tolerance to simulated herbivory compared to native and noninvasive exotic plants? (2) Do invasive plants demonstrate a greater competitive ability (competitive effects and response) than native and noninvasive exotic plant species? (3) Are the effects of competition and herbivory synergistic, reducing performance to a greater degree when both are present? If tolerance contributes to invasiveness, we predict that invaders will experience minimal effects from simulated herbivory, while native and noninvasive exotic species will be more negatively affected. If competitive ability contributes to invasiveness, we predict that invaders will both experience minimal effects from the presence of a competitor, while simultaneously reducing native species' performance to a greater degree than native and noninvasive exotic species. Finally, if competition and simulated herbivory have synergistic effects on performance, we expect plants grown in the presence of clipping and competition to have reduced performance below that predicted by the additive effects of both treatments.

Methods

Study Species

In our study, we included 19 old field plant species representing three of the four plant families ($n = 6$ Asteraceae, 6 Fabaceae, and 7 Poaceae) that have contributed most to invasive plant species in Michigan (Ahern et al. 2010) (Table 4). We categorized species as native,

Table 4: List of the 18 experimental species, and one competitor species, used in the experiment, along with their family and status designation. The competitor species, *Elymus canadensis*, is indicated with an *.

Family	Species Name	Abbrev.	Status
Asteraceae	<i>Centaurea cyanus</i>	CENCY	exotic
Asteraceae	<i>Coreopsis tinctoria</i>	CORTI	exotic
Asteraceae	<i>Sonchus oleraceus</i>	SONOL	exotic
Asteraceae	<i>Centaurea stoebe</i>	CENST	invasive
Asteraceae	<i>Coreopsis lanceolata</i>	CORLA	native
Asteraceae	<i>Erigeron annuus</i>	ERIAN	native
Fabaceae	<i>Coronilla varia</i>	CORVA	invasive
Fabaceae	<i>Lespedeza cuneata</i>	LESCU	invasive
Fabaceae	<i>Lotus corniculatus</i>	LOTCO	invasive
Fabaceae	<i>Melilotus officinalis</i>	MELOF	invasive
Fabaceae	<i>Desmodium canadense</i>	DESCA	native
Fabaceae	<i>Lespedeza capitata</i>	LESCA	native
Poaceae	<i>Bromus hordeaceus</i>	BROHO	exotic
Poaceae	<i>Poa trivialis</i>	POATR	exotic
Poaceae	<i>Bromus inermis</i>	BROIN	invasive
Poaceae	<i>Poa compressa</i>	POACO	invasive
Poaceae	<i>Bromus kalmii</i>	BROKA	native
Poaceae	<i>Elymus canadensis</i>	*ELYCA	native
Poaceae	<i>Sorghastrum nutans</i>	SORNU	native

noninvasive exotic, or invasive (n = 7, 5, and 7 species respectively) based on presence on local invasive species lists and herbarium records, and in consultation with local land managers (Schultheis et al. 2015). Invasive and noninvasive exotic species are both introduced to Michigan from outside the U.S. by human actions, either accidentally or intentionally (Reznicek et al. 2011). Noninvasive exotic plants assimilate into the native community with little effect, while invasive plants aggressively colonize natural areas and threaten biodiversity and human interests.

Experimental Design

To test tolerance to herbivory and competition, we initiated a greenhouse experiment at the W.K. Kellogg Biological Station, factorially manipulating simulated herbivory (clipping

treatment, control) and competition (competitor present, absent) ($n = 5$ replicates per species per treatment) ($N = 370$ pots). In addition, we included ten replicates of the competitor species, *Elymus canadensis*, grown alone, half of which were subjected to the clipping treatment. We germinated seeds and then directly transplanted experimental seedlings into 656ml pots (D40 Deepots, Stuewe & Sons, LLC.) containing a mixture of potting soil (Sunshine Mix #5; SunGro Horticulture Canada Ltd., Alberta, Canada), peat moss (Pro-Moss Hort, Premier Tech Ltd, Pennsylvania USA), sand (Tubesand Quikrete International, Inc, Georgia, USA) and perlite (Horticultural Perlite, Midwest Perlite, Wisconsin, USA) in a 3:3:3:1 ratio on 20 June 2013. We watered plants as needed during the course of the experiment. Three weeks after planting, we added 50 mL of water to each pot containing dissolved fertilizer (Miracle-Gro All Purpose Plant Food, NPK 24-8-16) at a concentration of 1.2 g/L. The location of each species and treatment was randomized at the pot level. Pots were spaced a minimum of 12cm apart to prevent shading and light competition between seedlings not growing within the same pot.

To manipulate competition, we grew half of our experimental seedlings in pots alone, while the other half grew with one individual of a competitor species, *Elymus canadensis* (Canada wild rye). *Elymus canadensis* is a grass native to Michigan, and was chosen as our competitor species because it overlaps in geographic range and habitat preference with all of our experimental species. On 12 August 2013 we administered a simulated herbivory treatment to half of our experimental seedlings. We measured the height of each seedling and clipped 50% of each individual's height, which was similar to herbivory from insect herbivores and mammalian browsers observed on these species in the field (Schultheis et al. 2015).

On 2 October 2013 we harvested the experiment and measured plant performance metrics on both the experimental species and the competitor *E. canadensis*, including height (cm) from

the soil surface to apical meristem, aboveground biomass (g), and flower number. Flower number analysis and results can be found in Appendix E, but are not presented in the main text because most species produced no flowers during the course of the experiment, and because flower number data could be misleading due to differences in phenology between experimental species. Harvested biomass was dried at 65°C for three days and weighed.

Statistical Analysis

Tolerance and Competitive Response: We performed all analyses in R (v. 3.2.0, R Core Team 2015). To determine whether our treatments influenced plant performance, we tested the effects of simulated herbivory and competition on plant biomass and height with mixed model ANOVA using the lmer function in the lme4 package in R (v. 1.1-7, Bates et al. 2015). Our models included plant biomass (g) or plant height (cm) as response variables and clipping treatment (clipped, unclipped), competition treatment (competitor present, absent), status (native, noninvasive exotic, invasive), family (Asteraceae, Poaceae, Fabaceae), and all possible interactions as fixed predictor variables. A significant negative effect of our competition treatment indicates a negative competitive response in our experimental species. A significant negative effect of our clipping treatment indicates that performance is reduced when clipped, indicating a negative tolerance value (undercompensation). Full compensation occurs when an individual's performance is the same in the presence and absence of clipping, and overcompensation results when clipping increases individual performance (Strauss and Agrawal 1999). Non-significant ($p > 0.05$) interaction terms were dropped from final models to increase our power to detect significant main effects.

The number of species in each status is the unit of replication for questions on whether

Table 5: Results from mixed model analysis of variance (ANOVA). Results show the effects of plant status (native, noninvasive exotic, or invasive), family (Asteraceae, Fabaceae, Poaceae), clipping treatment (clipped, control), and competition treatment (competitor present, no competition) on experimental plant biomass and height. Statistically significant ($P \leq 0.05$) effects are in bold.

Source	Biomass (g)				Height (cm)			
	df	F	χ^2	P	df	F	χ^2	P
<i>Native, Exotic, and Invasive Species</i>								
status	2, 18	2.51		0.11	2, 18	1.23		0.32
family	2, 18	0.04		0.96	2, 18	1.96		0.17
clipped	1, 54	20.35		< 0.001	1, 18	11.58		0.003
competition	1, 54	27.00		< 0.001	1, 36	6.09		0.02
clipped x competition	1, 54	0.31		0.58	1, 36	0.45		0.51
Random Effects								
(species)status			33.8	< 0.001			27.3	< 0.001
species x clipped			0.0	1.00			0.5	0.50
species x competition			0.0	1.00			0.0	1.00
species x competition x clipped			2.2	0.10			0.5	0.50

treatment effects differed between native, noninvasive exotic and invasive species, so we included species nested within status as a random factor in our models. To determine whether species responded differently to our treatments, we included species x clipping, species x competition, and species x competition x clipping interactions as random terms in our models. Because we were interested in proportional responses to our treatments, and to improve normality, height and biomass data were natural log transformed for analysis. P-values for fixed effects were obtained using the lmerTest package in R, and for random terms we used chi-squared tests and the rand function (v. 2.0-20, Kuznetsova et al. 2015).

Competitive Effects on *Elymus canadensis*: To determine whether invasive, noninvasive exotic, and native species differ in competitive effects, we tested the effects of competitor identity on *E. canadensis* performance with mixed model ANOVA. We measured competitive effect as the degree to which our experimental species reduced *E. canadensis* performance. Our model included *E. canadensis* biomass (g) or height (cm) as the response variable and competitor status (native, noninvasive exotic, invasive), competitor family (Asteraceae, Fabaceae, Poaceae),

Table 6: Results from mixed model analysis of variance (ANOVA). Results show the effect of competitor status, family, and whether the competitor was clipped for *Elymus canadensis* biomass and height. Statistically significant ($P \leq 0.05$) effects are in bold. Non-significant interaction terms were dropped from the final model.

Source	Biomass (g)				Height (cm)			
	df	F	χ^2	P	df	F	χ^2	P
<i>Elymus canadensis</i> <i>Competitor</i>								
competitor status	2, 22	4.67		0.02	2, 190	3.28		0.04
competitor family	2, 22	3.43		0.05	2, 190	2.74		0.07
competitor clipped	1, 173	0.21		0.65	1, 190	0.01		0.92
Random Effect								
(comp. species)comp. status			2.0	0.20			0.0	1.00
comp. species x comp. clipped			0.0	1.00			0.0	1.00

and whether the competitor species received the clipping treatment (clipped, unclipped), and all possible interactions as fixed predictor variables. No *E. canadensis* individuals flowered during the course of the experiment, so we were unable to determine competitive effects on fitness.

Non-significant ($p > 0.05$) interaction terms were dropped from final models. We included competitor species nested within competitor status and the species x clipping treatment interaction as random factors in our models. All *E. canadensis* performance data was natural log transformed for analysis.

Results

Tolerance and Competitive Response

Invasive, noninvasive exotic, and native species responded similarly to treatments, indicating that invasive species are no more tolerant to simulated herbivory and respond similarly to competition (Table 5, Fig. 10). Clipping and competition both reduced plant height and biomass (Fig. 10), but there was no interaction between the clipping and competition treatments, meaning that effects were additive (clipped x competition; biomass: $F_{1,54} = 0.31$, $p = 0.58$; height: $F_{1,36} = 0.45$, $p = 0.51$). Surprisingly, clipping and competition reduced plant

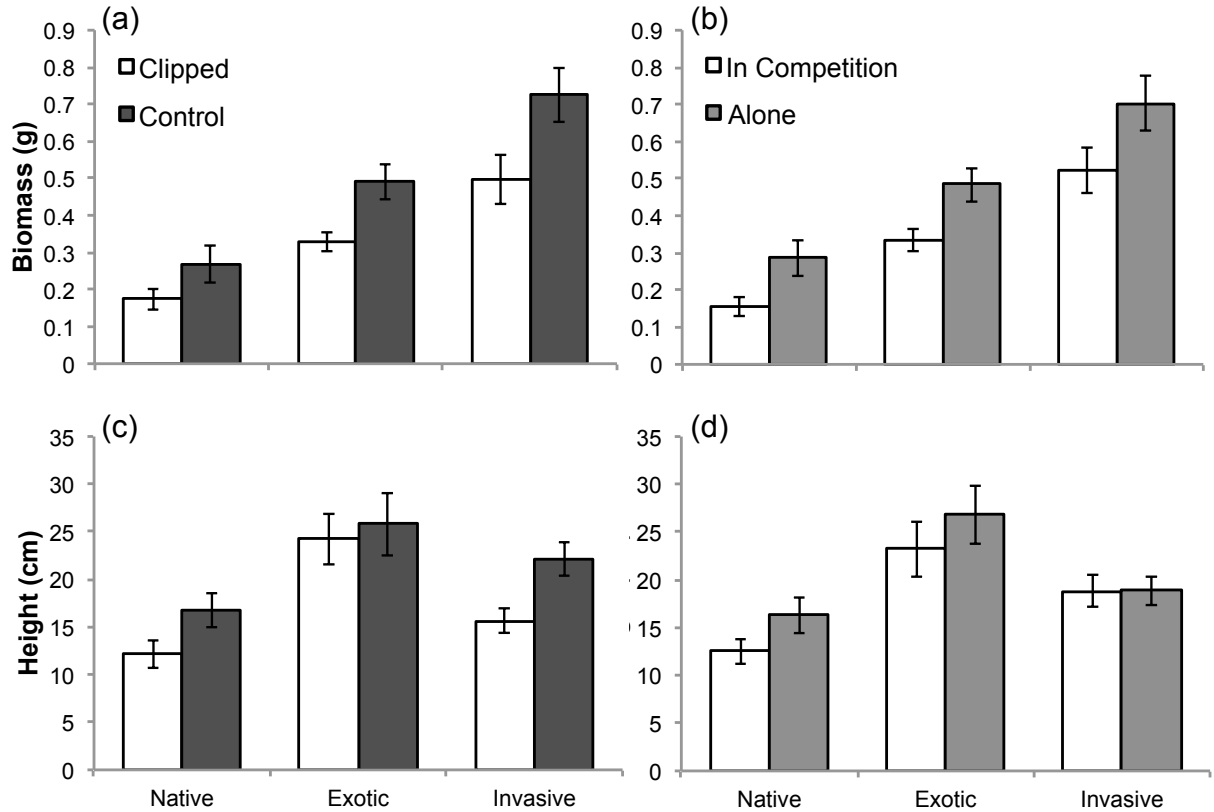


Figure 10: Biomass (a-b) and height (c-d) of native, noninvasive exotic, and invasive plants in clipped and unclipped treatments (a, c) or grown in the presence and absence of competition (b, d). Bars indicate mean $\pm$ SE.

biomass (species $\times$ clipped: $\chi^2 = 0.0$, $P = 1.0$; species $\times$ competition $\chi^2 = 0.0$, $P = 1.00$) and height (species $\times$ clipped: $\chi^2 = 0.5$, $P = 0.50$; species $\times$ competition $\chi^2 = 0.0$, $P = 1.00$) similarly for all study species, indicating that species did not differ in tolerance or competitive response.

Competitive Effects on Elymus canadensis

Competition marginally reduced *Elymus canadensis* biomass from $0.54\text{g} \pm 0.07$ to $0.40\text{g} \pm 0.02$ (mean $\pm$ SE) ($F_{1,188} = 2.87$, $p = 0.09$), and did not affect height ($F_{1,188} = 1.47$, $p = 0.23$). Invasive and noninvasive exotic species had the greatest competitive effects on *E. canadensis*, (significant effect of status on biomass: $F_{2,22} = 4.67$, $p = 0.02$; height: $F_{2,190} = 3.28$, $p = 0.04$; Fig.

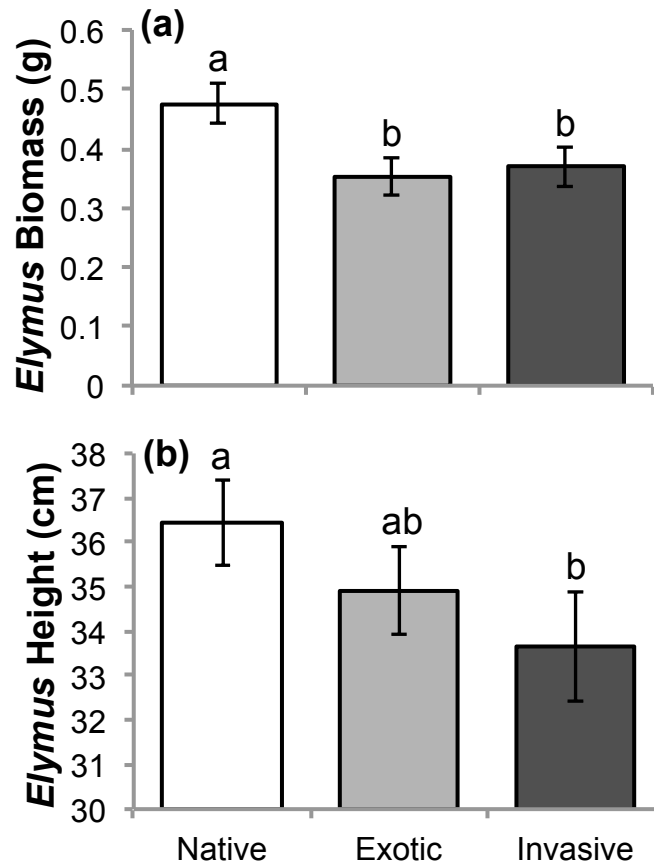


Figure 11: *Elymus canadensis* biomass (a) and height (b) when grown with native, noninvasive exotic, or invasive species competitors. Bars indicate mean $\pm$ SE. Means with different letters are significantly different ($P \leq 0.05$) based on post-hoc contrasts.

11). Notably, of the six species with the strongest competitive effect on *E. canadensis*, three were invasive and three were noninvasive exotic species (Fig. 12). Native, invasive, noninvasive exotic species exhibited similar competitive effects on *E. canadensis* when they were clipped or unclipped (competitor clipped x competitor status: $p > 0.05$), indicating that competitive ability was not affected by simulated herbivory. When grown with species in the Fabaceae, *E. canadensis* also tended to produce more biomass and was taller than when grown with species in the Asteraceae and Poaceae (Table 6). *Elymus canadensis* biomass, but not height, depended on

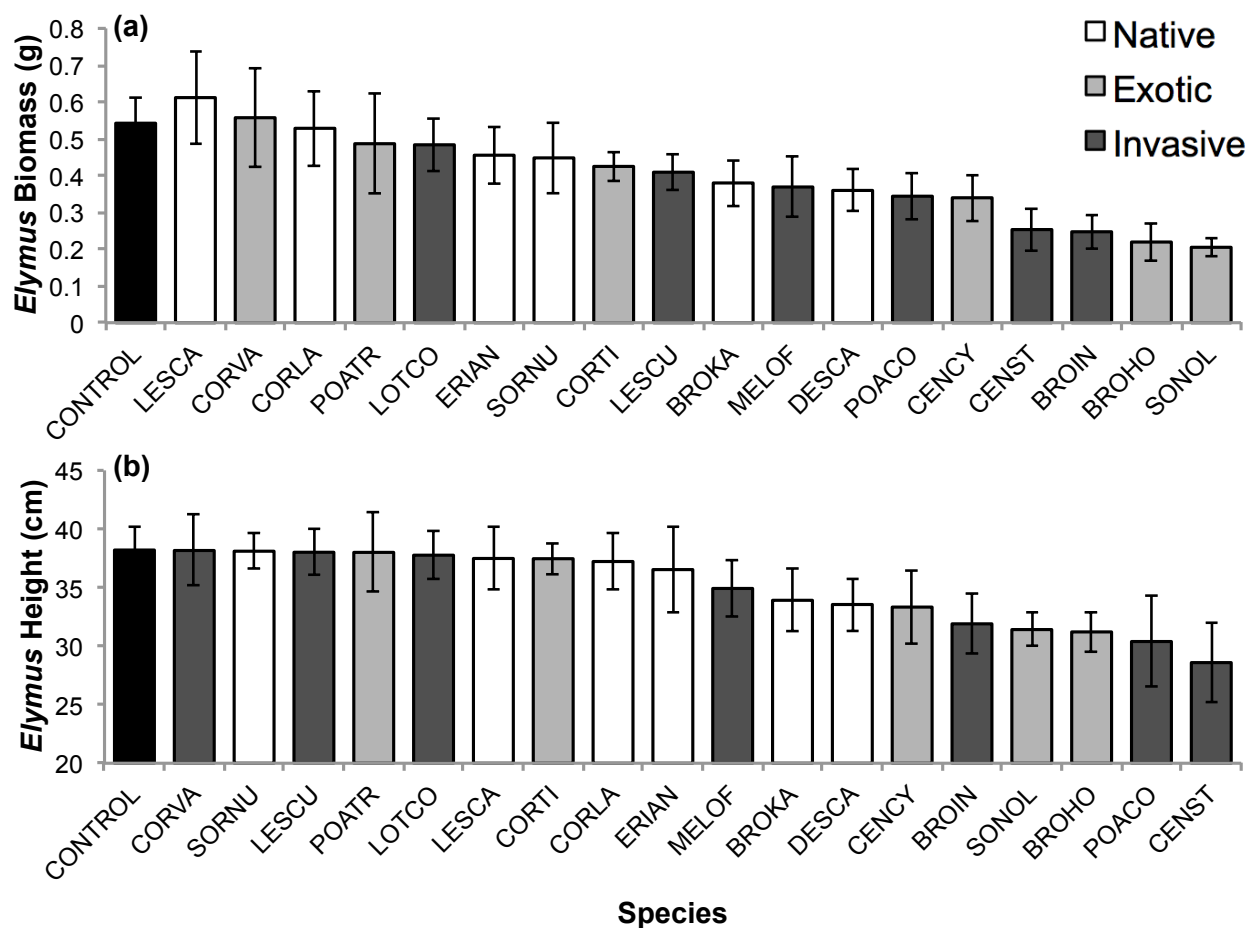


Figure 12: *Elymus canadensis* biomass (a) and height (b) when grown with different competing species. Bars are labeled by competing species status (native = white, noninvasive exotic = light gray, invasive = dark gray) and are ordered by descending mean values. The black bar indicates *E. canadensis* performance when grown alone. Bars indicate mean $\pm$ SE.

competitor species identity (biomass: $F_{18,171} = 2.17$, $p = 0.006$; height: $F_{18,171} = 1.50$, $p = 0.11$; Fig. 12).

Discussion

We found no evidence that invasive species were more tolerant to simulated herbivory or experienced less of a response to competition compared to native and noninvasive exotic species (Fig. 10). However, we found invasive and noninvasive exotic species exhibited the strongest

competitive effects on a native grass, *E. canadensis*. When grown with introduced competitors, *E. canadensis* produced less biomass and was shorter than when grown with natives, indicating that introduced species in this system may negatively effect native populations more so than other native competitors.

Competitive Ability of Invasive Species: Response and Effects

Invasive species often reduce native diversity and alter community structure through competitive effects on native species (Vilà et al. 2011, Levine et al. 2003), and our study is consistent with a review by Levine and colleagues (2003) that found when native and introduced species competed, introduced species often had stronger competitive effects on natives than natives on introduced species. These competitive effects could be driven by three mechanisms. First, because of lack of a shared evolutionary history between an introduced species and the new community (Verhoeven et al. 2009), antagonistic traits of the invader, such as allelopathy, often prove effective against native community members that lack previous experience with such tactics (Novel Weapons Hypothesis; Callaway and Ridenour 2004). Second, invasive species may be those that are simply more competitive for limiting resources, for example effectively driving down light and resources levels, excluding other species. Third, escape from enemies may increase introduced species growth and competitive effects on surrounding species (Keane and Crawley 2002, Klironomos 2002, Blair and Wolfe 2004).

Allelopathy, or chemically mediated plant interactions (Rice 1974, Meiners and Kong 2012), contributes to many successful plant invasions and may explain some of the strongest competitive effects observed in this experiment (Callaway and Aschehoug 2000, Callaway and Ridenour 2004). The success of the invasive plant, *Centaurea stoebe*, is partially attributed to the

novel weapon (–)-catechin, which it excretes from its roots serves as an allelochemical (Bias et al. 2003, but see Duke et al. 2009). Soils supporting populations of *C. stoebe* in the invasive range contain levels of (–)-catechin that are twice that found in the native range (Bias et al. 2003). This allelochemical negatively affects performance of natives in its invasive range (Ridenour and Callaway 2001), and to a lesser degree, natives in its native range (Bias et al. 2003). Interestingly, *C. stoebe* was still able to outcompete native species even when allelochemicals were inactivated using carbon, indicating that allelopathy only partially explains its competitive dominance in its invasive range (Ridenour and Callaway 2001). Invasive *Bromus inermis* has allelopathic effects on native and introduced species (Stowe 1979); native grass growth was halved in patches containing *B. inermis*, presumably due to the combined effects of competition and allelopathy (Dilleuth et al. 2009). In agricultural systems, fields planted with *Melilotus officinalis* had up to 97% lower unplanted weed densities due to direct competitive effects and release of allelochemicals by decomposing tissues (Blackshaw et al. 2001). These three invaders exhibited some of the strongest competitive effects observed in our experiment, suggesting that allelopathy may play an important role in invasiveness in our system.

Enemy Release and Tolerance

Interactions with native community members provide biotic resistance to introduced species, significantly reducing their performance (Levine et al. 2004), and often effect introduced species to an equal degree as natives (Chapter 5). Consistent with these findings, our simulated herbivory and competition treatments significantly reduced performance of invasive and noninvasive exotic species to a similar degree as natives. These results are also consistent with previous experiments in our system that determined that, in old field communities, invasive

species experienced similar performance effects of enemy damage and were no more tolerant of damage, compared to native and noninvasive exotic species (Schultheis et al. 2015). Similar to our own results, Ashton and Lerdau (2008) found that invasive temperate vine species were more damaged and were no more tolerant to browsing in the field, compared to native and noninvasive exotics. However, their simulated greenhouse manipulations revealed that invasive species were in fact more tolerant under controlled damage levels (Ashton and Lerdau 2008). Our clipping treatment was very similar theirs, where clipping stems removed 50% of all leaves, and we observed similar effects of our treatments on mean plant performance. The lack of higher tolerance exhibited by invasive species in our system could be due to the fact that invasive vines (Ashton and Lerdau 2008) and herbaceous species (this experiment) invade by different mechanisms.

Although our simulated herbivory and competition treatments reduced performance on average, most species in our experiment were able to maintain performance when experiencing simulated herbivory and competition, however some species were negatively affected by our treatments. Our tolerance measures represent just one growing season and should be interpreted with caution, as they do not represent species lifetime performance (Stowe et al. 2000). Compensation could be due to reallocation of belowground biomass to aboveground tissues, which may result in lower lifetime performance even though we observed no decrease in performance during the course of one growing season.

Conclusion

Due to unprecedented rates of transport of species across the globe, invasions are today common features shared by most ecosystems (Lonsdale 1999). Invaders threaten biodiversity,

often outcompeting and displacing native species. Here, we find evidence that competitive effects of introduced species on a native species likely contribute to their negative effects on native populations, but compared to native species, invasive and noninvasive exotic species were similarly affected by simulated herbivory and competition.

CHAPTER 5: MUTUALISM GAIN AND COMPETITIVE ABILITY, NOT ENEMY RELEASE, MAY EXPLAIN SUCCESS OF INVASIVE SPECIES: A META-ANALYSIS

Introduction

Identifying the mechanisms underlying the success of invasive species is one of the most challenging and pressing goals in the field of invasion biology. However, after almost 60 years of intensive study, no prevailing mechanism has yet been identified (Elton 1958). Dozens of hypotheses attempt to explain the increased population growth, size, and competitive ability of invasive species (van Kleunen et al. 2010), and many of these cite altered biotic interactions in the introduced range as potentially contributing to invasiveness (e.g. Enemy Release Hypothesis [Keane and Crawley 2002]). Here, we performed a meta-analysis of the literature to test whether altered biotic interactions generally contribute to invasive species' success, and investigated whether invasive species are released from antagonistic biotic interactions or benefit more from acquired mutualists compared to populations in their native range or co-occurring native and noninvasive exotic competitors in their introduced range.

Biotic interactions are major drivers of plant and animal community structure and population dynamics (Harper 1977, Crawley 1989, Louda 1982, Klironomos 2002, Morris et al. 2007). Thus, any alteration to these biotic interactions could have major effects on the populations of the species involved, be they native or introduced. For example, seed predators reduce plant population growth rates (Louda 1982), and herbivores limit species abundance and distributions to subsets of available habitat (Lau et al. 2008). In animals, predators greatly reduce the density of prey species (Krebs et al. 1995), and pathogens alter host population dynamics (Anderson and May 1981). Alternatively, mutualists increase individual performance, influencing local abundance and extending species' range sizes (Anacker et al. 2014).

When a species is introduced into a new community, it leaves behind native biotic interactions and encounters new mutualists, predators, herbivores, competitors, and diseases. Lack of a shared coevolutionary history between introduced species and new community members can lead to two potential outcomes (Elton 1958): (1) Biotic release – an introduced species may interact weakly with its invaded community, experiencing less damage from enemies, reduced suppression from competitors, and less benefit from mutualist partners. For example, native herbivores and pathogens may not recognize an introduced plant as a resource, which could result in reduced damage and increased fitness for the invader (Keane and Crawley 2002, Hallett 2006). Alternatively, (2) Biotic resistance – an introduced species may be equally, or even more, affected than native species by novel enemies and competitors, as it will have few evolved strategies to defend against them. For example, an introduced plant may lack defenses against unfamiliar herbivores, thus experiencing high attack rates and reduced fitness. In this case, intense novel antagonistic interactions may limit an introduced species' performance, preventing invasion (Levine et al. 2004).

Two complementary approaches have been used to compare effects of biotic interactions on native and introduced species (Liu and Stiling 2006): (1) Cross continental comparisons, which compare biotic interaction effects on populations of a single species in its native and introduced range, and (2) Native/introduced comparisons, which compare the effects of biotic interactions on co-occurring native and introduced species in a given location. Cross continental studies determine whether invasive species are doing something different in their new range compared to their native range (Hierro et al. 2005), while native/introduced comparison studies determine whether invasive species are doing something different compared to competing native or noninvasive exotics species in their new range.

To understand the role of biotic interactions in the invasion process, we must determine if and when positive and antagonistic interactions affect the fitness of introduced and native species, and whether differences in the magnitude of fitness effect can explain increased performance for invasive species, relative to native and noninvasive exotic species (Maron and Vila 2001). Ideally, experiments would manipulate biotic interactions to study their effects on individual performance or population growth rates (Maron and Vila 2001, Keane and Crawley 2002, Liu and Stiling 2006), although correlational approaches can also provide evidence on the fitness effects of biotic interactions. Many manipulative studies testing the effects of biotic interactions on native and introduced species have been conducted, yet no quantitative synthesis has been performed to determine whether biotic release is a prevailing mechanism shared by most invaders.

Previous meta-analyses have considered specific types of biotic interactions that may differ between native and invasive taxa (e.g., herbivory: Parker et al. 2006, Chun et al. 2010; plant-soil feedback: Kulmatiski et al. 2008, Suding et al. 2013). These studies all focused on one or two biotic interactions, or only considered introduced species without making comparisons to co-occurring native competitors or distinguishing between invasive and noninvasive exotics. For example, competitors, herbivores, and diversity of the resident community contribute to biotic resistance, reducing introduced species establishment and performance (Levine et al. 2004, Kimbro et al. 2013), but whether these effects are equivalent to those on native species was not considered. Another meta-analysis of nine studies found introduced species to be more affected by herbivores and disease than co-occurring native species, however invasive and noninvasive exotics were not considered separately (Chun et al. 2010). A meta-analysis on performance effects of herbivores by Parker and colleagues (2006) found no relationship between

invasiveness of introduced plants and herbivore effects, and compared these effects to those on native plants. Interestingly, native plants were most controlled by introduced herbivores, and introduced plants were most controlled by native herbivores (Parker et al. 2006). Meta-analysis of plant-soil feedback (PSF) studies manipulating entire soil communities found that native species were most negatively affected by PSF, invasive species least, and noninvasive exotic species to an intermediate degree (Kulmatiski et al. 2008). Similarly, introduced species were more likely to develop soil communities that facilitate their own growth (positive PSF), while native species cultivated soil communities that were detrimental to growth (negative PSF) (Suding et al. 2013). Even though many reviews and meta-analyses exist, no analysis has yet considered the multitude of biotic interactions experienced by introduced species in nature, nor compared the fitness effects of different types of mutualistic and antagonistic interactions on native, noninvasive exotic, and invasive species.

Here, we investigated whether altered biotic interactions during the process of introduction drive biological invasions. Our meta-analysis included studies that manipulated biotic agents to determine their effects on native, invasive, and noninvasive exotic species' performance (i.e., individual growth, fecundity, survival, and population growth). We included studies conducted in both the native and introduced range of a species (cross-continental), or conducted in the introduced range on native and introduced species (native/introduced comparison). Because introduced species commonly leave behind many strongly interacting species when they colonize new habitats, we predicted that introduced species (both invasive and noninvasive species) would be less strongly affected by biotic interactions, compared to co-occurring native species or populations in their historic range. Furthermore, if biotic release explains invasion success, we predicted that invasive species would be less affected by biotic

interactions than co-occurring noninvasive exotic species. Support for our latter prediction would provide evidence for biotic release as a general mechanism explaining invasion success.

Methods

We used traditional meta-analysis, a hierarchical framework (Appendix F), and vote counting approaches to test if and when altered biotic interactions facilitate biological invasions by comparing the fitness effects of biotic interactions among native, invasive, or noninvasive exotic species, type of organism (animal or plant), type of biotic interaction (competition, disease, herbivory, mutualism, predation, or plant-soil feedback) and performance metrics (hierarchical analysis only; individual growth, fecundity, survival, and population growth). Meta-analysis provided a tool to combine data from many individual studies and draw more general conclusions about whether the performance effects of biotic interactions differed for invasive species, compared to native and noninvasive exotic species across a wide range of taxa and types of biotic interactions. The multilevel framework allowed for testing how the magnitude of effect depended on the type of performance metric measured.

Literature Search and Data Collection

We searched ISI Web of Science for studies, published between 2000 and 2014, which manipulated biotic interactions on native and introduced species. Our searches included the topic search terms ([invasi*] OR [exotic*] OR [introduced]) AND ([enemy release] OR [enemy escape] OR [biotic resistance]) AND (*terms describing biotic interaction, see below*). We searched for studies covering the following types of biotic interactions: competition ([compet*]), disease ([disease] OR [fung*]), parasitism ([parasit*]), herbivory (folivory and browsing)

([herbivore] OR [herbivory] OR [brows*]), mutualism ([mutualis*]), plant-soil feedbacks (PSF) ([soil] OR [microb* community]), and predation (seed predation in plants) ([predate*]). We also searched for relevant reviews on the topic and used their bibliographies to crosscheck our own lists. These searches returned over 3,000 studies, from which we identified appropriate studies.

We included only: (1) Studies that experimentally manipulated the presence or intensity of biotic interactions under natural or realistic field conditions, or microcosm conditions if the manipulation could not be conducted in the field (mostly soil manipulations). For PSF, we included studies that grew plants in both live and sterilized soil, or in live soil conditioned by conspecific plants (home) and heterospecific plants (away). (2) Studies that performed manipulations on (a) both an introduced (noninvasive exotic or invasive) species and a co-occurring native species (native/introduced comparison studies), or (b) populations of an introduced species in its native and introduced range (cross-continental studies). If the genotype of the focal species was identified, we only included studies that collected data on local genotypes, such as native genotypes for manipulations conducted in the native range or introduced genotypes for manipulations conducted in the introduced range. (3) Studies that measured at least one performance metric (individual growth, fecundity, survival, or population growth).

From each study, we recorded species name, species status (native or introduced), and type of introduced species according to author classification in the text (invasive or noninvasive exotic). Though there can be some subjectivity about whether introduced species are described as noninvasive or invasive, we relied on the classification provided by the authors in the publication. Native species were generally defined as those occurring at a site without the aid of human introduction. Introduced species were those that occurred outside their native range, and were

classified as noninvasive exotic if they naturalized into the introduced community with little effect, or invasive if the authors listed the species as spreading rapidly or outcompeting native species. We also recorded whether the study was cross continental or a native/introduced species comparison, the type of organism (plant or animal), the performance metrics measured, and mean performance value and associated standard deviation and samples sizes within each treatment.

To extract data from figures, we used the software program xyExtract Graph Digitizer version 5.1 (developed by W.P. da Silva). If a paper reported standard errors, we transformed them into standard deviations using reported sample sizes. We recorded all performance metrics reported for each species in the study. If repeated measures of the same study were reported, we only included data from the final time point. We attempted to contact authors to fill in missing data if papers were missing key summary statistics. In total, we extracted a complete set of summary statistics (mean, standard deviation [SD], and sample size [n]) from figures, tables, and text of 98 studies that met our criteria for inclusion, resulting in a total of 1,030 effect sizes.

Statistical Analysis

We calculated the effect size (d) as Hedges' d (Rosenberg et al. 1999). Hedges' d performed well for our data as many studies had small sample sizes ($n < 10$), unequal sampling variances between experimental and control treatments, experimental and control groups with different signs (+ or -), and zeroes (Rosenberg et al. 1999). We conducted similar analyses using the log response ratio (Hedges et al. 1999), however this analysis yielded similar results so here we present only results based on Hedges' d . Hedges' d was calculated as:

$$d = \frac{\overline{X_E} - \overline{X_C}}{SD_{pooled}} J$$

where X_C is the mean performance in the presence of a biotic interaction (control treatment) and X_E is the mean performance without the biotic interaction (removal, experimental treatment). The effect size d represents the performance difference for a focal species in the presence and absence of a biotic interaction. A large positive or negative value for d represents a strong effect from a biotic interaction. For example, if competition strongly reduces performance, removing competitors would result in a positive d . Alternatively, if the presence of mutualists improves performance, removing mutualists would result in a negative d . J weighted each study by its sample size:

$$J = 1 - \frac{3}{4(n_E + n_C - 2) - 1}$$

The pooled standard deviation was calculated as:

$$SD_{pooled} = \sqrt{\frac{(n_E - 1)(SD_E)^2 + (n_C - 1)(SD_C)^2}{n_E + n_C - 2}}$$

where n is the study's sample size for each treatment, and SD is the standard deviation of the control (C) or experimental (E) treatment. This analysis results in large studies with small SD receiving the highest weights.

Using these effect sizes we calculated a cumulative effect size ($\bar{\bar{E}}$), as:

$$\bar{\bar{E}} = \frac{\sum_{i=1}^n w_i d_i}{\sum_{i=1}^n w_i}$$

where n is the number of studies and d_i is the effect size for the i th study. The d s from each study were weighted by the reciprocal of their sampling variance, $w_i = 1/v_{np\ i}$. We calculated nonparametric sampling variances (v_{np}) as:

$$v_{np} = \frac{n_i^E + n_i^C}{n_i^E n_i^C}$$

where n_i^C and n_i^E are the sample sizes from the experimental and control group of the i th study. Nonparametric sampling variances may be less constrained by the need for large sample sizes, compared to typical variances (Rosenberg et al. 1999).

To test whether the cumulative effect size ($\bar{E}$) for each status differs significantly from zero, we used 95% bias-corrected bootstrap confidence intervals (CI) obtained using 9999 iterations (Dixon 1993). When 95% CIs did not overlap zero, on average the removal of the biotic interaction significantly affected performance. To test for differences among native, invasive, and noninvasive exotic species, we performed a categorical random effects meta-analysis (Raudenbush 1994). The Q statistic assesses the homogeneity of effect sizes and determines whether all studies share a common effect size; the null hypothesis is that all effect sizes are equal and there is no difference between invasive, noninvasive, and native species (our status moderator variable) (Rosenberg et al. 1999). When the between-group heterogeneity (Q_B) was significant, status explained a significant portion of the overall variation in effect sizes, meaning that the mean effect size of biotic interaction removal differed between invasive, noninvasive, and native species. In cases where Q_B was significant, we tested for pairwise differences between native, noninvasive exotic, or invasive species by comparing 95% CIs.

We further explored our data to test for outliers and publication bias, or the tendency of authors to publish certain types of results over others (Begg 1994). We tested the relationship between the standardized effect size and sample size using funnel plots and Spearman rank correlations (Rosenberg et al. 1999). The graphical output showed decreasing variation around the cumulative effect size with increasing sample size and that the effect sizes were independent of the study sample sizes; our statistical tests revealed that these relationships were non-significant for each biotic interaction manipulated, consistent with a lack of publication bias

(Rosenberg et al. 1999). In addition, because we were concerned about several studies with extreme values of Hedges' d (# effects sizes < -3 or $> +3$), we conducted parallel analyses excluding those extreme effects sizes; results were qualitatively similar to our original analyses, so here we present the results from data analyses of our full range of effect sizes.

Initial data exploration revealed differences between (1) plant vs. animal studies, (2) native/introduced comparison studies vs. cross-continental studies, and (3) type of biotic interaction manipulated (Appendix F). We therefore conducted separate tests of status on each of these data subdivisions, or study groups. Due to insufficient replication ($n < 5$), we could not run analysis for cross continental studies on animals, on disease and mutualism for animals in native/exotic comparison studies, and on seed predation for plants in cross continental studies. Because animals do not experience herbivory or PSF, these data are also absent from our analysis. Analyses were performed using MetaWin version 2.0 (Rosenberg et al. 1999).

Vote Count

To further corroborate our results, we conducted two additional analyses, including a hierarchical framework (Appendix F) and vote count. Our hierarchical analysis allowed us to test whether biotic interaction removal had different effects depending on the performance metric measured in the study (i.e., fecundity, growth, population growth and survival), and our vote count allowed us to better explore the variation between our studies.

From each study included in the meta-analysis we recorded the (1) the directionality of responses to the removal of biotic interactions for native, noninvasive exotic, and invasive species, and (2) the proportion of our data that supports biotic release. We determined that a study supported biotic release when an introduced invasive or noninvasive species was less

Table 7: Total heterogeneity (Q_T) and between-group heterogeneity (Q_B) of effect sizes in studies comparing the effects of biotic interactions on native, noninvasive exotic, invasive species performance. A significant Q_B indicates that status explained a significant portion of the overall variation in effect sizes, meaning that mean effect size of biotic interaction removal differs between invasive, noninvasive, and native species. Significant p-values ($p \leq 0.05$) for Q_B are shown in bold. Missing cells represent categories with insufficient replication for analysis ($n < 5$).

Effect of Status (Moderator Variable)	Cross Continental Studies				Native/Introduced Comparison Studies							
	Plants				Plants				Animals			
	Q_T	Q_B	DF	p	Q_T	Q_B	DF	p	Q_T	Q_B	DF	p
Competition	33.1	6.4	13	0.23	596.8	12.7	133	0.28	1434.6	152.5	35	0.66
Disease	123.4	0.3	23	0.75	97.3	1.7	91	0.38				
Herbivory	426.0	0.25	40	0.895	802.7	16.9	219	0.11				
Mutualism	783.5	363.0	47	0.001	782.3	7.0	81	0.64				
Predation					120.4	0.2	86	0.76	42.1	6.1	37	0.07
PSF	1439.1	6.8	72	0.85	2994.3	15.1	117	0.71				

affected by the removal of a biotic interaction than was a native. We were only able to collect this data from studies that reported statistics on whether biotic interaction removal/addition treatments affected the performance of the focal species, and whether these performance effects differed by status (native, noninvasive exotic, invasive). Studies that included more focal species, or that manipulated more than one biotic interaction contribute more to our vote count data than do smaller studies, due to the fact that they reported a greater number of effect sizes. Therefore, we also calculated the proportion of studies that support biotic release, weighting each study the same.

Results

Cross Continental Studies – Plants

Statistically significant and large effect sizes for invasive and noninvasive exotic species indicate that competition, herbivory, and PSF all reduce performance of introduced species, however the magnitude of these effects did not differ from those on native taxa (Table 7, Fig. 13). Removing competition and herbivory increased performance of native, noninvasive exotic, and

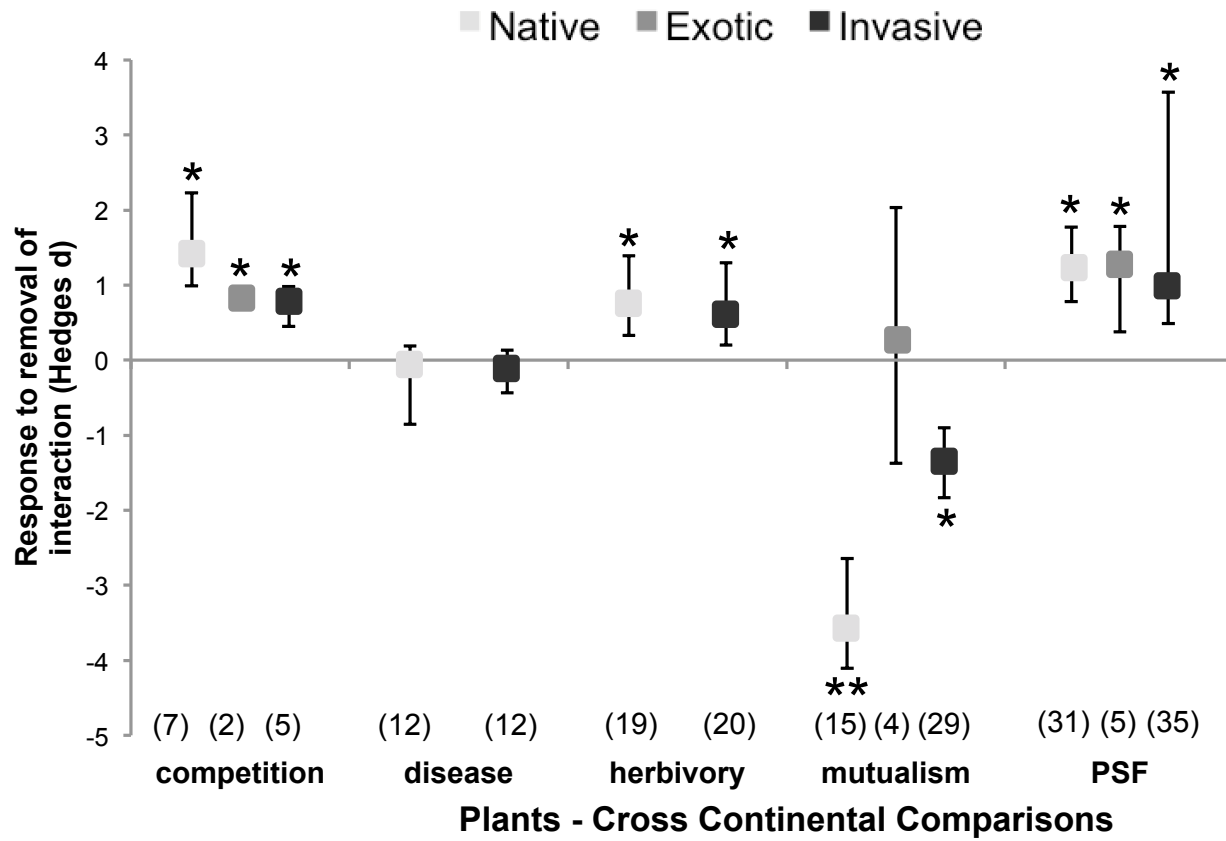


Figure 13: Mean $\pm$ 95% CI effect size of each type of biotic interaction on native, noninvasive exotic, and invasive plants for cross continental studies. One asterisk (*) indicates effect sizes that differ significantly from zero, and two stars (**) indicate significant effects of status. The number of studies in each category is indicated in parentheses. Positive and negative values indicate that removal of the interaction increases or decreases performance respectively.

invasive species, while removing mutualisms decreased performance of native and invasive species. We found that native species were most negatively affected by mutualism removal, that mutualism removal also reduced invasive species performance, but that exotic species were not generally affected by mutualisms (significant effect of status, $p = 0.001$). PSFs were generally negative for native, noninvasive exotic, and invasive species, meaning that species did best in sterilized soil and in soil conditioned by other species. Experimental reduction or removal of disease did not, in general, affect performance.

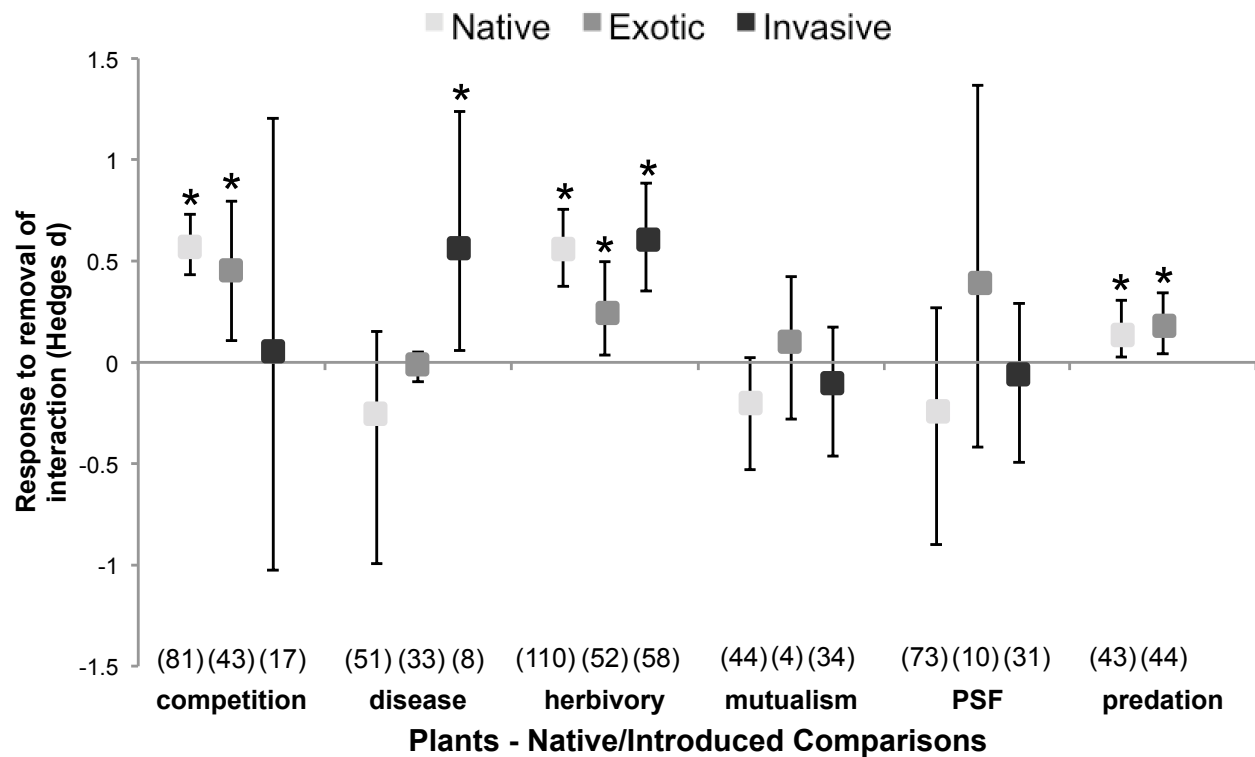


Figure 14: Mean +/- 95% CI effect size of each type of biotic interaction on native, noninvasive exotic, and invasive plants for native/introduced comparison studies. One star (*) indicates effect sizes that differ significantly from zero. The number of studies in each category is indicated in parentheses. Positive and negative values indicate that removal of the interaction increases or decreases performance respectively.

Native/Introduced Comparison Studies – Plants

The removal of competitors significantly increased the performance of native and noninvasive exotic species, but did not generally affect invasive species performance (Table 7, Fig. 14). The opposite pattern was present for disease – removal or reductions of pathogens significantly increased the performance of invasive species, while native and exotic species were not significantly affected (Fig. 14). Native, noninvasive exotic, and invasive species' performances were significantly increased when herbivores were removed. The removal of seed predators benefitted both native and noninvasive exotic species. PSF had no significant effect on any status (Fig. 14), but large CIs indicate substantial variation among studies and soil microbes

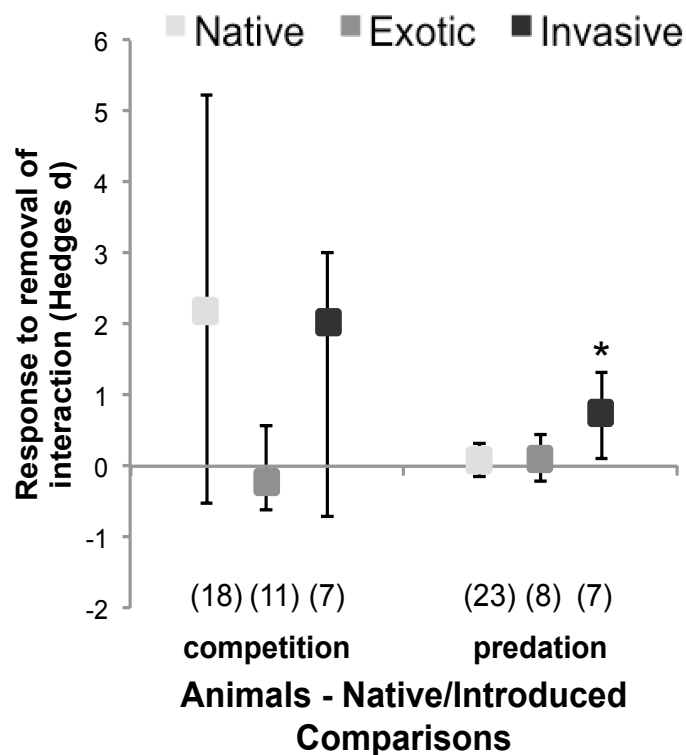


Figure 15: Mean $\pm$ 95% CI effect size of each type of biotic interaction on native, noninvasive exotic, and invasive animals for native/introduced comparison studies. One star (*) indicates effect sizes that differ significantly from zero. The number of studies in each category is indicated in parentheses. Positive and negative values indicate that removal of the interaction increases or decreases performance respectively.

may act as both antagonists and mutualists for plants. Native, noninvasive exotic, and invasive species' performances were not generally lowered by mutualist removal treatments.

Native/Introduced Comparison Studies – Animals

Competition removal treatments did not significantly influence native, noninvasive exotic, or invasive animal performance (Table 7). Invasive species' performance significantly increased

when predators were removed (Fig. 15), however there was no effect of removal of native or noninvasive exotic animals.

Vote Count

From the 98 studies included in our analysis, we collected 687 responses to treatments manipulating the presence of biotic interactions (Table 8). Of these, 42.4% (291 studies) found that focal species responded positively to the removal of antagonistic biotic interactions, and 43.6% (300 studies) showed no response. Some studies (8.4%, 58 studies) found that removing an antagonistic biotic interaction actually decreased performance, potentially due to some unmeasured factor. For example, if the removal of herbivores has a greater benefit to plant A than plant B, plant B may show a negative response to herbivore removal when in fact they are responding to increased competition from plant A. The remaining negative responses to biotic interaction removal were due to the removal of mutualists (5.5%, 38 studies).

When we look at the level of study (published paper), 28 found evidence in support of biotic release, while 58 found that biotic release did not drive the success of introduced plants in their study. We collected 146 data points from these 86 studies, recording whether an invasive or noninvasive introduced species was released, compared to native competitors in its introduced range or compared to conspecific populations in its native range. These measures give more weight to studies that manipulated more than one biotic interaction, or studied more than one introduced species, as these yielded separate data points for each. We found that 26.7% (39 studies) of these data points supported biotic release, while 73.3% (107 studies) did not. The finding that the majority of studies do not find evidence for biotic release supports the conclusions from our meta-analysis.

Table 8: Summary table of vote count results. For our vote count, significance is determined by statistics reported in the original papers. Significant effect sizes are indicated with + and -, where non-significant effects indicated by n.s.

Cross Continental Comparisons										
Plants	Native			Exotic			Invasive			Biotic Release?
	Effect of Biotic Interaction Removal									
All	(+)	n.s.	(-)	(+)	n.s.	(-)	(+)	n.s.	(-)	43.2% Y, 56.8% N
Competition	6	1		2			3	2		100% N
Disease	5	1	2					12		50% Y, 50% N
Herbivory	12	3			1		7	13		60.0% Y, 40.0% N
Mutualism	12				3	1	5	2		50% Y, 50% N
Predation										
PSF	23	6	3	5			16	12	8	42.1% Y, 57.9% N

Native/Introduced Comparisons										
Plants	Native			Exotic			Invasive			Biotic Release?
	Effect of Biotic Interaction Removal									
All	(+)	n.s.	(-)	(+)	n.s.	(-)	(+)	n.s.	(-)	20.1% Y, 79.9% N
Competition	33	30	4	14	17	3	8	4	5	23.5% Y, 76.5% N
Disease	4	13	1	1	2		1	5		100% N
Herbivory	23	30	2	9	16	3	21	15	1	17.6% Y, 82.4% N
Mutualism	12	19	10	1	2	1	7	9	9	33.3% Y, 66.7% N
Predation	4	4		5	4					66.7% Y, 33.3% N
PSF	29	20	12	4	4	2	6	13	9	100% N

Animals										
	(+)	n.s.	(-)	(+)	n.s.	(-)	(+)	n.s.	(-)	
All	(+)	n.s.	(-)	(+)	n.s.	(-)	(+)	n.s.	(-)	30.8% Y, 69.2% N
Competition	5	5			0		5		2	33.3% Y, 66.7% N
Disease	5			5	2					100% N
Mutualism										
Predation	4	18	1	1	7		5	2		83.3% Y, 16.7% N

Discussion

In our study, we find that biotic release is not a prevailing mechanism explaining invasive species success; antagonistic interaction effects did not differ significantly for invasive species, compared to populations in their native range, or native and noninvasive exotic competitors in their introduced ranges. These results were consistent across our traditional meta-analysis approach, hierarchical meta-analysis (Appendix F), and vote count. We found that exotic and invasive plants are negatively affected by competition, predation, PSF, disease (invasive plants only), and herbivory (invasive plants only). However, the removal of competitors in native/introduced comparison studies significantly improved performance of native and noninvasive exotic plants, while not improving performance of invasives. Additionally, we found that native and invasive plants relied substantially on mutualist partners, while noninvasive exotic plants did not. Therefore, we detected evidence that biotic interactions can limit introduced species performance and that invasiveness in plants may be driven by competitive release and the formation of successful mutualisms in the introduced range.

Our results are consistent with a previous meta-analysis, which found that introduced plant establishment and performance was reduced by competition and herbivory (Levine et al. 2004), and with previous studies and meta-analyses which found introduced species are not generally released from herbivores compared to natives (Parker et al. 2006, Chun et al. 2010, Schultheis et al. 2015). Chun and collaborators (2010) found that introduced and native species received equal damage from herbivores and disease. The same meta-analysis identified nine studies that manipulated the presence herbivores and disease, and consistent with our study, they found no difference in the performance response of native and introduced species to herbivores and diseases (Chun et al. 2010). Only one prior meta-analysis separated out the effects of

herbivores on invasive and noninvasive exotic species (Parker et al. 2006), and they too found that invasive and noninvasive exotics responded similarly to herbivore removal.

In our study, native and introduced species responded similarly to the soil community (Fig. 13 and 14), consistent with a study on an entire plant community's response to PSF (Anacker et al. 2014), but inconsistent with Klironomos (2002), which found native plants experience strong negative PSFs, while the most abundant invasive plants experience positive PSFs. While positive PSFs often correlate with greater field abundances, studies included in our analysis often did not report natural field densities, so we were unable to determine whether invasive species in these studies were in fact most abundant in the community.

Our results demonstrate invasive species are significantly harmed by disease and parasites, while native and exotic species were generally not affected by disease and parasite removal treatments (Fig. 14), supporting findings by Parker and Gilbert (2007), which found that invasive *Trifolium* and *Medicago* species had the highest levels of disease prevalence and greatest performance increases in response to disease removal. However, most enemy removal studies included in our analysis manipulated entire fungal communities with fungicide treatments. The lack of performance effects from disease in cross-continental studies could be driven by the fact that these treatments indiscriminately removed antagonistic and mutualistic fungi species, resulting in no net effect of removal on performance.

While invasive species were just as strongly affected by most antagonistic biotic interactions as native and noninvasive exotic species, we found evidence that competition may contribute to invasion success. While native and noninvasive exotic plants significantly benefitted from the removal of competition in native/introduced comparison studies, invasive species did not (Fig. 14). However, large CIs indicate that the effects of competition vary widely

for invasive species. Additionally, in cross-continental comparisons, invasive and noninvasive exotic populations tended to be less affected by competition than were natives (Fig. 13).

Therefore, release from competition may play a role in invasiveness.

Another key difference between introduced species that become invasive and those that do not may be the formation of successful mutualisms in the introduced range. In cross-continental studies, both native and invasive populations of introduced plants were negatively affected by the experimental removal of mutualist partners. This pattern was consistent with native/introduced comparison studies. In both study types, noninvasive exotic species were not affected by the removal of mutualists, suggesting that these species receive minimal benefit from mutualist partners in the introduced range and could even be limited by lack of mutualisms. Studies manipulating mycorrhizal mutualists dominated the literature, but we observed similar patterns across all mutualism types. Other mutualisms studied included rhizobia (Parker et al. 2007, Rodríguez-Echeverría et al. 2012, Horn et al. 2014), ants (Lach et al. 2010, Prior et al. 2015 *unpublished data*), earthworms (Wurst et al. 2011), frugivores (Zuel et al. 2012), and endophytes (Aschehoug et al. 2012). All mutualisms tested in our study were facultative, as missing obligate mutualisms would have prevented the establishment of introduced species, making further experimentation impossible.

Species Specific Case Studies and Context Dependency of Biotic Release

Although biotic interactions are not a general explanation for biological invasions, there is substantial variation around our cumulative effect sizes ($\bar{E}$); thus, altered biotic interactions may contribute to some invasions. Within the studies included in our analysis, several found evidence that enemy release contributed to the success of invaders in their system. For example,

herbivory by white-tailed deer (*Odocoileus virginianus*) increased the relative abundance of invasive *Alliaria petiolata* and *Microstegium vimineum*, while reducing native plant species abundance (Knight et al. 2009). In a coastal wetland, invasive *Lythrum salicaria* abundance was not significantly affected by the presence of herbivores, while many native species' abundances decreased (Barry et al. 2004). In a study of 30 native and introduced species, Agrawal and colleagues (2005) found that introduced species experienced half the negative performance effects from soil microbes compared to native species. Similarly, Klironomos (2002) found that invasive species in his system, which included *A. petiolata* and *L. salicaria*, had positive PSF, while rare, native species experienced negative PSF.

Species interactions are frequently context-dependent, varying in strength or even direction depending on environmental conditions (Chamberlain et al. 2014); thus, a particular invasive species may experience release under certain environmental conditions but not others, potentially accounting for some of the variation found between studies. For example, temperature can affect predator-prey interactions (Fey and Herren 2014), and release could occur in some climates but not others because of temperature-driven mismatch between interacting species. Additionally, plant interactions with microbes can vary from mutualism to parasitism depending on soil nutrient conditions (Hoeksema et al. 2010) or competitive environment experienced by a plant (Casper and Castelli 2007). The Resource-Enemy Release Hypothesis predicts that invaders growing in high nutrient conditions will benefit more from release from herbivory if higher levels of resource lead to faster growth and more poorly defended tissues in invaders (Blumenthal 2006). In this scenario, only studies manipulating herbivory in high nutrient environments would reveal evidence for enemy release.

Enemy and mutualist acquisition in the introduced range, over time and with increasing spread, also may explain some of the variation among studies. Introduced species leave behind biotic interactions from their native range, yet concurrently encounter a new suite of species in the introduced range. While release from negative biotic interactions may facilitate colonization and establishment during the early stages of an invasion, these benefits may be lost over time as introduced species accumulate enemies and competitors in their introduced range (Elton 1958; Mitchell et al. 2006, Mitchell et al. 2010). For example, studies on introduced plants find that release from herbivory and disease is lost over a period of a few hundred years (Mitchell et al. 2010). Processes affecting aboveground enemy acquisition might function similarly belowground as well; plants with longer residence times in New Zealand had more negative interactions with soil organisms than newly introduced plants (Diez et al. 2010). Unfortunately, very few studies on introduced species report information on introduction dates (Strayer et al. 2006), and for many species this data is unknown. Future studies on introduced species that elucidate the changing effects of biotic interactions over time will help determine the long-term effects of biological invasions (Mitchell et al. 2006).

Finally, biotic release is predicted to occur when an introduced species leaves behind coevolved antagonists and enter a community where co-evolved relationships are lacking (Hallett 2006). However, closely related native species often occur in the introduced community. Darwin's Naturalization Hypothesis predicts that species closely related to the invaded community are less likely to establish because they tend to have more similar traits, and as a result, are more likely to compete for resources (Darwin 1859). This hypothesis can be extended to traits that mediate interactions with antagonists and mutualists as well – if defense or mutualism traits are phylogenetically conserved between close relatives, introduced species may

be more likely to acquire biotic interactions from close relatives present in the community (Gilbert and Webb 2007). In support of this hypothesis, several recent studies have shown that phylogenetically dissimilar introduced species are more likely to establish and become invasive in novel communities, compared to introduced species with close relatives present (Strauss et al. 2006, Jiang et al. 2010, Schaefer et al. 2011; but see Duncan and Williams 2002).

Interactive and Synergistic Effects of Multiple Biotic Interactions

Many of our CIs included zero, resulting in the counter-intuitive interpretation that removal of a biotic interaction did not always significantly affect performance. Natural systems are complex, and survival and other performance metrics are typically simultaneously influenced by a multitude of abiotic and biotic factors. The vast majority of studies included in our analysis manipulated a single type of biotic interaction, but perhaps release from one interaction is not enough to cause significant shifts in fitness (potentially explaining why many of our CIs included zero) or drive invasiveness (potentially explaining the lack of significant differences between native, noninvasive and invasive species for most biotic interactions). Out of the 98 studies included in our analysis, 23 manipulated more than one biotic interaction, and nine of those found a significant interaction between treatments. Only two studies found that biotic interactions acted synergistically to suppress invaders, together reducing invader performance more than when acting alone. For example, competition from a native thistle and herbivory interact to resist invasion by the introduced thistle, *Cirsium vulgare* (Suwa and Louda 2012). In this case, release from multiple interactions might be necessary to drive invasiveness (Huang et al. 2012, Suwa and Louda 2012).

Conclusion

We found that predation, herbivory, disease, and PSFs generally decrease the fitness of native, noninvasive exotic, and invasive species similarly, providing little evidence that enemy release is a general mechanism facilitating invasions. However, invasive species were less affected by competition than native or noninvasive exotic species, and the removal of mutualists decreased performance for native and invasive plant species, but not noninvasive exotics, indicating that escape from competition and the formation of mutualisms in the introduced range may be important in promoting the success of introduced species.

The earliest writings on biotic release recognized that interactions gained in the introduced range might be just as limiting to performance as those lost (Elton 1958), and that release would not operate for all introduced species. Dozens of hypotheses attempt to explain invasiveness, and it is becoming clear that no one hypothesis will serve as a “magic bullet” explaining the diversity of strategies employed by invasive plants and animals (Gurevitch et al. 2011). Just as a variety of biotic and abiotic factors control the performance of native populations, introduced species likely succeed and fail due to a variety of mechanisms (Gurevitch et al. 2011). To determine if and when biotic release operates, future studies must focus on the features that may drive context dependency in release, and the factors influencing how introduced species acquire novel antagonistic biotic interactions in their introduced range.

APPENDICES

Appendix A: Supplemental Tables and Figures for Chapter 2

This appendix contains supplemental tables and figures for Chapter 2. Table A1 lists all experimental species and detailed information on their status, seed origin, years planted into the experiment, and GenBank accession numbers. Table A2 gives results from phylogenetic generalized least squares (PGLS) analysis of variance (ANOVA) testing the effects of plant status (native, noninvasive exotic, or invasive) and phylogeny on insect herbivory and mammal browsing. Table A3 gives results from analysis of covariance (ANCOVA) testing the effects of plant status and geographic spread (at three spatial scales) or time on herbivory and browsing. Fig. A1 gives the best scoring maximum likelihood (ML) phylogenetic tree, and Fig. A2 shows images of the experimental common garden.

Table A1: List of the 61 species planted into the 2011 and 2012-2013 common gardens. Species are color coded by plant status: native (white), exotic (gray), and invasive (black). In the columns for year, presence of a particular species is indicated with an 'X'. If the cell is grayed out, it indicates that survival was low and the species was not included in the analysis for that year. GenBank accession numbers of genes used for phylogeny construction are listed. When a species was not located in GenBank, a close relative was used and noted with (*).

Family	Species Name	Abbrev.	Status	Lists	Seed Source	2011	2012	2013	matK	ITS	rbcL
Asteraceae	<i>Achillea millefolium</i>	ACHMI	native		Michigan	X	X	X	EU385315.1	AY603185.1	JX848399.1
Poaceae	<i>Agropyron repens</i>	AGGRE	invasive	MSL	Michigan		X	X	FJ395421.1	GQ365145.1	KJ841296.1
Fabaceae	<i>Amorpha canescens</i>	AMCAN	native		Michigan	X				*AY426773.1	
Fabaceae	<i>Amorpha fruticosa</i>	AMFRU	exotic	none	Missouri	X			KC584927.1	GQ281030.1	KC584888.1
Poaceae	<i>Bromus hordeaceus</i>	BROHO	exotic	none	California	X	X		AM889695.1	KM077298.1	GQ248557.1
Poaceae	<i>Bromus inermis</i>	BROIN	invasive	WTP	Michigan		X	X	AF164398.1	KF713194.1	KJ841141.1
Poaceae	<i>Bromus kalmii</i>	BROKA	native		Michigan	X	X	X		AY367916.1	
Apiaceae	<i>Carum carvi</i>	CARCA	exotic	none	Pennsylvania	X			U58553.1	JQ792209.1	KF602102.1
Asteraceae	<i>Centaurea cyanus</i>	CENCY	exotic	none	Pennsylvania		X		JN894130.1	KC603919.1	AB530955.1
Asteraceae	<i>Centaurea stoebe</i>	CENST	invasive	MNFI, WTP	Michigan		X	X	KC969492.1	JF914072.1	KJ746252.1
Asteraceae	<i>Cichorium intybus</i>	CICIN	exotic	none	Pennsylvania	X	X	X	AJ633131.1	HM921413.1	HQ590035.1
Asteraceae	<i>Conyza canadensis</i>	CONCA	native		Michigan		X		HM850627.1	AY875695.1	HQ590045.1
Asteraceae	<i>Coreopsis lanceolata</i>	CORLA	native		Michigan	X	X	X	AY551495.1	KM347947.1	HM849915.1
Asteraceae	<i>Coreopsis palmata</i>	CORPA	native		Pennsylvania		X	X	AY551480.1	AY553673.1, AY553674.1	
Asteraceae	<i>Coreopsis tinctoria</i>	CORTI	exotic	none	Pennsylvania	X	X		HM989735.1	KM347935.1	GU724222.1
Asteraceae	<i>Coreopsis tripteris</i>	CORTR	native		Michigan	X	X	X	AY551499.1	KM347917.1	
Fabaceae	<i>Coronilla varia</i>	CORVA	invasive	WTP	Pennsylvania		X	X	HM049547.1	AF218537.1	U74222.1
Asteraceae	<i>Cosmos bipinnatus</i>	COSBI	exotic	none	Pennsylvania		X		HM989783.1	KM347948.1	GQ436474.1
Asteraceae	<i>Cosmos sulphureus</i>	COSSU	exotic	none	Ohio		X		EU049362.1	KM347949.1	
Apiaceae	<i>Daucus carota</i>	DAUCA	exotic	none	Kansas	X			HQ593265.1	KJ415356.1	KJ841260.1
Fabaceae	<i>Desmodium canadense</i>	DESCA	native		Michigan		X	X	HQ593266.1	KM098891.1	KJ841264.1
Asteraceae	<i>Erigeron annuus</i>	ERIAN	native		Michigan		X	X	HM989796.1	GU724302.1	KJ841309.1
Asteraceae	<i>Eupatorium perfoliatum</i>	EUPPE	native		Michigan	X	X	X	EU749317.1	DQ415741.1	KJ841315.1
Asteraceae	<i>Gaillardia pulchella</i>	GAIPU	exotic	none	Pennsylvania		X		HM989787.1	KF607074.1	HQ590105.1
Asteraceae	<i>Helianthus petiolaris</i>	HELAU	exotic	none	Michigan	X	X	X	*AY009458.1	JX121556.1	
Asteraceae	<i>Helianthus flexuosus</i>	HELFL	exotic	none	Ohio	X	X	X	*AY215804.1	KF607070.1	*AY215123.1
Brassicaceae	<i>Hesperis matronalis</i>	HESMA	invasive	MNFI, WTP	New York	X			HQ593319.1	DQ357547.1	HQ590129.1
Asteraceae	<i>Lactuca saligna</i>	LACSA	exotic	none	Michigan		X	X	*AJ633239.1	HQ161960.1	*JN893847.1
Asteraceae	<i>Lactuca serriola</i>	LACSE	exotic	none	Michigan		X	X	HQ593336.1	HQ172902.1	HQ590149.1
Fabaceae	<i>Lespedeza capitata</i>	LESCA	native		Michigan	X	X	X	GU572331	GU572172.1	
Fabaceae	<i>Lespedeza cuneata</i>	LESCU	invasive	none	Pennsylvania	X	X	X	EU717416.1	GU572175.1	EU717275.1
Asteraceae	<i>Leucanthemum vulgare</i>	LEUVU	exotic	none	Pennsylvania		X	X	HQ593344.1	EF091600.1	KJ841377.1
Fabaceae	<i>Lotus corniculatus</i>	LOTCO	invasive	PMW, WTP	Pennsylvania	X	X	X	HM049505.1	JN861076.1	KJ841388.1
Fabaceae	<i>Lupinus perennis</i>	LUPPE	native		Michigan		X	X		Z72162.1, Z72163.1	KF613009.1
Fabaceae	<i>Medicago lupulina</i>	MEDLU	exotic	none	Nebraska	X	X		HE966952.1	JQ858257.1	KJ841412.1
Fabaceae	<i>Melilotus albus</i>	MELAL	invasive	MNFI, PMW, WTP	Wisconsin	X	X	X	HE967441.1	DQ006009.1	DQ006095.1
Fabaceae	<i>Melilotus officinalis</i>	MELOF	invasive	MNFI, PMW, WTP	Pennsylvania	X	X	X	HE970723.1	KJ999362.1	KJ841414.1
Poaceae	<i>Panicum virgatum</i>	PANVI	native		Michigan		X	X	EU434294.1	DQ005062.1	EF125135.1
Poaceae	<i>Phleum pratense</i>	PHLPR	exotic	none	Michigan		X	X	HQ0593382.1	HQ600524.1	KJ841460.1
Poaceae	<i>Poa compressa</i>	POACO	invasive	WTP	Pennsylvania	X	X	X	KJ599232.1	KJ598896.1	KJ599121.1
Poaceae	<i>Poa nemoralis</i>	POANE	native		Canada	X	X	X	JN894815.1	GQ324529.1	KJ841479.1
Poaceae	<i>Poa pratensis</i>	POAPR	invasive	WTP	Pennsylvania		X	X	KJ599261.1	KJ598925.1	KJ599150.1
Poaceae	<i>Poa trivialis</i>	POATR	exotic	none	Pennsylvania	X	X	X	FJ395369.1	GQ324555.1	JN893080.1
Rosaceae	<i>Potentilla arguta</i>	POTAG	native		Michigan	X			HQ593397.1	U90787.1	HQ590221.1
Rosaceae	<i>Potentilla anserina</i>	POTAN	native		California	X			KJ840972.1	KF954772.1	KJ841496.1
Rosaceae	<i>Potentilla argentea</i>	POTAR	exotic	none	Canada	X			KJ840973.1	AB894151.1	KJ841497.1
Rosaceae	<i>Potentilla recta</i>	POTRE	exotic	none	Oregon	X			HQ593398.1	AB894160.1	HQ590222.1
Rosaceae	<i>Rosa setigera</i>	ROSSE	native		Michigan	X			AB048601.1	AB048596.1	
Poaceae	<i>Schizachyrium scoparium</i>	SCHSC	native		Michigan		X	X	FR832830.1	DQ005072.1	HE577863.1
Asteraceae	<i>Solidago canadensis</i>	SOLCA	native		Michigan		X	X	EU749415.1	HQ142591.1	EU677023.1
Asteraceae	<i>Solidago graminifolia</i>	SOLGR	native		Michigan		X	X	KM212072.1	HQ142624.1	HQ590098.1
Asteraceae	<i>Solidago rigida</i>	SOLRI	native		Michigan	X	X	X	HQ142603.1	JX848426.1	
Asteraceae	<i>Sonchus oleraceus</i>	SONOL	exotic	none	Michigan		X		JN894897.1	AY458002.1	KF196024.1
Poaceae	<i>Sorghastrum nutans</i>	SORNU	native		Michigan		X	X	EF137473.1	DQ005080.1	EF125121.1
Poaceae	<i>Sporobolus heterolepis</i>	SPOHE	native		Michigan		X	X	AF164429.1	*GJ359228.1	KJ740997.1
Asteraceae	<i>Symphotrichum pilosum</i>	SYMPI	native		Michigan		X	X	EU749444.1	UJ360419.1	EU677053.1
Asteraceae	<i>Taraxacum officinale</i>	TAROF	exotic	none	Michigan		X	X	FJ395377.1	HQ161934.1	FJ395571.1
Fabaceae	<i>Tephrosia virginiana</i>	TEPVI	native		Michigan	X				*AF467499.1	*KF511648.1
Fabaceae	<i>Trifolium hybridum</i>	TRIHY	exotic	none	Pennsylvania		X	X	AF522125.1	AF053159.1	KJ841632.1
Fabaceae	<i>Trifolium pratense</i>	TRIPR	exotic	none	Michigan		X	X	EU749448.1	AF053171.1	KJ841633.1
Fabaceae	<i>Trifolium repens</i>	TRIPE	exotic	none	Michigan		X	X	KJ841029.1	AF053172.1	KJ841634.1

Table A2: Results from phylogenetic generalized least squares (PGLS) analysis of variance (ANOVA) testing the effects of plant status (native, noninvasive exotic, or invasive) and phylogeny on insect herbivory and mammal browsing. Statistically significant ($P < 0.05$) effects are in bold.

Source	2011			2012			2013		
	df	<i>t</i>	<i>P</i>	df	<i>t</i>	<i>P</i>	df	<i>t</i>	<i>P</i>
<i>(a) Insect Herbivory PGLS</i>									
Status	2,27	2.20	0.04	2,43	0.24	0.81	2,31	1.20	0.24
	Blomberg's K = 0.13			Blomberg's K = 0.12			Blomberg's K = 0.03		
<i>(b) Mammal Browser PGLS</i>									
Status	2,27	-0.61	0.56	2,43	0.96	0.34	2,31	0.28	0.78
	Blomberg's K = 0.03			Blomberg's K = 0.12			Blomberg's K = 0.11		

Note: Significant effects of status ($P \leq 0.05$) are in bold.

Table A3: Results from analysis of covariance (ANCOVA) testing the effects of plant status (native, noninvasive exotic, or invasive) and geographic spread (at three spatial scales) or time on insect herbivory and mammal browsing. Statistically significant ($P < 0.05$) effects are in bold.

Source	MI Spread			Five State Spread			US Spread			Source	Time		
	df	χ^2	P	df	χ^2	P	df	χ^2	P		df	χ^2	P
(a) Insect Herbivory ANCOVA													
Status	1, 30	1.99	0.24	1, 30	1.64	0.16	1, 30	2.08	0.29	Status	1, 30	2.40	0.08
County	1, 31	2.04	< 0.001	1, 31	1.74	< 0.001	1, 31	2.16	0.001	Residence Time	1, 31	2.57	0.02
Status x County	1, 29	0.17	0.04	1, 29	1.58	0.28	1, 29	2.04	0.42	Status x Time	1, 29	1.76	< 0.001
(b) Mammal Browser ANCOVA													
Status	1, 30	9.52	0.22	1, 30	10.14	0.34	1, 30	10.52	0.40	Status	1, 30	9.72	0.59
County	1, 31	9.88	0.04	1, 31	10.34	0.10	1, 31	10.67	0.24	Residence Time	1, 31	9.82	0.06
Status x County	1, 29	7.83	0.009	1, 29	5.43	< 0.001	1, 29	5.86	< 0.001	Status x Time	1, 29	9.18	0.20

Note: Significant differences ($P \leq 0.05$) are in bold.

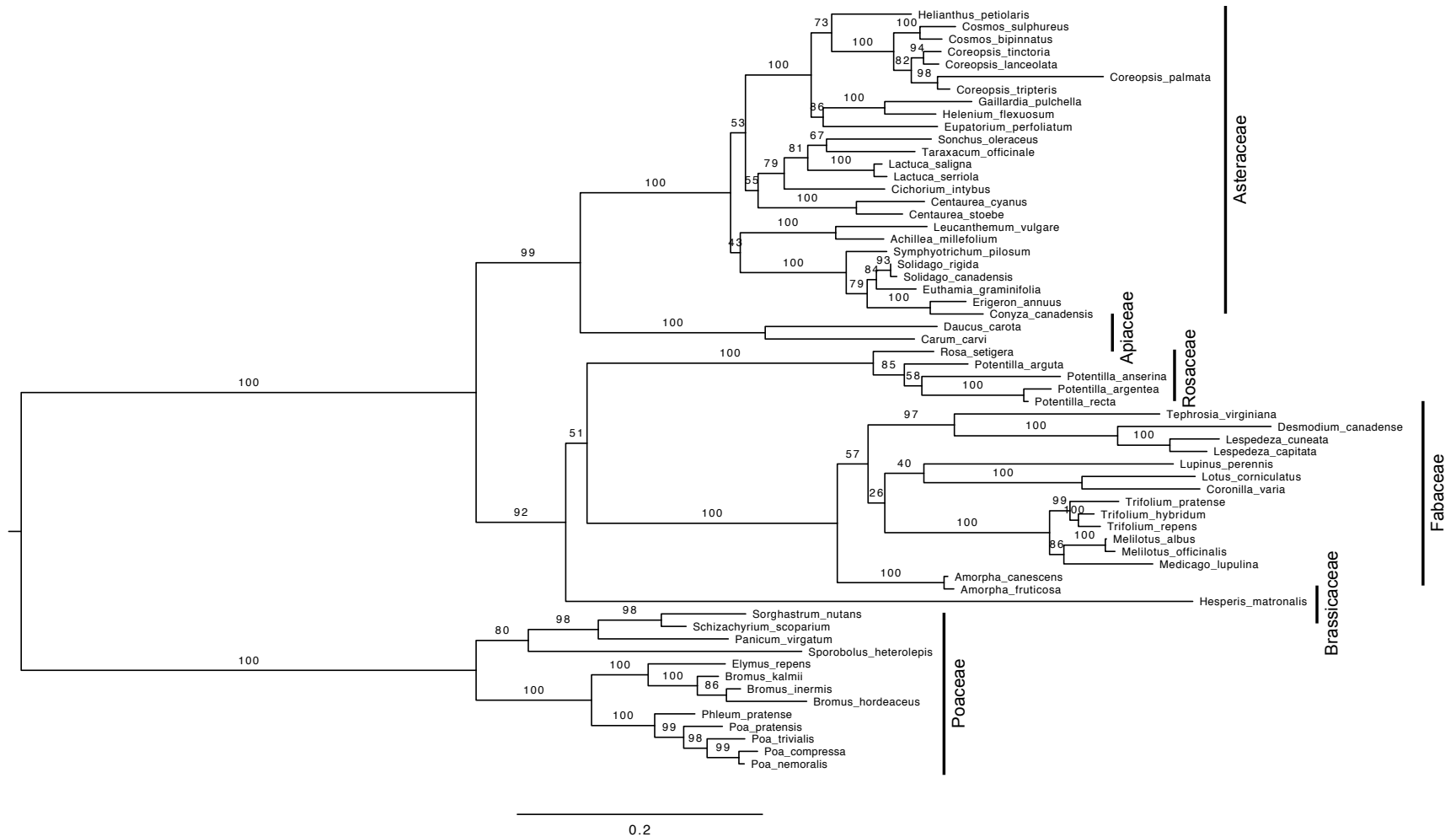


Figure A1: The best-scoring ML tree from a rapid bootstrap analysis in RAxML from the analysis of the concatenated sequences of matK, ITS, and rbcL. ML bootstrap frequencies are the numbers associated with nodes, and branch lengths are proportional to the number of nucleotide changes.

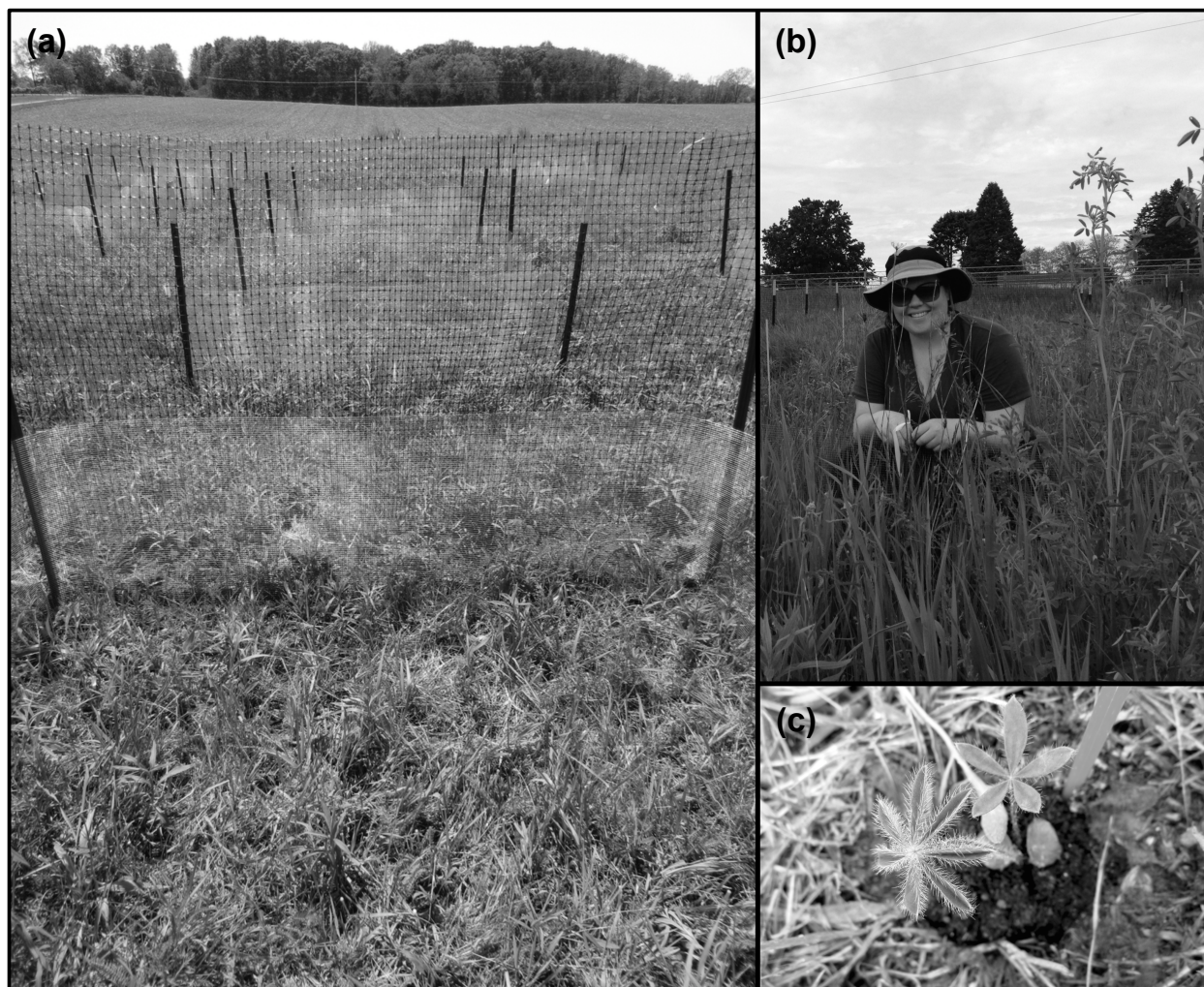


Figure A2: Images showing (a) the experimental common garden in 2012, (b) E.H. Schultheis in the field measuring insect herbivory and mammalian browsing on experimental seedlings, and (c) an experimental *Lupinus perennis* seedling.

Appendix B: Statistical Methods and Results for Plant Family Analysis

Statistical Analysis

To determine whether invasive, noninvasive exotic, or native species differ in herbivore damage, and whether plant family influenced damage, we performed ANOVA using the `aov` function in R. Proportion leaf area removed and proportion of stems browsed were included as response variables, and plant status (invasive, noninvasive exotic, or native), family, and the status x family interaction were included as fixed predictor variables. Analyses were conducted on within-year species averages; separate analyses were run for each year of data because species composition varied. In 2011 it was not possible to test for the interaction between status and family due to lack of replication of status within family. All non-significant interaction terms were dropped from the 2012 and 2013 models to increase power for testing main effects. Tukey-adjusted post-hoc contrasts were used to evaluate differences between treatment combinations when main effects or interaction terms were significant ($P \leq 0.05$). Response variables were not transformed because species mean data met ANOVA normality assumptions.

Results

Plant families received different amounts of herbivory and browsing damage (Table B1). Fabaceae tended to receive the most insect herbivore damage and Poaceae the least, with Asteraceae receiving intermediate amounts (Fig. B1a). In 2012, exotics and invasives in the Fabaceae tended to receive more insect herbivore damage than natives, and in the Asteraceae and Poaceae natives and invasives tended to receive more insect herbivore damage than exotics (Fig. B2). Plant families differed in susceptibility to browsing; in 2013, Fabaceae and Asteraceae

received more browsing damage than Poaceae, and though not statistically significant, similar patterns were observed in 2011 and 2012 (Fig. B1b).

Table B1: Results from analysis of variance (ANOVA) testing the effects of plant status (invasive, noninvasive exotic, or native), family, and their interaction on insect herbivory and mammal browsing. Statistically significant ($P < 0.05$) effects are in bold.

Source	2011			2012			2013		
	df	<i>F</i>	<i>P</i>	df	<i>F</i>	<i>P</i>	df	<i>F</i>	<i>P</i>
<i>(a) Insect Herbivory ANOVA</i>									
Status	2, 21	5.35	0.01	2, 36	4.23	0.02	2, 28	0.51	0.35
Family	5, 21	0.98	0.45	2, 36	23.6	< 0.001	2, 28	4.17	0.03
Status x Family				4, 36	6.55	< 0.001			
<i>(b) Mammal Browser ANOVA</i>									
Status	2, 21	2.07	0.15	2, 36	2.52	0.09	2, 28	3.09	0.06
Family	5, 21	0.60	0.70	2, 36	1.48	0.24	2, 28	6.92	0.004
Status x Family				4, 36					

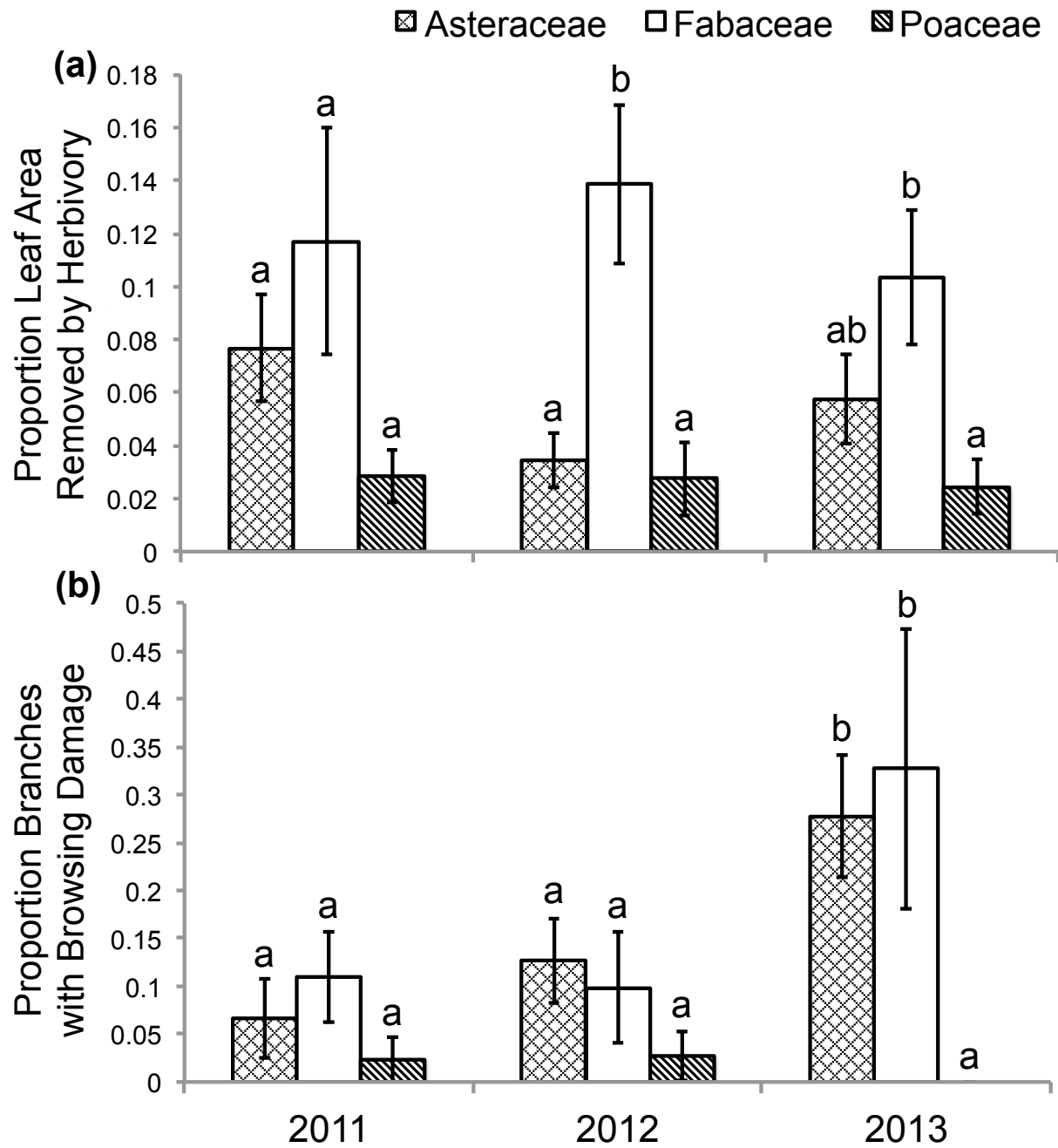


Figure B1: Three years of (a) insect herbivore and (b) mammal browser damage data on Asteraceae (hatched bars), Fabaceae (empty bars), and Poaceae (striped bars) plants. All analysis was performed within year. Bars indicate mean $\pm$ SE. Means with the same letter are not statistically different ($P \leq 0.05$) based on post-hoc contrasts.

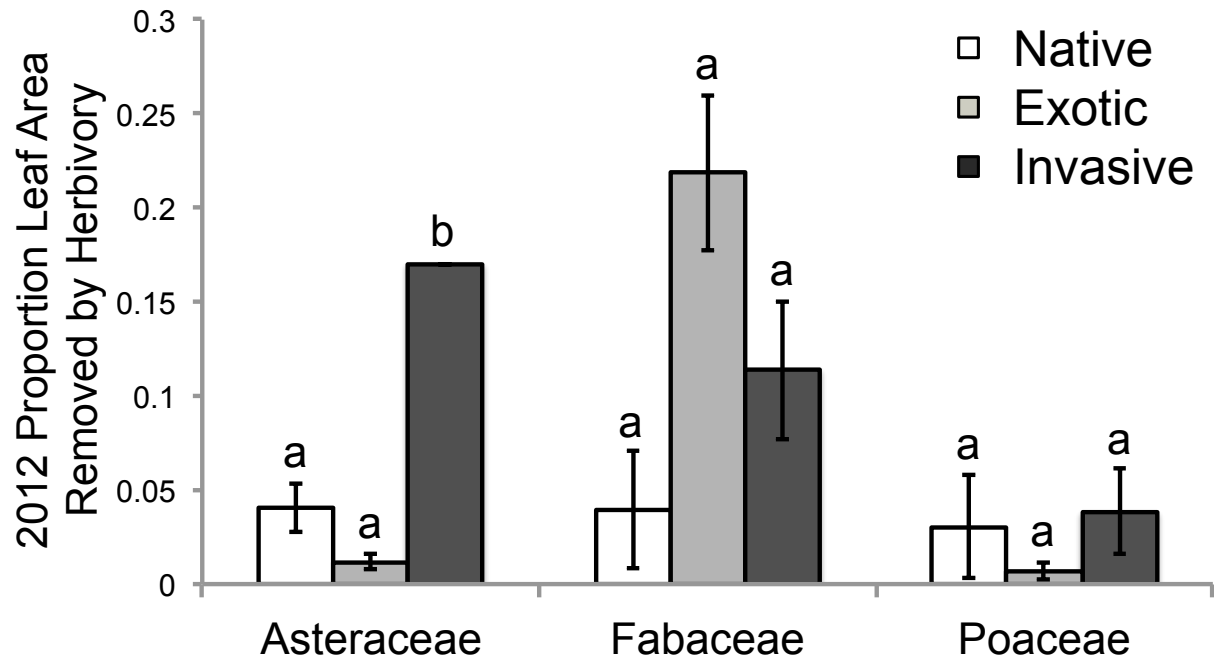


Figure B2: Data from 2012 showing the family by status interaction for insect herbivore damage data. Species statuses shown with different color bars: native (white bars), noninvasive exotic (gray bars), and invasive (black bars). Bars indicate mean $\pm$ SE. Means within family with the same letter are not statistically different ($P \leq 0.05$) based on post-hoc contrasts.

Appendix C: List of Experimental Species in the 2012-2014 experiment

Table C1: List of the 50 species planted into the 2012-2014 experimental plots. Species are color coded by plant status: native (white), noninvasive exotic (gray), and invasive (black). In the columns for year, presence of a particular species is indicated with an 'X'. For invasive species lists: WTP = Wild Type Plants, MNFI = Michigan Natural Features Inventory, MSL = Michigan Seed Law, PMW = Invasive Plants of the Upper Midwest (Czarapata 2005).

Family	Species Name	Abbrev.	Status	Lists	Seed Source	Perenniality	2012	2013	2014
Asteraceae	<i>Achillea millefolium</i>	ACHMI	native		Michigan	perennial	X	X	X
Asteraceae	<i>Conyza canadensis</i>	CONCA	native		Michigan	annual	X		
Asteraceae	<i>Coreopsis lanceolata</i>	CORLA	native		Michigan	perennial	X	X	X
Asteraceae	<i>Coreopsis palmata</i>	CORPA	native		Pennsylvania	perennial	X	X	X
Asteraceae	<i>Coreopsis tripteris</i>	CORTR	native		Michigan	perennial	X	X	X
Asteraceae	<i>Erigeron annuus</i>	ERIAN	native		Michigan	biennial	X	X	
Asteraceae	<i>Eupatorium perfoliatum</i>	EUPPE	native		Michigan	perennial	X	X	X
Asteraceae	<i>Solidago canadensis</i>	SOLCA	native		Michigan	perennial	X	X	X
Asteraceae	<i>Solidago graminifolia</i>	SOLGR	native		Michigan	perennial	X	X	X
Asteraceae	<i>Solidago rigida</i>	SOLRI	native		Michigan	perennial	X	X	X
Asteraceae	<i>Symphyotrichum pilosum</i>	SYMPI	native		Michigan	perennial	X	X	X
Asteraceae	<i>Centaurea cyanus</i>	CENCY	exotic	none	Pennsylvania	annual	X		
Asteraceae	<i>Cichorium intybus</i>	CICIN	exotic	none	Pennsylvania	perennial	X	X	X
Asteraceae	<i>Coreopsis tinctoria</i>	CORTI	exotic	none	Pennsylvania	annual	X		
Asteraceae	<i>Cosmos bipinnatus</i>	COSBI	exotic	none	Pennsylvania	annual	X		
Asteraceae	<i>Cosmos sulphureus</i>	COSSU	exotic	none	Ohio	annual	X		
Asteraceae	<i>Gaillardia pulchella</i>	GAIPU	exotic	none	Pennsylvania	annual	X		
Asteraceae	<i>Helenium flexuosum</i>	HELFL	exotic	none	Ohio	perennial	X	X	X
Asteraceae	<i>Helianthus petiolaris</i>	HELPE	exotic	none	Michigan	annual	X		
Asteraceae	<i>Lactuca saligna</i>	LACSA	exotic	none	Michigan	biennial	X	X	
Asteraceae	<i>Lactuca serriola</i>	LACSE	exotic	none	Michigan	biennial	X	X	
Asteraceae	<i>Leucanthemum vulgare</i>	LEUVU	exotic	none	Pennsylvania	perennial	X	X	X
Asteraceae	<i>Sonchus oleraceus</i>	SONOL	exotic	none	Michigan	annual	X		
Asteraceae	<i>Taraxacum officinale</i>	TAROF	exotic	none	Michigan	perennial	X	X	X
Asteraceae	<i>Centaurea stoebe</i>	CENST	invasive	MNFI, WTP	Michigan	biennial	X	X	
Fabaceae	<i>Desmodium canadense</i>	DESCA	native		Michigan	perennial	X	X	X
Fabaceae	<i>Lespedeza capitata</i>	LESCA	native		Michigan	perennial	X	X	X
Fabaceae	<i>Lupinus perennis</i>	LUPPE	native		Michigan	perennial	X	X	X
Fabaceae	<i>Medicago lupulina</i>	MEDLU	exotic	none	Nebraska	annual	X		
Fabaceae	<i>Trifolium hybridum</i>	TRIHU	exotic	none	Pennsylvania	perennial	X	X	X
Fabaceae	<i>Trifolium pratense</i>	TRIPR	exotic	none	Michigan	perennial	X	X	X
Fabaceae	<i>Trifolium repens</i>	TRIIR	exotic	none	Michigan	perennial	X	X	X
Fabaceae	<i>Coronilla varia</i>	CORVA	invasive	WTP	Pennsylvania	perennial	X	X	X
Fabaceae	<i>Lespedeza cuneata</i>	LESCU	invasive	none	Pennsylvania	perennial	X	X	X
Fabaceae	<i>Lotus corniculatus</i>	LOTCO	invasive	PMW, WTP	Pennsylvania	perennial	X	X	X
Fabaceae	<i>Melilotus albus</i>	MELAL	invasive	MNFI, PMW, WTP	Wisconsin	biennial	X	X	
Fabaceae	<i>Melilotus officinalis</i>	MELOF	invasive	MNFI, PMW, WTP	Pennsylvania	biennial	X	X	
Poaceae	<i>Bromus kalmii</i>	BROKA	native		Michigan	perennial	X	X	X
Poaceae	<i>Panicum virgatum</i>	PANVI	native		Michigan	perennial	X	X	X
Poaceae	<i>Poa nemoralis</i>	POANE	native		Canada	perennial	X	X	X
Poaceae	<i>Schizachyrium scoparium</i>	SCHSC	native		Michigan	perennial	X	X	X
Poaceae	<i>Sorghastrum nutans</i>	SORNU	native		Michigan	perennial	X	X	X
Poaceae	<i>Sporobolus heterolepis</i>	SPOHE	native		Michigan	perennial	X	X	X
Poaceae	<i>Bromus hordeaceus</i>	BROHO	exotic	none	California	annual	X		
Poaceae	<i>Phleum pratense</i>	PHLPR	exotic	none	Michigan	perennial	X	X	X
Poaceae	<i>Poa trivialis</i>	POATR	exotic	none	Pennsylvania	perennial	X	X	X
Poaceae	<i>Agropyron repens</i>	AGRRE	invasive	MSL	Michigan	perennial	X	X	X
Poaceae	<i>Bromus inermis</i>	BROIN	invasive	WTP	Michigan	perennial	X	X	X
Poaceae	<i>Poa compressa</i>	POACO	invasive	WTP	Pennsylvania	perennial	X	X	X
Poaceae	<i>Poa pratensis</i>	POAPR	invasive	WTP	Pennsylvania	perennial	X	X	X

Appendix D: Analysis of Background Community Changes

Methods

In September 2013 I estimated light competition and productivity of the background community in each experimental plot because it appeared that competition was becoming more intense in some of my enemy removal treatments. As an estimation of productivity, I harvested aboveground biomass and dead thatch in a $\frac{1}{2} \times \frac{1}{4}$ m sub-plot within my 40 2x2m experimental plots. Biomass was dried at 70°C for 72 hours and weighed. To assess light availability, I used a ceptometer (Decagon LP-80 AccuPAR) to measure photosynthetically active radiation (PAR) above the plant canopy and 10cm above the soil surface. I collected three measurements above and three below, averaged them, and took the difference between these two averages. Larger values represent potentially increased light competition in experimental field plots.

I tested the effects of enemy exclusion on background community, amount of thatch, and light competition with mixed model ANOVA using the aov function in R. PAR, thatch biomass, and aboveground biomass were included as response variables, and my three treatments (fencing, insecticide, and fungicide) and all interactions were included as fixed predictor variables. When a significant interaction between treatments was found, post hoc Tukey tests were used to determine which treatment combinations differed from one another ($P \leq 0.05$). Data was untransformed as it satisfied normality assumptions.

Results

I found evidence that enemy removal treatments affected standing stock of the background community, thatch biomass, and light availability (PAR). The fencing treatment

increased the productivity of the background community ($F_{1,28} = 6.38$, $p = 0.02$), raising biomass 18.6% from $692.0 \pm 47.6 \text{ g/m}^2$ to $820.5 \pm 69.1 \text{ g/m}^2$ [mean $\pm$ SE]. There was also a marginally significant interaction between fencing and insecticide treatments ($F_{1,28} = 3.7$, $p = 0.06$); biomass was lowest at $621.8 \pm 45.5 \text{ g/m}^2$ in control plots, and removal of either insects or browsers was enough to raise biomass up to levels found in plots where both types of herbivores were excluded ($806.5 \pm 92.7 \text{ g/m}^2$).

The fungicide treatment significantly increased the amount of thatch present in experimental plots ($F_{1,28} = 4.8$, $p = 0.04$). Thatch increased 8.5% from $86.0 \pm 6.2 \text{ g/m}^2$ in untreated plots, to $93.3 \pm 7.6 \text{ g/m}^2$ in treated plots. There was also a significant interaction between fungicide and insecticide treatments ($F_{1,28} = 4.8$, $p = 0.04$); thatch was highest in plots that received the fungicide, but not insecticide, treatment ($103.5 \pm 12.7 \text{ g/m}^2$).

Fencing and fungicide treatments significantly increased light availability in experimental plots (fencing: $F_{1,32} = 18.9$, $p < 0.001$; fungicide: $F_{1,32} = 4.3$, $p = 0.04$). PAR increased from $1,234.4 \pm 20.9 \text{ } \mu\text{mol/m}^2\text{s}$ to $1,385.0 \pm 7.1 \text{ } \mu\text{mol/m}^2\text{s}$ in fenced plots, and from $1,288.3 \pm 28.9 \text{ } \mu\text{mol/m}^2\text{s}$ to $1,331.1 \pm 14.1 \text{ } \mu\text{mol/m}^2\text{s}$ in plots that received the fungicide treatment.

Appendix E: Flower Number Analysis

Methods

At the end of the experiment we measured plant performance metrics, including height (cm) from the soil surface to apical meristem, aboveground biomass (g), and flower number. To determine whether our treatments influenced plant performance, we tested the effects of simulated herbivory and competition on plant biomass and height with mixed model ANOVA using the lmer function, and flower number with the glmer function, in the lme4 package in R (v. 1.1-7, Bates et al. 2015). To test treatment effects, our model included plant biomass (g), plant height (cm), or flower number as the response variable and clipping (clipped, unclipped), competition (competitor present, absent), status (native, noninvasive exotic, invasive), family (Asteraceae, Poaceae, Fabaceae), and all possible interactions as fixed predictor variables.

Flower number data was analyzed using the Poisson distribution, and because only a small number of individuals flowered during the course of the experiment, we analyzed only data for those individuals and species that flowered. To test significance fixed and random effects for flower number, we used chi-squared tests.

Results

No native species flowered during the experiment (Fig. E1a), and only noninvasive exotic *Centaurea cyanus*, *Sonchus oleraceus*, and *Bromus hordeaceus*, and invasive *Lotus corniculatus*, *Melilotus officinalis*, and *Poa compressa* flowered; only one individual of *M. officinalis* and *P. compressa* produced any flowers. Flower number depended on the interaction between status, clipping, and the competition treatment (Table E1); invasive species in unclipped competition

pots produced significantly more flowers than did exotic species where either competition or clipping treatments were applied (Fig. E1a). This pattern was driven by invasive *L. corniculatus*, which produced significantly more flowers when grown in competition and without clipping compared to the control (Fig. E1b).

Table E1: Results from mixed model analysis of variance (ANOVA) showing the effects of status, family, clipping, and competition on experimental plant flower number. Statistically significant ($P \leq 0.05$) effects are in bold. All non-significant interaction terms were dropped from the final model.

Source	Flower Number			
	df	<i>F</i>	χ^2	<i>P</i>
<i>Native, Exotic, and Invasive Species</i>				
status	1, 10	0.02	6.6	0.01
family	2, 10	4.34	6.2	0.04
clipped	1, 10	12.84	6.5	0.01
competition	1, 10	0.98	0.2	0.63
status x clipped	1, 12	0.57	0.3	0.58
status x competition	1, 12	13.45	8.5	0.004
clipped x competition	1, 13	1.79	1.7	0.19
status x clipped x competition	1, 14	19.15	19.7	< 0.001
Random Effects				
(species)status			1.1	0.59
species x clipped			0.0	1.00
species x competition			8.0	0.005

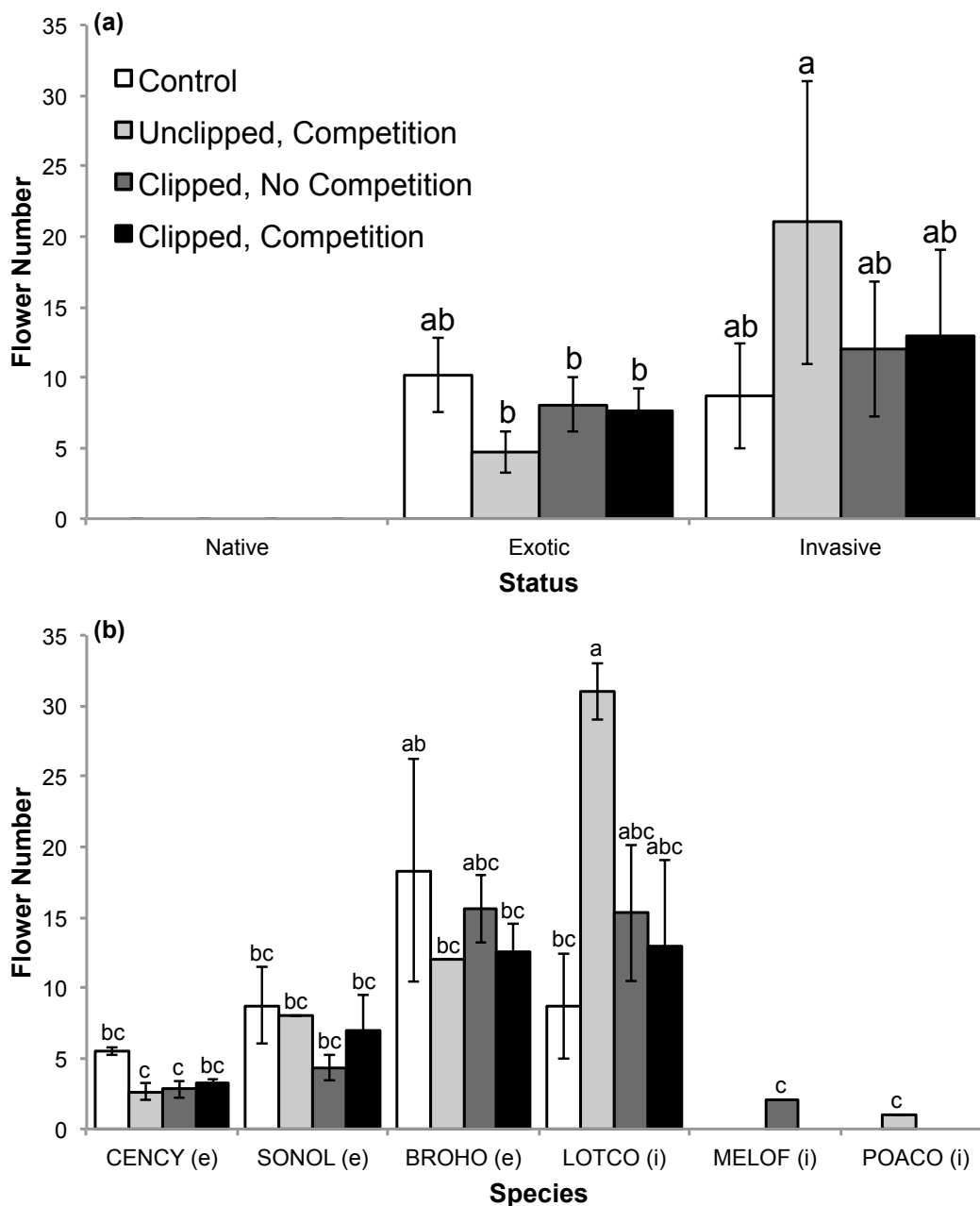


Figure E1: Flower number data for native, noninvasive exotic, and invasive plants that flowered during the course of the experiment. Graph a displays data by status, while graph b displays data by species. Different colored bars represent the clipping and competition treatments. Bars indicate mean $\pm$ SE. Means with different letters are significantly different ($P \leq 0.05$) based on post-hoc contrasts.

Appendix F: Hierarchical Meta-analysis

Methods

To further explore how biotic interactions influence performance and also to better account for the low number of observations in some categories, we analyzed the data following a multilevel, or hierarchical, framework where the different categories of the data were nested within each other (Clark 2007, Ibanez et al. 2014). By using a multilevel/hierarchical framework we thoroughly document if altered biotic interactions are driving the invasion process by assessing difference among species status (native, invasive, or noninvasive exotic), type of organism (animal or plant), type of biotic interaction (competition, disease, herbivory, mutualism, predation, or plant-soil feedback), and among fitness metrics (fecundity, growth, population growth, and survival). We used the same effect size (Hedges d) estimated for the main analysis, and their associated SD. In this case, instead of using non-parametric variance, we included study random effects to account for any bias that could have been associated with any particular study and used variance estimates calculated as:

$$v_d = \frac{N^C + N^E}{N^C N^E} + \frac{d^2}{2(N^C + N^E)}$$

where N^E is the sample size of the experimental group, N^C the sample size of the control group, and d is the measure of Hedges d (Rosenberg et al. 1999).

Unlike the traditional meta-analysis approach, our hierarchical approach allowed us analyze data with smaller sample sizes, such as studies that measured disease effects on animals, and to further explore differences among fitness metrics. The significance of the effect size values (different from zero or not) were first estimated for each status (native, invasive, or noninvasive exotic); within each status, we then separated data by organism type (animal or

plant), followed by type of biotic interaction manipulated (competition, disease/parasite, herbivory, mutualism, predation or plant-soil feedbacks [PSF]), and finishing with fitness metric as the lowest level of our hierarchical analysis (fecundity, growth, population growth, or survival). Given the multilevel structure of the data, and the large number of parameters involved, we used a Bayesian framework to estimate parameter values from non-informative distributions. We independently analyzed cross continental studies from native/introduced comparison studies. Observation i , $ES_{obs\ i}$, of status(i), organism type(i), biotic interaction(i) and fitness metric(i) was modeled as:

$$ES_{obs\ i} \sim Normal(ES_{status(i),organism(i),interaction(i),fitness(i)} + SRE_{(i)}, \sigma_{obs(i)}^2)$$

where $ES_{status(i),organism(i),interaction(i),fitness(i)}$ is the mean ES for the combination of fitness metric, biotic interaction, type of organism and species status that observation i belongs to. SRE represents study random effects, $SRE_* \sim Normal(0, \sigma_{SRE}^2)$ and $\sigma_{SRE} \sim Uniform(0,10)$. The variability around ES_{obs} was the estimated variability in the original study, σ_{obs}^2 . Mean parameters were then estimated from hyperparameter values that follow the multilevel structure of the data.

-Species status, organism type, biotic interaction and fitness metrics ES:

$$ES_{status*,organism*,interaction*,fitness*} \sim Normal(ES_{status*,organism*,interaction*}, \sigma_{status*,organism*,interaction*}^2)$$

-Species status, type of organism and biotic interaction ES:

$$ES_{status*,organism*,interaction*} \sim Normal(ES_{status*,organism*}, \sigma_{status*,organism*,interaction*}^2)$$

-Species status and type of organism ES:

$$ES_{status*,organism*} \sim Normal(ES_{status*}, \sigma_{status*,organism*}^2)$$

-Species status, overall, ES:

$$ES_{status*} \sim Normal(0, 10000)$$

All variances were estimated from non-informative prior distributions, $\sigma_* \sim Uniform(0, 10)$.

Analyses were performed in OPENBUGS (Thomas et al. 2006) and ran for 100000 iterations, after the 25000 initial burn-in period parameter values were estimated by thinning every 100th iteration.

Results

Species Status and Type of Organism Levels

Overall predicted effect sizes for each species status (native, exotic and invasive) in the native/introduced comparisons show no effect of the removal of the biotic interaction (Fig. F1). When we divided studies by type of organism (animals or plants), effect sizes were mainly positive but none was significantly different from zero. For the cross continental comparisons, noninvasive exotics had effect sizes significantly different from zero, driven by the animal studies (Fig. F1).

Biotic Interactions Level

Among native/introduced comparisons studying animals only, three biotic interactions were reported: competition, disease and predation. Removal of competition only significantly affected the performance of native animals (Fig. F2a). In cross continental studies of animals, only two interactions were reported, competition for native species and disease for exotic species and only ES associated with the latest was significantly different from zero (Fig. F2b).

Among plant studies for native/introduced comparisons, none of the effect sizes at this level were statistically different from zero (Fig. F3a). Effect sizes for cross continental

comparisons were significantly positive for disease, herbivory and PSF removal in native species, PSF removal in exotic species, and disease, herbivory and PSF removal for invasive species (Fig. F3b).

Fitness Metric Level

Animal studies in the native/introduced comparisons showed positive effect sizes, significantly different from zero for population growth of native species and invasive species when released from competition, for survival of invasive species when released from competition, and for population growth of invasive species when release from predation (Fig. F4a). Across cross continental studies, only survival of noninvasive exotic species when released from disease was statistically significant (Fig. F4b).

Among plant native/introduced comparison studies, release from competition had a positive effect on the fecundity, growth, and survival of native species and the population growth and survival of noninvasive exotic species. Competition had a negative effect on the growth of invasive species (Fig. F5). Release from disease only benefited growth of invasive species, while release from herbivory benefited growth of native and invasive species and survival of noninvasive exotic species (Fig. F5). Released from predation also had a positive effect on survival of native and noninvasive exotic species (Fig. F5).

From the analyses of the cross continental comparisons, native and invasive species experienced significant release from disease and herbivory and experienced increased fecundity, individual growth, and survival (Fig. F6). Disease also significantly reduced population growth of native and invasive species, while herbivory only reduced population growth of native species. PSF significantly reduced individual growth of native and invasive species. The removal of

mutualists had a negative effect on growth of native species (Fig. F6). Noninvasive exotic species experienced improved fecundity when herbivores were removed, and increased growth when growing in sterilized soil or soil trained by heterospecifics (Fig. F6).

Table F1: To ease the process of comparing results from our vote count, traditional meta-analysis, and hierarchical meta-analysis we summarized the results of all three analyses here. Summary table of effect sizes from studies comparing the effects of biotic interactions on status performance. Symbols represent positive (+), negative (-), and neutral (n.s.) effects of biotic interaction removal. For our traditional (a) and hierarchical meta-analyses (b), effect sizes are calculated as Hedges' d, and significance is determined as whether 95% CIs cross zero. For our vote count, significance is determined by statistics reported in the original papers. Significant effect sizes are indicated with + and -, where non-significant effects indicated by n.s.

(a) Traditional Meta-analysis

Cross Continental Comparisons

Plants	Effect of Biotic Interaction Removal		
	Native	Exotic	Invasive
All	n.s.	n.s.	n.s.
Competition	+	+	+
Disease	n.s.		n.s.
Herbivory	+		+
Mutualism	-	n.s.	-
Predation			
PSF	+	+	+

Native/Introduced Comparisons

Plants	Effect of Biotic Interaction Removal		
	Native	Exotic	Invasive
All	n.s.	+	n.s.
Competition	+	+	n.s.
Disease	n.s.	n.s.	+
Herbivory	+	+	+
Mutualism	n.s.	n.s.	n.s.
Predation	+	+	
PSF	n.s.	n.s.	n.s.

Animals

All	n.s.	n.s.	n.s.
Competition	n.s.	n.s.	n.s.
Disease			
Mutualism			
Predation	n.s.	n.s.	+

(b) Hierarchical Meta-analysis

Cross Continental Comparisons

Plants	Effect of Biotic Interaction Removal		
	Native	Exotic	Invasive
All	n.s.	n.s.	n.s.
Competition	n.s.	n.s.	n.s.
Disease	+		+
Herbivory	+	n.s.	+
Mutualism	n.s.	n.s.	n.s.
Predation			
PSF	+	+	+

Native/Introduced Comparisons

Plants	Effect of Biotic Interaction Removal		
	Native	Exotic	Invasive
All	n.s.	n.s.	n.s.
Competition	+	n.s.	n.s.
Disease	n.s.	n.s.	+
Herbivory	+	n.s.	n.s.
Mutualism	n.s.	n.s.	n.s.
Predation	n.s.	n.s.	
PSF	n.s.	n.s.	n.s.

Animals

All	n.s.	n.s.	n.s.
Competition	n.s.	+	n.s.
Disease	n.s.	n.s.	
Mutualism			
Predation	n.s.	n.s.	n.s.

(c) Vote Count

Cross Continental Comparisons

Plants	Effect of Biotic Interaction Removal									Biotic Release?
	Native	Exotic	Invasive	Native	Exotic	Invasive	Native	Exotic	Invasive	
All	(+)	n.s.	(-)	(+)	n.s.	(-)	(+)	n.s.	(-)	43.2% Y, 56.8% N
Competition	6	1		2			3	2		100% N
Disease	5	1	2						12	50% Y, 50% N
Herbivory	12	3			1		7	13		60.0% Y, 40.0% N
Mutualism	12				3	1	5	2		50% Y, 50% N
Predation										
PSF	23	6	3	5			16	12	8	42.1% Y, 57.9% N

Native/Introduced Comparisons

Plants	Effect of Biotic Interaction Removal									Biotic Release?
	Native	Exotic	Invasive	Native	Exotic	Invasive	Native	Exotic	Invasive	
All	(+)	n.s.	(-)	(+)	n.s.	(-)	(+)	n.s.	(-)	20.1% Y, 79.9% N
Competition	33	30	4	14	17	3	8	4	5	23.5% Y, 76.5% N
Disease	4	13	1	1	2		1	5		100% N
Herbivory	23	30	2	9	16	3	21	15	1	17.6% Y, 82.4% N
Mutualism	12	19	10	1	2	1	7	9	9	33.3% Y, 66.7% N
Predation	4	4		5	4					66.7% Y, 33.3% N
PSF	29	20	12	4	4	2	6	13	9	100% N

Animals

All	(+)	n.s.	(-)	(+)	n.s.	(-)	(+)	n.s.	(-)	30.8% Y, 69.2% N
Competition	5	5			0		5		2	33.3% Y, 66.7% N
Disease	5			5	2					100% N
Mutualism										
Predation	4	18	1	1	7		5	2		83.3% Y, 16.7% N

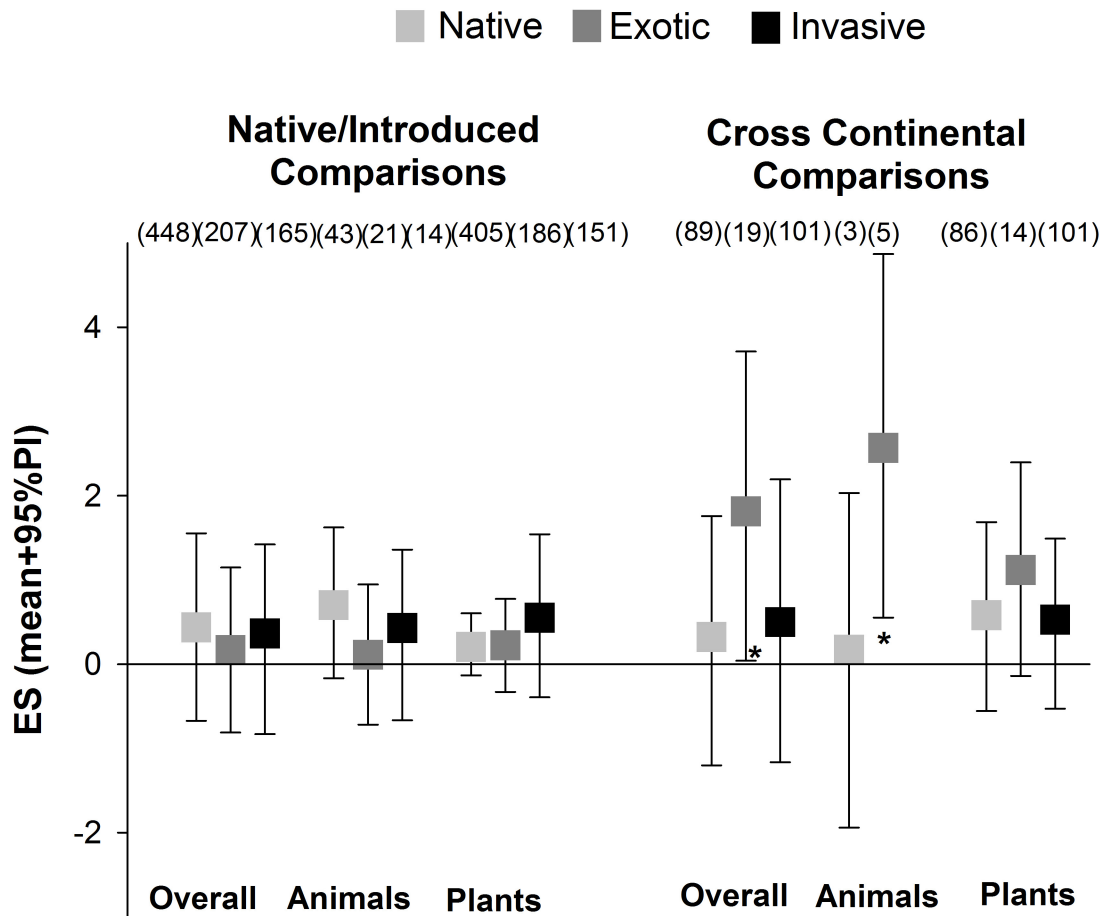


Figure F1: Effects of biotic interactions on native (light gray), exotic (medium gray), and invasive (black) species for cross continental studies, and native/introduced species comparison studies. Points show means bracketed by 95% confidence intervals. Asterisk represents significant effect when 95% confidence intervals did not cross zero. The number of studies in each category is given in parenthesis.

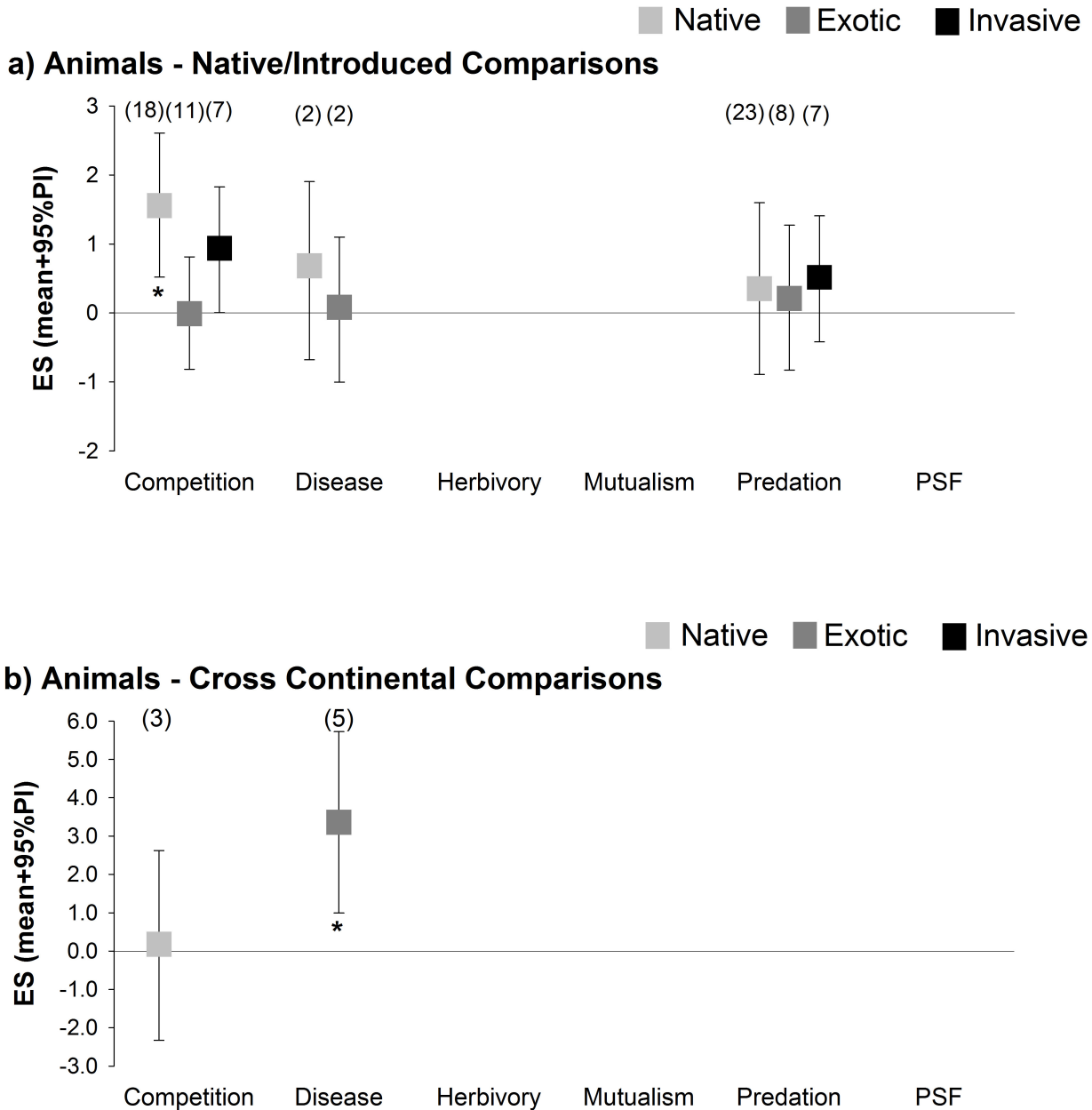
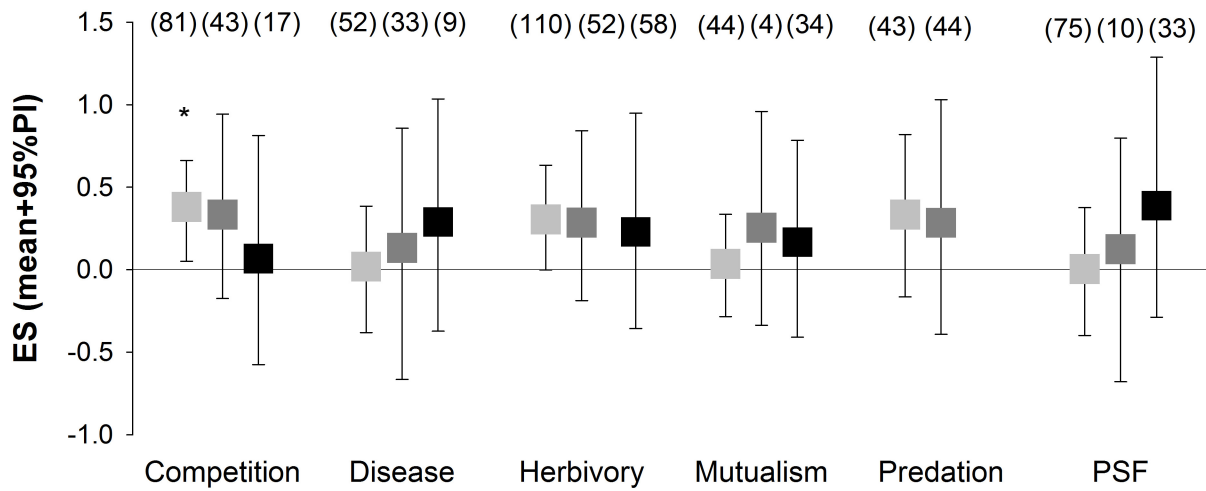


Figure F2: Effects biotic interactions on native, exotic, and invasive animals for (a) native/introduced comparison studies, and (b) cross continental studies. Results are split up by biotic interaction manipulated. Points show means bracketed by 95% confidence intervals. Black asterisks represent effects are significant and 95% confidence intervals do not cross zero. The number of studies in each category is given in parenthesis.

a) Plants - Native/Introduced Comparisons



b) Plants - Cross Continental Comparison

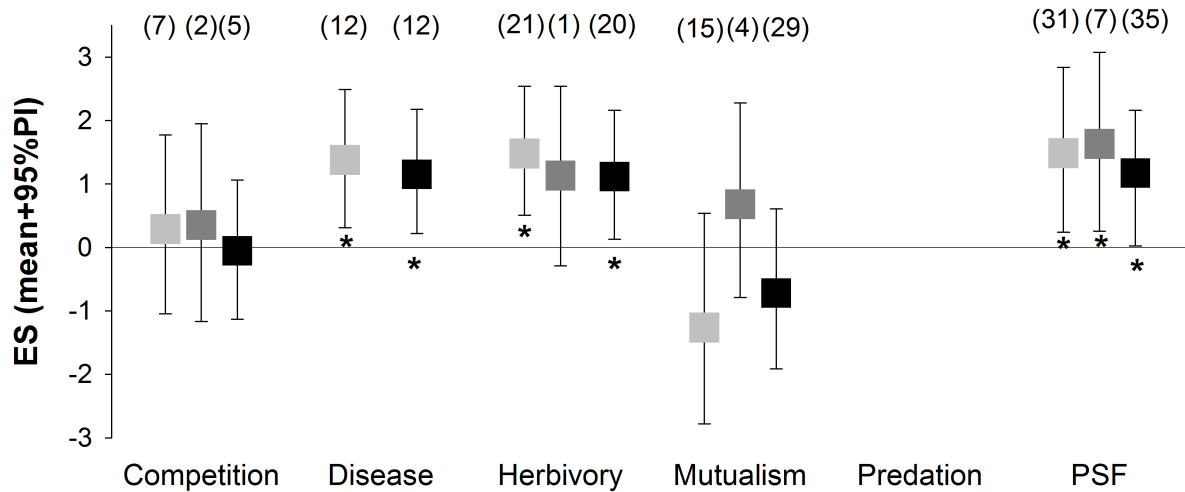
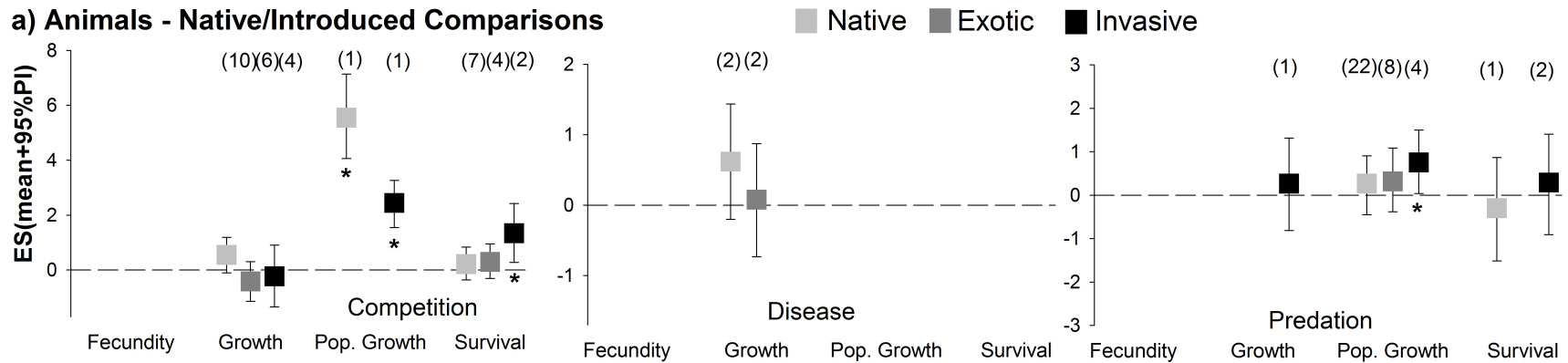


Figure F3: Effects biotic interactions on native, exotic, and invasive plants for (a) native/introduced comparison studies, and (b) cross continental studies. Results are split up by biotic interaction manipulated. Points show means bracketed by 95% confidence intervals. Black asterisks represent effects are significant and 95% confidence intervals do not cross zero. The number of studies in each category is given in parenthesis.

a) Animals - Native/Introduced Comparisons



b) Animals - Cross Continental Comparisons

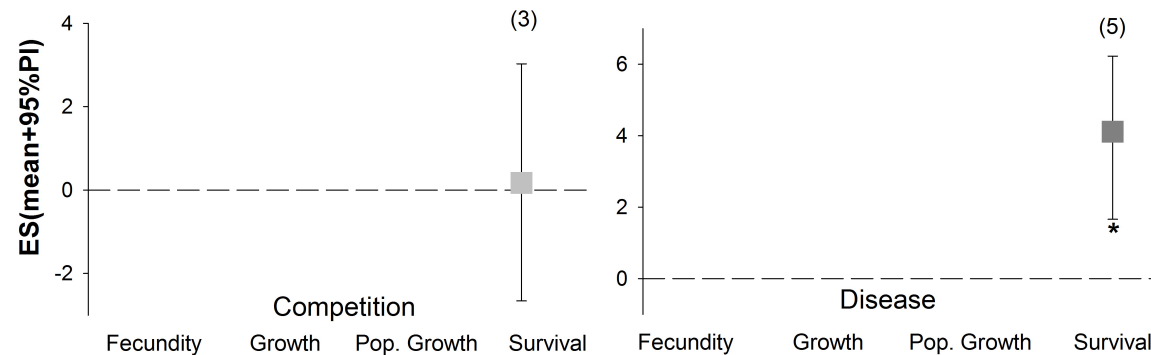


Figure F4: Effects biotic interactions on native, exotic, and invasive animals for (a) native/introduced comparison studies, and (b) cross continental studies. Results are split up by biotic interaction manipulated and performance response variable measured (fecundity, growth, population growth, and survival). Points show means bracketed by 95% confidence intervals. Black asterisks represent effects are significant and 95% confidence intervals do not cross zero. The number of studies in each category is given in parenthesis.

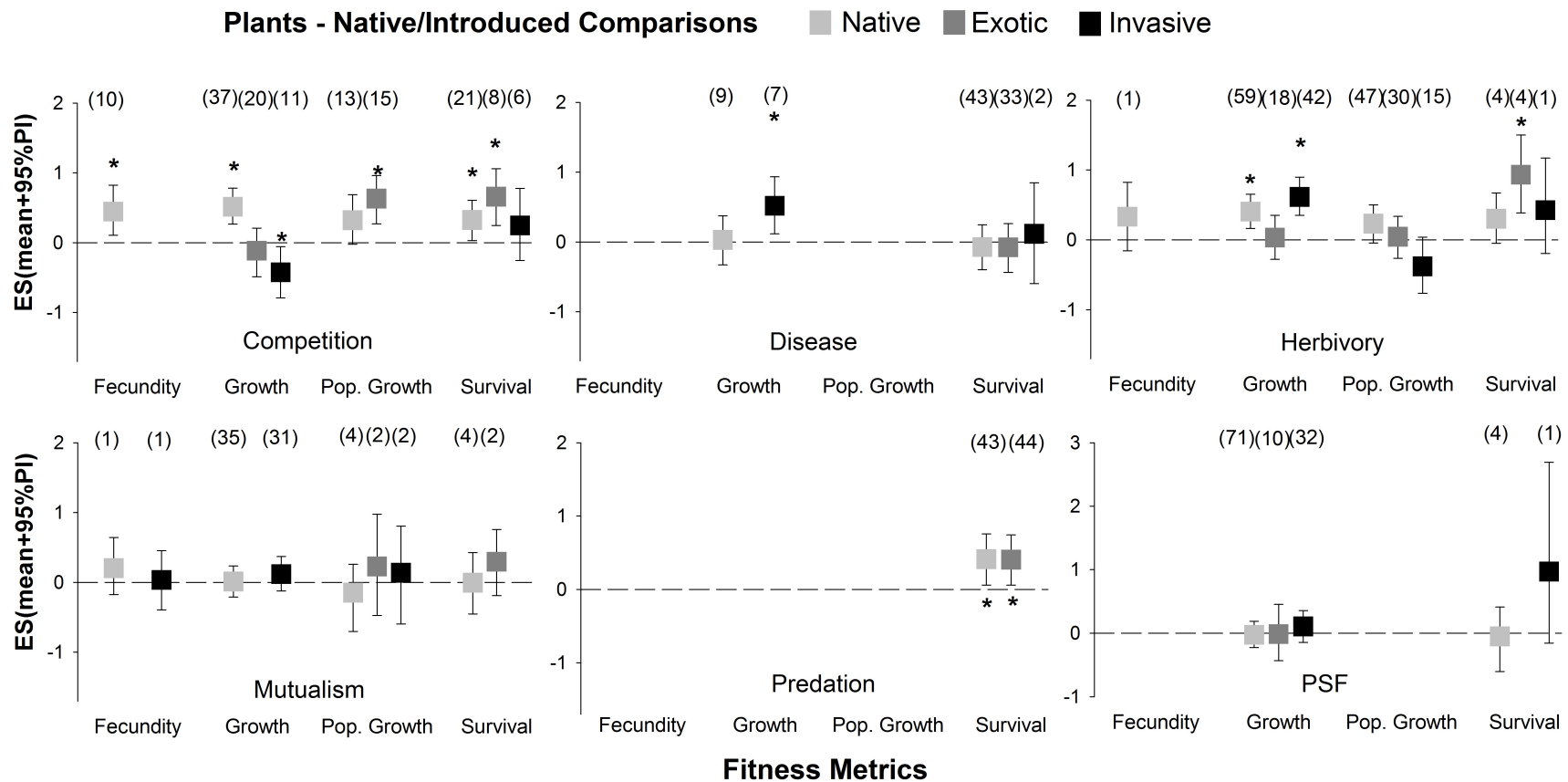


Figure F5: Effects biotic interactions on native, exotic, and invasive plants for native/introduced comparison studies. Results are split up by biotic interaction manipulated and performance response variable measured (fecundity, growth, population growth, and survival). Points show means bracketed by 95% confidence intervals. Black asterisks represent effects are significant and 95% confidence intervals do not cross zero. The number of studies in each category is given in parenthesis.

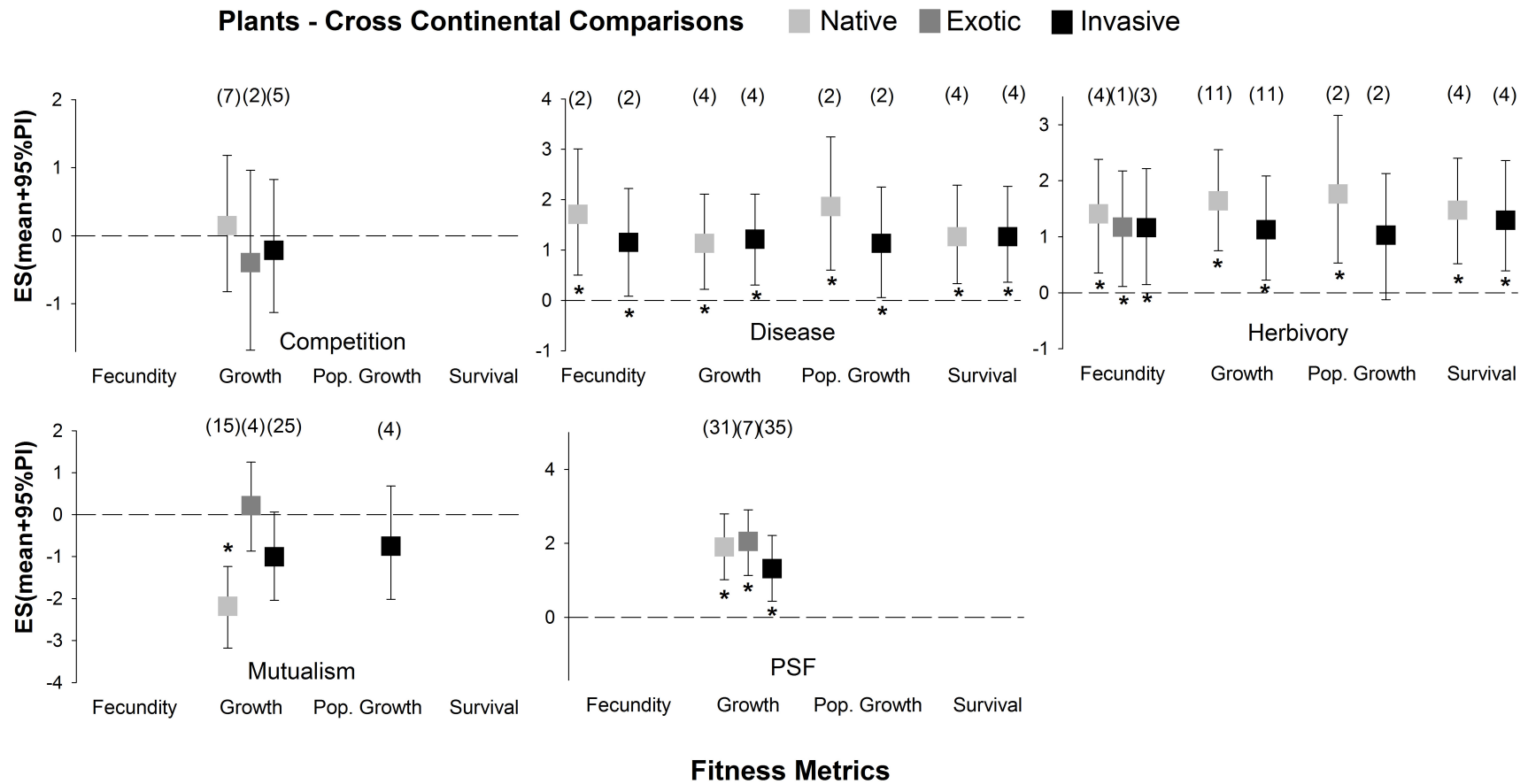


Figure F6: Effects biotic interactions on native, exotic, and invasive plants for cross continental studies. Results are split up by biotic interaction manipulated and performance response variable measured (fecundity, growth, population growth, and survival). Points show means bracketed by 95% confidence intervals. Black asterisks represent effects are significant and 95% confidence intervals do not cross zero. The number of studies in each category is given in parenthesis.

REFERENCES

REFERENCES

- Agrawal, A.A. and P.M. Kotanen. 2003. Herbivores and the success of exotic plants: a phylogenetically controlled experiment. *Ecology Letters* 6:712-715.
- Agrawal, A.A., P.M. Kotanen, C.E. Mitchell, A.G. Power, W. Godsoe, and J. Klironomos. 2005. Enemy release? An experiment with congeneric plant pairs and diverse above- and belowground enemies. *Ecology* 86:2979-2989.
- Agrawal, A.A. 2007. Macroevolution of plant defense strategies. *Trends in Ecology and Evolution* 22:103-109.
- Agrawal, A.A., D.D. Ackerly, F. Adler, A.E. Arnold, C. Caceres, D.F. Doak, E. Post, P.J. Hudson, J. Maron, K.A. Mooney, M. Power, D.W. Schemske, J. Stachowicz, S.Y. Strauss, M.G. Turner, and E. Werner. 2007. Filling key gaps in population and community ecology. *Frontiers in Ecology and the Environment* 5:145-152.
- Ahern, R.G., D.A. Landis, A.A. Reznicek, and D.W. Schemske. 2010. Spread of exotic plants in the landscape: the role of time, growth habit, and history of invasiveness. *Biological Invasions* 12:3157-3169.
- Anacker, B.L., J.N. Klironomos, H. Maherali, K.O. Reinhart, and S.Y. Strauss. 2014. Phylogenetic conservatism in plant-soil feedback and its implications for plant abundance. *Ecology Letters* 17:1613-1621.
- Anderson, R.M. and R.M. May. 1979. Population biology of infectious diseases. Part1. *Nature* 280:361-367.
- Anderson, R.M. and R.M. May. 1981. The population dynamics of microparasites and their invertebrate hosts. *Philosophical Transactions B* 291:451-524.
- Ashton, I.W. and M.T. Lerdau. 2008. Tolerance to herbivory, and not resistance, may explain differential success of invasive, naturalized, and native North American temperate vines. *Diversity and Distributions* 14:169-178.
- Augustine, D.J. and S.J. McNaughton. 1998. Ungulate effects on the functional species composition of plant communities: herbivore selectivity and plant tolerance. *Journal of Wildlife Management* 62:1165-1183.
- Baker R. 1983. *Michigan Mammals*. Michigan State University Press, East Lansing, MI.
- Barry, M.J., R. Bowers, and F.A. De Szalay. 2004. Effects of hydrology, herbivory and sediment disturbance on plant recruitment in a Lake Erie coastal wetland. *The American Midland Naturalist* 151:217-232.

- Bates, D., M. Maechler, B. Bolker, S. Walker, R.H.B. Christensen, H. Singmann, and B. Dai. 2015. lme4: Linear mixed-effects models using Eigen and S4 . R package version 1.1-7, Available at: <http://lme4.r-forge.r-project.org/>.
- Bais, H.P., R. Vepachedu, S. Gilroy, R.M. Callaway, and J.M. Vivanco. 2003. Allelopathy and exotic plant invasion: from molecules and genes to species interactions. *Science* 301:1377-1380.
- Begg, C.B. 1994. Publication bias. In H. Cooper and L. V. Hedges (eds.). *The Handbook of research synthesis*. (pp. 399-409). Russell Sage Foundation, New York.
- Bever, J.D. 1994. Feedback between plants and their soil communities in an old field community. *Ecology* 75:1965-1977.
- Blackshaw, R.E., J.R. Moyer, R.C. Doram, and A.L. Boswell. 2001. Yellow sweetclover, green manure, and its residues effectively suppress weeds during fallow. *Weed Science* 49:406-413.
- Blair, A.C. and L.M. Wolfe. 2004. The evolution of an invasive plant: An experimental study with *Silene latifolia*. *Ecology* 85:3035-3042.
- Blaney, C.S. and P.M. Kotanen. 2001a. Effects of fungal pathogens on seeds of native and exotic plants: a test using congeneric pairs. *Journal of Applied Ecology* 38:1104-1113.
- Blaney, C.S. and P.M. Kotanen. 2001b. Post-dispersal losses to seed predators: an experimental comparison of native and exotic old field plants. *Canadian Journal of Botany* 79:284-292.
- Blaney, C.S. and P.M. Kotanen. 2002. Persistence in the seed bank: the effects of fungi and invertebrates on seeds of native and exotic plants. *Ecoscience* 9:509-517.
- Blomberg, S.P., T. Garland Jr., and A.R. Ives. 2003. Testing for phylogenetic signal in comparative data: behavioral traits are more labile. *Evolution* 57:717-745.
- Blossey, B. and R. Nötzold. 1995. Evolution of Increased Competitive Ability in Invasive Nonindigenous Plants: A Hypothesis. *Journal of Ecology* 83:887-889.
- Blumenthal, D. M. 2006. Interactions between resource availability and enemy release in plant invasion. *Ecology Letters* 9:887-895.
- Bolker, B.M. 2008. *Ecological Data and Models in R*. Princeton University Press
- Borland, K., S. Campbell, R. Schillo, and P. Higman. 2009. A field identification guide to invasive plants in Michigan's natural communities, Michigan Natural Features Inventory. Lansing, MI. Available at: <http://mnfi.anr.msu.edu/invasivespecies/InvasivePlantsFieldGuide.pdf>

- Burdon, J.J. and G.A. Chilvers. 1982. Host density as a factor in plant disease ecology. *Annual Review of Phytopathology* 20:143-166.
- Callaway, R.M. and E.T. Aschehoug. 2000. Invasive plants versus their new and old neighbors: A mechanism for exotic invasion. *Science* 290:521-523.
- Callaway R.M. and W.M. Ridenour. 2004. Novel weapons: invasive success and the evolution of increased competitive ability. *Frontiers in Ecology and the Environment* 2:436-443.
- Cappuccino, N. and D. Carpenter. 2005. Invasive exotic plants suffer less herbivory than non-invasive exotic plants. *Biology Letters* 1:435-438.
- Carpenter, D. and N. Cappuccino. 2005. Herbivory, time since introduction and the invasiveness of exotic plants. *Journal of Ecology* 93:315-321.
- Casper, B.B. and J.P. Castelli. 2007. Evaluating plant–soil feedback together with competition in a serpentine grassland. *Ecology Letters* 10:394-400.
- Catford, J.A., R. Jansson, and C. Nilsson. 2009. Reducing redundancy in invasion ecology by integrating hypotheses into a single theoretical framework. *Diversity and Distributions* 15:22-40.
- Cebrian, J. and C.M. Duarte. 1994. The dependence of herbivory on growth rate in natural plant communities. *Functional Ecology* 8: 518-525.
- Chamberlain, S.A., J.L. Bronstein, and J.A. Rudgers. 2014. How context dependent are species interactions? *Ecology Letters* 17:881-890.
- Chun, Y.J., M. Van Kleunen, and W. Dawson. 2010. The role of enemy release, tolerance and resistance in plant invasions: linking damage to performance. *Ecology Letters* 13:937-946.
- Clark, J.S. 2007. *Models for ecological data*. Princeton University Press, Princeton, NJ.
- Cleveland, C.C., A.R. Townsend, D.S. Schimel, H. Fisher, R.W. Howarth, L.O. Hedin, S.S. Perakis, E.F. Latty, J.C.V. Fischer, A. Elseroad, and M.F. Wasson. 1999. Global patterns of terrestrial biological nitrogen (N₂) fixation in natural ecosystems. *Global Biogeochemical Cycles* 13:623-645.
- Colautti, R.I., A. Ricciardi, I.A. Grigorovich and H.J. MacIsaac. 2004. Is invasion success explained by the enemy release hypothesis? *Ecology Letters* 7:721-733.
- Crawley, M.J. 1987. What makes a community invisable? Colonization, Succession, and Stability (eds A.J. Gray, M.J. Crawley & P.J. Edwards), pp. 429 – 453. Blackwell, Oxford.

- Crawley, M.J. 1989. Insect herbivores and plant population dynamics. *Annual Review of Entomology* 34:531-534.
- Crawley, M.J. 2007. *The R Book*. John Wiley & Sons, Ltd. West Sussex, England.
- Czarapata, E.J. 2005. *Invasive Plants of the Upper Midwest*. University of Wisconsin Press, Madison, WI.
- Daehler, C.C. 2003. Performance comparisons of co-occurring native and alien invasive plants: implications for conservation and restoration. *Annual Review of Ecology, Evolution, and Systematics* 34:183-211.
- Darriba, D., G.L. Taboada, R. Doallo, and D. Posada 2012. jModelTest 2: more models, new heuristics and parallel computing. *Nature Methods* 9:772.
- Darwin, C. 1859. *On the origin of species by means of natural selection*. London.
- Dawson, W., A. Bottini, M. Fischer, M. van Kleunen, and E. Knop. 2014. Little evidence for release from herbivores as a driver of plant invasiveness from a multi-species herbivore-removal experiment. *Oikos* 123:1509-1518.
- DeWalt S.J., J.S. Denslow and K. Ickes. 2004. Natural-enemy release facilitates habitat expansion of the invasive tropical shrub *Clidemia hirta*. *Ecology* 85:471-483.
- Dietz H., L.R. Wirth, H. Buschmann. 2004. Variation in herbivore damage to invasive and native woody plant species in open forest vegetation on Mahe, Seychelles. *Biological Invasions* 6:511-521
- Dietz, H. and P. Edwards. 2006. Recognition that causal processes change during plant invasion helps explain conflicts in evidence. *Ecology* 87:1359-1367.
- Diez, J.M., I. Dickie, G. Edwards, P.E. Hulme, J.J. Sullivan, and R. P. Duncan. 2010. Negative soil feedbacks accumulate over time for non-native plant species. *Ecology Letters* 13:803-809.
- Dillemuth, F.P., E.A. Rietschier, and J.T. Cronin. 2008. Patch dynamics of a native grass in relation to the spread of invasive smooth brome (*Bromus inermis*). *Biological Invasions* 11:1381-1391.
- Dixon, P.M. 1993. The bootstrap and the jackknife: Describing the precision of ecological indices. In S. M. Scheiner and J. Gurevitch (eds.). *Design and analysis of ecological experiments*. (pp. 290-318). Chapman and Hall, New York.
- Dostál, P., E. Allan, W. Dawson, M. van Kleunen, I. Bartish, and M. Fischer. 2013. Enemy damage of exotic plant species is similar to that of natives and increases with productivity. *Journal of Ecology* 101:388-399.

- Duke, S.O., F.E. Dayan, J. Bajsa, K.M. Meepagala, R.A. Hufbauer, and A.C. Blair. 2009. The case against (-)-catechin involvement in allelopathy of *Centaurea stoebe* (spotted knapweed) *Plant Signaling and Behavior* 4:422-424.
- Duncan, R.P. and P.A. Williams. 2002. Ecology: Darwin's naturalization hypothesis challenged. *Nature*, 417:608-609.
- Elton, C.S. 1958. The ecology of invasions of animals and plants. Methuen, London.
- Emery, S. M. and J.A. Rudgers. 2012. Impact of Competition and Mycorrhizal Fungi on Growth of *Centaurea stoebe*, an Invasive Plant of Sand Dunes. *The American Midland Naturalist* 167:213-222.
- Endara, M.J. and P.D. Coley. 2011. The resource availability hypothesis revisited: a meta-analysis. *Functional Ecology* 25:389-398.
- Feeny, P.P. 1976. Plant apparency and chemical defense. Pages 1–40 in J. W. Wallace and R. L. Mansell, editors. *Biochemical interaction between plants and insects*. Plenum, New York, New York, USA.
- Fey, S.B. and C.M. Herren. 2014. Temperature-mediated biotic interactions influence enemy release of nonnative species in warming environments. *Ecology* 95:2246-2256.
- Firn, J., J.L. Moore, A.S. MacDougall, E.T. Borer, E.W. Seabloom, J. HilleRisLambers, W.S. Harpole, E.E. Cleland, C.S. Brown, J.M. Knops, et al. 2011. Abundance of introduced species at home predicts abundance away in herbaceous communities. *Ecology Letters* 14:274-281.
- Flory, S.L. and K. Clay. 2013. Pathogen accumulation and long-term dynamics of plant invasions. *Journal of Ecology* 101:607-613.
- Garland, T., A.W. Dickerman, C.M. Janis, and J.A. Jones. 1993. Phylogenetic analysis of covariance by computer simulation. *Systematic Biology* 42:265-292.
- Gilbert, G.S. and C.O. Webb. 2007. Phylogenetic signal in plant pathogen-host range. *Proceedings of the National Academy of Sciences* 104:4979-4983.
- Goßner, M.M., A. Chao, R.I. Bailey, and A. Prinzing. 2009. Native fauna on exotic trees: phylogenetic conservatism and geographic contingency in two lineages of phytophages on two lineages of trees. *The American Naturalist* 173: 599-614.
- Gurevitch, J., G.A. Fox, G.M. Wardle, Inderjit, and D. Taub. 2011. Emergent insights from the synthesis of conceptual frameworks for biological invasions. *Ecology Letters* 14:407-418.
- Hallett, S.G. 2006. Dislocation from coevolved relationships: a unifying theory for plant invasion

- and naturalization? *Weed Science* 54:282-290.
- Harper, J.L. 1977. *Population Biology of Plants*. Academic Press, London.
- Hawkes, C.V. 2007. Are invaders moving targets? The generality and persistence of advantages in size, reproduction, and enemy release in invasive plants species with time since introduction. *The American Naturalist* 170: 832-843.
- Hayes, K.R. and S.C. Barry. 2008. Are there any consistent predictors of invasion success? *Biological Invasions* 10:483-506.
- Heard, M.J. and D.F. Sax. 2013. Coexistence between native and exotic species is facilitated by asymmetries in competitive ability and susceptibility to herbivores. *Ecology Letters* 16:206-213.
- Hedges, L.V., J. Gurevitch, and P.S. Curtis. 1999. The meta-analysis of response ratios in experimental ecology. *Ecology* 80:1150-1156.
- Heger, T. and L. Trepl. 2003. Predicting biological invasions. *Biological Invasions* 5:313-321.
- Heger, T. and J.M. Jeschke. 2014. The enemy release hypothesis as a hierarchy of hypotheses. *Oikos* 123:741-750.
- Herrera, A.M., R.I. Carruthers, and N.J. Mills. 2011. Introduced populations of *Genista monspessulana* (French broom) are more dense and produce a greater seed rain in California, USA, than native populations in the Mediterranean Basin of Europe. *Biological Invasions* 13: 369-380.
- Hierro, J.L., J.L. Maron, and R.M. Callaway. 2005. A biogeographical approach to plant invasions: the importance of studying exotics in their introduced and native range. *Journal of Ecology* 93:5-15.
- Hill, S.B. and P.M. Kotanen. 2010. Phylogenetically structured damage to Asteraceae: susceptibility of native and exotic species to foliar herbivores. *Biological Invasions* 12:3333-3342.
- Hinz, H.L. and M. Schwarzlaender. 2004. Comparing Invasive Plants from Their Native and Exotic Range: What Can We Learn for Biological Control? *Weed Technology* 18:1533-1541.
- Hochwender C., R. Marquis, and K. Stowe. 2000. The potential for and constraints on the evolution of compensatory ability in *Asclepias syriaca*. *Oecologia* 122:361-370.
- Hoeksema, J.D., V.B. Chaudhary, C.A. Gehring, N.C. Johnson, J. Karst, R.T. Koide, A. Pringle, C. Zabinski, J.D. Bever, J.C. Moore, et al. 2010. A meta-analysis of context-dependency in plant response to inoculation with mycorrhizal fungi. *Ecology Letters* 13:394-407.

- Hokkanen, H.M.T. and D. Pimentel. 1989. New associations in biological control – theory and practice. *The Canadian Entomologist* 121: 829-840.
- Hothorn, T., F. Bretz, P. Westfall, R.M. Heiberger, A. Schuetzenmeister, and S. Scheibe. 2015. multcomp: Simultaneous Inference in General Parametric Models. R package version 1.4-0, Available at: <http://multcomp.R-forge.R-project.org>.
- Huang, W., J. Carrillo, J. Ding, and E. Siemann. 2012. Interactive effects of herbivory and competition intensity determine invasive plant performance. *Oecologia* 170:373-382.
- Ibáñez, I., D.W. Katz, D. Peltier, S.M. Wolf, and B.T. Connor Barrie. 2014. Assessing the integrated effects of landscape fragmentation on plants and plant communities: The challenge of a multiprocess-multiresponse dynamics. *Journal of Ecology* 102:882-895.
- Jiang, L., J. Tan, and Z. Pu. 2010. An Experimental Test of Darwin's Naturalization Hypothesis. *The American Naturalist* 175:415-423.
- Jogesh, T., D. Carpenter, and N. Cappuccino. 2008. Herbivory on invasive exotic plants and their noninvasive relatives. *Biological Invasions* 10:797-804.
- Keane, R.M. and M.J. Crawley. 2002. Exotic plant invasions and the enemy release hypothesis. *Trends in Ecology and Evolution* 17:164-170.
- Kearse, M., R. Moir, A. Wilson, S. Stones-Havas, M. Cheung, S. Sturrock, S. Buxton, A. Cooper, S. Markowitz, C. Duran, et al. 2012. Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics* 28:1647-1649.
- Kempel, A., T. Chrobock, M. Fischer, R.P. Rohr, and M. van Kleunen. 2013. Determinants of plant establishment success in a multispecies introduction experiment with native and alien species. *Proceedings of the National Academy of Sciences of the United States of America* 110:12727-12732.
- Kimbro, D.L., B.S. Cheng, and E.D. Grosholz. 2013. Biotic resistance in marine environments. *Ecology Letters* 16:821-833.
- Klironomos, J.N. 2002. Feedback with soil biota contributes to plant rarity and invasiveness in communities. *Nature* 417:67-70.
- Knapp A. and T. Seastedt. 1986. Detritus accumulation limits productivity of tallgrass prairie. *Bioscience* 36:662-668.
- Knight, T.M., J.L. Dunn, L.A. Smith, J. Davis, and S. Kalisz. 2009. Deer facilitate invasive plant success in a Pennsylvania forest understory. *Natural Areas Journal* 29:110-116.

- Kolar C.S. and D.M. Lodge. 2001. Progress in invasion biology: Predicting invaders. *Trends in Ecology and Evolution* 164:199-204.
- Kowarik, I. and P. Pyšek. 2012. The first steps towards unifying concepts in invasion ecology were made one hundred years ago: revisiting the work of the Swiss botanist Albert Thellung. *Diversity and Distributions* 18:1243-1252.
- Krebs, C.J., S. Boutin, R. Boonstra, A.R.E Sinclair, J.N.M Smith, M.R.T. Dale, K. Martin, and R. Turkington. 1995. Impact of Food and Predation on the Snowshoe Hare Cycle. *Science* 269:1112-1115.
- Kulmatiski, A., K.H. Beard, J.R. Stevens, and S.M. Cobbold. 2008. Plant-soil feedbacks: a meta-analytical review. *Ecology Letters* 11:980-992.
- Kuznetsova, A., P. Bruun Brockhoff, and R.H.B. Christensen. 2015. lmerTest: Tests in Linear Mixed Effects Models R package version 2.0-20 , <http://cran.r-project.org/package=lmerTest>.
- Lau, J.A., A.C. McCall, K.F. Davies, J.K. McKay, and J.W. Wright. 2008. Herbivores and edaphic factors constrain the realized niche of a native plant. *Ecology* 89:754-762.
- Lau, J.A., and E.H. Schultheis. 2015. When two invasion hypotheses are better than one. *New Phytologist* 205:958-960.
- Levine, J.M. 2000. Species diversity and biological invasions: relating local process to community pattern. *Science* 288:852-854.
- Levine, J.M., M. Vila, C.M.D. Antonio, J.S. Dukes, K. Grigulis, and S. Lavorel. 2003. Mechanisms underlying the impacts of exotic plant invasions. *Proceedings of the Royal Society B: Biological Sciences* 270:775-781.
- Levine, J.M., P.B. Adler, and S.G. Yelenik. 2004. A meta-analysis of biotic resistance to exotic plant invasions. *Ecology Letters* 7:975-989.
- Lind, E.M. and J.D. Parker. 2010. Novel weapons testing: are invasive plants more chemically defended than native plants? *PloS one* 5:e10429.
- Liu, H. and P. Stiling. 2006. Testing the enemy release hypothesis: a review and meta-analysis. *Biological Invasions* 8:1535-1545.
- Liu, H., P. Stiling, and R. Pemberton. 2007. Does enemy release matter for invasive plants? evidence from a comparison of insect herbivore damage among invasive, noninvasive and native congeners. *Biological Invasions* 9:773-781.
- Lonsdale, W.M. 1999. Global patterns of plant invasions and the concept of invisibility. *Ecology* 80:1522-1536.

- Louda, S. M. 1982. Distribution Ecology: Variation in plant recruitment over a gradient in relation to insect seed predation. *Ecological Monographs* 51:25-41.
- Lowman, M.D. and H. Heatwole. 1992. Spatial and temporal variability in defoliation of Australian eucalypts. *Ecology* 73:129-142.
- Maron, J.L. and M. Vila. 2001. When do herbivores affect plant invasion? Evidence for the natural enemies and biotic resistance hypotheses. *Oikos* 95:361-373.
- Marquis R.J. and C.J. Whelan. 1994. Insectivorous birds increase growth of white oak through consumption of leaf-chewing insects. *Ecology* 75:2007-2014.
- Martin, P.H., C.D. Canham, and R.K. Kobe. 2010. Divergence from the growth-survival trade-off and extreme high growth rates drive patterns of exotic tree invasions in closed-canopy forests. *Ecology* 98:778-789.
- Martins, E.P. and T.F. Hansen. 1997. Phylogenies and the comparative method: a general approach to incorporating phylogenetic information into the analysis of interspecific data. *The American Naturalist* 149:646-667.
- Maschinski J. and T.G. Whitham. 1989. The continuum of plant responses to herbivory: the influence of plant association, nutrient availability, and timing. *The American Naturalist* 134:1-19.
- McCarthy-Neumann, S. and R.K. Kobe. 2008. Tolerance of soil pathogens co-varies with shade tolerance across species of tropical tree seedlings. *Ecology* 89:1883-1892.
- McNaughton, S.J. 1983. Compensatory plant growth as a response to herbivory. *Oikos* 40:329-336.
- Meiners S.J. and C.-H. Kong. 2012. Introduction to the special issue on allelopathy. *Plant Ecology* 213:1857-1859.
- Michigan Department of Natural Resources and Environment [MDNR]. 2010. Michigan Deer Management Plan. Lansing, Michigan. Available at: http://www.michigan.gov/dnr/0,4570,7-153-10363_41153---,00.html
- Miller, G.A., and J.P. Chapman. 2001. Misunderstanding Analysis of Covariance. *Journal of Abnormal Psychology* 110:40-48.
- Mitchell, C. E., and A. G. Power. 2003. Release of invasive plants from fungal and viral pathogens. *Nature* 421:625-627.
- Mitchell, C.E., A.A. Agrawal, J.D. Bever, G.S. Gilbert, R.A. Hufbauer, J.N. Klironomos, J.L. Maron, W.F. Morris, I.M. Parker, A.G. Power, E.W. Seabloom, M.E. Torchin, and D.P.

- Vazquez. 2006. Biotic interactions and plant invasions. *Ecology Letters* 9:726-740.
- Mitchell, C.E., D. Blumenthal, V. Jarošík, E.E. Puckett, and P. Pyšek. 2010. Controls on pathogen species richness in plants' introduced and native ranges: roles of residence time, range size and host traits. *Ecology Letters* 13:1525-1535.
- Morris, W.F., R.A. Hufbauer, A.A. Agrawal, J.D. Bever, and V.A. Borowicz. 2007. Direct and interactive effects of enemies and mutualists on plant performance: a meta-analysis. *Ecology* 88:1021-1029.
- Morrison, W.E. and M.E. Hay. 2011. Herbivore preference for native vs. exotic plants: generalist herbivores from multiple continents prefer exotic plants that are evolutionarily naïve. *PloS one* 6:e17227.
- Munger, J.C. and J.H. Brown. 1981. Competition in desert rodents: an experiment using semipermeable exclosures. *Science* 211:510-512.
- Olson B.E. and J.H. Richards. 1988. Tussock regrowth after grazing: intercalary meristem and axillary bud activity of tillers of *Agropyron desertorum*. *Oikos* 51:374–82
- Paige K.N. 1992. Overcompensation in response to mammalian herbivory from mutualistic to antagonistic interactions. *Ecology* 73:2076-2085.
- Paige K.N., and T.G. Whitham TG. 1987. Overcompensation in response to mammalian herbivory: the advantage of being eaten. *The American Naturalist* 129:407-416.
- Paradis, E. 2012. *Analysis of Phylogenetics and Evolution with R* (Second Edition). New York: Springer.
- Parker, I.M. and G.S. Gilbert. 2007. When There Is No Escape: The Effects of Natural Enemies on Native, Invasive, and Noninvasive Plants. *Ecology* 88:1210-1224.
- Parker, I.M., M. Saunders, M. Bontrager, A.P. Weitz, R. Hendricks, R. Magarey, K. Suiter, and G.S. Gilbert. 2015. Phylogenetic structure and host abundance drive disease pressure in communities. *Nature* 520:542-544.
- Parker, J.D., and M.E. Hay. 2005. Biotic resistance to plant invasions? Native herbivores prefer non-native plants. *Ecology Letters* 8:959-967.
- Parker, J.D., D.E. Burkepile, and M.E. Hay. 2006. Opposing Effects of Native and Exotic Herbivores on Plant Invasions. *Science* 311:1459-1461.
- Pearse, I.S. and A.L. Hipp. 2009. Phylogenetic and trait similarity to a native species predict herbivory on non-native oaks. *Proceedings of the National Academy of Sciences* 106:18097-18102.

- Pinheiro, J., D. Bates, S. DebRoy, D. Sarkar and R Core Team. 2015. nlme: Linear and Nonlinear Mixed Effects Models. R package version 3.1-119, Available at: <http://CRAN.R-project.org/package=nlme>.
- Powell, K.I., J.M. Chase, and T.M. Knight. 2011. A synthesis of plant invasion effects on biodiversity across spatial scales. *American Journal of Botany* 98:539-548.
- Powell, K.I., J.M. Chase, and T.M. Knight. 2013. Invasive plants have scale-dependent effects on diversity by altering species-area relationships. *Science* 339:316-318.
- Quader, S., K. Isvaran, R.E. Hale, B.G. Miner, and N.E. Seavy. 2004. Nonlinear relationships and phylogenetically independent contrasts. *Journal of Evolutionary Biology* 17:709-715.
- R Core Team. 2015. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. Available at: <http://www.R-project.org/>
- Raudenbush, S.W. 1994. Random effects models. In H. Cooper and L. V. Hedges (eds.). *The Handbook of research synthesis*. (pp. 301-321). Russell Sage Foundation, New York.
- Revell, L.J. (2012) phytools: An R package for phylogenetic comparative biology (and other things). *Methods in Ecology and Evolution* 3:217-223.
- Reznicek, A.A., E.G. Voss, and B.S. Walters. 2011. Michigan Flora Online. University of Michigan. Available at: <http://michiganflora.net/home.aspx>
- Rhoades, D.F. and R.G. Cates. 1976. Towards a general theory of plant antiherbivore chemistry. *Recent Advances in Phytochemistry*. 10:168-213.
- Richardson D.M., P. Pysek, M. Rejmanek, M.G. Barbour, F.D. Panetta, and C.J. West. 2000. Naturalization and invasion of alien plants: Concepts and definitions. *Diversity and Distributions* 6:93-107.
- Rice E.L. 1974. Allelopathy. Academic Press, New York
- Ridenour, W.M. and R.M. Callaway. 2001. The relative importance of allelopathy in interference: the effects of an invasive weed on a native bunchgrass. *Oecologia* 126:444-450.
- Robertson, B.A., C. Porter, D.A. Landis, and D.W. Schemske. 2011. Agroenergy Crops Influence the Diversity, Biomass, and Guild Structure of Terrestrial Arthropod Communities. *BioEnergy Research* 5:179-188.
- Rodriguez, L.F. 2006. Can invasive species facilitate native species? Evidence of how, when, and why these impacts occur. *Biological Invasions* 8:927-939.
- Rosenberg, M.S., D.C. Adams, and J. Gurevitch. 1999. *MetaWin: statistical software for meta-analysis*. Version 2.0. Sinauer Associates, Sunderland.

- Sala, O.E., F.S. Chapin, J.J. Armesto, E. Berlow, J. Bloomfield, R. Dirzo, E. Huber-Sanwald, L.F. Huenneke, R.B. Jackson, A. Kinzig, et al. 2000. Global biodiversity scenarios for the year 2100. *Science* 287:1770-1774.
- Schultheis, E.H., A.E. Berardi, and J.A. Lau. 2015. No release for the wicked: enemy release is dynamic and not associated with invasiveness. *Ecology in press* (Chapter 2)
- Seabloom, E.W., W.S. Harpole, O.J. Reichman, and D. Tilman. 2003. Invasion, competitive dominance, and resource use by exotic and native California grassland species. *Proceedings of the National Academy of Sciences* 100:13384-13389.
- Shea, K. and P. Chesson. 2002. Community ecology theory as a framework for biological invasions. *Trends in Ecology & Evolution* 17:170-176.
- Siemann, E., W.E. Rogers, and S.J. Dewalt. 2006. Rapid adaptation of insect herbivores to an invasive plant. *Proceedings of the Royal Society B: Biological Sciences* 273:2763-2769.
- Smith, S.A. and C.W. Dunn. 2008. Phyutility: a phyloinformatics tool for trees, alignments, and molecular data. *Bioinformatics* 24:715-716.
- Stamatakis, A. 2014. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* 30: 1312–3.
- Stowe, L.G. 1979. Allelopathy and its Influence on the Distribution of Plants in an Illinois Old-Field. *Journal of Ecology* 67:1065-1085.
- Stowe, K.A., R.J. Marquis, C.G. Hochwender, and E.L. Simms. 2000. The evolutionary ecology of tolerance to consumer damage. *Annual Review of Ecology, Evolution and Systematics* 31:565–595.
- Strauss, S.Y. and A.A. Agrawal. 1999. The ecology and evolution of plant tolerance to herbivory. *Trends in Ecology and Evolution* 14:179-185.
- Strauss, S.Y., C.O. Webb, and N. Salamin. 2006. Exotic taxa less related to native species are more invasive. *Proceedings of the National Academy of Sciences* 103:5841-5845.
- Strayer, D.L., V.T. Eviner, J.M. Jeschke, and M.L. Pace. 2006. Understanding the long-term effects of species invasions. *Trends in Ecology and Evolution* 21:645-651.
- Stricker, K.B. and P. Stiling. 2014. Release from herbivory does not confer invasion success for *Eugenia uniflora* in Florida. *Oecologia* 174:817-826.
- Strong, D.R., E.D. McCoy, and J.R. Rey. 1977. Time and the number of herbivore species: the pests of sugarcane. *Ecology* 58:167-175.

- Stowe, K.A., R.J. Marquis, C.G. Hochwender, and E.L. Simms. 2000. The evolutionary ecology of tolerance to consumer damage. *Annual Review of Ecology, Evolution and Systematics* 31:565–595.
- Stricker, K.B. and P. Stiling. 2012. Herbivory by an introduced Asian weevil negatively affects population growth of an invasive Brazilian shrub in Florida. *Ecology* 93:1902-1911.
- Strauss, S.Y. and A.A. Agrawal. 1999. The ecology and evolution of plant tolerance to herbivory. *Trends in Ecology & Evolution* 14:179-185.
- Strayer, D.L., V.T. Eviner, J.M. Jeschke, and M.L. Pace. 2006. Understanding the long-term effects of species invasions. *Trends in Ecology and Evolution* 21:645-651.
- Suding, K.N., W. Stanley Harpole, T. Fukami, A. Kulmatiski, A.S. MacDougall, C. Stein, W.H. van der Putten, and M. Hutchings. 2013. Consequences of plant-soil feedbacks in invasion. *Journal of Ecology* 101:298-308.
- Suwa, T. and S.M. Louda. 2012. Combined effects of plant competition and insect herbivory hinder invasiveness of an introduced thistle. *Oecologia* 169:467-476.
- Swenson, N.G. 2014. *Functional and Phylogenetic Ecology* in R. Springer, New York.
- Thebaud, C. and D. Simberloff. 2001. Are plants really larger in their introduced ranges? *The American Naturalist* 157:231-236.
- Thellung, A. 1912. La flore adventice de Montpellier. *Mémoires de la Société nationale des sciences naturelles de Cherbourg* 38:57-728.
- Theoharides, K.A. and J.S. Dukes. 2007. Plant invasion across space and time: factors affecting nonindigenous species success during four stages of invasion. *New Phytologist* 176:256-273.
- Thomas, A., R. O'Hara, U. Ligges, and S. Sturts. 2006. Making BUGS Open. *R News* 6:12-17.
- Torchin M.E., K.D. Lafferty, A.P. Dobson, V.J. McKenzie, and A.M. Kuris. 2003. Introduced species and their missing parasites. *Nature* 421: 628-630.
- Torchin, M.E. and C.E. Mitchell. 2004. Parasites, Pathogens, and Invasions by Plants and Animals. *Frontiers in Ecology and the Environment* 2:183-190.
- Tylianakis, J.M., R.K. Didham, J. Bascompte, and D.A. Wardle. 2008. Global change and species interactions in terrestrial ecosystems. *Ecology Letters* 11:1-13.
- Underwood, A.J. 1997. *Experiments in Ecology*. Cambridge, UK.
- van Kleunen, M., E. Weber, and M. Fischer. 2010. A meta-analysis of trait differences between

- invasive and noninvasive plant species. *Ecology Letters* 13:235-245.
- Vavra, M., C.G. Parks, and M.J. Wisdom. 2007. Biodiversity, exotic plant species, and herbivory: The good, the bad, and the ungulate. *Forest Ecology and Management* 246:66-72.
- Verhoeven, K.J.F., A. Biere, J.A. Harvey, and W.H. van der Putten. 2009. Plant invaders and their novel natural enemies: who is naïve? *Ecology Letters* 12:107-117.
- Vilà, M., J.L. Espinar, M. Hejda, P.E. Hulme, V. Jarosik, J.L. Maron, J. Pergl, U. Schaffner, Y. Sun, and P. Pysek. 2011. Ecological impacts of invasive alien plants: a meta-analysis of their effects on species, communities and ecosystems. *Ecology Letters* 14:702-708.
- Williams, J.L., H. Auge, and J.L. Maron. 2010. Testing hypotheses for exotic plant success: parallel experiments in the native and introduced ranges. *Ecology* 91:1355-1366.
- Williamson, M. and A. Fitter. 1996. The varying success of invaders. *Ecology* 77:1661-1666.
- Wootton, J.T. 1994. The nature and consequences of indirect effects in ecological communities. *Annual Review of Ecology and Systematics* 25:443-466.
- Zou, J., W.E. Rogers, and E. Siemann. 2008. Increased competitive ability and herbivory tolerance in the invasive plant *Sapium sebiferum*. *Biological Invasions* 10:291-302.