INTRODUCED PLANT SPECIES, HERBIVORES AND PATHOGENS, AND THE HOST-ENEMY RELATIONSHIPS THAT ACCOMPANY INVASIONS

by

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DISSERTATION ABSTRACT

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Invasions by introduced plant species cost billions of dollars each year in the United States and threaten native habitat. The primary goal of my dissertation research was to examine the role that natural enemies (pathogens and herbivores) play in these invasions in both unmanaged and restored plant communities.

In two related studies in seasonal wetland prairies in the Willamette Valley, Oregon, USA, I surveyed natural enemy attack on common native and introduced plant species in a restoration experiment designed to test the effects of site preparation techniques on plant community composition. Restoration treatments had little influence on enemy attack rates. Attack rates depended on idiosyncratic differences in the relationships between host species and plant community characteristics, suggesting that existing theories concerning these relationships have limited predictive power.

Another field experiment tested the potential for enemy spillover from introduced to native species and dilution of natural enemy attack on introduced species by native species. I examined natural enemy attack on three native and three perennial grasses that commonly co-occur in the Willamette Valley. The native species are commonly used in restoration. The introduced species are common throughout North America and potentially harbor enemies that could affect both crops and natural communities. There was no compelling evidence of enemy spillover from the introduced to the native species, but dilution of enemies on the introduced species by the native species was evident in year 2 and even stronger in year 3 for two of the three introduced species.

Using the same three introduced species from the spillover/dilution study, I tested the enemy release hypothesis, which proposes that introduced species lose natural enemies upon introduction and are thus “released” from population control. I surveyed populations of the three grass species across a wide geographic area in their native and naturalized ranges in Europe and the United States, respectively. I also compared my results to those of a previously published literature survey. My field survey supported release from herbivores but not from fungal pathogens. In contrast, the literature survey found evidence of release from fungal pathogens.

This dissertation includes unpublished co-authored material.

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TABLE OF CONTENTS

Chapter Page

LIST OF FIGURES

LIST OF TABLES

Table Page

CHAPTER I

INTRODUCTION

Background and significance

Charles Elton (Elton 1958) pioneered the science of community invasions by introduced species, and was the first to clearly synthesize multiple explanations of those invasions (Richardson and Pyšek 2008). Elton correctly predicted that as intercontinental travel and trade increased, so would problems of species introductions via increased human traffic. Some of these ideas had previously been visited by Darwin (1859) and by Candolle and Sprengel (1821), but were not widely considered by other scientists before Elton. In the United States 5,000 plant species from other regions have become naturalized, compared with 17,000 native plant species (Pimentel et al. 2005). Fifty-seven percent of native plant species in the United States that are imperiled are threatened by introduced species (Wilcove et al. 1998). Efforts to maintain and restore native plant species often need to focus primarily on the control of introduced plant species.

Herbivores can affect plant community structure by preferentially feeding on and regulating the populations of their favored host species. In restoration efforts of rare native plants, controlling herbivore attack, especially at certain vulnerable life stages, can increase population growth of those plants (Bevill et al. 1999). Also, in some cases where herbivores have been introduced for control of an invasive plant species, those herbivores also feed on multiple co-occurring native plant species that are of conservation concern (Louda and O'Brien 2002).

Similar to herbivores, pathogens also can affect plant community structure, because they can infect only some plant species and differentially affect host fitness among species that they infect (Dobson and Crawley 1994). Plant pathogens can alter competitive interactions among plant species, and have been experimentally used to regulate invasive plant species (Carsten et al. 1998, Carsten et al. 2001). Plant pathogens have also been implicated in facilitating the invasion of introduced plant species by affecting the fitness of native more than introduced species (Borer et al. 2007).

Several factors may affect the abundance of herbivores and pathogens (referred to as natural enemies throughout this dissertation) in a plant community (reviewed in Alexander 1992, Burdon 1993, Agrawal et al. 2006). Some studies have found evidence that natural enemy attack decreases as plant species diversity increases (Mitchell et al. 2002), but this relationship has been most strongly supported at very low plant diversity levels such as agricultural settings (Power 1987). As total plant density or litter increase, the sheltered, more humid microclimate may lead to increased disease and herbivore attack (Folgarait et al. 1995, Facelli et al. 1999). Individual plant traits, such as nutrient status, may also affect enemy attack. The plant vigor hypothesis (Price 1991) predicts that natural enemy attack will be greater on larger, healthier plants (reviewed in Cornelissen et al. 2008). These patterns have been examined primarily only on one species of interest in a given plant community (but see Mitchell et al. 2002), and more community-wide studies are needed.

Multiple, somewhat contradictory explanations for the invasiveness of introduced plant species focus on the potential roles of herbivores and pathogens. First, enemy spillover occurs when natural enemy levels in one host population are due to the high abundance of that natural enemy in another more competent reservoir host species. A competent host species is defined as being easily colonized by a natural enemy, able to maintain high populations of that natural enemy, and readily able to transmit that natural enemy to other hosts. Some studies have found evidence that pathogen spillover from introduced to native grass species augmented the invasion by those introduced grass species (Malmstrom et al. 2005, Beckstead et al. 2010). These studies, however, compared native perennial grasses to introduced annual grasses, and their findings may have been confounded with life history differences between the native and introduced grasses (Borer et al. 2009).

Second, dilution of natural enemies on a particular host species may occur when it is surrounded by less competent host species, leading to that plant species' increased survival and reproduction. Dilution of natural enemies has been found in the context of diseases of animals (Ostfeld and Keesing 2000), but has not been extensively studied in plant communities (but see Borer et al. 2010).

Finally, the enemy release hypothesis is probably the most popular explanation of invasions by introduced plant species. According to this hypothesis, plants experience a decrease in regulation by natural enemies upon introduction to a new geographic range, and therefore increase rapidly in distribution and abundance (Keane and Crawley 2002). Many studies have set out to test enemy release (reviewed in Keane and Crawley 2002, Colautti et al. 2004, Liu and Stiling 2006), with varying results. Few studies, however, have effectively combined multiple approaches of testing enemy release (but see Vermeij et al. 2009), or simultaneously tested enemy release and other competing explanations of invasions (but see Williams et al. 2010).

To better understand and manage plant communities, the roles that herbivores and pathogens play in shaping plant community structure and mediating species invasions need to be better understood. For my dissertation research, I combined multiple approaches to address several hypotheses about plant community structure, invasions by introduced species, and the host-enemy patterns that are associated with species introductions.

Overview of research

For my dissertation I performed four related research projects, described in Chapters II, III, IV, and V, in which I examined the role that natural enemies play in invasions by introduced plant species. My objective was to test the enemy release hypothesis and alternative roles of natural enemies in invasions, including spillover, dilution, and effects of plant community structure. My research also applies to restoration ecology of the wetland prairie native plant communities in the Willamette Valley in Oregon, where less than 1% of previously existing wetland prairies remain intact today. My field experiments were located in the Willamette Valley, in a seasonal wetland prairie. For Chapters IV and V, I focused on six perennial grass species, which combined comprise 64% of the total plant cover in the area where my field site was.

All data chapters were co-authored. Chapters II and III, which are very closely linked, were co-authored with Drs. Bitty Roy, Scott Bridgham, and Laurel Pfeifer-Meister. Chapter IV was co-authored with Drs. Bitty Roy and Scott Bridgham, and Chapter V was co-authored with Dr. Bitty Roy.

In Chapter II, our objective was to test several currently debated hypotheses about patterns of natural enemy attack and plant community characteristics, including (i) plant species diversity may reduce vulnerability of plants to attack, (ii) enemy spillover may occur, in which an enemy increase in one host species is due to transmission from another host species, (iii) dilution may occur, in which an enemy population is reduced by one or more plant species in the community, leading to reduced attack on an otherwise more heavily infested host, (iv) physical traits of the plant community, such as total plant cover, may influence enemy abundance, dispersal, and attack rates, and (v) traits of individual plants, such as size or nutrient content, may affect enemy populations and attack rates on those plants. To investigate these hypotheses, herbivore and pathogen attack were surveyed on the six most common native plant species, which combined comprised eighty percent of the total plant cover in the community, in a wetland prairie restoration experiment in Eugene, Oregon (Pfeifer-Meister 2008). Additionally, leaf chlorophyll content and shoot biomass were measured at the time of the survey as measures of plant vigor. We then used multi-model inference to examine the associations between enemy attack and plant community structure, based on detailed plant cover data that was collected concurrently with the natural enemy survey.

Our goals in Chapter III were to test the enemy release hypothesis by comparing natural enemy attack on native versus introduced species, and to compare natural enemy attack among different restoration treatments. In the same restoration experiment that we surveyed for Chapter II, herbivore and pathogen damage were surveyed on the two most common native grass species, *Agrostis exarata* and *Deschampsia cespitosa*, and the most common introduced grass, *Lolium multiflorum*, in plots that had been treated with five different experimental site preparation techniques, the adjacent agricultural field planted with *L. multiflorum,* and an intact seasonal wetland prairie. Enemy attack rates were compared between the native and introduced species to test enemy release, and among the different restoration treatments to test response of enemy attack to different site preparation techniques.

In Chapter IV, we experimentally tested enemy spillover and dilution among three native (*Agrostis exarata, Danthonia californica*, and *Deschampsia cespitosa*) and three introduced (*Anthoxanthum odoratum, Holcus lanatus,* and *Schedonorus arundinaceus*) perennial bunchgrass species. The native species are commonly used in restoration. The introduced species are common throughout North America and potentially harbor enemies that could affect both crops and natural communities. We designed this study to test enemy spillover from introduced to native species, and dilution of natural enemies by native species on introduced species. Our eight experimental communities were as follows: three introduced species, three native species, three native species plus one introduced species, and three introduced species plus one native species. Communities were replicated five times in a randomized block design, for 40 plots total. We carried out this study over three growing seasons, surveying natural enemy attack on each species in each experimental treatment five times during the three years. Based on these surveys, we were able to evaluate differences in enemy attack on each species among the different treatment communities, how enemy attack varied over the course of three growing seasons, and how enemy attack varied over the course of one growing season.

In Chapter V, we used a biogeographical survey to test the enemy release hypothesis. We then compared our findings to existing compilations of information gathered from host indices of fungal plant pathogens to determine whether the two methods would yield similar or conflicting results. Herbivore and pathogen damage were surveyed in several populations of each of the three introduced grass species studied in

Chapter IV, across a wide geographic area in both their native (Europe) and naturalized (United States) ranges. We then compared the results of our field survey to those of a published literature survey (Mitchell and Power 2003).

In Chapter VI, I review and synthesize the findings of Chapters II, III, IV, and V.

CHAPTER II

IDIOSYNCRATIC DIFFERENCES IN THE RELATIONSHIPS BETWEEN HOST SPECIES AND PLANT COMMUNITY CHARACTERISTICS DOMINATE HERBIVORE AND PATHOGEN ATTACK RATES

A paper submitted to *Plant Ecology* and co-authored with Bitty A. Roy, Laurel E. Pfeifer-Meister, and Scott D. Bridgham

Author contributions: The restoration experiment on which this study was based was designed and implemented by Laurel E. Pfeifer-Meister, Bitty A. Roy, Scott D. Bridgham, and Bart Johnson. The disease survey was designed by G. Kai Blaisdell, Bitty A. Roy, and Scott D. Bridgham. G. Kai Blaisdell wrote the paper with contributions from Bitty A. Roy, Scott D. Bridgham, and Laurel E. Pfeifer-Meister.

Introduction

Conservation efforts to restore and protect native plant communities should consider the role of natural enemies (herbivores and pathogens), which reduce host fitness and can affect restoration and conservation success (Bevill et al. 1999). There is evidence that introduced natural enemies can spread ahead of introduced plants, and may facilitate invasions (Malmstrom et al. 2007, Borer et al. 2007). The effects of host community composition on natural enemies, and the reciprocal, the effects of natural enemies on host community composition have been hotly debated. In this study, we observed natural enemy attack rates on the most common native host species in an experimental restoration of a seasonal wetland prairie, across a range of host community characteristics.

One community pattern that has received support is that enemy attack tends to decrease as host diversity increases (Elton 1958, Mitchell et al. 2002, LoGiudice et al. 2003, Keesing et al. 2006, Johnson et al. 2008). Many studies, however, focused only on host species richness, which is not always representative of diversity when the evenness of species varies, especially if dominant or rare species are present. A number of studies focused on the effects of diversity on one focal species, ignoring enemy attack on the rest of the community (e.g., Root 1973, Schellhorn and Sork 1997). Furthermore, this pattern has been most strongly supported at low diversity and species richness levels, with less clear results at higher diversity (Mitchell et al. 2002). The relationship between host diversity and enemy attack is not always negative or simple (Letourneau 1987, Andow 1991, Joshi et al. 2004, Dobson 2004).

Low host density may limit natural enemies due to the difficulty of dispersal among host plants (Alexander 1992, Folgarait et al. 1995). Numerous hypotheses relating to host density and herbivore attack rates generally predict increased attack rates as host density increases (reviewed by Agrawal et al. 2006). Many studies have focused on density of one particular host species and enemy attack on that species, with mixed findings (Root 1973, Schellhorn and Sork 1997, Harmon et al. 2003). Few studies, however, have examined community-wide patterns of density of multiple hosts and enemy attack on those host species. Additionally, studies and discussion of host communities often fail to distinguish between the effects of host diversity and host density. For example, Keesing and others (2006) indicated that the “diversity effect” found by Mitchell and others (2002) was actually an artifact of the density of one particular host species.

As a particular natural enemy population increases on its favored host species, the natural enemy may then spill over onto neighboring species, which would suffer more attack than in the absence of the favored host species (Power and Mitchell 2004). Conversely, as the favored host species becomes less common, the other less competent species can dilute the natural enemy in the community. Enemy spillover from introduced to native plant species has been implicated in facilitating invasions in plant communities (Tompkins et al. 2003, Malmstrom et al. 2007, Borer et al. 2007, Beckstead et al. 2010).

Abiotic factors can affect populations of herbivores and pathogens, and are influenced by the physical structure of the plant community. Total vegetation cover affects microclimate, including light, temperature, and humidity. High humidity in dense stands usually favors infection and sporulation, but can impede dispersal of newly formed inoculum (Burdon 1987, Kranz 1990). Sun and shade have species-specific effects on enemies (Collinge and Louda 1988, Stanton et al. 2004). A buildup of dead plant material (thatch) can harbor pathogenic fungi. For example, in our study area in Oregon, USA, farmers use field burning after harvest to reduce crop residue and pathogens harbored there (Young et al. 1999).

The plant vigor hypothesis (Price 1991) predicts that larger, more vigorous plants will experience more attack by herbivores than smaller, stressed plants. Larger plants can be a more desirable target for natural enemies (Folgarait et al. 1995, Bradley 2003). Higher nitrogen content per leaf area has been found to increase foliar fungal disease (Neumann et al. 2004, Throop and Lerdau 2004), and the carrying capacity and population growth rate of aphids (Zehnder and Hunter 2008). Alternatively, the plant stress hypothesis predicts that unhealthy plants will experience more herbivore attack (White 1974). In a meta-analysis of herbivore studies, Cornellisen and others (2008) found higher abundances of insect herbivores on larger plants, in support of the plant vigor hypothesis, but did not find strong support for the plant stress hypothesis.

Patterns of natural enemies in plant community restoration have not been extensively studied, and that was a goal of our project. We surveyed damage by natural enemies on six native plant species in a seasonal wetland prairie restoration experiment in Eugene, Oregon, USA (Pfeifer-Meister 2008). The restoration experiment provided an ideal setting for our study because it created different communities with some shared species in an area that has a relatively homogeneous physical environment. The same 15 native plant species were seeded in each of 50 plots following implementation of 10 different site preparation treatments designed to remove the extant vegetation and reduce the introduced seed bank. Following seeding of the experiment, natural succession of the plant communities was allowed to occur, without plant removal or additional seeding. We examined the effects of diversity, relative abundance of introduced species, relative abundance of affected species, physical attributes of the community structure, and individual plant traits on enemy attack rates by herbivores and pathogens on six native grass and forb species. Detailed plant cover data enabled us to address several patterns of plant community effects on natural enemies. We hypothesized that (i) herbivore and pathogen attack would decrease as plant species diversity increased, (ii) if enemy spillover from introduced to native species was occurring, damage to native species would increase as relative abundance of introduced species increased, (iii) we might find evidence of dilution by one or more species of enemy attack on other plant species, (iv) high total plant cover would lead to increased pathogen attack, and (v) larger plants and those with higher nutrient content would sustain more damage by natural enemies.

Methods

Study site

Our survey was performed in a restoration experiment that was designed to test how site preparation techniques affect the relative success of native and introduced plant species (Pfeifer-Meister 2008). Prior to the restoration experiment, the area had been planted in *Lolium multiflorum* Lam. (annual ryegrass) for agricultural production. The 4.5-hectare experiment included ten experimental land preparation techniques, replicated five times in randomized 15-m^2 plots, for a total of 50 plots. Ten-m mown buffers separated plots, and a 23-m mown buffer surrounded the experimental restoration site. The treatments included various combinations of tilling, herbicide application, thermal application, and solarization: till only, herbicide only, herbicide + thermal, two herbicide applications, till + herbicide, till + two herbicide applications, till + solarization, till + thermal, till + herbicide + solarization, and till + herbicide + thermal. Application of all treatments and seeding of 15 native grass and forb species were completed in October 2004, and natural succession was allowed to occur without more seeding or plant removal. We also surveyed a nearby intact reference wetland prairie for comparison to the restoration treatments. The reference wetland prairie was 4 km from the restoration experiment, and had the same soil type and similar hydrology to the restoration experiment.

Description of restoration treatments

Tilling can reduce the introduced seed bank in recently cultivated fields. As a result of tilling, seeds present in the soil are moved to the surface and germinate, thus reducing future germination of introduced species (Fitzpatrick 2004). Thermal treatment is applied using an infrared burner, which produces temperatures ranging from 540-1090° C. Plants' cells are ruptured due to the resulting heat exposure. Thermal treatment is most effective with grasses and small forbs (Fitzpatrick 2004). Solarization is the generation of heat and humidity over an extended time period by covering a large area of the ground with plastic. Solarization causes seeds in the seed bank to germinate and then die, thus reducing future germination (Fitzpatrick 2004). In this restoration experiment, the herbicide applied was the broad spectrum glyphosate. Herbicide application reduces existing vegetation in the short term, but its effects diminish within a few growing seasons. Practitioners and researchers have found that combining multiple site preparation techniques that mitigate the introduced seeds and plants at different stages can improve results (Fitzpatrick 2004).

Collection of data

We collected plant cover data in June 2006 using the point-intercept method (Jonasson 1988) in one randomly located 1-m^2 subplot per 15-m^2 plot, for a total of 5 replicates per treatment. In the reference wetland prairie, five 15-m^2 plots were randomly chosen, and one 1-m^2 subplot was placed in each 15-m^2 plot. Percent cover was recorded for each species using a 1-m^2 frame with 25 pins. Species that were present but did not contact a pin were allocated 1% cover. Additionally, dead plant material was recorded as present or absent with each pin, counting each pin hit at 4% cover. Standing thatch was defined as dead plant material that was still standing, and ground thatch was defined as dead plant material lying horizontally on the ground. In 2006, the mean number of plant species richness per plot was 14, and the range was 8-26 species per plot.

To examine how the restoration treatments and plant community structure affected enemy attack rates on the native plants in the site, we assessed natural enemy damage to the six most common native plant species *Agrostis exarata* Trin. (spike bent grass), *Deschampsia cespitosa* (L.) P. Beauv. (tufted hair grass), *Madia glomerata* Hook. (tarweed), *Prunella vulgaris* L. (common selfheal), *Epilobium densiflorum* (Lindl.) Hoch & P. H. Raven (willow herb), and *Grindelia integrifolia* DC (gumweed) in all ten restoration treatments and the reference wetland prairie. These species combined comprised 80% of total plant cover in the study. The timing of sampling, from June through August 2006, was based on the phenology of each species, after flowering but before mature seed set or substantial senescence. To associate detailed information about plant community composition and cover with enemy attack, natural enemy attack was surveyed on one plant of each native species, collected from within 10 cm of the subplot used to measure cover in each plot (Table 2.1).

Percent visible foliar herbivore and pathogen damage were assessed on each of three randomly selected leaves from each plant, and the average of the three leaves was used. Percent damage was scored as a continuous variable. Percent damage caused by each type of symptom on each leaf was also scored. Herbivore attack was scored as chew, rasp, mine, or sucking damage. Pathogen attack was scored as blotch, spot, or rust pustules. Above-ground biomass of each plant at the time of sampling was measured by clipping and drying at 60°C for 48 hours.

Leaf nitrogen content was estimated by measuring chlorophyll content in the field using a hand held portable SPAD-502 chlorophyll meter (Spectrum Technologies, Inc., Plainfield, Illinois, USA). For each plant, one measurement was taken for each of three randomly selected leaves, and the average of the three measurements was used. Chlorophyll content is highly correlated with nitrogen content, and is a quick, non-destructive and inexpensive estimate of nitrogen content (Gáborčík 2003).

Data analysis

All analyses were performed using JMP version 7.0.1. We performed two-way ANOVAs for herbivore and pathogen damage, with plant species, treatment, and their interactions as independent variables. We considered treatment a fixed variable and species a random variable. Because of inadequate replication of *E. densiflorum* and *G. integrifolia* in two of the treatments, two ANOVAs were run for herbivore and pathogen damage. One included the four remaining species and all treatments, and the other included all six species but excluded the reference and till + solarization treatments. Post hoc Tukey’s HSD tests were performed to determine differences among individual treatments and plant species.

The proportion of variance in herbivore and pathogen attack explained by each predictor variable was calculated for the random variables species, location, and their interaction. Location was included to account for possible spatial autocorrelation. For this analysis, sampling locations were grouped into eleven blocks of five adjacent plots.

Our decision to survey the six species was partially motivated by our observation of large plant community differences among the restoration treatments in 2005. However, the differences in plant community composition among the restoration treatments dampened over time, and the restoration treatments became more similar to the composition of the reference sites in 2006 (Pfeifer-Meister 2008). Therefore we found little to no difference among the treatments with respect to the variables that we measured. However, there were community differences among plots (Table 2.1), and the lack of treatment effects allowed us to more directly address how herbivore and pathogen attack varied with plant community composition and other factors.

To determine which potential explanatory factors were associated with natural enemy attack, we used Akaike's Information Criterion (AIC) multi-model inference (Burnham and Anderson 2002) to select groups of equivalent models that would explain herbivore and pathogen attack on the six native species. The models included the relative abundance of each of the six native species sampled, relative abundance of the introduced *Lolium multiflorum*, relative abundance of introduced species, total plant cover, Simpson's diversity index, percent ground thatch, percent standing thatch, above-ground biomass, and chlorophyll content (Table 2.1). We selected models for which the corrected AIC value differed from the minimum corrected AIC value by less than two (Burnham and Anderson 2002). Using the corrected AIC values adjusted for our relatively small sample sizes (Table 2.1). Because some species surveyed did not occur in all plots sampled, the values of the community factors varied somewhat among the six species.

Table 2.1. Community factors that were measured and entered into AIC analysis, (average relative abundance of each species sampled, and number of plants of each species sampled.) Mean and Standard Error represent the mean value and standard error among all 55 plots in the survey. The Minimum and Maximum represent the plot with the lowest and highest values for each species or factor, and n represents the number of plots sampled for that species. The species that were surveyed for natural enemy attack are totaled.

Species or Factor	**Mean**	**Standard Error**	**Minimum**	**Maximum**	**n**
Agrostis exarata	41.0%	3.29	0.0%	88.2%	55
Deschampsia cespitosa	23.8%	2.75	0.2%	95.0%	55
Madia glomerata	7.0%	1.09	0.0%	30.0%	53
Prunella vulguaris	5.9%	0.87	0.0%	27.0%	55
Epilobium densiflorum	1.8%	0.42	0.0%	13.9%	39
Grindelia integrifolia	0.3%	0.07	0.0%	2.2%	32
Total	**80.2%**				**289**
Lolium multiflorum	5.5%	1.15	0.0%	46.27%	
Introduced species	14.6%	2.26	0.1%	65.42%	
Total cover	790%	25.41	422%	1389%	
Standing Thatch	85%	1.82	40%	100%	
Ground Thatch	43%	2.60	8%	96%	
Diversity (Simpson’s)	0.63	0.02	0.10	0.91	

Each variable entered into our AIC analyses was selected a priori and addressed a specific hypothesis. Some variables showed pair-wise correlations and variance inflation factors high enough to raise concern (Mac Nally 2000, Graham 2003). For example, in our worst case scenario, the partial correlation of the relative abundance of introduced species with the relative abundance of *L. multiflorum* was r = 0.87 for the analysis of *E. densiflorum*. When pair-wise correlations were worrisome, and removing one variable could potentially hurt our analysis in other ways (O'Brien 2007), we used stepwise regression with both variables and each variable excluded to test for model stability. Furthermore, others have shown that AIC multi-model inference is robust against error with variable pair-wise correlations up to 0.9 (Smith 2009) or 0.94 (Burnham and Anderson 2002). Another potential pitfall is that the relative abundances of *L. multiflorum* and introduced species contain overlapping information because *L. multiflorum* was the most common introduced species in many plots. However, in a previous survey of the three most common grasses in a subset of the restoration treatments, we found that the introduced *L. multiflorum* had more pathogen damage than the two native grasses *A. exarata* and *D. cespitosa* (data not shown). Based on these results we hypothesized that the pathogen may spread from the *L. multiflorum* to the two native grasses, and we decided that the benefit of keeping both *L. multiflorum* and introduced species abundance in the analysis outweighed the costs of potential bias. Others have included variables that contain overlapping information using AIC (e.g., Seabloom et al. 2009). The strength of our analysis is the number and breadth of hypotheses that we were able to test about the effects of community variables on natural enemy attack across the dominant species in the community.

Results

Pathogen attack varied among plant species ($F_{5,41} = 19.26$, $P < 0.0001$) but not among the ten restoration treatments and reference wetland prairie (Figure 2.1). There was an interaction between the two factors only when *Grindelia integrifolia* and *Epilobium densiflorum* were excluded from the analysis and all treatments were included, because these two species were absent from some treatments ($F_{30,174} = 1.70$, $P = 0.019$).

Herbivore attack varied among plant species ($F_{5,40} = 9.03$, $P < 0.0001$) and among treatments ($F_{8,49} = 2.37$, $P = 0.035$) (Figure 2.1). Attack was not the same for each plant species in each treatment (species by treatment interaction $F_{48,225} = 2.85$, $P < 0.001$). Herbivore damage was higher in the herbicide treatment than in the till + thermal treatment (Tukey's HSD: $P < 0.05$); otherwise herbivory did not vary among the restoration treatments or reference wetland prairie. Overall, there was not a strong effect of treatment on pathogen or herbivore attack.

Examination of the proportion of variance explained by plot location or plant species revealed that little of the variation in enemy attack was explained by plant species, location, or an interaction between the two variables, especially for pathogen attack rates. For herbivory, plant species explained 31.4%, spatial location 0.1%, species by location 15.5%, and there was 60.0% residual variance. There was more unexplained variance in pathogen damage, with plant species explaining 3.3%, spatial location 0.0%, species by location 29.0%, and there was 67.6% residual variance.

AIC multi-model inference

Multiple regression analysis using AIC on 14 potential explanatory factors revealed that different factors influenced herbivores versus pathogens, and the importance of individual factors varied among the six native plant species (Figure A.1 in the Appendix). In general, the models selected explained more of the variation in pathogen damage than herbivore damage.

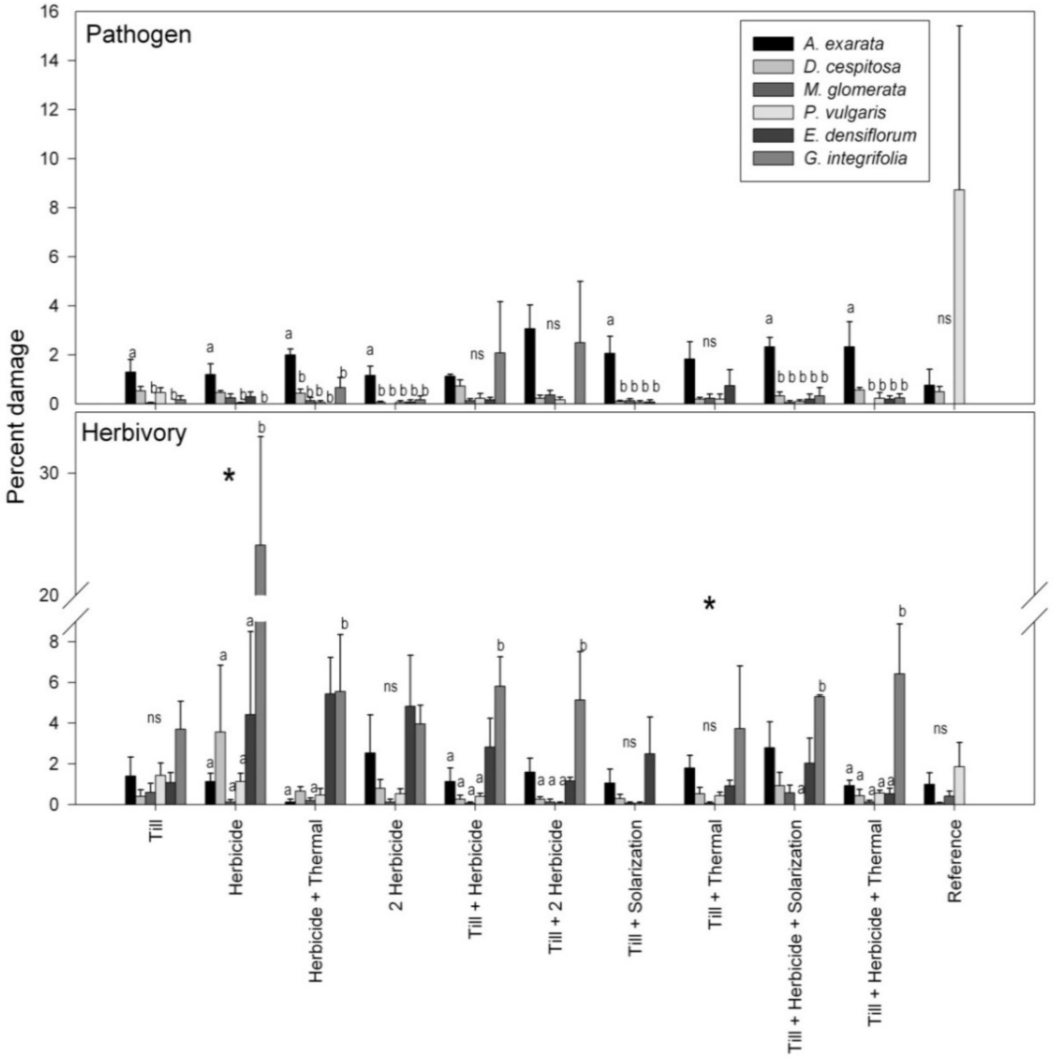

Figure 2.1. Herbivore and pathogen damage to six native perennial grasses and forbs among restoration treatments and reference wetland prairie. We found no significant differences among treatments for pathogens. Small letters represent significant differences in herbivore and pathogen damage ($P < 0.05$) among plant species within treatments. Asterisks represent significant differences in herbivory among restoration treatments.

As plant species diversity increased, there were no large increases or decreases in enemy attack on any one of the six plant species (Figure 2.2a). There was, however, a slight negative partial correlation with plant species diversity and pathogen attack on five of the six species; *Deschampsia cespitosa, Madia glomerata, Prunella vulgaris, Epilobium densiflorum,* and *Grindelia inetrifolia*. This relationship, though small, appeared in all selected models for two of the plant species, *D. cespitosa* and *P. vulgaris.* There was no strong or consistent relationship between herbivore attack and plant species diversity.

As the relative abundance of target host species increased, there were no clear community-wide trends (Figure 2.2b). As *E. densiflorum*'s relative abundance increased, herbivore attack on *E. densiflorum* also increased, and *E. densflorum* relative abundance appeared in all models selected.

As relative abundance of the most common introduced plant species *Lolium multiflorum* increased, pathogen attack on the native grass *D. cespitosa* and the native forb *G. integrifolia* increased (Figure 2.2c). The variable *L. multiflorum* appeared in all selected models for these two species. In contrast, as relative abundance of *L. multiflorum* increased, pathogen attack on *P. vulgaris* decreased. *Lolium multiflorum* appeared in 89% of the models selected for *P. vulgaris.* Other partial correlations for *L. multiflorum* were very small or appeared in a small fraction of the selected models. As relative abundance of introduced plant species increased, herbivore attack decreased on *P. vulgaris* but increased on *M. glomerata* (Figure 2.2d). This variable appeared in all models selected for these two species.

There was no clear community-wide response to physical attributes of the community (Figs. 2.2e, 2.2f, 2.2g). As total plant cover increased, pathogen attack on *E.*

densiflorum and *M. glomerata* increased, and appeared in all models selected (Figure 2.2e).

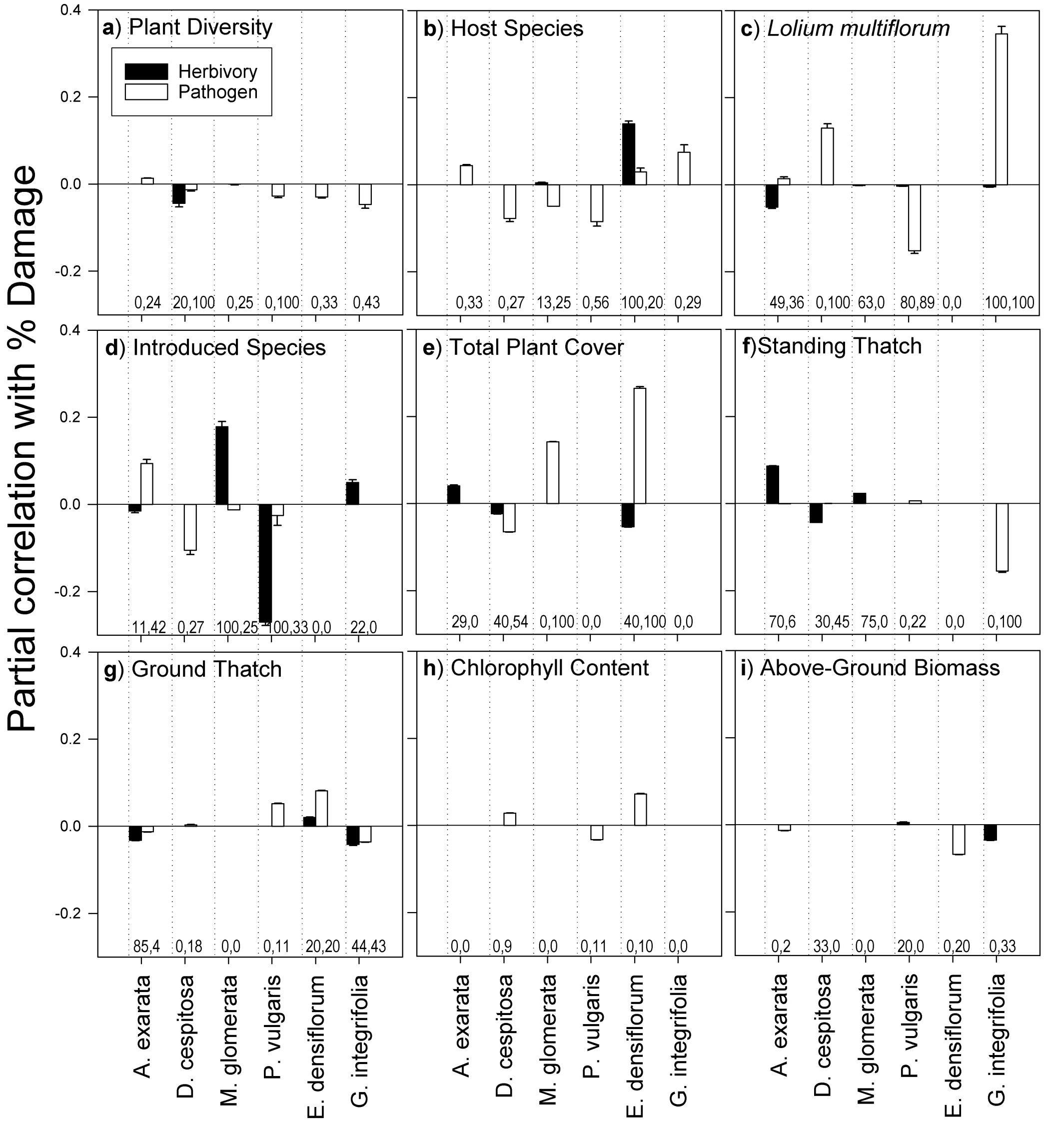

Figure 2.2. Partial correlations of variables with herbivore and pathogen attack to six native species. Title of each panel is the variable of interest for that panel. Panels **b**, **c**, and **d** represent relative abundance of the variable of interest. Panels **f** and **g** represent percent cover of the variable of interest. Partial correlations with the variable of interest and percent herbivore or pathogen attack on each of six species are represented by bars. Error bars represent variation (standard error) in magnitude of partial correlation among selected models. Numbers along x axes below each bar represent the percent of models in which that variable was selected.

Pathogen attack on *G. integrifolia* decreased as standing thatch increased, and this variable appeared in all selected models (Figure 2.2f). No plant species showed a strong change in enemy attack with respect to ground thatch (Figure 2.2g). There were no strong community-wide or individual plant species changes in enemy attack with respect to the individual plant traits shoot biomass (Figure 2.2h) or chlorophyll content (Figure 2.2i).

Pathogen attack on *P. vulgaris* decreased as relative abundance of *L. multiflorum, M. glomerata,* and *A. exarata* increased, suggesting a possible dilution effect by the other species (Figure 2.3). Pathogen attack on *M. glomerata* increased as *P. vulgaris* relative abundance increased, but decreased as *G. integrifolia* increased. As *E. densiflorum* relative abundance increased, herbivory on itself and on *G. integrifolia* also increased, suggesting possible spillover of an herbivore from *E. densiflorum* to *G. integrifolia.* The most common herbivore damage on both species was caused by chewing insects (Table 2.2), which supports the possibility that the two host species shared a common herbivore. Pathogen attack on *D. cespitosa* and *G. integrifolia* increased as *L. multiflorum* relative abundance increased, suggesting possible spillover from the introduced grass to the two native species. The native grass *A. exarata* seemed to have a dilution effect on pathogen attack on *D. cespitosa* and *P. vulgaris.*

Discussion

The AIC models generally explained more variation in pathogen attack than in herbivory, perhaps because more variation in herbivory was explained by host species identity. Of the variables that we addressed, the only consistent community-wide pattern we found was a small negative correlation between pathogen attack and plant species

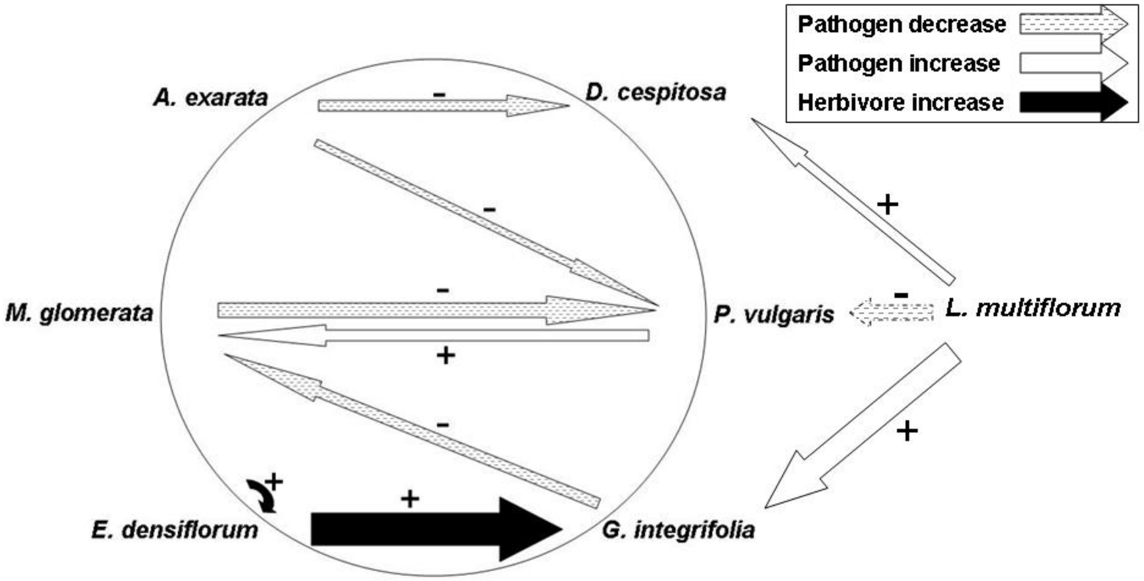

Figure 2.3. Interpretive diagram: partial correlations of changes in herbivore and pathogen attack with respect to abundance of each plant species. Width of arrows indicates magnitude of partial correlation, ranging from 0.06 to 0.40. The arrow with a dashed border was selected in 89% of the models selected. All other relationships shown were selected in 100% of the models.

diversity, which is consistent with the findings of other studies (Mitchell et al. 2002, Lau et al. 2008). The diversity effect did not appear to be an artifact of the abundance of any one particular plant species in the community. Interestingly, we did not find the same effect for herbivore attack. Past studies that have found reduced herbivores with increased host diversity have typically focused on agricultural systems with lower species richness than in our study. Lau and others (2008) found increased herbivory by specialists, but decreased herbivory by generalists with low host diversity.

Table 2.2. Types of pathogen and herbivore symptoms found on plant species surveyed, and percent of plants surveyed on which each symptom type was found.

Plant Species	Blotchy Pathogen	Spot Pathogen	Rust pustules	Chew	Rasp	Mine	Suck
Agrostis exarata	3.7	3.7	83.3	7.4	5.6	1.9	68.5
Deschampsia cespitosa	0	80.0	3.6	7.2	3.6	0	41.8
Epilobium densiflorum	10.2	35.9	18.0	66.7	35.9	0	7.7
Grindelia integrifolia	46.9	18.8	0	96.9	31.3	12.5	3.1
Madia glomerata	11.1	0	50.0	20.4	14.8	0	1.9
Prunella vulgaris	20.8	94.3	3.8	28.3	49.1	13.2	0

Consistent with enemy spillover and dilution, we found host species-specific correlations among the relative abundance of one species and attack on another. This result held for both herbivore and pathogen attack, and both native and introduced host species were correlated with increased or decreased enemy attack on another plant species. This suggests the potential for enemy spillover and dilution, both among the native species sampled and from introduced species to the native species. While we do not know the identities of the majority of the enemies, the patterns that we found are consistent with spillover and dilution. To clearly discern spillover and dilution, there is a need for future community-wide studies that identify the herbivores and pathogens on all hosts.

Variation in a host's ability to maintain the population of a particular natural enemy is a necessary condition of enemy spillover. For example, in tallgrass prairies in Kansas, Garrett and others (2004) found wide variation in host competency among four related co-occurring host species for the generalist pathogen barley/cereal yellow dwarf virus. In communities with manipulated host species composition, Power and Mitchell (2004) found that the presence of the highly susceptible host *Avena fatua* increased the prevalence of barley/cereal yellow dwarf virus infection in several other species in the community. Alternatively, the patterns of attack relative to abundance of certain species that we observed could be due to some influence of a particular plant species on the physical structure of the plant community, but our data do not indicate that responses of enemy attack are similar with respect to the community physical characteristics that we measured versus relative abundance of any one plant species. We found that pathogens and herbivores on some host species responded to variation in physical attributes of the plant community, but not to individual plant traits within host species.

Our results support the idea that host species diversity in communities reduces attack by pathogens, although this effect was not large. Other recent studies of pathogens and host diversity have also supported this finding. Dizney and Ruedas (2009) found that as mammalian diversity in forests declined, the proportion of deer mice infected with sin nombre virus increased. High diversity in ecological communities has also been shown to reduce the frequency of Lyme disease (Keesing et al. 2006), frog malformations caused by parasites (Johnson et al. 2008), and foliar pathogens of plants (Mitchell et al. 2002, Lau et al. 2008). While the effect of host diversity was consistent across our study, species-specific interactions were stronger. With respect to restoration of native plant communities, achieving species diversity should be a priority, but consideration should also be given to each species' interaction with other species and with natural enemies. Our results indicate stronger effects of individual host species identity, enemy spillover, and dilution.

Bridge to Chapter III

In this chapter, we examined a variety of community variables and their associations with enemy attack on several native plant species in the community. We found that no single pattern dictated enemy attack rates across the native plant community. In the following chapter, we focused on the three most common grass species only, and included an introduced species in our study. This enabled us to further address enemy spillover, and to test enemy release by comparing natural enemy attack on the native versus introduced plant species. Additionally we examined both nitrogen and phosphorus leaf content, for a more detailed examination of the relationship between natural enemy attack and plant nutrient status.

CHAPTER III

RESTORATION TREATMENTS, PLANT NUTRIENT STATUS, AND NATURAL ENEMIES

Author contributions: The restoration experiment on which this study was based was designed and implemented by Laurel E. Pfeifer-Meister, Bitty A. Roy, Scott D. Bridgham, and Bart Johnson. The disease survey was designed by G. Kai Blaisdell, Bitty A. Roy, and Scott D. Bridgham. Julie Stewart processed the nitrogen content samples. G. Kai Blaisdell wrote the paper with contributions from Bitty A. Roy, Scott D. Bridgham, and Laurel E. Pfeifer-Meister.

Introduction

In this study, we observed natural enemy attack rates on the three most common grass species in the wetland prairie restoration described in the previous chapter, to compare enemy attack on the introduced versus native species, and to examine the relationships among restoration treatments, plant nutrient status, and natural enemy attack. If herbivores and pathogens reduce the vigor or cover of one host, another may benefit, as occurred when chestnut blight reduced the cover of chestnuts, maples and oaks filled in forests in the Eastern United States (Day and Monk 1974). Also, natural enemies may be affected by host community structure. For example, very simple communities, such as monocultures, are more susceptible to disease (Mitchell et al. 2002). However, debate regarding the importance of host community composition with respect to natural enemies has not been fully resolved (Harmon et al. 2003, Agrawal et al. 2006, Keesing et al. 2006, Johnson et al. 2008, Jiang et al. 2008).

Enemy spillover occurs when natural enemy populations in one host species are driven by another host species that acts as a reservoir (Power and Mitchell 2004). Evidence of enemy spillover from introduced to native plant species has been found in grasslands and other plant communities (Malmstrom et al. 2007, Borer et al. 2007, Beckstead et al. 2010). The enemy release hypothesis, which is probably the most popularly cited explanation for invasions by introduced species, proposes that introduced species flourish because they leave their natural enemies behind and are no longer regulated by them (Keane and Crawley 2002, Colautti et al. 2004).

Higher nutrient content per leaf area has been found to increase foliar fungal disease (Neumann et al. 2004, Throop and Lerdau 2004), and the carrying capacity and population growth rate of aphids (Zehnder and Hunter 2008). Due to fertilizing, nutrient availability is higher agricultural systems, which would lead to higher nutrient content in the plants relative to natural and restored communities. Different site preparation techniques may affect nutrient availability, which could affect the nutrient status of the plants in each restoration treatment.

Our goal was to examine the effects of different restoration treatments and plant nutrient status on natural enemy attack on the most common introduced and native plant species in a restoration experiment in a wet prairie. To test enemy release, we also compared enemy attack rates among the native and introduced species that we surveyed. We performed a survey of damage by natural enemies on plants in the same seasonal wetland prairie restoration experiment described in the previous chapter (Pfeifer-Meister

2008). Grasses comprised the largest portion of plant biomass in the community, and we surveyed foliar herbivore and pathogen damage on the two most common native and the one most common introduced grass species to compare natural enemies among them. We hypothesized (i) that if enemy release were occurring, we would find less damage on the introduced grass than on the native grasses, (ii) that the different restoration treatments may lead to different nutrient status in the plants, and (iii) that this nutrient status would be associated with different rates of enemy attack.

Methods

Study site

Prior to the restoration experiment, the area had been planted in *Lolium multiflorum* Lam. (annual ryegrass) for agricultural production. The treatments were primarily designed to reduce germination of introduced species including the previously planted *Lolium multiflorum*. We also surveyed the adjacent *L. multiflorum* field, which continued to be actively fertilized, for comparison to the restoration treatments. This study was performed across the farm, reference prairie, and a subset of five restoration treatments (till only, two herbicide applications, till + two herbicide applications, till + solarization, and till + thermal). We chose these treatments based on data from 2005 that indicated maximally divergent plant community responses (Pfeifer-Meister 2008).

Collection of data

Our goal was to examine natural enemies of the dominant native and introduced species in the most divergent site preparation treatments. During May 2006, foliar herbivore and pathogen damage to the introduced grass *L. multiflorum* and native grasses *Agrostis exarata* Trin. (spike bent grass) and *Deschampsia cespitosa* (L.) P. Beauv. (tufted hair grass) were measured. Within each 15-m^2 plot for the five site preparation treatments surveyed, three locations were randomly selected. The closest plant of the three species to those random locations was collected and measured. Percent visible foliar herbivore and pathogen damage were assessed on each of three randomly selected leaves from each plant. The values for the leaves of each species were averaged per plot, and each plot counted as one replicate in data analysis. We determined leaf phosphorus status by digesting foliage with 30% hydrogen peroxide and 100% sulfuric acid (Lowther, 1980), and measuring phosphorus concentration on a Genesys 5 light spectrometer (Thermo Spectronic, Rochester, New York, USA). Foliar nitrogen content was measured with a CN elemental combustion analyzer (Costech Analytical, Valencia, California, USA).

Data analysis

All analyses were performed using JMP version 7.0.1. To ascertain species and restoration treatment effects for the three species survey, we performed two-way ANOVAs for herbivory, pathogen damage, phosphorous content, and nitrogen content, with host species, treatment, and their interaction as the independent variables. Species was considered a random variable, while treatment was fixed. It was necessary to partition analyses because *Lolium multiflorum* was not present in the reference prairie, and the two native grasses were not present in the farm field. For each response variable, three ANOVAs were run: (i) all three plant species in only the five restoration treatments,

(ii) *L. multiflorum* only in the five restoration treatments plus the farm field, and (iii) *A. exarata* and *D. cespitosa* only in the five restoration treatments and the reference prairie. Post hoc Tukey's HSD tests were performed to determine differences among individual treatments and species. For the *L. multiflorum* only, an a priori least squares contrast comparing the farm vs. the five restoration treatments was performed. We used least squares regression to compare phosphorous content and nitrogen content against herbivore and pathogen damage individually for each plant species.

Results

Natural enemy attack and restoration treatments

Within the five restoration treatments, pathogen damage varied among grass species ($F_{2,8} = 41.36$, $P < 0.0001$), but not restoration treatments, farm, or reference prairie, and the effects of the two factors were not interdependent. The introduced *Lolium multiflorum* had more pathogen damage than either of the natives *Agrostis exarata* or *Deschampsia cespitosa* (Tukey's HSD: $P < 0.05$) (Figure 3.1).

Within the restoration treatments, the amount of herbivore damage depended on grass species ($F_{2,8} = 5.67$, $P = 0.029$) and, marginally, on treatment ($F_{4,8} = 3.68$, $P = 0.055$) (Figure 3.1). The effects of species and treatment were not interdependent. The introduced *L. multiflorum* had more herbivory than the native *D. cespitosa*, with the native *A. exarata* being intermediate (Tukey's HSD: $P < 0.05$). When testing the two native species only, we found that herbivory on *A. exarata* and *D. cespitosa* did not significantly differ among the reference wetland prairie or five restoration treatments.

Similarly, when tested by itself, herbivory on *L. multiflorum* did not significantly vary among the farm or restoration treatments.

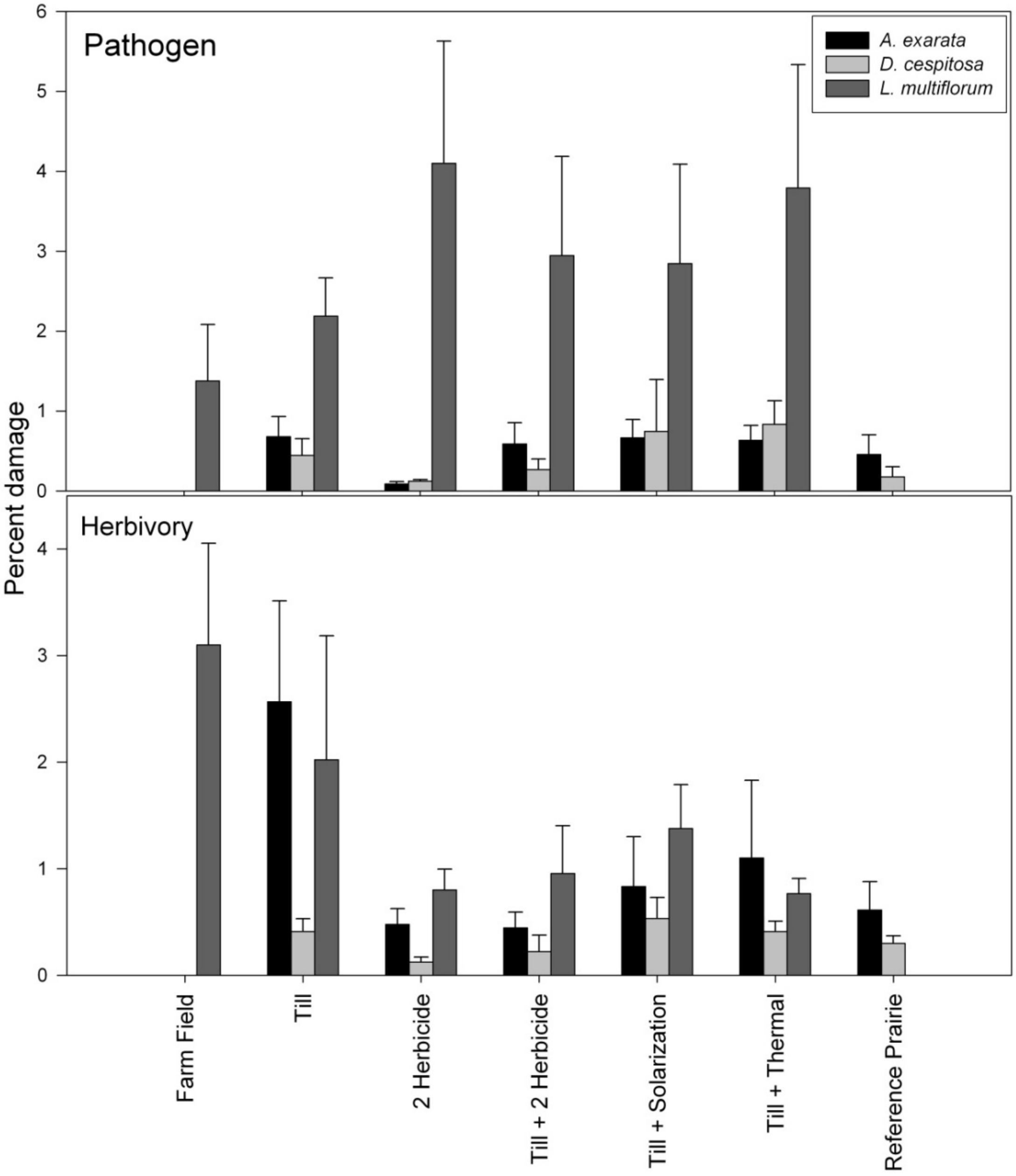

Figure 3.1. Herbivore and pathogen damage to *Agrostis exarata*, *Deschampsia cespitosa*, and *Lolium multiflorum* among restoration treatments, farm, and reference wetland prairie (+ standard error).

Foliar nitrogen and phosphorus content among restoration treatments

There were no significant differences in phosphorous content among treatments, and few differences in nitrogen content among treatments. Within the five restoration treatments, foliar nitrogen content differed among plant species ($F_{2,8} = 16.76$, $P = 0.0014$) and treatments ($F_{4,8} = 6.03$, $P = 0.015$) (Figure 3.2). The effects of the two factors were not interdependent. *Agrostis exarata* (Mean: 1.37 ± 0.09%) and *D. cespitosa* (Mean: 1.30 ± 0.07%) had significantly higher nitrogen content than *L. multiflorum* (Mean: 0.84 ± 0.06%) (Tukey's HSD: $P < 0.05$). The plants in the till + thermal treatment had higher nitrogen content than the plants in the two herbicide applications treatment, with no other differences among the restoration treatments (Tukey's HSD: $P < 0.05$). *Lolium multiflorum* in the farm field had higher nitrogen content than in any of the restoration treatments ($F_{5,24} = 25.69$, $P < 0.0001$, Tukey's HSD: $P < 0.05$). Nitrogen content of *A. exarata* and *D. cespitosa* varied among the restoration treatments and reference wetland prairie ($F_{5,5} = 17.79$, $P = 0.003$). Plants in the till + thermal treatment had higher nitrogen content than plants in the reference wetland prairie, two herbicide applications, till + two herbicide applications, and till + solarization treatments, with the till only treatment being intermediate (Tukey's HSD: $P < 0.05$).

Phosphorus content differed among plant species ($F_{2,8} = 33.22$, $P < 0.0001$) but not restoration treatments, and the effects of the two factors were not interdependent. *Agrostis exarata* had the highest phosphorous content (Mean: 0.19 ± 0.005%), *D. cespitosa* was intermediate (Mean: 0.15 ± 0.006%), and *L. multiflorum* had the lowest phosphorous content (Mean: 0.14 ± 0.007%, Tukey's HSD: $P <0.05$). Phosphorus content of *A. exarata* and *D. cespitosa* varied among the restoration treatments and the reference wetland prairie ($F_{5,5} = 6.55$, $P = 0.030$); there was no interaction between species and treatment. Phosphorus content was lower in the reference wetland prairie than in till + 2 herbicide applications, till + thermal, and till + solarization, with 2

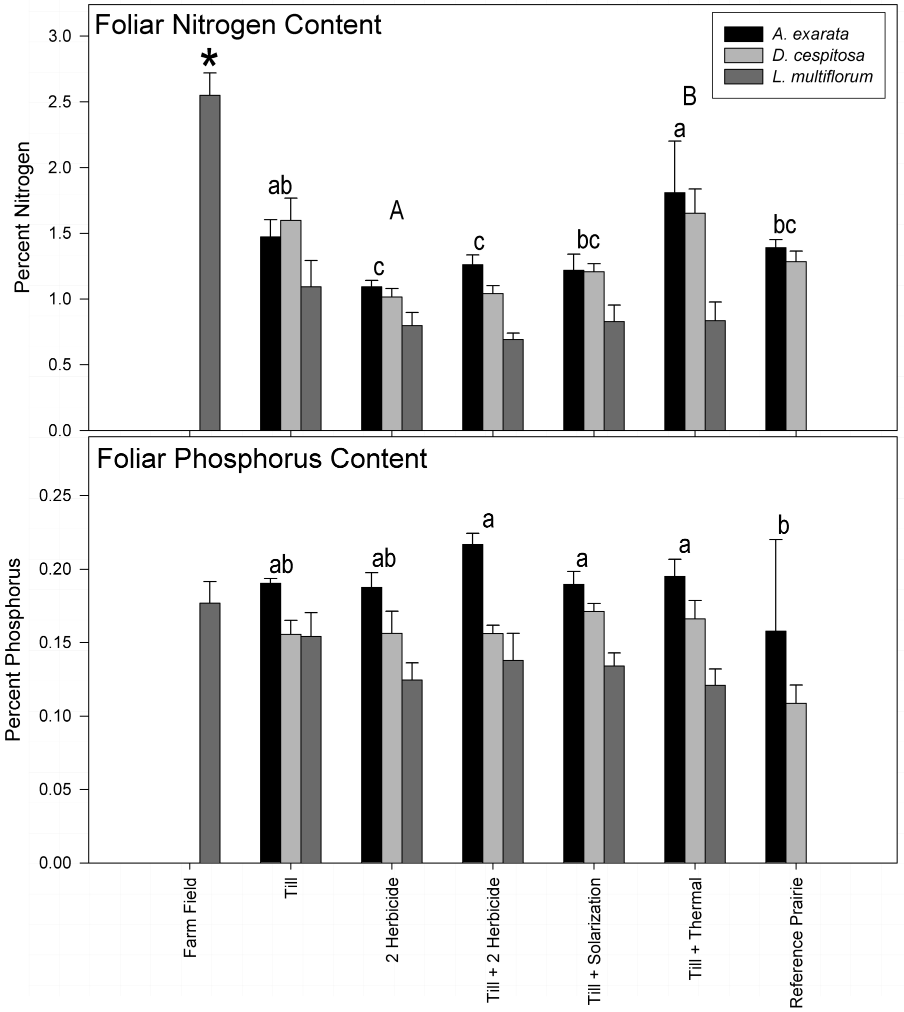

Figure 3.2. Foliar nitrogen and phosphorus content among treatments for *Agrostis exarata*, *Deschampsia cespitosa*, and *Lolium multiflorum*. The asterisk indicates a significant difference in nitrogen among treatments for *L. multiflorum* only. Uppercase letters indicate differences among restoration treatments for all three grass species. Lowercase letters represent significant differences among restoration treatments and reference wetland prairie for *A. exarata* and *D. cespitosa* only.

herbicide applications and till being intermediate (Tukey's HSD: $P < 0.05$). Phosphorus content of *L. multiflorum* in the farm field trended toward being higher than in the restoration treatments (ANOVA: $F_{5,24} = 2.29$, $P = 0.084$, Least Squares Contrast: $F_{1,20} = 8.31$, $P = 0.009$).

Within plant species, we found only marginally significant correlations between enemy attack and nitrogen content. Pathogen attack on *A. exarata* increased as nitrogen content increased ($r^2 = 0.10$, $P = 0.088$). Herbivore attack on *L. multiflorum* also increased with nitrogen content ($r^2 = 0.12$, $P = 0.065$). Pathogen attack on *L. multiflorum* tended to decrease as phosphorus increased ($r^2 = 0.13$, $P = 0.068$), but there was no correlation between phosphorus content and herbivory.

Discussion

The strongest pattern that we found was higher pathogen attack rates on the *Lolium multiflorum* than on the other two grass species. We did not find compelling evidence of effects of restoration treatment on herbivore or pathogen attack. As expected, *L. multiflorum* had higher nitrogen content in the farm field than in the restoration experiment, but this was not the case for phosphorus. We found weak evidence of a positive correlation between nitrogen content and enemy attack on *Agrostis exarata* and *L. multiflorum.* Interestingly, there was no difference in enemy attack in the farm field, where nutrient levels were higher, relative to the restoration experiment.

This study was motivated by our observation of large plant community differences among the five restoration treatments in the previous growing season. However, these differences dampened over time, and the restoration treatments became more similar to the composition of the reference sites in the year of our survey (Pfeifer-Meister 2008). Therefore the treatments may not have differed enough in plant community composition or other factors for us to detect an effect of treatment on natural enemy attack at the time of this study.

Our finding that the introduced grass had more pathogen damage than the two native grasses is contrary to expectations of the enemy release hypothesis, in which we would expect introduced, “released” plants to have less damage by natural enemies than the native species. These results, however, are consistent with pathogen spillover from the introduced *Lolium multiflorum* to the native *Agrostis exarata* and *Deschampsia cespitosa*. The potential for spillover suggests that when considering restoration and eradication of introduced species, it may be helpful to focus eradication efforts on the unwanted plant species that are also competent hosts of generalist natural enemies. Local community context may play a role in the greater damage on *L. multiflorum*, as the majority of grass seed in the Willamette Valley region is from *L. multiflorum*, and the neighboring farm field was planted in this species. Also, enemy release tends to be more pronounced with recently introduced species(Hawkes 2007), and *L. multiflorum* has been common in the region for several decades. Our finding of potential spillover from the introduced to the native species is consistent with other recent studies of grassland communities (Malmstrom et al. 2007, Borer et al. 2007, Beckstead et al. 2010) and warrants further investigation of spillover as a mechanism of invasions by introduced species in restoration efforts and natural plant communities.

When comparing enemy attack among plant species, life history traits of each species need to be considered. *Agrostis exarata* and *D. cespitosa* are perennial bunchgrasses, but *L. multiflorum* is a winter annual. Because *L. multiflorum* leaves are short-lived, they may be less tough, less defended, and more vulnerable to enemy attack (Borer et al. 2009). Alternatively, because the plants only live for a maximum of one year, they may have less time to accumulate damage by natural enemies. Because of this and the small number of species surveyed, the result of more attack on the introduced species than the two native species should be interpreted with caution. Two other recent studies that included grasses found differing results. One included two native and three introduced grass species at each of three sites in Oregon, in which Roy and others (unpublished data) found no overall pattern of greater damage on the native versus introduced species. The second study, of tallgrass prairie grass, forb, and legume species in Kansas found more herbivore and more overall damage on native species than on introduced species (Han et al. 2008).

The weak positive relationship between plant nitrogen status and enemy attack that we found may be due to the low variation in plant nutrient status among the restoration treatments, farm field, and reference site. Studies of the association between enemy attack and plant nutrient content have produced mixed findings (e.g., Borer et al. 2006, 2009). The plant vigor hypothesis (Price 1991) predicts that larger, more vigorous plants will experience more attack by herbivores than smaller, stressed plants, while the plant stress hypothesis predicts that unhealthy plants will experience more herbivore attack (White 1974). In a meta-analysis of herbivore studies, Cornellisen and others (2008) found support for the plant vigor hypothesis, but not for the plant stress hypothesis. Rasmussen and others (2007) found that higher nutrient content in *L. multiflorum* led to weaker endophytic associations and lower alkaloid productions, indicating reduced defense against natural enemy attack. Our findings are consistent with the plant vigor hypothesis or with weakened endophytic associations with plants having more nutrients.

Bridge to Chapter IV

In the first studies, we examined correlations between natural enemy attack and restoration treatments, plant community variables, and individual plant traits. We did not find compelling evidence for community-wide patterns between community or individual plant variables and natural enemy attack, with the exception of a consistent but weak negative correlation between plant species diversity and enemy attack. We found compelling evidence for enemy spillover from the introduced to the native plant species, and wanted to further examine this relationship. In the next chapter, we used experimental communities of the six most common native and introduced perennial bunchgrasses, in order to further examine enemy spillover and dilution. We carried this experiment out for three growing seasons, and surveyed enemy attack on the six grass species each year, as well as over the course of the growing season during the second year. Our primary goal was to examine enemy spillover and dilution among the six native and introduced grass species.

CHAPTER IV

A TEST OF THE RELATIVE STRENGTHS OF COMPETITION, ENEMY SPILLOVER, AND DILUTION AMONG NATIVE AND INTRODUCED PERENNIAL BUNCHGRASSES

Author contributions: This study was designed by G. Kai Blaisdell, with substantial input from Bitty A. Roy and Scott D. Bridgham. The field experiment was carried out by G. Kai Blaisdell, with Bitty A. Roy's contribution throughout the three growing seasons, and this chapter was written by G. Kai Blaisdell with contributions from Bitty A. Roy and Scott D. Bridgham.

Introduction

Natural enemies (pathogens and herbivores) can change population and community structure, because they reduce host survival and reproduction (Burdon et al. 2006, Alexander 2010). Introduced plants sometimes bring generalist enemies with them to their new territory, and native plants, not having evolutionary history with the novel enemies, may be more susceptible than the introduced plants (Parker and Gilbert 2007). Some dramatic examples of abrupt community change due to pathogen introductions have involved plant pathogens, such as the current *Phytophthora ramorum* epidemic in California, which causes sudden oak death. *Phytophthora ramorum* infects several tree species, but is lethal only to some (Meentemeyer et al. 2004, 2008). This is an example of pathogen spillover, which occurs when a potential host is infected at higher levels when in the presence of another host that acts as a reservoir (Power and Mitchell 2004).

Recent studies in prairie systems suggest that pathogens play a key role in determining community composition and ecosystem function in grasslands (Garrett et al. 2004, Malmstrom et al. 2005, 2007, Borer et al. 2007, Beckstead et al. 2010). Malmstrom and others (2005) found that the native grass *Elymus glaucus* in California is more often infected with barley/cereal yellow dwarf viruses in the presence of the introduced grass *Avena fatua* than in its absence. Furthermore, the diversity of barley/cereal yellow dwarf viruses in California proliferated at the approximate time that introduced grasses arrived, indicating that the hosts and pathogens were introduced together (Malmstrom et al. 2007). More recently, Beckstead and others (2010) found that the presence of the introduced grass *Bromus tectorum* led to increased infection of co-occurring native grasses with the seed pathogen *Pyrenophora semeniperda.* These examples of pathogen spillover in grasslands involved introduced annual species and native perennial species, and life history was therefore confounded with geographic origin. In our study, we utilized three native and three introduced perennial grass species, to minimize life history differences between native and introduced plant species.

In addition to pathogens, spillover of herbivores from one species to another has been documented in plant communities, especially in the case of the non-target effects of biological control. For example, Rand and Louda (2004) found more attack on the native thistle *Cirsium undulatum* by the biocontrol weevil *Rhinocyllus conicus* as the abundance of the introduced thistle *Carduus nutans* increased in the community.

In contrast, dilution of natural enemies in a community can also occur, leading to the protection of a potentially susceptible host when in a community with unfavorable hosts. This phenomenon has been studied very little in natural plant communities (but see

Mitchell et al. 2002, Borer et al. 2010), but it is a familiar concept with animal diseases (e.g., Ezenwa et al. 2006). For example, Ostfeld and Keesing (2000) found that the presence of wildlife hosts in a community with low rates of infection and transmission can reduce community pathogen loads of the tick-borne transmission of *Borrelia burgdorferi* (Lyme's disease), and may reduce the risk of infection to humans. An introduced plant species in a novel community may suffer less attack by natural enemies simply because those enemies are less abundant in that novel community relative to a typical plant community in the introduced plant species' native range.

Separating out the effects of natural enemy attack from the effects of competition is an issue in field experiments, as removing neighboring plants may also lead to the removal of natural enemies that resided on them. In a study of meadow species in Canada, Reader (1992) used a combination of competitor removal and herbivore exclusion to tease out the effects of herbivory versus competition, and found that both were important factors. Another study found that the effect of herbivory relative to the effect of competition weakened as site biomass decreased (Bonser and Reader 1995). In a review and meta-analysis of the relative strength of competition and predation effects, Gurevitch and others (2000) found that herbivores generally have a stronger effect than competition on plant survival. Chase and others (2002) found a more complicated story, which depended largely on the measures of intensity of competition and predation that were used. When the competitive outcome of two species is due to the effects of shared natural enemies rather than direct resource competition, this is called apparent competition (Holt et al. 1994).

In addition to natural enemies, mutualisms can confound the effects of resource competition. Infection of *Schedonorus* with *Neotyphodium* endophytes has been studied extensively (Bacon and White 2000, Antunes et al. 2008), and other grass species can be infected as well (Cooke 2007, Rasmussen et al. 2007, Omacini et al. 2009). Alkaloids produced by *Neotyphodium* are toxic deterrents against herbivores (Tanaka et al. 2005), although they have been associated with increased survival of some herbivores (Saikkonen et al. 1999). *Neotyphodium* endophytes have allelopathic impacts on other plant species in the community (Antunes et al. 2008). These endophytes can also increase a plant's tolerance for nutrient and water stress (Ravel et al. 1997, Hesse et al. 2003), although there is not always strong evidence of this in natural communities (Schulthess and Faeth 1998).

In our study, we used different combinations of native and introduced species to test for enemy spillover and dilution among native and introduced plant species. To control for life history differences, we selected six species of perennial bunchgrasses that commonly co-occur. First, we hypothesized that, if enemy spillover from the introduced to the native grasses were occurring, enemy attack on the native species would be lowest when they were in a community with only other native species, and highest when in a community with the most introduced species. Second, we hypothesized that, if dilution of natural enemies on the introduced species by the native species were occurring, enemy attack on the introduced species would be lowest when in a community with the most native species, and highest when in a community with only other introduced species. We also tested the frequency of infection with *Neotyphodium* for each grass species. The primary goal of our study was to test enemy spillover and dilution by quantifying variation in natural enemy attack among different plant communities on each native and introduced species. Because total plant cover (Alexander 1992, Folgarait et al. 1995) and density of each individual species (Burdon 1987, Kranz 1990) could affect natural enemy attack, we also measured these variables in the experiment. This information enabled us to make inferences about competition among all six grass species.

Methods

Experimental design and data collection

The field site was located in a seasonal wetland prairie in West Eugene, Oregon, USA, at the border of the restoration experiment and farm field planted in *Lolium multiflorum* discussed in Chapters II and III. The vegetation in the immediate area consisted primarily of *Deschampsia cespitosa.* Eugene has a Mediterranean climate, with average annual temperature 11.4° C, average maximum temperature 17.4° C, average minimum temperature 5.3° C, and average annual rainfall 0.99 m (Western Regional Climate Center 2009). To prepare the site, the experimental area was treated with glyphosate herbicide in October 2006. One week later, vegetation was removed from the plots by tilling. Following tilling, we seeded all plots by hand scattering seed.

The three native species, *Agrostis exarata* Trin. ("spike bentgrass"), *Danthonia californica* Bol. ("California oatgrass"), and *Deschampsia cespitosa* (L.) P. Beauv. ("tufted hairgrass"), are native across most of the United States and Canada west of the Mississippi (Barkworth et al. 2007). The three introduced species, *Anthoxanthum odoratum* L. ("sweet vernal grass"), *Holcus lanatus* L. ("common velvetgrass"), and *Schedonorus arundinaceus* (Schreb.) Dumort ("tall fescue"), are native to Europe and naturalized in the United States, and all are common in most of the 48 continental states. *Schedonorus arundinaceus* was introduced to the United States in the late 1800s, and became a widely used forage in the 1940s (Pedersen et al. 1990). *Anthoxanthum odoratum* and *Holcus lanatus* were also probably introduced in the 1800s, but are not widely used commercially. All seeds were obtained from local seed companies, and all were collected from the Southern Willamette Valley of Oregon, near the research site, or produced in nurseries that used seed sources from local populations. Seeds of these species are typically planted between September-January, and plant growth begins April-May. Plants reach peak biomass in mid-late June, and then dry season senescence occurs in July-August.

Our eight treatments were (i) a community of three native species, (ii) a community of three introduced species, (iii-v) three communities each with all of the native species plus one of the introduced species, and (vi-viii) three communities each with all of the introduced species plus one of the native species (Table 4.1). To minimize differences in plant species diversity, which may affect natural enemy attack (Mitchell et al. 2002), we chose to use communities that had either three or four plant species. Each plot was two by two meters, with five replicates in a randomized block design for a total of 40 plots. Each block contained one full replicate of the eight treatments, and consisted of eight adjacent plots.

In October 2006, each treatment was planted with 1,500 viable seeds m^{-2}. In each community, we planted an equal number of viable seeds of each plant species, calculated after adjusting for seed purity and germination rate, which varied among the six species. In Spring 2007 (year one) germination was sparse, and we reseeded all plots at 750 seeds

m^{-2}. In two of the plots planted with *A. odoratum*, it was not found, so we reseeded these plots with 200 seeds m^{-2} of *A. odoratum* only in July of year one. In September of year one, we reseeded all plots again with 750 seeds m^{-2} of all species except *D. cespitosa* and *S. arundinaceus*, as these two species had ample germination. All species planted were present, and plots filled in to a desirable density in year two. Throughout all growing seasons we mowed between plots every two weeks and kept the plots well weeded of all species except those planted. The third and final growing season of this experiment was 2009.

Table 4.1. Eight different communities were planted to test for spillover and dilution of natural enemies among six native and introduced perennial grass species. Each species was planted in five of the eight treatments.

3 Native Species	3 Introduced Species
3 Native Species + *A. odoratum*	3 Introduced Species + *A. exarata*
3 Native Species + *H. lanatus*	3 Introduced Species + *D. californica*
3 Native Species + *S. arundinaceus*	3 Introduced Species + *D. cespitosa*

We surveyed for damage in June 2007, May 2008, June 2008, August 2008, and June 2009. For each sampling date, we closely examined five randomly selected plants of each species in each plot for visible damage. Randomization was accomplished by throwing five markers into each plot, and selecting the plant of each species that was closest to that marker. We visually estimated percent of overall herbivore and pathogen damage on each plant, and visually estimated percent of each type of damage on one middle-aged leaf from a randomly selected tiller of each plant. Plants surveyed ranged in number of leaves from about three leaves to over one hundred leaves per plant. We also counted number of types of visibly distinct herbivore and pathogen symptoms per plant, as a proxy for natural enemy richness. Immediately following the sampling in June of each growing season, at peak standing biomass, we collected plant cover data in each plot using the point intercept method (Jonasson 1988). To collect cover data, a 1-m^2 pin frame with 25 pins was placed in the middle of the plot, and we counted each time a plant part touched a pin as one count. These counts were totaled for each plot and multiplied by four to calculate total cover of each species and thatch (dead plant material) in each plot. In July of year three, we measured above-ground biomass of each species and thatch in a 0.2*0.6-m quadrat, placed 10 cm inside the northeast corner of each plant cover subplot, by clipping at the plant base and oven drying at 60° C for 48 hours.

We cultured and identified fungal pathogens on all six grass species in the experiment. Leaves of each grass species with the most common symptoms were collected, surface sterilized in 3% hydrogen peroxide, and placed on crude agar plates. Alternatively, some leaves were incubated in humid chambers, or fungi were collected using tape mounts. Fungi were identified using morphological characteristics. In year three, we used sticky traps, pan traps, and pitfall traps to collect and identify arthropods in the experiment. In May 2010, we used an immunoblot assay (Agrinostics, Watkinsville, GA, USA) to test 25 plants of each grass species for the infection with the symbiotic *Neotyphodium* endophytes.

Analyses

Variation among treatments during three growing seasons

All analyses were performed in JMP Version 8. Plot was the unit of replication, and for all analyses, values for each species were averaged within each plot. Because each of the six grass species was in a different subset of five of the eight treatments, each grass species was analyzed separately. To comply with the assumption of normality in ANOVA, we arcsine transformed percentage data, and square root transformed count data. All data were back-transformed after analysis for presentation.

We were concerned that our two response variables for enemy attack rates, whole plant percent damage and number of symptoms per plant, may be redundant. To address this concern, we examined their correlations. Across all surveys, whole plant percent damage and number of symptoms per plant were positively correlated for each plant species (*A. exarata:* $F_{1,131} = 224.04$, $P < 0.0001$, $r^2 = 0.63$, *A. odoratum:* $F_{1,135} = 112.47$, $P < 0.0001$, $r^2 = 0.45$, *D. californica:* $F_{1,134} = 73.30$, $P < 0.0001$, $r^2 = 0.35$, *D. cespitosa:* $F_{1,148} = 259.84$ $P < 0.0001$, $r^2 = 0.64$, *S. arundinaceus:* $F_{1,146} = 76.89$, $P < 0.0001$, $r^2 = 0.34$, *H. lanatus:* $F_{1,145} = 142.31$, $P < 0.0001$, $r^2 = 0.50$). Furthermore, we performed the analyses described below for both response variables and found similar trends. To avoid redundancy and because we feel that number of types of symptoms per plant is a better measure of enemy attack, we focus here on that variable as a proxy for natural enemy richness. Number of species or types of natural enemies is commonly used as an estimate of natural enemy pressure in studies regarding invasions and natural enemies (Mitchell and Power 2003, van Kleunen and Fischer 2009). Finally, the process of identifying and counting distinct symptoms is a more objective measure than visually estimating percent damage. Here we focus on number of symptoms per plant.

We used repeated measures to test the effect of plant community composition treatments on natural enemy attack over the three growing seasons, using the three June surveys to control for seasonality in enemy attack. Then, to testfor seasonal variation, we used repeated measures to test the effects of treatments from May through August during the second growing season. For our repeated measures tests, we first used a multivariate approach to repeated measures. When the assumption of sphericity was not violated and missing values reduced our statistical power, we proceeded with an equivalent univariate split-plot approach for repeated measures, which has more power when some values are missing, because, unlike the multivariate repeated measures, it can retain individual subjects that are missing a value for one or more time points (Cole and Grizzle 1966). We used the univariate split-plot approach for *S. arundinaceus* only. When the assumption of sphericity was violated (*D. cespitosa* and *H. lanatus* only), we reported the Greenhouse-Geyser adjusted statistics. When the assumption of sphericity was not violated, we reported the unadjusted statistics.

In cases where the repeated measures analysis indicated a significant treatment effect or treatment by time interaction, we performed mixed model ANOVA's within each year, including block as a random factor and using restricted maximum likelihood to eliminate it as a nuisance factor (Corbeil and Searle 1976). When block explained < 1% of the variance, it was excluded from the analysis (Underwood 1981). When the overall ANOVA for any one season was not significant, we performed an a priori least squares contrast to compare the treatment with all introduced species to the treatment with all native species plus the focal introduced species, based on our hypothesis that if dilution by the native species were occurring, the most clear difference among treatments would be between these two treatments. In no case for the native species did the repeated measures analyses indicate significant differences among the treatments or treatment by time interactions during the three growing seasons, so no a priori least squares contrasts were performed to test spillover from the introduced to the native species. We used Tukey's HSD tests to determine differences among the five treatments at individual time points.

We considered using plant cover as a covariate, but found a treatment by cover interaction with respect to natural enemy attack, which precludes use of cover as a covariate. We checked for correlations between abundance of each grass species and enemy attack on that species by regressing enemy attack on cover of that species during each survey. We also explored the correlation between total plant cover and enemy attack on each species in this manner.

Variation among treatments during second growing season

Analyses of seasonal variation among treatments were performed using repeated measures ANOVA in a similar manner to that of variation over the three year period, using the three surveys from year two. Because of late season senescence, there were substantial missing values. For *A. exarata* only, the natives + *S. arundinaceus* treatment was excluded from the repeated measures analysis, and the univariate split plot analysis was used. For all other species, the multivariate repeated measures analysis was used.

Abundance of each plant species

To confirm that our cover data was representative of total above-ground biomass of each species, we regressed total per plot plant cover in year three against total above-ground biomass collected in year three. We then used multivariate repeated measures analysis of the cover data to examine the abundance of each species over the three growing seasons. When we found a treatment effect or treatment by time interaction, we followed up with individual univariate analyses, including block as a random factor only when block explained > 1% of variation in plant cover.

Results

Fungi, arthropods, and herbivores found

Spores of *Alternaria spp.* and rust pustules were found on all six grass species in the experiment. *Nigrospora sp.* and *Mastigosporium sp.* were found only on *Schedonorus arundinaceus*, and a *Drechslera sp.*was found on *Danthonia californica.* We found evidence of rodent herbivory on all grass species except for *Agrostis exarata*. Potential arthropod herbivores found in the experiment included Coleoptera (beetles), Diptera (flies), Hemiptera (true bugs), Homoptera (aphids and leafhoppers), Lepidoptera (moths and butterflies), Orthoptera (grasshoppers), and Thysanoptera (thrips).

Neotyphodium endophytes were not found in *Danthonia californica* or *Schedonorus arundiaceus*. We detected *Neotyhodium* in one of 25 *Agrostis exarata* and *Holcus lanatus*, three of 25 *Anthoxanthum odoratum*, and 15 of 25 *Deschampsia cesptiosa* plants.

Across all sampling dates and plant species, percent herbivore and percent pathogen attack were significantly negatively correlated, but the relationship was weak ($F_{1,879} = 45.2$, $P < 0.0001$, $r^2 = 0.05$).

Variation among treatments during three growing seasons

Spillover

We found no compelling evidence of spillover from the introduced to the native plant species. Over the three growing seasons, number of symptoms per plant on all three native species significantly increased (Table 4.2, Figure 4.1a-c), but there were no significant treatment effects on natural enemy attack of any of the three native species, and the treatment effect did not change with time (Table 4.2).

Dilution

We found trends of reduced natural enemy attack on the introduced species when in treatments with the native species that are generally consistent with dilution of natural enemies by the native species. Number of symptoms on *H. lanatus* increased over time, and differed with marginal significance among treatments (Figure 4.1e). The effects of treatment varied over time. During the first growing season, differences among treatments in number of types of symptoms per plant on *H. lanatus* were marginally significant ($F_{4,20} = 2.66$, $P = 0.063$). There was weak evidence that enemy attack on *H. lanatus* was higher in the natives + *H. lanatus* than in the introduced species (a priori least squares $F_{1,20} = 8$, $P = 0.10$), the reverse of dilution of natural enemy attack on *H. lanatus* by the native grasses. During the second season, differences among treatments were marginally significant again ($F_{4,16} = 2.32$, $P = 0.10$), and enemy attack was higher in the introduced species treatment than in the natives + *H. lanatus* (a priori least squares $F_{1,16} = 7.20$, $P = 0.016$), a reversal of the weak trend from the first growing season and consistent with dilution of natural enemy attack by the native grasses.

Table 4.2. Statistical results of repeated measures tests for variation among treatments in enemy attack and grass species abundance.

Test	***Term***	***A. exarata***	***D. californica***	***D. cespitosa***	***A. odoratum***	***H. lanatus***	***S. arundinaceus***
# Symptoms 3 years	Time	$F_{2,40} = 54.29$ $P < 0.0001$	$F_{2,28} = 66.2$ $P < 0.0001$	$F_{1.6,31.1} = 240.4$ $P < 0.0001$	$F_{2,36} = 53.97$ $P < 0.0001$	$F_{1.5,29.8} = 60.02$ $P < 0.0001$	$F_{2,39.55} = 74.00$ $P < 0.0001$
# Symptoms 3 years	Treatment	$F_{4,20} = 1.21$ $P = 0.34$	$F_{4,14} = 0.41$ $P = 0.80$	$F_{4,20} = 0.68$ $P = 0.61$	$F_{4,18} = 1.21$ $P = 0.12$	$F_{4,20} = 2.71$ $P = 0.06$	$F_{4,20.11} = 0.90$ $P = 0.48$
# Symptoms 3 years	T * T	$F_{8,40} = 0.62$ $P = 0.75$	$F_{8,28} = 1.43$ $P = 0.23$	$F_{6.21,31.07} = 1.38$ $P = 0.25$	$F_{8,40} = 0.62$ $P = 0.75$	$F_{7.68,36.49} = 2.11$ $P = 0.0048$	$F_{4,39.51} = 2.29$ $P = 0.04$
# Symptoms Year 2	Time	$F_{2,22.82} = 3.75$ $P = 0.039$	$F_{1.5,28.7} = 29.53$ $P < 0.0001$	$F_{2,40} = 5.44$ $P = 0.0082$	$F_{1.3,13.3} = 11.29$ $P = 0.0030$	$F_{1.4,23.4} = 25.51$ $P < 0.0001$	$F_{1.2,29.7} = 34.60$ $P < 0.0001$
# Symptoms Year 2	Treatment	$F_{3,15.78} = 1.61$ $P = 0.23$	$F_{4,19} = 0.42$ $P = 0.79$	$F_{4,20} = 0.60$ $P = 0.67$	$F_{4,10} = 2.00$ $P = 0.17$	$F_{4,17} = 3.24$ $P = 0.038$	$F_{4,20} = 1.60$ $P = 0.21$
# Symptoms Year 2	T * T	$F_{6,22.38} = 2.42$ $P = 0.059$	$F_{6.04, 28.7} = 0.85$ $P = 0.54$	$F_{8,40} = 0.38$ $P = 0.92$	$F_{5.33,13.13} = 0.44$ $P = 0.83$	$F_{5.52,23.44} = 1.43$ $P = 0.25$	$F_{4.78.23.91} = 0.74$ $P = 0.60$
Cover 3 years	Time	$F_{1.2,24.8} = 46.28$ $P <0.0001$	$F_{2,40} = 3.89$ $P = 0.0018$	$F_{2,40} = 165.21$ $P < 0.0001$	$F_{1.4,28.3} = 21.99$ $P < 0.0001$	$F_{1.3,25.7} = 32.25$ $P < 0.0001$	$F_{2,40} = 25.54$ $P = < 0.0001$
Cover 3 years	Treatment	$F_{4,20} = 20.17$ $P < 0.0001$	$F_{4,20} = 1.31$ $P = 0.30$	$F_{4,20} = 1.30$ $P = 0.30$	$F_{4,20} = 7.32$ $P = 0.0008$	$F_{4,20} = 11.96$ $P < 0.0001$	$F_{4,20} = 5.46$ $P = 0.0039$
Cover 3 years	T * T	$F_{4.95,24.77} = 7.47$ $P = 0.0002$	$F_{8,40} = 3.89$ $P = 0.0018$	$F_{8,40} = 0.61$ $P = 0.77$	$F_{5.7, 28.3} = 2.97$ $P = 0.024$	$F_{5.1, 25.7} = 8.60$ $P < 0.0001$	$F_{8,40}= 4.28$ $P = 0.0009$

During the third growing season, there were highly significant differences in attack on *H. lanatus* among the five treatments ($F_{4,20} = 6.30$, $P = 0.0019$). Natural enemy attack was highest in the introduced species and introduced species + *D. californica* treatments, lowest in the natives + *H. lanatus,* and intermediate in the remaining two treatments (Tukey's HSD $P < 0.05$). The results of the second two growing seasons are consistent with dilution of natural enemy attack on the introduced *H. lanatus* by the native grass species.

Number of symptoms on *S. arundinaceus* increased over time. There was no main effect of treatments, but treatment effects varied over time (Table 4.2, Figure 4.1f). During the first growing season, number of types of symptoms per plant on *S. arundinaceus* varied among treatments with marginal significance ($F_{4,20} = 2.21$, $P = 0.10$), and there was weak evidence that enemy attack in the introduced species treatment was lower than in the natives + *S. arundianceus* treatment (a priori least squares $F_{1,20} = 3.60$, $P = 0.072$), indicating a possible trend toward the reverse of natural enemy dilution by the native grasses. During the second growing season, number of types of symptoms on *S. arundinaceus* did not differ significantly among treatments ($F_{4,16} = 0.95$, $P = 0.46$), but attack was marginally higher in the introduced species treatment than in the natives + *S. arundinaceus* treatment (a priori least squares contrast $F_{1,16} = 3.64$, $P = 0.075$), indicating a reversal of the weak trend from the first growing season. During the third growing season, variation among treatments in enemy attack on *S. arundinaceus* was marginally significant ($F_{4,15.23} = 2.81$, $P = 0.063$), and enemy attack was higher in the introduced species treatment than the natives + *S. arundinaceus* treatment, with the three other communities being intermediate (Tukey's HSD $P < 0.05$, Figure 4.1f).

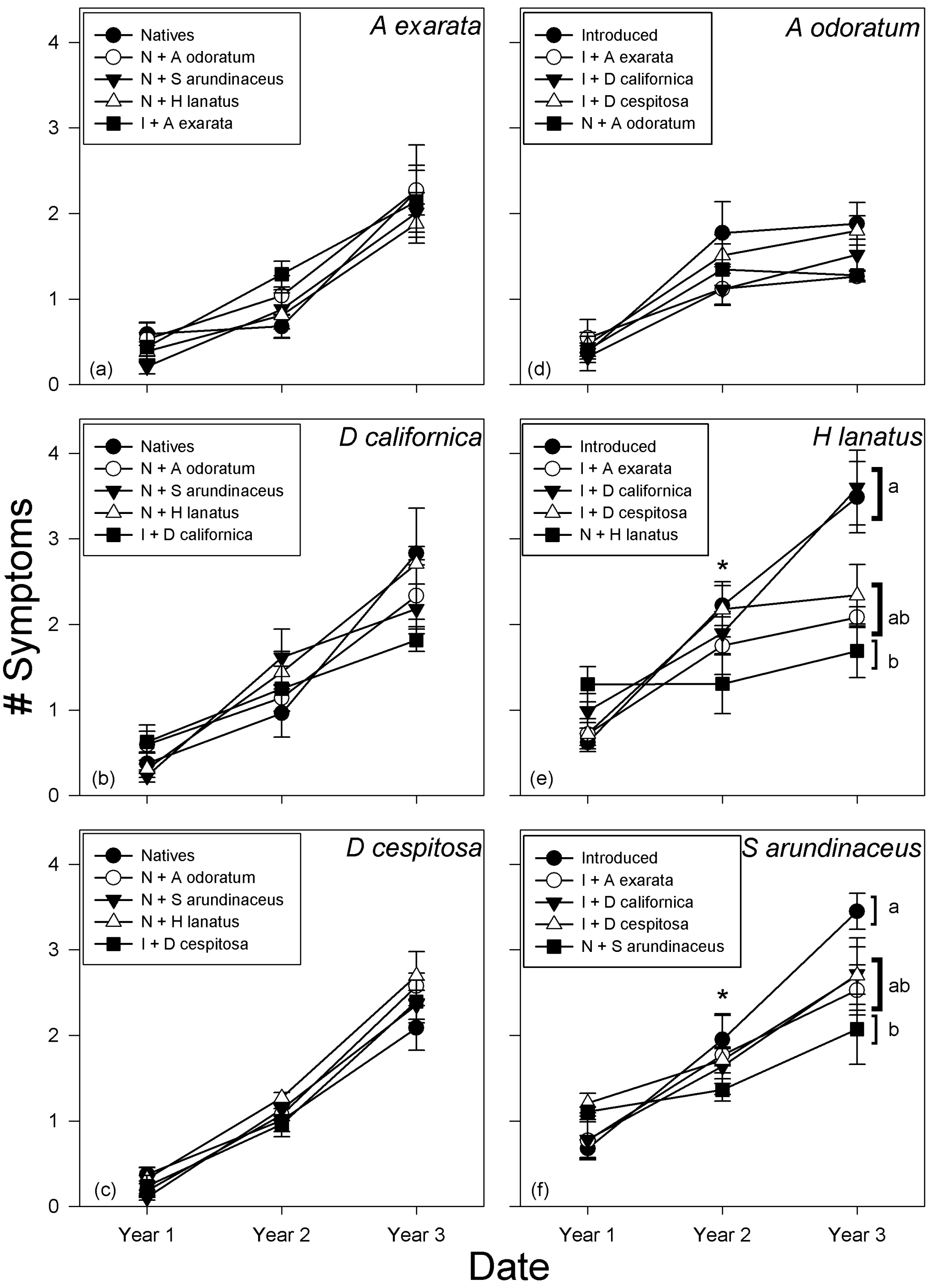

Figure 4.1. Enemy attack (number of symptom types per plant) on the three native grass species *Agrostis exarata*, *Danthonia californica, and Decschampsia cespitosa* (a-c) and the three introduced grass species *Anthoxanthum odoratum, Holcus lanatus,* and *Schedonorus arundinaceus* (d-f) over the course of three growing seasons. Asterisks indicate a significant a priori least squares contrast of the two most diffferent treatments at one particular time point. Lowercase letters indicate differences in enemy attack among treatments in the final growing season (Tukey's HSD $P < 0.05$).

The results of the second two growing seasons are consistent with dilution of natural enemy attack on the introduced *S. arundinaceus* by the native grass species.

Number of symptoms on *A. odoratum* increased over time, but did not vary significantly among treatments, and the effects of treatment did not differ over time (Table 4.2). Natural enemy attack on *A. odoratum* shows a suggestive though not statistically significant trend of divergence among the treatments, and enemy attack in year three was highest in the introduced species treatment (Figure 4.1d).

Variation among treatments over the second growing season

Spillover

Overall, we still found no support for enemy spillover from the introduced to the native species during the second growing season when all three sampling dates were considered. With natives + *S. arundinaceus* excluded from the repeated measures analysis due to low replication in August, enemy attack on the native *A. exarata* increased over the course of the growing season, and the treatment effect varied marginally over time, but there was no main treatment effect (Table 4.2, Figure 4.2a). In May of the second growing season, enemy attack on *A. exarata* differed among the four remaining treatments when natives + *S. arundinaceus* was excluded from the analysis ($F_{3,12} = 3.95$, $P = 0.036$), but this trend weakened when all five treatments were included ($F_{4,16} = 2.30$, $P = 0.10$). There was weak evidence of the reverse trend of enemy spillover, with more enemy attack in the natives treatment than in the introduced species + *A. exarata* treatment (a priori least squares contrast $F_{1,16} = 4.87$, $P = 0.042$).

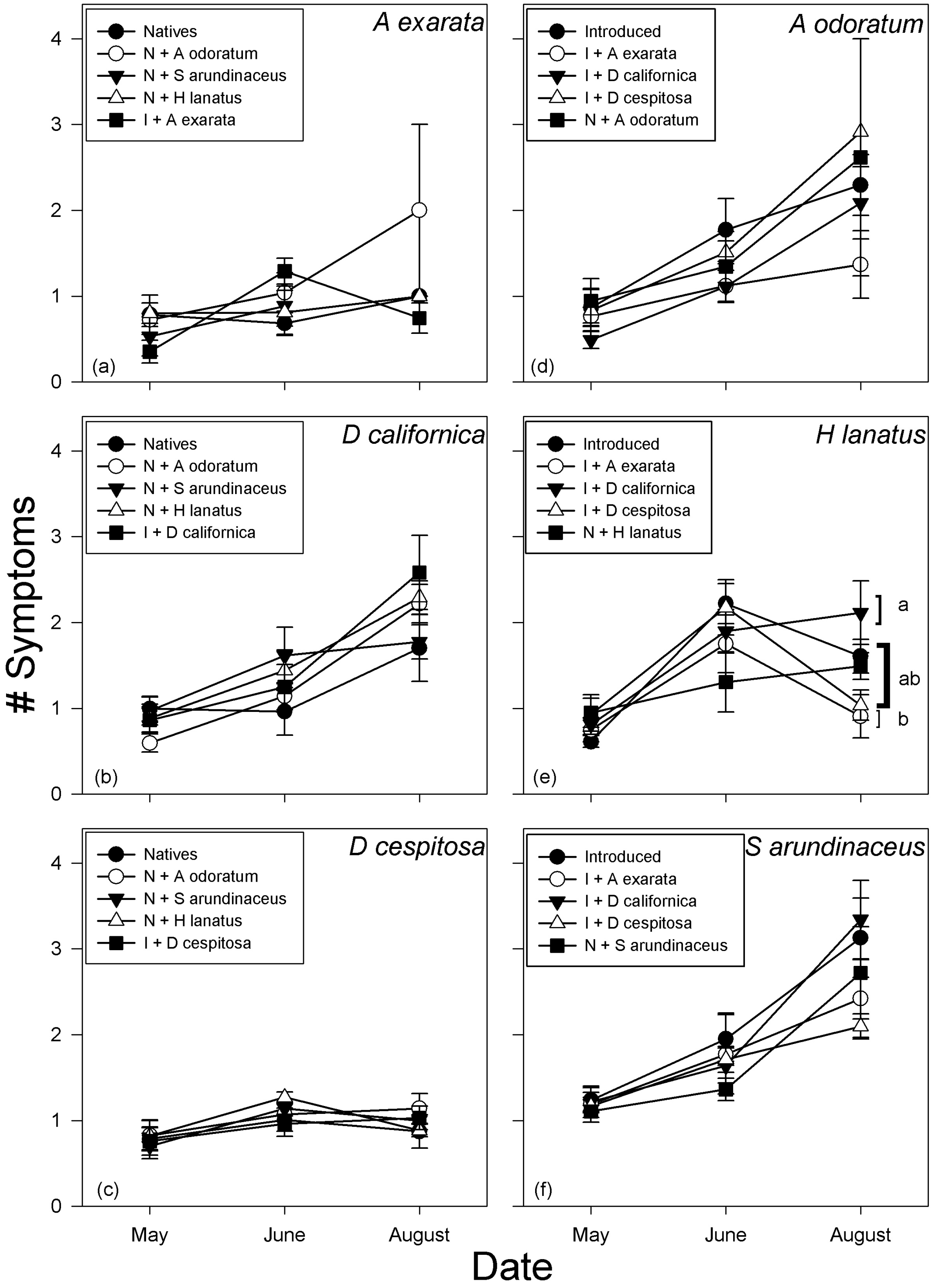

Figure 4.2. Enemy attack (number of symptom types per plant) on the three native grass species *Agrostis exarata, Danthonia californica, and Decschampsia cespitosa* (a-c) and the three introduced grass species *Anthoxanthum odoratum, Holcus lanatus,* and *Schedonorus arundinaceus* (d-f) over the course of the second growing season. Lowercase letters indicate differences in enemy attack among treatments in August (Tukey's HSD $P < 0.05$).

There was no significant variation among treatments in June ($F_{3,16} = 1.86$, $P = 0.17$) or August ($F_{3,4} = 1.14$, $P = 0.43$). Enemy attack on the natives *D. californica* and *D. cespitosa* varied over the growing season, but did not differ among treatments, and the effect of treatment did not vary over time (Table 4.2, Figure 4.2b-c).

Dilution

Enemy attack on the introduced species *A. odoratum* and *S. arundinaceus* increased over the growing season. Treatments did not differ, and the effect of treatment did not change over time (Table 4.2, Figure 4.2d and 4.2f).

Enemy attack on the introduced *H. lanatus* varied over the growing season, and differed among treatments, with no change in effect of treatment over time (Table 4.2) (Figure 4.2e). Enemy attack on *H. lanatus* showed a general trend of stronger variation among the treatments over the growing season, with no significant variation in May ($F_{4,16} = 0.90$, $P = 0.49$), marginally significant variation in June ($F_{4,16} = 2.32$, $P = 0.10$), and highly significant variation in August ($F_{4,13.43} = 5.37$, $P = 0.0084$). In August, there was more natural enemy attack on *H. lanatus* in the introduced species + *D. californica* treatment than in the introduced species + *A. exarata* treatment, with enemy attack being intermediate in the other three treatments (Tukey's HSD $P < 0.05$).

Total cover and abundances of each plant species

In year three, plot above-ground biomass was positively correlated with plot cover ($F_{1,38} = 51.25$, $P < 0.0001$, $r^2 = 0.57$). Because our cover data were collected over a larger area than biomass in each plot, and collected in the same manner each year, we report cover data here to describe abundances over the duration of the experiment.

Total plant cover increased over the three growing seasons ($F_{2,64} = 413.80$, $P < 0.0001$), differed among the eight treatments ($F_{7,32} = 22.62$, $P < 0.0001$), and the effect of treatment varied over time ($F_{14,64} = 8.33$, $P < 0.0001$) (data not shown). In the first growing season, total cover differed among the eight treatments ($F_{7,28} = 3.23$, $P = 0.012$); total cover was highest in the natives + *H. lanatus* treatment and lowest in the introduced species + *A. exarata* treatment, with the other six treatments being intermediate (Tukey's HSD $P < 0.05$; data not shown). In the second growing season total plant cover differed significantly among treatments ($F_{7,28} = 35.08$, $P < 0.0001$), and the five treatments containing *D. cespitosa* had significantly higher cover than the other three treatments (Tukey's HSD $P < 0.05$, data not shown). In the third growing season (Figure 4.3), variation in total plant cover among treatments was again significant ($F_{7,28} = 16.57$, $P < 0.0001$), with differences among treatments showing a similar trend to that in the second growing season. In treatments with *D. cespitosa,* it established as the dominant species, comprising from 20-99% of the total per plot cover in year one, 45-94% in year two, and 60-99% in year three.

Abundances of the native species *A. exarata* and *D. californica* varied over the three growing seasons (Table 4.2). *Agrostis exarata* abundance differed among the five treatments, but there was no main effect of treatment on abundance of *D. californica*. Effect of treatment varied over time for abundances of both species (Figure 4.4a-b). Abundances of *A. exarata* and *D. californica* did not vary among treatments during the first (*A. exarata:* $F_{4,16} = 0.87$, $P = 0.50$, *D. californica:* $F_{4,20} = 0.95$, $P = 0.46$) or second

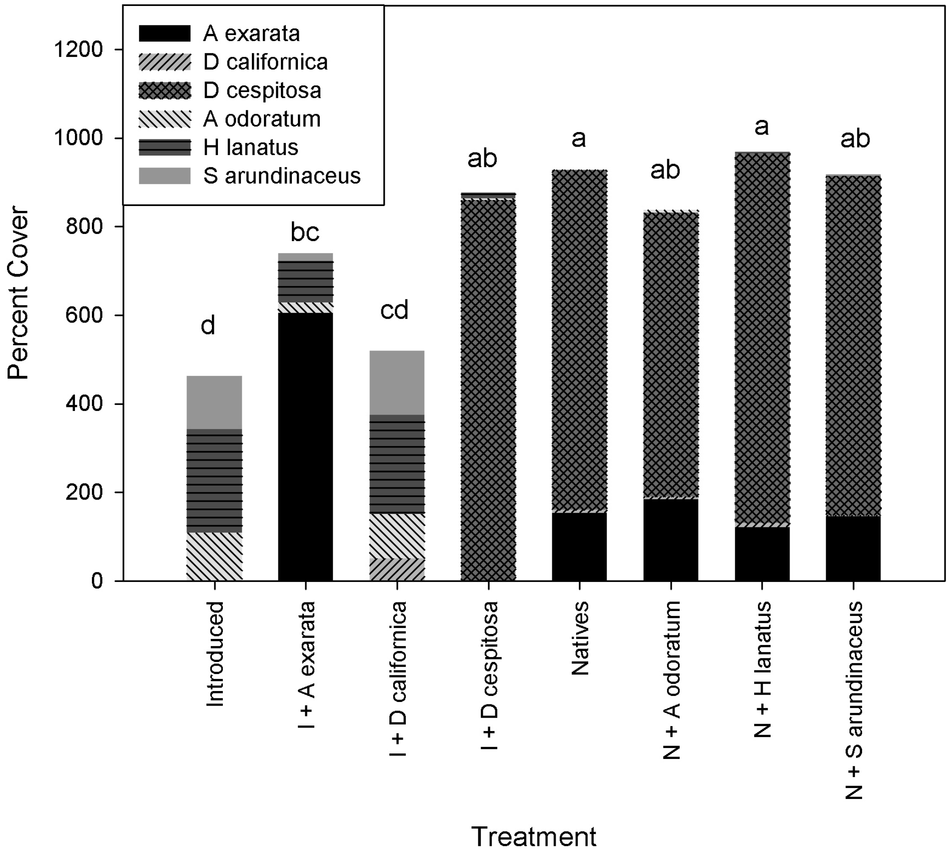

Figure 4.3. Total plant cover in the final growing season among the eight treatments. Lowercase letters indicate significant differences among treatments (Tukey's HSD $P <$ 0.05).

growing season (*A. exarata:* $F_{4,16} = 1.50$, $P = 0.25$, *D. californica:* $F_{4,16} = 1.09$, $P = 0.39$), but treatments had diverged by the third growing season (*A. exarata:* $F_{4,16} = 14.41$, $P <$ 0.0001, *D. californica:* $F_{4,16} = 4.34$, $P = 0.014$). In the final growing season, the abundance of *A. exarata* was greater in the introduced species + *A. exarata* treatment than in the other four treatments, which were equivalent (Tukeys HSD $P < 0.05$). Similar to the *A. exarata, D. californica* was more abundant in the introduced species + *D. californica* treatment than in the other four treatments, which were equivalent (Tukeys

HSD $P < 0.05$), in the final growing season. Abundance of the native *D. cespitosa* varied over time, first increasing and then plateauing, but there was no main treatment effect, and effect of treatment did not depend on time (Table 4.2, Figure 4.4c).

Abundance of all three introduced species, *A. odoratum, H. lanatus,* and *S. arunidinaceus* varied over the three growing seasons and among treatments. Treatment effect varied over time (Table 4.2, Figure 4.3d-f). Abundance of *A. odoratum* did not differ among treatments during the first ($F_{4,16} = 0.49$, $P = 0.74$) or second ($F_{4,16} = 1.94$, $P = 0.15$) growing season, but diverged by the third growing season ($F_{4,16} = 8.37$, $P = 0.0008$). Abundance of *A. odoratum* was highest in the introduced species and introduced species + *D. californica* treatments, the introduced species + *D. californica* treatment was equivalent to the introduced species + *A. exarata* treatment, and *A. odoratum* abundance was lowest in the introduced species + *A. exarata*, natives + *A. odoratum,* and introduced species + *D. cespitosa* treatments (Tukey's HSD $P < 0.05$).

Abundance of *S. arundinaceus* varied with marginal significance among treatments in the first growing season ($F_{4,16} = 2.52$, $P = 0.083$), with cover in the introduced species and introduced species + *D. californica* trending toward being higher than in the other three treatments. The pattern of abundance of *S. arundinaceus* among the five treatments followed a similar trend during the second growing season ($F_{4,16} = 3.04$, $P = 0.048$), although a Tukey's HSD test did not distinguish differences among the five treatments. During the final growing season, this trend strengthened ($F_{4,20} = 11.68$, $P < 0.0001$), and, similar to *A. odoratum,* abundance of *S. arundinaceus* was greater in the introduced species and introduced species + *D. californica,* which were equivalent, than in the other three treatments, which did not differ (Tukey's HSD $P < 0.05$).

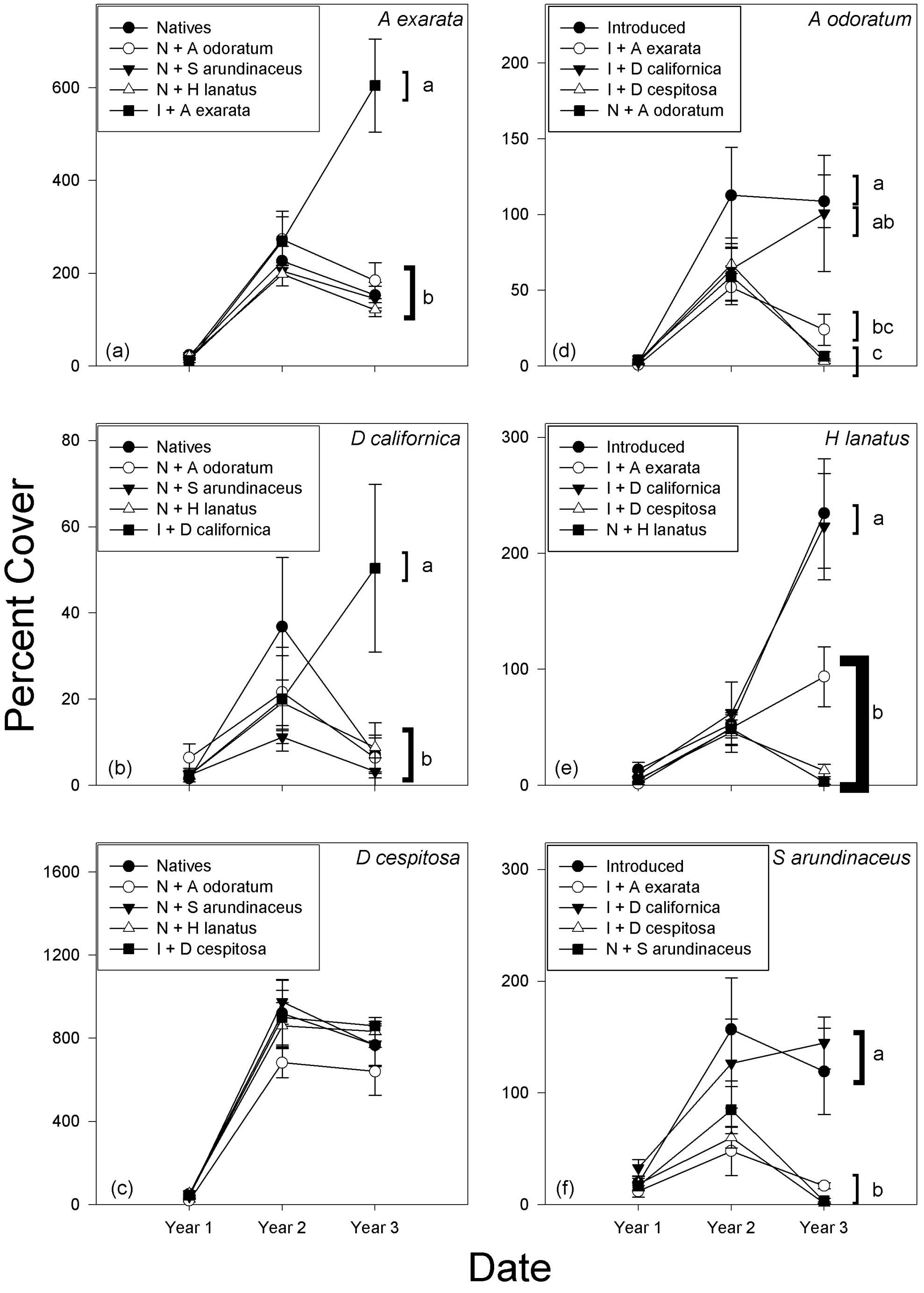

Figure 4.4. Percent cover of the three native grass species *Agrostis exarata, Danthonia californica, and Decschampsia cespitosa* (a-c) and the three introduced grass species *Anthoxanthum odoratum*, *Holcus lanatus*, and *Schedonorus arundinaceus* (d-f) over the course of three growing seasons. Lowercase letters indicate differences in plant cover among treatments in the final growing season (Tukey's HSD $P < 0.05$). Note scale difference in y axes among the six panels.

In the first growing season, abundance of *H. lanatus* differed with marginal significance among treatments ($F_{4,16} = 2.39$, $P = 0.094$), with cover in the introduced species treatment trending toward being higher than in the other four treatments. Abundance of *H. lanatus* did not differ among treatments in the second growing season. In the third growing season, *H. lanatus* differed strongly among the five treatments ($F_{4,16} = 15.64$, $P < 0.0001$), and, similar to the trend in abundance of *A. odoratum* and *S. arundinaceus, H. lanatus* was more abundant in the introduced species and introduced species + *D. californica* treatments than in the other three treatments (Tukey's HSD $P < 0.05$).

Because natural enemy attack can vary with host density, we checked for correlations between abundance of each grass species and enemy attack on that species. Enemy attack on *A. odoratum* and *A. exarata* did not change with their own abundance during any of the five surveys. Enemy attack on the native *D. californica* increased as its abundance increased in year one only ($F_{1,23} = 7.97$, $P = 0.0097$, $r^2 = 0.26$), but did not differ among treatments during any surveys. Enemy attack on the native *D. cespitosa* increased with marginal significance as its own abundance increased in August of year two ($F_{1,23} = 3.25$, $P = 0.084$, $r^2 = 0.12$) and in year three ($F_{1,23} = 3.06$, $P = 0.094$, $r^2 = 0.12$), but did not differ among treatments during any surveys. Enemy attack on *S. arundinaceus* increased as its own abundance increased in August of year two only ($F_{1,23} = 4.96$, $P = 0.036$, $r^2 = 0.18$), but did not differ among treatments during that survey. Enemy attack on *H. lanatus* increased as its own abundance increased in year three only ($F_{1,23} = 12.30$, $P = 0.0019$, $r^2 = 0.35$), which could be associated with the treatment effects on enemy attack that were found.

Total plant cover may also influence natural enemy attack rates. Enemy attack increased on *D. cespitosa* as total cover increased in August of year two only ($F_{1,23}$ = 6.53, P = 0.018, r^2 = 0.22), but did not differ among treatments. Enemy attack on *D. californica* increased as total cover increased in year three only ($F_{1,17}$ = 6.73, P = 0.019, r^2 = 0.28), but did not differ among treatments during that year. Enemy attack on *A. exarata* increased with marginal significance as total cover increased in May of year two ($F_{1,23}$ = 3.06, P = 0.094, r^2 = 0.12), but decreased with marginal significance as total plant cover increased in June of year two ($F_{1,23}$ = 4.00, P = 0.057, r^2 = 0.15). Enemy attack on *H. lanatus* decreased as total cover increased in June of year two with marginal signifance ($F_{1,23}$ = 3.78, P = 0.064, r^2 = 0.14) and in year three with stronger significance ($F_{1,23}$ = 13.37, P = 0.0013, r^2 = 0.37), which could be associated with treatment differences in enemy attack. Enemy attack on *S. arundinaceus* increased as total plant cover increased in year one ($F_{1,23}$ = 6.59, P = 0.017, r^2 = 0.22). Enemy attack on *A. odoratum* increased as total plant cover increased in year one ($F_{1,23}$ = 4.45, P = 0.040, r^2 = 0.09), and followed the same trend with marginal significance in August of year two ($F_{1,13}$ = 3.71, P = 0.076, r^2 = 0.22), which could have obscured our ability to detect dilution on that species. In year three, however, enemy attack on *A. odoratum* reversed trends, decreasing with marginal significance as total plant cover increased ($F_{1,23}$ = 3.32, P = 0.082, r^2 = 0.13).

Discussion

We found compelling evidence of dilution of natural enemy attack on two of the three introduced species, *Holcus lanatus* and *Schedonorus arundinaceus,* with increasing divergence in enemy attack among treatments over the course of the three growing seasons (Figure 4.1). *Anthoxanthum odoratum* followed a similar suggestive trend, although a treatment effect on enemy attack was not found in our repeated measures analysis over the three growing seasons. It should be noted, however, that enemy attack on *H. lanatus* and *S. arundinaceus* were higher in treatments that also had higher abundance of these two species, and enemy attack on a particular host can increase as abundance of that host increases (Alexander 1992, Folgarait et al. 1995, Agrawal et al. 2006). In contrast, abundance of the native species *Agrostis exarata* and *Danthonia californica* also varied significantly among treatments, while enemy attack on those two species did not, suggesting that host abundance may not have affected enemy attack in this experiment. Our finding of dilution of natural enemies on introduced grasses by native grasses warrants further study.

We found no evidence of enemy spillover from the introduced to the native plant species. Natural enemy attack on the three native species did not vary among treatments in any of the three growing seasons. Our results are contrary to past findings of enemy spillover from introduced to native grasses (Malmstrom et al. 2005, 2007, Beckstead et al. 2010), which may have been confounded with the life history differences of annual versus perennial plants. Borer and others (Borer et al. 2009) found that aphids, the exclusive vectors of barley/cereal yellow dwarf viruses, preferred and had higher fecundity on annual than perennial host species, while host geographic origin and nutrient status were less influential. More recently, Cronin and others (2010) found that a host's physiological phenotype, described in terms of metabolic rate, leaf nutrient concentration, and leaf mass per unit area, predicted its level of reservoir potential, while host life span, provenance, and phylogeny did not. In addition to dilution and spillover, future studies of patterns of natural enemy attack should consider these other variables. We controlled for life history and phylogenetic variables by using closely related species with similar life histories. In two previous studies in the same location, foliar nitrogen and phosphorus concentrations had no or minimal effect on pathogen and herbivore attack (Blaisdell 2011 - Chapter II and Chapter III).

We can make some inferences about the effects of enemy attack versus the effects of plant species competition by examining the plant cover data over the course of the three growing seasons. Abundance of the native *Deschampsia cesptiosa* and enemy attack on *D. cespitosa* were unaffected by treatment (Figure 4.4). *Deschampsia cespitosa* increased steeply in abundance as determined by cover between the first two growing seasons, then plateaued between the second and third growing seasons when it dominated all plots in which it was planted (Figure 4.3), which suggests that intraspecific competition was the dominant factor controlling abundance of this species. Pfeifer-Meister (2008) found that *D. cespitosa* was the dominant competitive plant species in the nearby seasonal restored wetland prairies, with its dominance increasing over several years. This dominance may be due directly to its competitive ability, or to its relatively high rate of infection with the endophyte *Neotyphodium.* The other two native species *A. exarata* and *D. californica* increased over all three growing seasons only in the treatments where *D. cespitosa* was absent, but actually decreased from the second to third growing seasons in the treatments that contained *D. cespitosa.* This observation combined with the lack of treatment effect on natural enemy attack of these two species indicates that competition with *D. cespitosa* was the primary factor controlling their abundance.

In all treatments, the introduced *A. odoratum, H. lanatus,* and *S. arundinaceus* increased in abundance from the first to the second growing season (Figure 4.4). In the second growing season, all three introduced grass species shared similar abundances, but only *H. lanatus* increased between the second and third growing seasons (Figure 4.4). All three introduced species were much less abundant when the native *D. cespitosa* was present, indicating strong interspecific competition (Figure 4.4). *Agrostis exarata* also appeared to somewhat influence abundance of the introduced species in the final growing season. In the final year, the three introduced grass species achieved their highest abundance in the introduced species and introduced species + *D. californica* treatments, suggesting that *D. californica* is either a weak competitor or had a positive effect on the introduced species through enemy dilution. *Danthonia californica* had little to a modest effect on enemy dilution for the three introduced grasses (Figure 4.1), suggesting that competition was the dominant effect in their abundances.

Sometimes the presence of one plant species can affect another species by harboring natural enemies, and this distinction between competition versus increased enemy populations has been addressed in past experiments by using exclosures to exclude natural enemies (Reader 1992, Bonser and Reader 1995, Hulme 1996, Van Der Wal et al. 2000, Borer et al. 2009). When one species affects another via shared natural enemies rather than via direct resource competition, this is called apparent competition (Holt et al. 1994). Our study did not directly manipulate natural enemy abundance in this manner, and we therefore cannot discern between the effects of enemy attack and competition with certainty. However, *D. cespitosa* had a dilution effect on *H. lanatus* and *S. arundinaceus* (Figure 4.1), and it also led to a reduction in abundance of both of these species (Figure 4.4), which suggests that resource competition was the dominant influence.

Overall, natural enemy attack increased on all six grass species in all treatments over time, indicating an accumulation of natural enemies over the three growing seasons. Plant resistance to insect herbivores has been found to be higher in younger plants than in middle-aged plants, and then increase again in older plants (Stein and Price 1995). In the Northwestern United States, periodic field burning has traditionally been used to reduce pathogen load, which otherwise can build up in plant material over multiple years (Hardison 1980). This may explain the accumulation of natural enemies over the three growing seasons in this study. Plant size is also positively associated with enemy attack in some cases (Burdon 1987), but this has not been the case for these grasses in closely related studies (Blaisdell 2011- Chapter II and Chapter V).

Our findings indicate dilution of natural enemy attack on introduced species by native species, rather than spillover of natural enemies from the introduced to the native species. In our study, the native *D. cespitosa* was the dominant species, and appeared to be unaffected by the introduced species. Despite the positive effect of native species on introduced species via enemy dilution, our results indicate that the primary effect of the native species on introduced species was negative through competitive interactions. Interestingly, abundance of the native species and enemy attack on the native species during this three-year experiment were not affected by the presence of the introduced species, suggesting that there may be larger effects of the native species on the introduced species. The relative strength of competitive interactions almost certainly will vary with the particular species involved, and in grass communities without such a strong competitor as *Dechampsia cespitosa,* enemy dilution may play a more pronounced role in the success of introduced species. Invading genotypes in some cases are larger with higher reproductive output, but with relatively poorly defended leaves relative to genotypes in the plants' native range, and could therefore benefit disproportionately from dilution of enemy attack (Siemann and Rogers 2001).

Bridge to Chapter V

In this chapter, we experimentally tested our earlier findings from Chapters II and III that were suggestive of enemy spillover from introduced to native plant species, controlling for life history by using only perennial bunchgrasses. We also explored dilution of natural enemy attack on introduced species by native species. Our experiment yielded no evidence of enemy spillover from the introduced species, but instead we found evidence of dilution of natural enemies. This and the previous chapters did not directly address the enemy release hypothesis, which is the most popular proposed mechanism of invasions by introduced species. According to the enemy release hypothesis, plant species experience a decrease in regulation by herbivores and pathogens, resulting in a rapid increase in the plants' distribution and abundance. In Chapter V, we tested enemy release of our three introduced species used in Chapter IV, by comparing natural enemy attack on existing populations from a broad geographic area in both their native and naturalize ranges, Europe and the United States.

CHAPTER V

A TEST OF ENEMY RELEASE OF THREE COMMONLY CO-OCCURRING PERENNIAL BUNCHGRASS SPECIES NATIVE TO EUROPE AND INTRODUCED IN THE UNITED STATES

Author contributions: This study was designed and implemented by G. Kai Blaisdell, and this chapter was written by her with contributions from Bitty A. Roy. Bitty A. Roy also contributed to the study by locating some populations, and collecting samples with G. Kai Blaisdell at one site.

Introduction

Invasive introduced plants threaten native biodiversity, alter ecosystem structure, and cost approximately $120 billion per year in the United States (Pimentel et al. 2005). The popularly cited enemy release hypothesis (Keane and Crawley 2002, Colautti et al. 2004) proposes that introduced plants fare better than native plants because they are “released” from pathogens and herbivores that exist only in their native range. With fewer enemies, these introduced plants have a competitive advantage over native plants and become invasive.

Various approaches have been used to test enemy release. For example, some studies tested for differences in herbivore and/or pathogen attack between closely related native and introduced species, or between co-occurring native and introduced species, on the premise that the introduced species, if indeed released from natural enemies, would have less enemy attack than their native congeners or neighbors (Agrawal and Kotanen

2003, Dietz et al. 2004, Vilà et al. 2005, Carpenter and Cappuccino 2005, Agrawal et al. 2005, Han et al. 2008). These studies found mixed results with respect to supporting or not supporting the enemy release hypothesis. Recently, Vermeij and others (2009) showed that comparison of herbivory on native and introduced algae in the naturalized range showed more herbivory on the introduced algae, yet a biogeographical comparison of the introduced algae in its native versus naturalized ranges showed reduced herbivory in its naturalized range relative to its native range. This finding indicates that comparing native and introduced plants in one area could lead to erroneous conclusions about enemy release.

Alternative to contrasting native and introduced species in one geographic range, some scientists have compared natural enemy attack on plant species between their native and naturalized ranges. Consistent with enemy release, Adams and others (Adams et al. 2009) found more leaf herbivory and fungal attack on *Acer platanoides* (L.) (Norway maple) in Europe, the native range, than in North America, the naturalized range. Similarly, Vermeij and others (Vermeij et al. 2009) found less grazing on algae in their naturalized range, Hawaii, than in their native range, the Caribbean. Genton and others (Genton et al. 2005) found evidence of enemy release from herbivores but not pathogens in populations of *Ambrosia artimisiifolia* (ragweed) native to North America and invasive in France. Roy and others (in press) found reduced pathogen and mollusk attack on *Brachypodium sylvaticum* in the United States, the naturalized range, versus Europe, the native range, but an increase in insect herbivory in the naturalized range. These biogeographical studies are small in number, and the majority surveyed a small geographic area in the native and naturalized ranges of their study species (but see Adams et al 2009). Most studies focused only on one particular plant species (but see Vermeij et al 2009).

Yet another approach besides the field studies mentioned above is to mine existing information by comparing species richness of already documented natural enemies on plant species in their native and naturalized ranges (i.e., host index compilations).A host index lists pathogens known to attack particular plant species or hosts. Mitchell and Power (Mitchell and Power 2003) used this approach, compiling data from existing host indices of viral and fungal pathogens on 471 plant species native in Europe and naturalized in the United States, and generally found higher numbers of viruses and fungal pathogens on plant species in Europe than in the United States. One potential weakness of this approach is that diseases are better documented in Europe than in the United States (Mitchell and Power 2003). Van Kleunen and Fischer (2009) used a correction for this literature bias, and found that the trend of enemy release, though reduced in magnitude, still held. Furthermore, these authors compiled information on fungal pathogens of 140 plant species native in North America and naturalized in Europe, and found a similar but weaker trend of enemy release, as shown by fewer species of fungal pathogens per host species in the plants' naturalized range than in the native range. This approach of measuring species richness of natural enemies per plant species assumes that greater amounts of attack are correlated with greater numbers of enemy species, which may not always be accurate. Van Kleunen and Fischer (2009) also examined the correlation of pathogen species richness per host species with geographic expansion of each host species in their naturalized range, and, contrary to the prediction of enemy release, found that release from foliar pathogens was negatively correlated with the geographic expansion of the plant species in their naturalized range.

To test the enemy release hypothesis, we used a combination of two approaches. First, we surveyed herbivore and pathogen attack on individual plants in populations of three commonly co-occurring perennial bunchgrasses, native in Europe and naturalized in North America. Multiple populations across a wide geographic area in both the native and naturalized ranges were surveyed. We measured both percent leaf area damaged by herbivores and pathogens, and, as a proxy for enemy species richness, number of types of herbivore and pathogen symptoms per plant. As an estimate of fitness, above-ground per plant biomass was measured. Second, for a comparison of field results to host index compilations, we then compared our findings from our field survey to the data compiled by Mitchell and Power (2003) for each of the three plant species we surveyed.

Methods

We surveyed three species of perennial bunchgrasses, *Anthoxanthum odoratum* L. (“sweet vernal grass”), *Holcus lanatus* L. (“common velvetgrass”), and *Schedonorus arundinaceus* (Schreb.) Dumort (“tall fescue”), synonym = *Festuca arundinacea*, (Flora of North America Editorial Committee 1993), that are native to Europe and naturalized in the United States. All are found widely across both Europe and the United States, and commonly co-occur in both their native and naturalized ranges.

Populations were selected from as wide an area as logistically possible within each range, and were found using Nature Conservancy species lists, prior knowledge of populations by authors and colleagues, and surveys of areas likely to have one or more of the three grass species. Sampling in the United States occurred in May, June, and August 2008. Sampling in Europe occurred in July 2008. All populations that were found and could be surveyed during the 2008 growing season were sampled, which resulted in unequal replication. In the United States, populations were sampled in Oregon, Washington, Wisconsin, North Carolina, and New York, with seven populations of *A. odoratum*, eight of *H. lanatus*, and six of *S. arundinaceus* (Figure 5.1, Table A.1 in the Appendix). In Europe, populations were sampled in Switzerland, Austria, Germany, and the Netherlands, with eight populations each of *A. odoratum* and *H. lanatus,* and three of *S. arundinaceus.*

To sample each population, a 30-m transect was laid through the population. Density of each of the three grass species was estimated at three 1-m sections of the transect (4-5 m, 10-11 m, and 25-26 m): At each 1-m space, the number of stems of each plant touching the transect tape was counted. As an estimate of overall community vigor, three 20-cm^2 quadrats of total above-ground community biomass were collected from 50 cm away from the transect tape at 5, 15, and 25 m. The collected community biomass was oven-dried at 60°C for 72 hours and weighed. At each site, ten plants of each host species present were collected and surveyed for attack by natural enemies at each population. For this sampling, the nearest plant to the meter mark was taken every three meters along the transect. Percent foliar herbivore and pathogen damage were estimated visually for one randomly selected leaf from each plant. Plants surveyed ranged approximately from having three to fifty leaves.

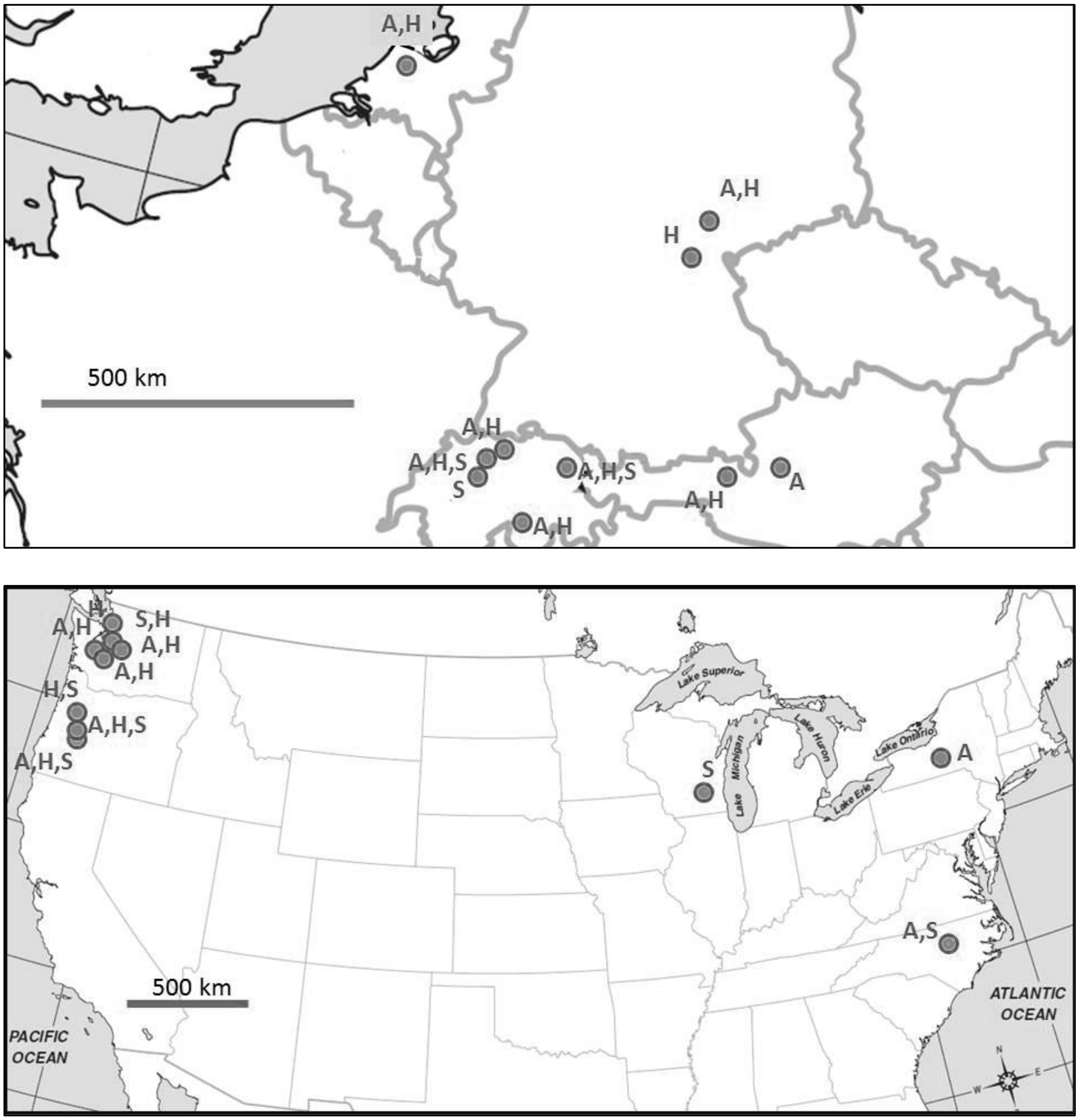

Figure 5.1. Populations of three commonly co-occurring perennial bunchgrasses were sampled across a wide geographic range in both the native range (Europe) and the naturalized range (United States) in 2008. Each white dot represents a location sampled. The letters at each location correspond to which of the three plant species were sampled at that location: A = *Anthoxanthum odoratum*, H = *Holcus lanatus*, S = *Schedonorus arundinaceus.*

Additionally, the entire plant was examined for number of types of visibly distinct herbivore and pathogen attack, and percent total damage to the plant was estimated visually. Furthermore, chlorophyll content as an estimate of nutrient status (Gáborčík 2003) and percent total senescence were recorded for each plant. Above-ground biomass of each plant at the time of sampling was measured by clipping and drying at 60°C for 72 hours.

All analyses were performed in JMP version 8. All percentage data were arcsine square root transformed, count data were square root transformed, and shoot biomass data were log transformed to better meet the assumptions of ANOVA. The two ranges, Europe and the United States, were compared as fixed factors, and population nested within range was included as a random factor for the following variables: percent herbivore damage per leaf, percent pathogen damage per leaf, total percent damage per leaf, number of types of herbivore per plant, types of pathogen per plant, total number per plant, total percent damage per plant, and total above-ground biomass per plant. All data were back-transformed for presentation.

To explore potentially confounding factors, we first compared the plant level and site level variables between the native and naturalized ranges, using the same ANOVA model used to test differences in natural enemy attack between the two ranges. When we found a difference in variables between the two ranges, we explored further by performing least squares regressions of the plant or site variables versus percent leaf herbivore and pathogen damage.

We admittedly performed many statistical tests, yet the results yielded tended to corroborate each other rather than being contradictory. The statistical model that we used is conservative because site is treated as a random variable, and, especially in the case of *S. arundinaceus*, our low replication of sites led to relatively weak statistical power. Similar biogeographic studies have used a Student's t-test (Wolfe 2002), which is a much less conservative model, to compare natural enemy attack between ranges. Student's t-tests fail to treat site within ranges as a random variable, limiting the ability to correctly extrapolate results from individual sites to the entire native and naturalized ranges. More recently, Adams and others (2009) and Roy and others (in press) have employed a statistical approach similar to what we used in this study.

To compare the field and literature survey approaches to each other, we compared our field observations to data compiled from existing recorded information about pathogen attack on the three grass species (Mitchell and Power 2003). These authors shared their data on *Schedonorus arundinaceus* and *Holcus lanatus* with us. Additionally, we followed their protocol to collect similar data for *Anthoxanthum odoratum*. For *A. odoratum*, in addition to compiling data for rusts, smuts, and mildews as done by Mitchell and Power, we also compiled data for all other fungal pathogens found in the same sources (Gäumann 1959, Wilson and Henderson 1966, Gleason and Cronquist 1991, Hickman 1993, Farr and Rossman 2010) used by Mitchell and Power.

Results

Percent damage per leaf

Consistent with enemy release, both *Holcus lanatus* ($F_{1,13.95} = 4.27$, $P = 0.03$) and *Schedonorus arundinaceus* ($F_{1,6.90} = 8.61$, $P = 0.02$) had significantly more percent herbivore damage per leaf in the native than in the naturalized range (Figure 5.2). Percent herbivore damage did not significantly differ between the two ranges on *Anthoxhanthum odoratum* ($F_{1,13} = 0.25$, $P = 0.63$). Pathogen damage did not significantly differ between the two ranges on any of the three grass species (*A. odoratum:* $F_{1,13} = 0.17$, $P = 0.69$, *H. lanatus:* ($F_{1,13.97} = 0.23$, $P = 0.64$, *S. arundinaceus:* ($F_{1,6.95} = 1.07$, $P = 0.34$, Figure 5.2). Total percent leaf damage was higher in the native than naturalized range with marginal significance on *H. lanatus* ($F_{1,13.91} = 3.05$, $P = 0.10$), but not on *A. odoratum* ($F_{1,13} = 1.67$, $P = 0.22$) or *S. arundinaceus* ($F_{1,6.96} = 1.05$, $P = 0.33$). Percent leaf herbivore damage was weakly, but significantly negatively correlated with percent leaf pathogen damage for the three grass species ($F_{1,392} = 45.93$, $P < 0.0001$, $r^2 = 0.10$).

Whole plant measurements

Across all three grass species, number of types of symptoms per plant showed a positive correlation with percent leaf damage. Percent herbivore damage was correlated with number of types of herbivore symptoms ($F_{1,392} = 144.30$, $P < 0.0001$, $r^2 = 0.27$), and percent pathogen damage was also positively correlated with number of types of pathogen symptoms ($F_{1,392} = 98.15$, $P < 0.0001$, $r^2 = 0.20$).

Number of types of symptoms per plant generally followed a similar pattern to that of percent leaf damage between ranges, but with weaker statistical significance (number of herbivore symptoms per plant: *A. odoratum:* $F_{1,13} = 0.058$, $P = 0.81$, *H. lanatus:* $F_{1,14.13} = 3.35$, $P = 0.08$, *S. arundinaceus:* $F_{1,6.96} = 3.30$, $P = 0.11$; number of pathogen symptoms per plant: *A. odoratum:* $F_{1,13} = 0.53$, $P = 0.48$, *H. lanatus:* $F_{1,14.31} = 0.060$, $P = 0.81$, *S. arundinaceus:* $F_{1,6.96} = 0.68$, $P = 0.44$); total number of symptoms per plant: *A. odoratum:* $F_{1,13} = 0.50$, $P = 0.49$, *H. lanatus:* $F_{1,14.19} = 1.42$, $P = 0.25$, *S. arundinaceus:* $F_{1,6.97} = 0.23$, $P = 0.65$).

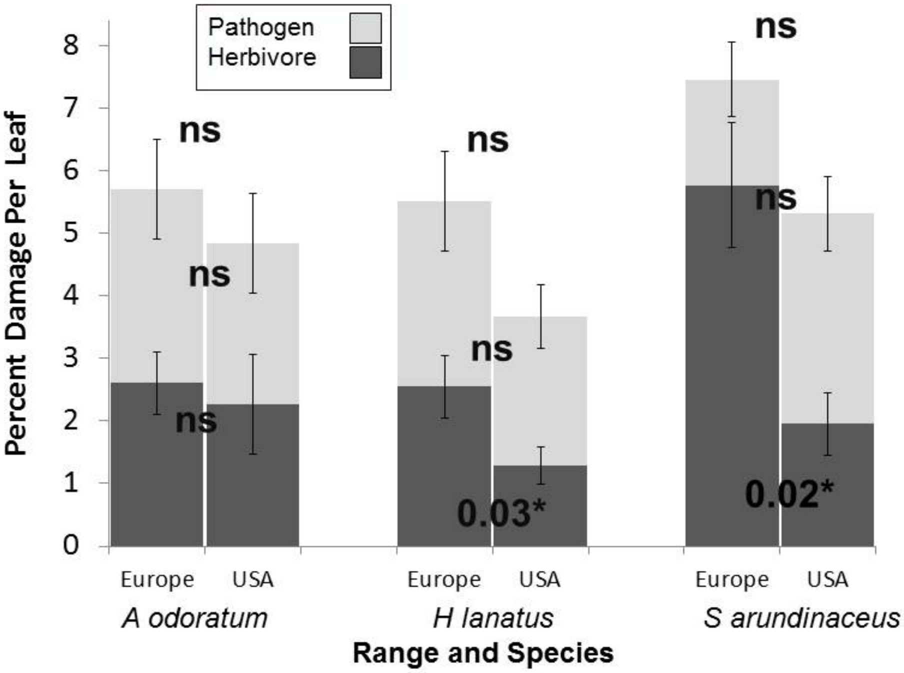

Figure 5.2. Percent herbivore, pathogen, and total damage per leaf on three plant species (*Anthoxanthum odoratum*, *Holcus lataus*, *Schedonorus arundinaceus*), native in Europe and naturalized in the United States. *P* values are shown for all significant differences in herbivore, pathogen, and total damage per leaf between ranges. ns = not significant.

Total percent damage per plant was significantly higher in the native range than the naturalized range on *A. odoratum* ($F_{1,13,36} = 8.41$, $P = 0.01$), and *H. lanatus* ($F_{1,12} = 40.68$, $P < 0.0001$), but not on *S. arundinaceus* ($F_{1,7.09} = 2.61$, $P = 0.15$). For all three grass species, the difference between ranges trended in the same direction (Figure 5.3). Per plant above-ground biomass of *A. odoratum* was significantly higher in the naturalized range than the native range ($F_{1,13} = 12.53$, $P = 0.004$), and *H. lanatus* followed the same trend, though marginally significant ($F_{1,13.94} = 3.75$, $P = 0.07$).

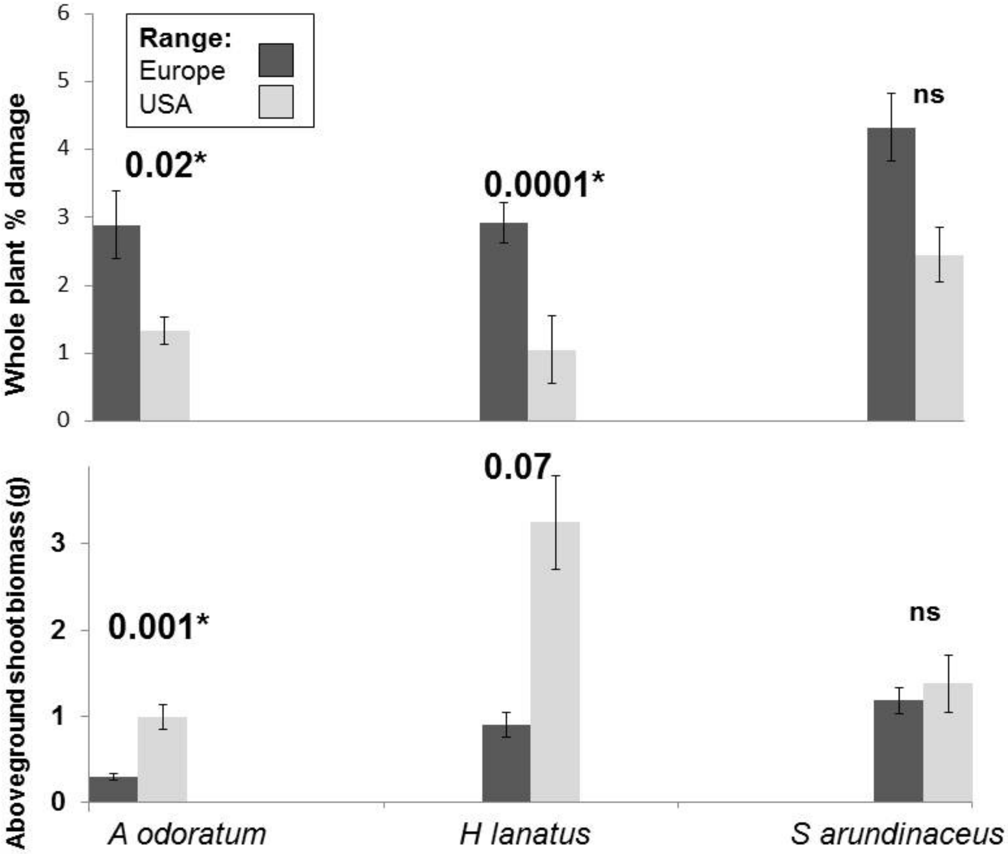

Figure 5.3. (a) Whole plant percent damage and (b) per plant above-ground biomass in the naturalized and native ranges on *Anthoxanthum odoratum, Holcus lataus,* and *Schedonorus arundinaceus*. *P* values are shown for differences in whole plant percent damage and per plant above-ground biomass between the native and naturalized ranges for each plant species.

Potentially confounding plant level variables

The plant vigor hypothesis (Price 1991, Cornelissen et al. 2008) predicts that natural enemy attack will increase as plant health increases, and we explored shoot biomass (Folgarait et al. 1995, Bradley 2003), chlorophyll content (Gáborčík 2003), and whole plant percent senescence as potentially confounding variables. Percent leaf herbivore damage ($F_{1,389} = 0.69$, $P = 0.41$, $r^2 = 0.002$) and percent leaf pathogen damage ($F_{1,389} = 0.80$, $P = 0.24$, $r^2 = 0.004$) were not significantly correlated with whole plant biomass. Chlorophyll content did not significantly vary between the two ranges for any of the three grass species (*A. odoratum:* $F_{1,13.24} = 0.0021$, $P = 0.96$, *H. lanatus:* $F_{1,13.91} = 0.33$, $P = 0.57$, *S. arundinaceus:* $F_{1,7.16} = 0.29$, $P = 0.60$). *Anthoxanthum odoratum* ($F_{1,13.03} = 6.29$, $P = 0.026$) and *H. lanatus* ($F_{1,13.32} = 24.21$, $P = 0.0003$) had significantly higher percent per plant senescence in the US than in Europe. Percent senescence did not differ significantly between the two ranges on *S. arundinaceus* ($F_{1,6.98} = .33$, $P = 0.58$). Percent leaf herbivore damage ($F_{1,391} = 0.14$, $P = 0.71$, $r^2 = 0.0004$) and percent leaf pathogen damage ($F_{1,391} = 0.44$, $P = 0.51$, $r^2 = 0.001$) were not significantly correlated with percent senescence. Therefore, shoot biomass, chlorophyll content, and plant senescence would not have confounded our results.

Potentially confounding site level variables

Overall, community plant biomass had some weak associations with herbivory that may have confounded our finding of release from herbivory. Community plant biomass per m^2 differed with marginal significance between ranges at sites where *A. odoratum* was sampled, trending toward being higher in Europe than in the US ($F_{1,13} = 4.14$, $P = 0.063$). Community plant biomass followed a similar trend between the native and naturalized ranges for sites where *H. lanatus* ($F_{1,14} = 2.20$, $P = 0.16$) and *S. arundinaceus* ($F_{1,7} = 1.90$, $P = 0.21$) were sampled, but with no statistical significance.

Across all three plant species, community biomass had a weak, positive correlation with herbivory in both ranges ($F_{1,38}$ = 4.22, P = 0.047, r^2 = 0.10). Therefore, the trend toward higher community biomass in Europe and its positive association with herbivory could have contributed to our finding of less herbivory in the US. Across all three plant species and both ranges, community biomass had no association with leaf pathogen attack ($F_{1,38}$ = 0.27, P = 0.61, r^2 = 0.0007), indicating that community biomass would not have confounded our results with respect to pathogen attack.

Across all three plant species, elevation of sites sampled in Europe was significantly higher than elevation of sites sampled in the US ($F_{1,20.32}$ = 14.71, P = 0.0010). Across all plant species, elevation had a low, marginally significant positive correlation with leaf herbivore damage ($F_{1,38}$ = 4.09, P = 0.0501, r^2 = 0.097). This positive correlation of elevation and herbivory could reduce our ability to detect less herbivory in the naturalized than the native range. Across all three grass species, elevation was not significantly correlated with pathogen damage ($F_{1,38}$ = 0.0029, P = 0.96, r^2 = 0.000008), indicating that elevation would not have confounded our results with respect to pathogen attack.

Each site in this study was sampled on one date only, and, on average, the sites in Europe were sampled at a later date in the growing season ($F_{1,38}$ = 79.32, P < 0.0001). Across all three grass species, percent leaf pathogen damage had a weak, marginally significant positive association with sampling date ($F_{1,38}$ = 3.72, P = 0.061, r^2 = 0.089), indicating that slightly more pathogen damage may have been found later in the growing season. This association may have obscured our ability to detect enemy release from pathogens. Sampling date was not correlated with herbivory ($F_{1,38}$ = 0.22, P = 0.64, r^2 =

0.006), indicating that our results with respect to herbivore damage were not confounded by sample date.

Mean density of *A. odoratum* ($F_{1,11} = 7.79$, $P = 0.018$) and *H. lanatus* ($F_{1,9} = 6.12$, $P = 0.035$) were significantly higher in the US than in Europe, and *S. arundinaceus* followed a similar but non-significant trend ($F_{1,2} = 3.80$, $P = 0.19$). On all three grass species, percent leaf herbivory was not significantly correlated with density of *A. odoratum* ($F_{1,26} = 1.72$, $P = 0.20$, $r^2 = 0.062$) or *S. arundinaceus* ($F_{1,18} = 0.009$, $P = 0.92$, $r^2 = 0.00005$). Across all three species, leaf herbivory decreased as density of *H. lanatus* increased ($F_{1,28} = 6.16$, $P = 0.019$, $r^2 = 0.18$). Within individual grass species, when a trend in correlation between herbivory and density of any single grass species was present, the direction of the relationship was negative. As densities of the three grass species were higher in the US than in Europe, this correlation of more herbivory at lower densities of the host species may have reduced our ability to detect release from herbivores. On all three grass species combined, percent leaf pathogen damage did not vary significantly with mean density of *A. odoratum* ($F_{1,26} = 1.32$, $P = 0.26$, $r^2 = 0.048$), *H. lanatus* ($F_{1,28} = 0.064$, $P = 0.80$, $r^2 = 0.002$), or *S. arundinaceus* ($F_{1,18} = 1.40$, $P = 0.25$, $r^2 = 0.072$). Therefore, density of each plant species would not have confounded our findings for pathogen attack.

Comparison with literature survey

Our finding that number of types of pathogen symptoms had a modest positive correlation with percent pathogen damage ($r^2 = 0.20$) provides limited support that species richness of pathogens, as used by Mitchell and Power (2003) and Van Kleunen and Fischer (2009), may be a reasonable predictor of degree of damage caused. For all three plant species, number of types of pathogen per plant was positively correlated with leaf percent pathogen damage (*A. odoratum:* $F_{1,149} = 39.91$, $P < 0.0001$, $r^2 = 0.21$, *H. lanatus:* $F_{1,151} = 26.30$, $P < 0.0001$, $r^2 = 0.15$, *S. arundinaceus:* $F_{1,89} = 58.61$, $P < 0.0001$, $r^2 = 0.40$). For all three plant species, percent herbivore damage was also positively correlated with number of types of herbivore symptoms (*A. odoratum:* $F_{1,149} = 42.37$ $P < 0.0001$, $r^2 = 0.22$, *H. lanatus:* $F_{1,151} = 70.08$, $P < 0.0001$, $r^2 = 0.32$, *S. arundinaceus:* $F_{1,89} = 34.45$, $P < 0.0001$, $r^2 = 0.28$).

In our field survey, ANOVA revealed no significant differences in number of types of pathogen attack between the native and naturalized ranges on any of the three host species (Figure 5.4a), indicating that there was no evidence of enemy release with respect to pathogens. According to Mitchell and Power’s data compilation (2003), *A. odoratum* and *H. lanatus* had a net release from fungal pathogens, as indicated by lower species richness of pathogens on each plant species in the naturalized than in the native range, while there was no difference in fungal species richness between the two ranges for *S. arundinaceus.* (Figure 5.4b). Following Mitchell and Power’s protocol, we also found a strong net release of *A. odoratum* from mildews, smuts, and rusts combined. However, for other fungi that we compiled from the same sources, we found 13 other pathogen species in Europe versus 19 other pathogens in the United States, indicating the reverse trend expected by enemy release for these other pathogens not considered by Mitchell and Power’s compilation.

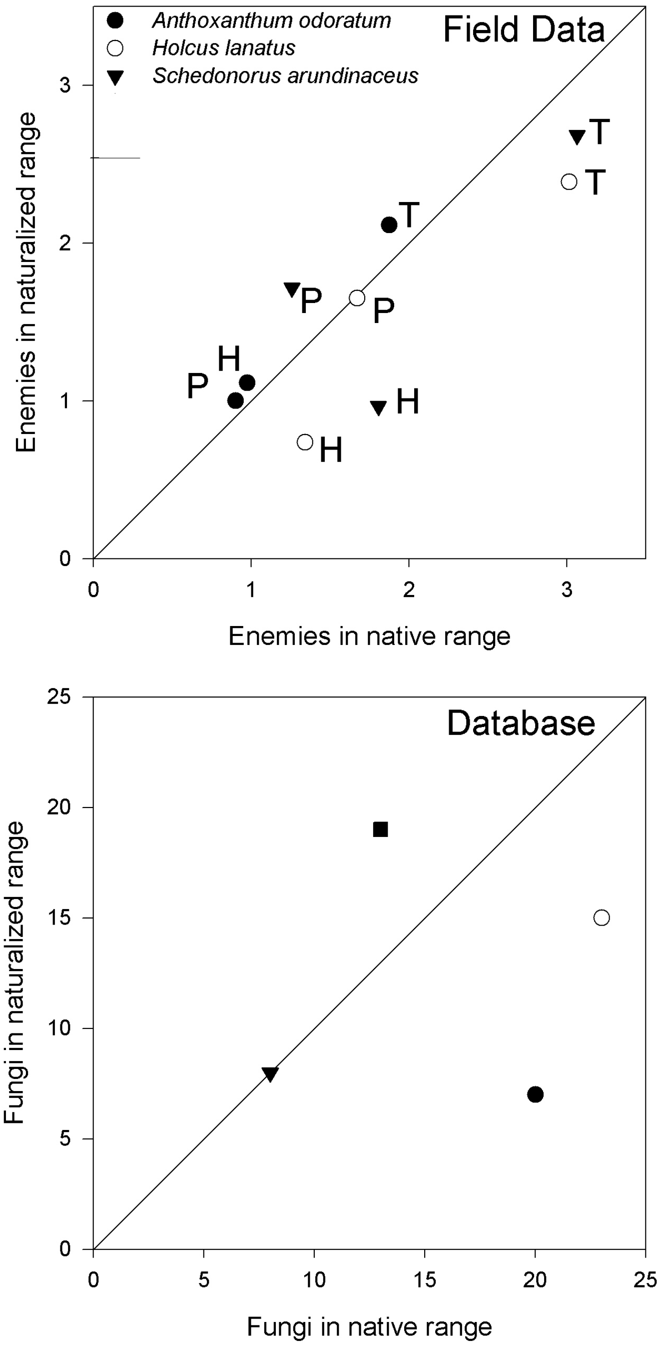

Figure 5.4. (a) Results of field survey: axes represent number of types of symptoms per plant. H = Herbivores, P = Pathogens, T = Total. (b) Results of data compilation from literature survey: axes represent total number of rusts, smuts, and mildews found on each plant species in each range, except that the dark square represents the number of other pathogens on *A. odoratum* besides rusts, smuts, and mildews not considered by Mitchell and Power's study. The diagonal lines across each graph represent the one to one ratio for which the number of enemies in both ranges would be equal.

Discussion

We found a general trend of reduced enemy attack and increased per plant biomass in the naturalized range versus the native range that is consistent with enemy release, especially from herbivores, of the three grasses that we studied. On *Holcus lanatus* and *Schedonorus arundinaceus,* there was less percent herbivore damage per leaf in the naturalized range, while *Anthoxantum odoratum* showed no clear difference. However, *A. odoratum* and *H. lanatus* both had significantly less whole plant percent damage in the naturalized than in the native range, and *S. arundinaceus* followed the same pattern. Shoot biomass of *A. odoratum* was significantly higher in the naturalized range, and the other two grass species followed the same trend. We found no clear evidence that foliar pathogen attack on any of the three grass species differed between the two ranges.

Our finding of release from herbivores may be due to many contributing factors. Loss of one or more specialist herbivores in the plants' native range upon introduction, which is an assumption of enemy release, could explain this pattern. Generalist herbivores already present in the naturalized range would account for the remaining herbivore attack. Another possible explanation is that associations between the grasses and beneficial mutualists may differ between the two ranges. *Schedonorus arundinaceus* is known to be commonly infected with endophytes in the genus *Neotyphodium,* which could act as a deterrent against herbivores (David P. Belesky and Charles W. Bacon 2009). Williams and others tested for both the effects of small-scale disturbance and enemy release, and found that each factor contributed to the invasiveness of *Cynoglossum officinale* (houndstongue) in its naturalized range. Selection and genetic drift that occur during the introduction of species into a new range could lead to either an increase or decrease in resistance to natural enemy attack. Other variables that could influence invasions currently under debate include enemy spillover (Power and Mitchell 2004, Malmstrom et al. 2005, 2007, Beckstead et al. 2010), enemy dilution, allelopathy, disruption of mutualistic relationships (Antunes et al. 2008, Thieltges et al. 2009), phylogenetic relatedness of native to introduced species (Strauss et al. 2006), resource competition (Funk and Vitousek 2007), and the pre-invaded productivity (Davies et al. 2007), diversity (Harrison et al. 2006, Crutsinger et al. 2008), or species composition (Emery and Gross 2007) of a given native community. Our findings could potentially be due to dilution of enemy attack by the novel plant community encountered in the naturalized range relative to that in the native range. Blaisdell (2011- Chapter 4) found evidence of dilution of natural enemy attack on both *H. lanatus* and *S. arundinceus* by co-occurring native grass species. More studies are needed that simultaneously test enemy release and other competing hypotheses (Liu and Stiling 2006).

While we found evidence of enemy release from herbivores, we found no differences in pathogen attack between the native and naturalized ranges. This may be due to a lack of specialist pathogens present on these three grass species relative to the herbivores. If predominately generalist pathogens were attacking the plants in both ranges, this could lead to equal attack rates in both ranges.

Interestingly, the findings of Mitchell and Power (2003) and Van Kleunen and Fischer (2009) in support of enemy release from pathogens based on their compilations differed from our findings with respect to pathogen attack based on our field survey. With respect to the identity of pathogens considered, our field survey was more general than Mitchell and Power’s data compilation (2003), which may contribute to our differences in findings. Mitchell and Power’s study included only rusts, smuts, and mildews, while we considered all fungal and herbivore symptoms that we found in the field. Many smut fungi are seed transmitted (Neergaard 1977), but rusts and mildews are not as commonly transmitted by seed. Because many plant introductions occur via seed, this selection of a subset of pathogens that are not commonly seed-borne may have led to a bias in Mitchell and Power’s (2003) and van Kleunen and Fischer’s (2009) findings of reduced pathogen species richness in the plants’ naturalized range. Van Kleunen and Fischer (2009) found that the pathogens lost upon introduction tended to be relatively rare pathogens, which could have led to a weak relationship between pathogen species richness and population fitness effects.

Many methods of testing enemy release have been used, but for the most part these multiple approaches have not been combined into any one study. Future studies concerning biological invasions should carefully choose their method or methods of testing enemy release, but should also test other hypotheses concerning invasions with respect to the role of natural enemies, mutualisms, competition, and other potential mechanisms of invasion.

Our comparison of natural enemy attack on populations of three species in their native and naturalized ranges as measured in the field to a literature compilation yielded conflicting results; our field survey found support for release from herbivores but not pathogens, while the literature compilation found evidence of enemy release from pathogens. The strength of the literature compilation is that primary information was gathered over many decades, using the resources of many scientists. The strengths of our field survey are its empirical nature, consideration of the fitness effects, and the study of a broader taxonomic range of fungi. A plant may be released from a number of rare natural enemies that would all carry equal weight based on the literature compilation, but that would not matter in the field if that plant did not escape from the one or few natural enemies that cause major damage and are widespread.

CHAPTER VI

SUMMARY, SYNTHESIS, AND IMPLICATIONS OF RESULTS

In his book about the ecology of species invasions (1958), Charles Elton provided a collection of examples of species invasions and potential explanations for mechanisms of those invasions. His book is the most cited source in invasion ecology literature, and its citation frequency has been increasing each year since it was published (Richardson and Pyšek 2008), which indicates that the discipline of invasion ecology is growing. Some topics that Elton discussed, such as dispersal and spread of invasive species, their impact on biodiversity, and the role that disturbance and enemy release have with respect to invasions, are still being studied today. More recently in invasion ecology, there has been a stronger emphasis on using multiple methods to simultaneously test multiple explanations for species invasions (Liu and Stiling 2006), and to examine species invasions at multiple geographical scales (Byers and Noonburg 2003, Fridley et al. 2007). In my research, I used observational studies at very different geographical scales, a manipulated field experiment, and compilation of existing data about pathogens in their hosts' native and naturalized ranges to test enemy release, spillover, and dilution, as well as other potential variables related to species invasions.

Summary of results

First, examination of a variety of community variables (Chapter II) and their associations with enemy attack on several native plant species in the community showed that (i) plant diversity had a weak but consistent negative correlation with pathogen attack across the host community, (ii) there was no relationship between enemy attack and the individual plant traits shoot biomass or chlorophyll content, and (iii) the strongest effects of community characteristics on attack rates varied among plant species with no strong community-wide patterns, suggesting that no single hypothesis, such as spillover, dilution, total plant cover, or litter abundance, successfully predicted attack rates across this community. We did not find a strong effect of site preparation technique in the restoration experiment on natural enemy attack.

Second, in a comparison of natural enemy attack on the most common introduced grass species (Chapter III) to the two most common native grass species, we learned that, contrary to predictions of enemy release, there was significantly more pathogen attack on the introduced species than on the two native species. Again, we found no strong effect of site preparation technique on natural enemy attack.

Third, using experimental communities of six common native and introduced perennial bunchgrasses (Chapter IV), we found no evidence of enemy spillover from the introduced species, but instead we found evidence of dilution of natural enemies by the native species on the introduced species. This dilution effect becomebecame stronger over the course of three growing seasons. While enemy attack on two of the three introduced species was significantly lower in the treatments with native species, abundance of those introduced species did not increase in the treatments with less enemy attack, indicating that resource competition instead may have been the dominant force determining abundance of all six species.

Finally, from our comparison of natural enemy attack on the same three introduced grasses used in our experimental communities (Chapter V), in both their native and naturalize ranges, we found evidence of enemy release from herbivore attack for two of the three grass species, but not pathogen attack. In contrast to the field survey, a literature survey of pathogens on the same three grass species found evidence of release from pathogens for two of the three grass species.

Synthesis and broader context

Natural enemies have been found to alter plant community structure by differentially affecting the fitness of different host species. In our experimental communities, we found some evidence of a reduction in abundance of two out of three introduced grass species by natural enemies (Chapter IV), but the other four species seemed to be affected more strongly by competition. In our biogeographical survey (Chapter V), one of the three grass species that we surveyed had significantly higher shoot biomass in its naturalized range relative to the native range, while the other two followed the same suggestive pattern. Additionally, populations of two grass species in that survey had higher density in their naturalized range than in their native range, and the third followed a similar pattern. This increase in individual plant size and population density could be a response to reduced natural enemy attack. Further studies, perhaps manipulating enemy attack using exclosures or pesticides, are needed to tease apart the effects of competition and enemy attack on these grasses.

Pathogen spillover from introduced to native grasses has been detected in grassland communities, (Malmstrom et al. 2005, Borer et al. 2007, Beckstead et al. 2010) and is thought to be a potential driver of invasions by those introduced species. Our study is the first to compare enemy attack on native and introduced grasses that share a similar life history (Chapter IV), and we did not find clear evidence of enemy spillover from the introduced to native grass species. Instead, in our experimental communities of native and introduced grasses, we found stronger evidence of dilution of enemy attack on the introduced species by the native species. The results from our biogeographical survey (Chapter V) of reduced herbivore attack in the plants' naturalized range could be due either to enemy release, or to this same dilution effect, as presumably the plants exist in a different plant community context in their naturalized range from that of their native range. Some might argue that dilution is equivalent to enemy release, but occurs at a much smaller scale.

Our findings of evidence for enemy release from herbivores or a dilution effect exemplify the necessity to simultaneously test multiple hypotheses in invasion ecology. If we had performed only the biogeographical survey without the manipulated field experiment, we may not have thought to consider dilution as a possible mechanism of species invasions.

The need for multiple approaches to testing enemy release or other mechanisms of species invasions is illustrated by the difference of findings between our field surveys and a literature survey. The results from our survey of enemy attack on three common native and introduced grasses in a restoration experiment (Chapter III) and from our biogeographical survey (Chapter V) were not consistent with enemy release from pathogens. Results from compilations of host indices, however, did point to enemy release of pathogens. Yet, the correlation between actual rates of enemy attack and enemy richness is not particularly strong, which may be a limitation of approach. host compilations Other approaches, such as a manipulative study, should be used to corroborate or refute our finding of enemy release from herbivores in our biogegraphical survey (Chapter V).

Invasion ecology, which is a growing discipline, is often applied to restoration and management of plant communities. Practitioners and researchers alike often consider only the effects of competition against invading species, but in many cases other factors may drive the success or failure of native plant community restoration. Here we have considered various associations that herbivores and pathogens may have with species invasions. We found weak evidence that enemy spillover was a factor in shaping the grassland communities in our studies. Our results point to a stronger role of enemy release and dilution of natural enemies on introduced plant species as potential players in community invasions.

APPENDIX

SUPPLEMENTAL FIGURE AND TABLE

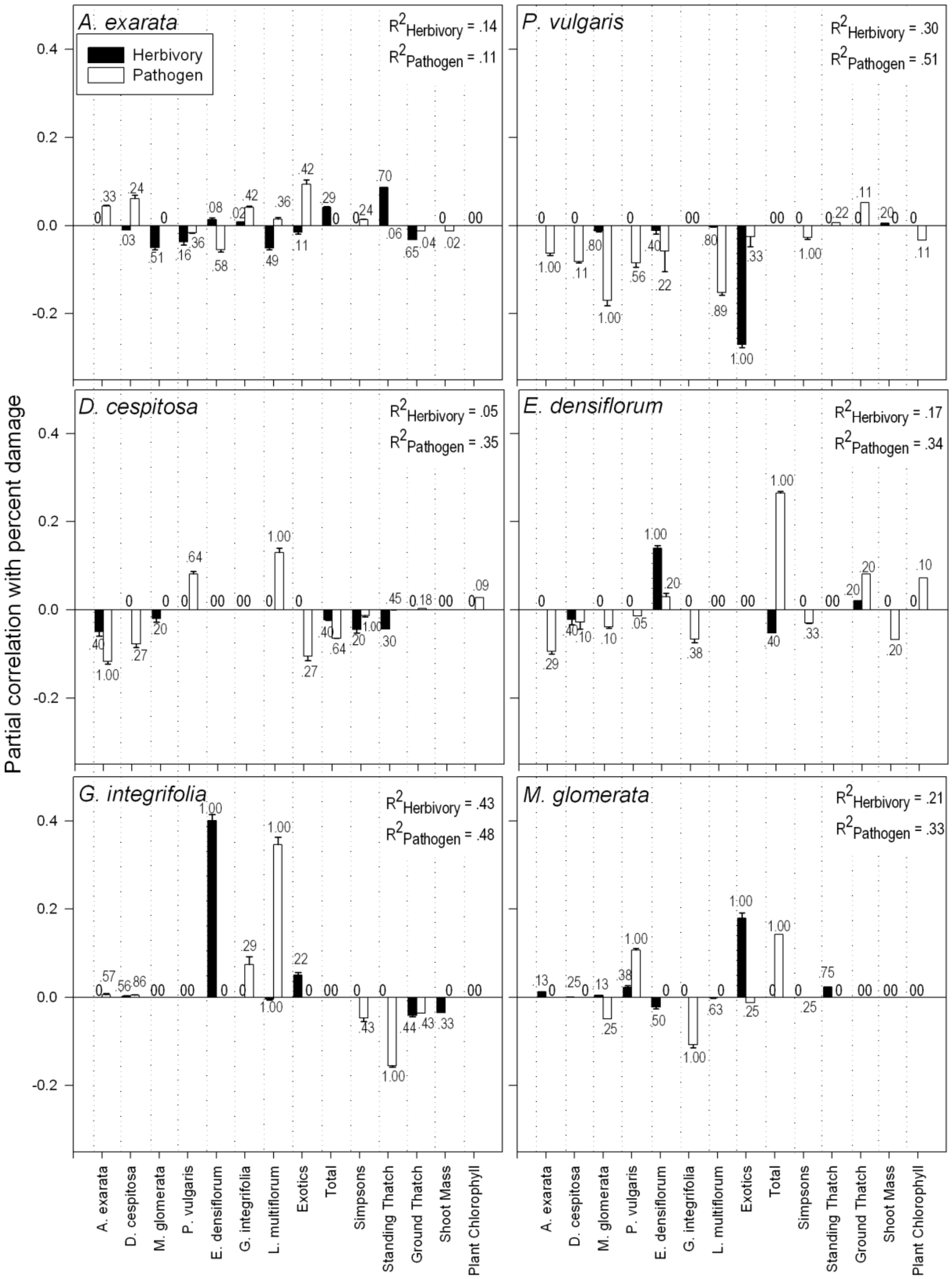

Figure A.1. Detailed presentation of all results of AIC multi-model inference analysis: Average partial correlations from multiple regression models using AIC of 14 predictor variables regressed against percent herbivore and pathogen damage. Error bars (standard error) are shown among partial correlations of each variable across all models in which that variable was selected. Numbers above each bar represent the proportion of models in which that variable was selected. Error bars represent variation in magnitude of partial correlation among selected models.

Table A.1. List of sites sampled and geographic information

Site name	**Range**	**State**	*A odoratum*	*H lanatus*	*S arundinaceus*	**Date**	**Lat**	**Long**	**Elev (m)**	**Comm biomass (g/m2)**
Acqua Rossa	EU[1]	Switzerland	1	1	0	24-Jul-2008	46.467	8.921	886	260
Bayreuth	EU	Germany	1	1	0	17-Jul-2008	49.922	11.583	351	389
Crooked Creek	US	Wisconsin	0	0	1	8-Aug-2008	42.833	-88.477	276	394
Flaach	EU	Switzerland	0	0	1	8-Jul-2008	47.589	8.607	347	778
Gais	EU	Switzerland	1	1	0	9-Jul-2008	47.380	9.484	1137	298
Glacial Heritage	US	Washington	1	1	0	5-Jun-2008	46.866	-123.049	43	137
Hertogenbosch	EU	Netherlands	1	1	0	22-Jul-2008	51.673	5.308	1	485
Hirzel	EU	Switzerland	1	1	1	10-Jul-2008	47.217	8.625	665	310
Innsbruck	EU	Austria	1	1	0	28-Jul-2008	47.214	11.376	938	429
Laufamholz	EU	Germany	0	1	0	16-Jul-2008	49.461	11.184	325	99
Mima Mounds	US	Washington	1	1	0	5-Jun-2008	46.891	-123.051	70	188
Morgan Property	US	Washington	0	1	0	4-Jun-2008	46.900	-122.732	138	109

Mount Pisgah	US	Oregon	1	1	1	29-May-2008	43.993	-122.951	175	295
Carrboro	US	North Carolina	1	0	1	12-Jun-2008	35.913	-79.056	148	107
Pigeon Butte	US	Oregon	0	1	1	22-May-2008	44.390	-123.323	83	570
Postalm	US	Austria	1	0	0	26-Jul-2008	47.661	13.434	1300	61
Rumlang	EU	Switzerland	1	1	1	15-Jul-2008	47.444	8.520	494	383
South Weir	US	Washington	1	1	0	4-Jun-2008	46.904	-122.735	160	93
Triangle Prairie	US	Washington	0	1	1	5-Jun-2008	47.013	-122.429	134	228
Whipple Farm	US	New York	1	0	0	25-Jun-2008	42.492	-76.429	519	364
Willow Corner	US	Oregon	1	1	1	26-Jun-2008	44.037	-123.166	125	233

[1] Note that EU does not refer to the European Union, but is simply an abbreviation for Europe. *A odoratum = Anthoxanthum odoratum, H lanatus = Holcus lanatus, S arundinaceus = Schedonorus arundinaceus,* Lat = Latitude, Long = Longitude, Elev = Elevation, Comm biomass = Community biomass.

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