Invasion consequences and ecology: evaluation of community and environment interactions of an exotic, invasive plant, garlic mustard (*Alliaria petiolata*)

A thesis

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Tegan A.L. Morton

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ABSTRACT

Globally, exotic, invasive species are the second leading cause of native species decline. I aimed in this thesis to explore first the resource ecology of a "strong invader" of New England forests, the exotic weed garlic mustard (*Alliaria petiolata*). Particularly, I aimed to understand to what extent performance differences among populations were explained by limitations imposed by soil nutrient abundance and availability. Secondly, I aimed to understand the potential of a native herbivore (*Pieris napi oleracea*) to adapt to community disturbances following garlic mustard and exotic parasitoid invasion to escape an evolutionary trap. Specifically, I focused on the adaptation of larvae to complete development to pupation on the novel host plant that has previously caused population decline.

With regards to the first aim, I surveyed several populations of garlic mustard from a variety of habitats in Massachusetts and measured a variety of variables related to soil nutrient availability and plant performance. Overall, soil variables have limited explanatory and predictive power with regards to plant performance. There is some evidence of stronger relative effects of soil pH and potassium availability on performance, however it does not resolve much of the variation among populations. It is likely that other environmental factors, such as light or soil moisture, impose greater limits on performance.

To address the second aim, I modified an existing stochastic simulation model to generate a subpopulation of adapted individuals. I applied treatments addressing two issues: (1) the source of genetic variation for the adaptive trait,

and (2) the role of top-down regulation by exotic parasitoids in limiting adaptation. Results show that even under high rates of mutation, persistence of adapted individuals is low, and any adaptation observed in natural populations is likely due to residual variation. Secondly, parasitism significantly decreased the likelihood of adaptation, and persistence of adapted individuals was possible at substantial levels only under conditions of enemy free space.

Cumulatively, my research shows that for garlic mustard, the abiotic soil environment imposes little limitations on performance and likely this plant can successfully colonize a variety of environments, provided there is a suitable availability of light and water. When it comes to understanding the effect of such colonization and invasion on native community members, my research shows that the community context, particularly the third trophic level, influences greatly predictions for native species persistence and adaptation.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER ONE

Introduction: Global Change & Exotic, Invasive Species

The world is currently undergoing a period of rapid, human-induced changes to the climate, ecosystem nutrient and hydrological cycling, and biodiversity abundance, composition, and distributions (Vitousek et al. 1997). An unprecedented number of species are transported intentionally or unintentionally to completely new regions, where they have the opportunity to flourish in environments in which they are free of coevolved predators, pests and pathogens (i.e., Enemy Release Hypothesis, Keane & Crawley 2002), and are released from competitive pressure, allowing evolutionary changes to resource allocation (i.e., Evolution of Increased Competitive Ability Hypothesis, Blossey & Notzold 1995). Exotic species are also often freed from co-evolved defenses of competitors and prey (i.e., Novel Weapons Hypothesis, Callaway & Aschehoug 2000), and likely able to reproduce with previously genetically isolated members of the same species (Dlugosch & Parker 2008). Those exotic species that have an advantageous combination of pre-evolved traits, conditions conducive to rapid adaptive evolution following establishment, and/or favorable recipient community conditions, can increase in abundance, and disperse to adjacent or further removed areas. In doing so, often these species disrupt native community interactions, and directly or indirectly, negatively affect native community members, thus becoming invasive species. Globally, exotic, invasive species are

the second highest contributor, after habitat loss, to biodiversity decline (Wilcove et al. 1998).

Only a portion of the total exotic species introduced to a region surpass multiple barriers to become invasive (Williamson & Fritter 1996). Many exotic species are less than perfect habitat generalists, and during increases in abundance and spread, many encounter unsuitable environments for growth and reproduction. Often the "lag time" between establishment and spread accounts for some of a species' ability to overcome such inhospitable environments through adaptive evolution to such environments (Sakai et al. 2001). In other cases, to some extent the challenges posed by inhospitable environments to population growth can be overcome by propagule pressure (Lockwood et al. 2005), however, many of those that can invade following a flood of introduced individuals, are relatively "weak" invaders and become only minor components of the recipient communities (Ortega & Pearson 2005). Those that are "strong" invaders do not necessarily show rapid post-invasion adaptive evolution, (such as *Silene latifolia*, Blair & Wolfe 2004) but rather have pre-adapted traits that are particularly advantageous in that environment (e.g., *Senecio inaequidens*, Bossdorf et al. 2008; *Prunus serotina*, Closset-Kopp et al. 2011), or strong phenotypic plasticity for important characters and performance (e.g., Funk 2008). Understanding the limits posed by environments to exotic species performance and population growth can provide insight into how these invasive species become dominants in their recipient communities.

Those exotic species that do surpass barriers to become "strong" invaders (Ortega & Pearson 2005) cause native species decline by assuming a variety of roles in their recipient environments, including acting as predators, pathogens, competitors and hosts (Carroll 2007). Additionally, exotic invasives may cause native decline by acting as evolutionary traps (Schlaepfer et al. 2005). Broadly, an evolutionary trap is when previously reliable cues used by an organism to increase fitness are disrupted, instead creating a population sink (Schlaepfer et al. 2002). Unless the native species can respond positively to the selection pressure (Mooney & Cleland 2001), evolutionary traps can result in local extinction. When faced with a novel selection pressure, how can native species escape from potential extinction vortices caused by evolutionary traps? What types of community conditions allow for adaptations in response to such traps?

I aimed in this thesis to answer questions related to (1) performance of an exotic, invasive species, following establishment and invasion, across a heterogeneous landscape, and (2) the conditions allowing adaptive evolution by a native in response to an exotic, invasive species acting as an evolutionary trap.

Study systems & research goals

Garlic mustard (*Alliaria petiolata*) is an obligate biennial introduced from Eurasia to North America in the 1880s. Garlic mustard is assumed to be a strong competitor that is responsible for displacement of native herbaceous species (Anderson, Kelley & Dhillion 1996). Garlic mustard has been shown to decrease mycorrhizal fungi community abundance (Wolfe et al. 2008) and diversity

(Rodgers et al. 2008b), contributing specifically to native tree seedling decline (Meekins & McCarthy 1999, Stinson et al. 2006). Allelopathy, as well as unique chemical compounds discussed above, contribute to a release from predators and pathogens in the invasive range (Rodgers et al. 2008a).

Furthermore, garlic mustard is unique compared to many exotic, invasive plants in its ability to invade sites without a disturbance, facilitating establishment in diverse habitat types (Meekins & McCarthy 2001). Moreover, Rodgers and colleagues (2008b) documented significant changes to the nutrient cycling and availability in sites invaded by garlic mustard, suggesting plant invasion facilitates further site occupancy. The soil environment, or the nutrients and factors affecting availabilities, has been suggested to cause variation in secondary chemistry (Cipollini 2002), and overall garlic mustard has a high degree of phenotypic plasticity in response to soil nutrients, as well as water availability (Hillstrom & Cipollini 2011). Nutrient increases in manipulative experiments have shown to increase plant performance (Meekins & McCarthy 2000, Hyatt & Hewins 2010), however little research has investigated the effect of field soil environment conditions on garlic mustard performance. I aimed, using field research, to study how, *in situ*, soil nutrients and factors controlling their availabilities, predicted plant performance following invasion (Chapter 2). Bossdorf et al. (2004b) suggested garlic mustard has undergone evolution of reduced competitive ability in the invasive range, and shifting from resource competition to growth and reproduction, has conferred greater benefits. However, with reduced resource competitive ability, this suggests garlic mustard, in the

monospecific stands which develop following invasion, may experience performance costs associated with the soil environment and intraspecific resources competition.

Once established, garlic mustard acts an evolutionary trap (Keeler et al. 2006, Keeler & Chew 2008) for the native North American mustard white butterfly (*Piers napi oleracea* Harris), which is a member of the Holarctic *P. napi* L. species complex. Documented decline of *P. n. oleracea* has occurred since the 1880s (Scudder 1889, Longstaff 1912, Klots 1951, Opler and Krizek 1984, unpubl. data from Massachusetts Natural Heritage and Endangered Species Program) and several hypotheses have been advanced for the apparent decline. Competition with the introduced exotic butterfly *P. rapae* was proposed (Scudder 1889) but ultimately rejected, and instead it has been suggested that land use changes decreasing habitat, and specifically host plant availability, have driven the decline (Chew 1981). Benson and colleagues (2003b) instead suggested that introduction of an exotic parasitoid *Cotesia glomerata* has contributed to the decline. More recently, the exotic weed garlic mustard (*Alliara petiolata* M. Bieb, Cavara & Grande) has been shown to be an evolutionary trap for this native butterfly and likely contributes to decline (Keeler et al. 2006). Specifically, chemical similarity to native host plants stimulate oviposition in females (Feeny & Rosenberry 1982, Huang et al. 1995, Renwick 2002), but larvae survive poorly and develop slowly on this exotic, invasive plant (Courant et al. 1994, Huang, Renwick & Chew 1995, Keeler & Chew 2008) due to at least two feeding deterrents (Renwick et al. 2001), though also possibly due to the existence of high

levels of endogenous cyanogenic compounds in the leaves (Cipollini & Gruner 2007).

Recently, Keeler and colleagues (2006) evaluated, using a stochastic simulation model, the potential effect of garlic mustard invasion, coupled with decreasing host plant availability, as well as parasitoid top-down regulation on population persistence of the butterfly. Their results suggest that garlic mustard invasion and habitat destruction most likely explain the decline of this species. Predation by the parasitoid does not in, and of itself, cause extinction, but can exacerbate garlic mustard invasion and cause extinction at lower levels of ground cover (Keeler et al 2006).

Interestingly, *Pieris napi oleracea* presents intriguing evidence of a species climbing out of an evolutionary trap (Courant et al. 1994, Keeler & Chew 2008, R.A. Steward & F.S. Chew unpublished data). Larvae have recently shown in laboratory rearing experiments, to sometimes complete development to pupation on garlic mustard, and there are significant effects of maternal families, suggesting it is a hereditary trait (Courant et al. 1994, Keeler & Chew 2008). If sufficient variation exists, we expect selection to favor (1) increased larval performance, or (2) possibly decreased oviposition preference as (Keeler 2005), though evidence links increased larval performance to increased oviposition preference (Keeler & Chew 2008).

I aimed to determine, using a stochastic simulation model, ecological variables that would promote or inhibit adaptation of increased larval performance, determined as the increase in the number of adapted individuals in

the population (Chapter 3). I evaluated the likely way in which the trait appeared in the populations, either as a spontaneous mutation within a maternal family, or as residual neutral variation due to dissected polymorphism. Furthermore, I aimed to evaluate the possible effects of the presence and absence of top-down regulation on the proliferation of adapted individuals within the population.

CHAPTER TWO

Performance of the exotic weed garlic mustard (*Alliaria petiolata*) across a heterogeneous soil landscape

Introduction

Many exotic species become invasive when they colonize and become established in nutrient-rich, disturbed areas. Whether this is due to environmental conditions or to traits of the species is debated. The Fluctuating Resource Availability hypothesis suggests increases in nutrient availability due to disturbance explain the invasion of species-rich habitats by weak competitors (Davis et al. 2000). Alternatively, exotic species may be those that are broadly tolerant of their environments (i.e. Jack of All Trades strategy, Richards et al. 2006) and approach the niche of an "ideal weed" (Baker 1965). These species have an advantage across a wide range of abiotic conditions, which facilitates establishment and population growth.

Though many species are able to invade ecosystems by capitalizing on increases in resource availability, as suggested by Davis and colleagues (2000), the exotic weed garlic mustard (*Alliaria petiolata*, M.Bieb, Cavara & Grande, Brassicaceae), introduced from Eurasia in the 1800s, is unusual for its ability to invade undisturbed forest understories (Meekins & McCarthy 2001). While there is evidence that garlic mustard performance is limited by at least some soil nutrients, (e.g., nitrogen, Hewins & Hyatt 2010), whether soil nutrients are a significant barrier to invasion is unclear. In fact, Rodgers et al. (2008b) found

that garlic mustard can manipulate soil pH and nutrient cycling, increase soil nutrient availability and thereby increase site occupancy, and Stinson et al. (2006) suggests that alleopathy reduces competitive pressure, with native tree seedlings (presumably increasing access to nutrients). Light does not appear to be an important constraint to garlic mustard. Myers et al. (2005) suggest that garlic mustard thrives in shady forest understories due to its photosynthetic plasticity and non-overlapping early spring phenology, in which the majority of photosynthesis occurs prior to canopy closure in the early spring (Myers & Anderson 2003). Given such patterns in the invaded range, it is surprising that in the native range garlic mustard is restricted to small populations (Blossey et al. 2001), often in forests edges or adjacent to rivers (Grime et al. 1988), suggesting reliance on disturbance.

Cumulatively, these results suggest garlic mustard is tolerant of a wide range of conditions, yet little research has evaluated the effect of the soil nutrient environment specifically. Because garlic mustard is non-mycorrhizal (Rodgers et al. 2008a), we predicted that phosphorus abundance and availability would be the most limiting to performance and explain the most variation in performance among populations. In this study, we aimed to investigate the effect of the soil nutrient environment, including both nutrient abundance and factors controlling its availability, on garlic mustard performance. To do so, we surveyed forest understory populations in Massachusetts, and analyses focused on both determining to what extent soil resources explain variation in performance among populations, as well determining which resources provide explanatory power of

plant performance. Previous research indicates that secondary chemistry varies among populations, possibly as a function of soil environments (Cipollini 2002), and we hypothesized that though garlic mustard is able to invade nutrient poor understories, performance would vary as a result of variation in soil conditions, with specific limitations caused by phosphorus deficiencies.

Methods

Field site characterization

Seven well established populations of garlic mustard in Massachusetts, located under moderate to full canopy cover, were identified. Each contained a mostly monospecific stand of garlic mustard of at least 10 m^2 in area. Sites differ in a variety of soil characteristics including bedrock, expected pH, cation exchange capacity (CEC), sodium absorption ratio (SAR) and salinity, as well as common tree species (Table 2.1). Furthermore, geographically these sites represented a variety of habitats in Massachusetts including primary forest (HMF and HMF2), forest communities that were formerly cultivated (FF) coastal habitat upland of a beach (CB), swamp forest inland of a beach (CC), and urban preservation areas (AA and FE).

Transect sampling

Within each population, two 10 m transects were laid within portions of the population that had 1 m^2 of monospecific ground cover on both sides of the measuring tape. At three intervals along each transect, at 0, 5, and 10 m, a photo of the canopy was taken from approximately 0.5 m above groundlevel. Photos

were analyzed using ImageJ to determine percent canopy cover. At 1 m intervals along the tape, for 10 intervals, the plant closest to the point was sampled, as well as the soil directly below the individual. The soil sample consisted of approximately 1 kg from the top soil layer.

Sampling occurred in two separate years, with transects laid in the same approximate locations within each population. In middle-late August of 2010, soil and rosette plants were sampled. In early-mid June of 2011, bolting plants were sampled prior to senescence, but after flowering and fruit maturation; no soil samples were taken in 2011. Two field sites (HMF2 and FE) were sampled for soil, but not for plants due to temporary population extinction (discussed below) during the year of rosette sampling (2010); only soil data from these sites are presented.

Sample analysis

Soil samples were analyzed for both nutrient abundance and factors controlling soil nutrient availability. Soil pH was measured in a 1:10 soil:water solution with an acid-calibrated pH meter (Hendershot et al. 1993a). Available nitrogen (NO_3^- and NH_4^+) and phosphate were extracted in 2 M KCl (Maynard & Kalra 1993). Cations (K^+, Ca^{2+}, Mg^{2+}, Mn^{2+}, Al^{3+}, and Na^+) were extracted in 2 M $BaCl_2$ (Hendershot et al. 1993b). Total sulfur (SO_3^- and organic sulfur) was extracted in 0.016 M K_2HPO_4 (Zhao & McGrath 1994). Nitrogen extracts were quantified using a Lachat autoanalyzer; phosphorus, cations and total sulfur were quantified using ICP-AES. The sodium absorption ratio (SAR) (Brady & Weil 2004) and cation exchange capacity (CEC) (Ross 1995) were calculated following

quantification of cations.

Individual plants were separated into above- and below-ground sections and weighed separately. Frozen fresh mass was recorded. Siliques and associated stems of bolting individuals were also separated and weighed. The number and lengths of siliques were quantified.

Data analysis

All data were non-normal and transformation did not improve homoscedasticity. Therefore, differences among sites in canopy cover, soil characteristics and plant traits were analyzed using Kruskal-Wallis rank sum tests, followed by pairwise Wilcoxon comparisons.

To determine if differences among sites in soil and plant traits resolved as emergent site and population characteristics and groups, cluster and principal component analysis (PCA) were performed. PCA also aimed to determine which soil characteristics are associated with plant traits. All of the variables which were compared among sites were used in cluster analysis and PCA.

To determine any explanation of variation in rosette biomass (response variable) due to specific individual soil variables (predictors), linear regressions were used. Single linear regressions, as well as a series of multiple linear regressions, were performed in order to determine significant predictors working both singly and in tandem, as well as variable interactions. For multiple regression, all possible models were considered, including single variables and all two-way interactions, and competing models are reported. Only rosette biomass was considered in the regression models due to lack of predictive relationship

between rosette and bolting biomass (r^2 = -0.004, P=0.4553), in addition to controlling for the possibility of variation in soil processes, as affected by weather differences, between years and during seasons significantly affecting soil nutrient cycling and availability (Gil-Sores et al. 2005). All statistical analyses were performed using JMP (version 9.0.2, The SAS Institute).

Results

Sites did not significantly differ in canopy cover (Kruskal-Wallis rank sums, χ^2 = 8.46, df = 4, P = 0.0761). The sites did differ significantly in median soil (Table 2.2) and plant (Table 2.3) performance values (Kruskal-Wallis rank sums at $P \leq 0.05$). Coefficients of variation indicate that soil pH and iron were the least variable, whereas other soil factors showed a larger degree of variation (Table 2.2). For plant traits, rosette biomass showed lower variation than bolting biomass or the average number of siliques per plant (Table 2.3).

Sites resolve into distinct clusters for soil data (Figure 2.1), however, there were no emergent population groupings delineated for plant traits (Figure 2.1).

Using both soil and plant data, we found that the sites segregated into separate character space in principal component analysis, with significant overlap of three sites, and more distinct clustering for the remaining two sites (Figure 2.2). Plant performance variables are positively associated with soil pH, calcium, potassium and CEC according to PCA (Figure 2.3). Principal components 1 and 2 account for 42% (25.6 and 16.4 % respectively) of the total variation across sites (Figures 2.2 & 2.3).

Soil variables associated with plant traits in PCA positively correlate with

rosette biomass in individual linear regressions (Table 2.4), however these correlations explain only a small portion (<15%) of the variation (adjusted r^2 values, Table 2.4). Many of the significantly associated soil variables are significantly correlated with each other (Table 2.5), indicating possible non-independent effects.

Multiple regression analysis yielded several competing models (Table 2.6). The only consistent predictor of rosette biomass (i.e., in all competing models) is potassium. Soil pH, magnesium, aluminum, CEC, and SAR were significant predictors in the majority of models. Predictors unique to only one of the competing models were sulfur and nitrate. Significant two-way interactions vary by model (Table 2.6) and likely reflect correlations among soil variables (Table 2.5). Overall, the amount of variation in rosette biomass explained by soil variables in any of the competing models was less than 40% (Table 2.6). Surprisingly, variables significantly, positively correlated with rosette biomass (Table 2.4) did not always appear in competing, multiple regression models (Table 2.6). Additionally those variables that do significantly, positively predict rosette biomass (Table 2.4) are not necessarily positively associated in multiple regression models (e.g., soil pH, Table 2.6).

Discussion

We found significant differences in soil nutrient availability, and factors controlling such availability, across the seven sites. While the five populations of garlic mustard considered differed significantly in plant performance, contrary to our expectations, individual plant data points did not mirror soil assemblages in

the cluster analysis, suggesting a limited role of the soil environment in individual performance. However, plant variables aligned with a subset of variables in principle component analysis, and such associations were substantiated with both single and multiple regression models. Overall, the explanatory effect of soil variables is less than 40%, suggesting primary determinants of plant performance are soil moisture (Meekins & McCarthy 2001, Hillstrom & Cipollini 2011; see below), and possibly light (Myers & Anderson 2003, Myers et al. 2005) and that soil nutrients are secondary in effect.

Contrary to our predictions, phosphorus is not a significant predictor of garlic mustard performance. Garlic mustard is non-mycorrhizal (Rodgers et al. 2008a) and decreases the abundance of mycorrhizal fungi in the soil (Stinson et al. 2006, Wolfe et al. 2008), and thus phosphorus availability to competitors, suggesting this plant is phosphorus limited. However, Elk (2010) documented root phosphatase activity of garlic mustard as the highest of all plants considered in the study, suggesting that garlic mustard accesses organic and insoluble pools of phosphorus through high enzymatic activity. It is possible this high enzymatic activity compensates for the lack of mycorrhizal symbioses, or low inorganic phosphorus availability in general. Phosphorus values represent a conservative estimate of availability due to challenges associated with extraction and quantification of available phosphorus. It is possible that with more comprehensive data this variable could be a significant predictor of plant performance.

Of the three major macronutrients, potassium is the most consistently

significant predictor of plant performance across all multiple regression models. In general, potassium nutrition is linked to the tolerance of environmental stresses, and improves winter hardiness, resistance to fungal pathogens, and increases tolerance of insect pests (Brady & Weil 2004). Rosette mortality is a large determinant of garlic mustard demography (Davis et al. 2006), and improving over-wintering survival of rosettes could be an important effect on population persistence. Cation exchange capacity (CEC) is a reflection of, in part, potassium availability (Ross 1995), and is greater in less acidic soils. In acidic soils, potassium, along with calcium and magnesium, are readily leached from the soil, and potassium is more readily lost in leaching than phosphorus (Brady & Weil 2004).

Soil pH has the highest explanatory power of any single regression. Furthermore, it also appears in three of the four competing multiple regression models, though it is negatively associated with rosette performance in these models. This could possibly be driven by the negative pH by magnesium interaction effect. As noted above, magnesium is negatively associated with rosette biomass, which could itself be driving the interaction, or that magnesium buffers the pH above approximately 6.5 (Brady & Weil 2004), which could potentially also limit plant growth as it falls outside the "ideal" soil pH range (5.5-6.5) for plant growth. Soil pH is possibly the most limiting soil factor to nutrient availability, and by extension, garlic mustard performance; Anderson and Kelley (1995) documented evidence of a lower soil acidity threshold, below which garlic mustard does not grow. Rodgers and colleagues (2008b) documented significant

increases in soil pH following garlic mustard invasion, facilitating site occupancy. The importance of soil pH further emphasizes the surprising lack of effect of phosphorus, since phosphorus is highly variable in solubility according to pH. However, as discussed above, this could likely be circumvented by the high root enzymatic activity (Elk 2010). At lower pH levels, potassium and calcium are less exchangeable, and thus garlic mustard shows a positive performance association with high pH, possibly due to the higher availability of potassium.

Garlic mustard is characterized as a nitrophile (Marschner 2002), and has flexible, indiscriminate uptake of both ammonium and nitrate (Hewins & Hyatt 2010). However our results do not indicate nitrogen to be a significant predictor of plant performance, excepting the presence of nitrate in one of the multiple regression models, and an interaction with ammonium in another. Perhaps due to the flexible uptake (Hewins & Hyatt 2010) and broad tolerance of soil nitrogen availability this is not a significant limitation to plant performance. Experimental increases in nitrogen, in the presence of other complementary nutrients, increased plant growth (Hewins & Hyatt 2010) indicating that it can be sufficiently limiting, however potentially not at the time of sampling in our study, or nitrogen is not the most limiting macronutrient for our populations.

Despite general patterns, our results indicate that soil traits only partially explain garlic mustard performance. That soil nutrients have limited explanatory power for plant performance is consistent with previous work by Meekins and McCarthy (2000). They found, in a controlled experiment using the application of soil nutrients in addition to control of light and water availability, that soil

nutrients have effects secondary in importance to light and population density on garlic mustard growth. However, soil nutrients were found to potentially indirectly affect performance: increases in chlorophyll content followed nutrient increases, thus allowing greater capture of carbon (Meekins & McCarthy 2000). In our study, magnesium, the key component of chlorophyll, is negatively associated with rosette growth, suggesting this interaction might not hold for our populations. This suggests other traits such as soil moisture may be important (Meekins & McCarthy 2001; Hillstrom & Cipollini 2011). Hillstrom & Cipollini (2011) found that soil moisture, along with nutrients, has been shown to cause, in a manipulative study, a large degree of variation among populations in plant characters including a suite of enzymatic defense traits, number of rosette leaves, and specific leaf mass.

Anecdotal data from two of the original seven field sites supports the importance of soil moisture, rather than nutrient availability in performance, particularly rosette performance and persistence. Prior to rosette and soil sampling at the end of the summer (2010), approximately one month passed without rainfall, possibly causing the observed complete mortality of all individuals at sites HMF2 and FE. Soils sampled were dry relative to the other sites. Soil nutrient environment medians are not extreme values within the dataset (Table 2.2). Inclusion of soil data from these sites does not alter soil variable associations of field site clustering by soil traits (Appendix A). Cumulatively, data from the soil nutrient environment suggest that these limitations were not substantial enough to cause extinction at these sites, while other populations with

more extreme values persisted, supporting the assertion of water availability driving the high mortality.

Soil moisture (Meekins & McCarthy 2001; Hillstrom & Cipollini 2011) exerts substantial performance limitations, likely at a scale smaller than the soil nutrient heterogeneity observed in our studies. Performance, specifically biomass, is also determined in part by light available (Meekins & McCarthy 2000). The primacy of effect of these two factors potentially explains the lack of significant prediction of bolting biomass from rosette biomass within sites. Bolting plants sampled represent the subset of individuals that survived the mortality imposed by overwinter events, and furthermore likely represent a subset that survived the previous summer's water limitations. There was also a substantial amount of variation among individuals, within sites, in plant performance variables (Table 2.3), suggesting a scale of abiotic factors, such as light and moisture, that is finer in than the soil nutrient environment. Given the importance of soil moisture (Meekins & McCarthy 2001, Hochstedler & Gorchov 2007), the predictions of a higher occurrence of mini-droughts and lower soil moisture in New England due to climate change (Union of Concerned Scientists Northeast Climate Impacts Assessment Report 2006) suggest that these factors could cause substantial limitations to further garlic mustard persistence via high rosette mortality during summers.

Based on the limited explanatory power of our results, we suggest that soil nutrients are likely more important during establishment; Nuzzo (1999) found that periodic disturbance of high quality forests are needed for populations to increase

in number, though the study did not evaluate the role of disturbance in initial establishment. The role of soil nutrients in facilitating establishment is potentially important with regards to improving species distribution modeling (e.g., Welk et al. 2002). Our results indicate that once established there is likely not a performance decrease due to soil nutrient conditions, but if these factors inhibit establishment in the absence of disturbance, this could potentially improve models, especially at finer scales.

Table 2.1 Field site location, soil bedrock, expected and typical soil environments, and common tree species. Tree species found at all seven sites and highlighted in bold, and species unique to one of the seven sites are underlined for emphasis.

Field site	Location	Bedrock*	pH†	CEC/CECe (meq/100g)†‡	Sodium Absorption Ratio (SAR)†	Salinity (mmhos/com)†	Common trees †
HMF	Williamstown, MA 42°43'28.61"N 73°13'32.98"W	Marble-metamorphic	4.5 – 8.4	0.6 – 22/ 2 - 25	0	0	American basswood, American beech, American elm, balsam fir, Eastern hemlock, **Eastern white pine**, hickory, Northern red oak, paper birch, **red maple**, red pine, sugar maple, tuliptree, **white ash**, white oak, white spruce, yellow birch
FF	Williamstown, MA 42°40'3.07"N 73°15'16.35"W	Marble-metamorphic	3.6 – 8.4	0 – 59/ 2 - 25	0	0	American basswood, American beech, American elm, balsam fir, <u>black spruce</u>, Eastern hemlock, **Eastern white pine**, hickory, Northern red oak, paper birch, **red maple**, red pine, red spruce, sugar maple, tamarack, tuliptree, **white ash**, white oak, white spruce, yellow birch
HMF 2	Williamstown, MA 42°43'33.92"N 73°13'13.75"W	Marble-metamorphic	4.5 – 8.4	0.9 – 22/ N/A	0	0	American basswood, American beech, American elm, Eastern hemlock, **Eastern white pine**, Northern red oak, **red maple**, sugar maple, **white ash**
CB	Ipswich, MA 42°41'19.70"N 70°46'25.73"W	Granite	3.6 – 7.8	0 – 26/ 0 - 10	0	2 - 60	American basswood, American elm, Atlantic white cedar, balsam fir, <u>bitternut hickory</u>, <u>Eastern cottonwood</u>, Eastern hemlock, **Eastern white pine**, gray birch, green ash, Northern red oak, **red maple**, red pine, red spruce, shagbark hickory, sugar maple, **white ash**
AA	Jamaica Plain, MA 42°17'51.12"N 71° 7'13.06"W	Metamorphic	3.6 - 7.8	0 – 15/ 0 - 12	0	0	American elm, Atlantic white cedar, balsam fir, black cherry, Eastern hemlock, <u>Eastern white cedar</u>, **Eastern white pine**, <u>elm</u>, gray birch, green ash, Northern red oak, **red maple**, red spruce, shagbark hickory, sugar maple, tamarack, **white ash**, white spruce
CC	Eastham, MA 41°49'11.81"N 69°57'52.93"W	Sediments	3.6 – 7.8	26 – 130/ 0 - 44	0	0 - 16	Atlantic white cedar, **Eastern white pine**, **red maple**, red pine, **white ash**
FE	Stoneham, MA 42°27'29.75"N 71° 5'0.16"W	Granite-mafic rocks	3.5 – 6.5	0.7 – 12/ 0.3 -9.9	0	0	balsam fir, black cherry, **Eastern white pine**, green ash, Northern red oak, **red maple**, red pine, red spruce, sugar maple, **white ash**

* UMass, Department of Geosciences, based on USGS Open-File Report 03-225

†USDA NRCS Custom Soil Resource Reports,

‡ Cation exchange capacity is measured at neutral pH (7.0). Effective cation exchange capacity (CECe) is calculated for soils of a pH less than 5.5.

Table 2.2 Field site location and soil environment descriptions (medians reported). Differences in letter superscripts next to mean values indicate significant differences (at $P<0.05$, Kruskall-Wallis rank sums test, Wilcoxon post-hoc pairwise comparisons), beginning with the largest site value for the soil variable. The coefficient of variation (denoted as C.V.) is provided for each variable, and was calculated across sites. Field sites HMF2 and FE are highlighted for emphasis, but excluded from the statistical analysis. Analyses performed using JMP.

Field site	Soil pH	Nitrate (NO_3^- mg/L)	Ammonium (NH_4^+ mg/L)	Phosphorus (PO_4^{3-} mg/L)	Potassium (K^+ mg/L)	Sulfur (SO_4^{2-} + Organic-S mg/L)	Calcium (Ca^{2+} mg/L)	Magnesium (Mg^{2+} mg/L)	Iron (Fe^{3+} mg/L)	Mananese (Mn^{2+} mg/L)	Aluminum (Al^{3+} mg/L)	Sodium (Na^+ mg/L)	CECe (meq/100 g)	SAR
HMF	5.26^{b}	1.07^{a}	0.06^{c}	1.06^{cd}	1.06^{d}	3.85^{c}	100.00^{b}	9.56^{b}	1.46^{d}	2.55^{a}	1.17^{d}	0.00^{b}	5.91^{b}	0.00^{b}
FF	5.33^{b}	0.54^{b}	0.24^{d}	1.18^{ab}	8.25^{a}	3.72^{c}	62.53^{d}	5.83^{d}	1.48^{b}	1.48^{c}	1.21^{b}	0.00^{b}	3.99^{c}	0.00^{b}
HMF2	5.26	1.43	0.12	1.08	1.09	4.41	85.47	10.63	1.48	1.99	1.19	0.00	5.44	0.00
CB	6.22^{a}	0.77^{c}	0.46^{b}	1.10^{bc}	4.52^{b}	3.23^{d}	161.94^{a}	6.61^{cd}	1.47^{c}	1.39^{c}	1.13^{c}	0.00^{b}	8.97^{a}	0.00^{b}
AA	5.28^{b}	0.62^{ab}	0.23^{d}	1.03^{d}	3.95^{bc}	5.47^{b}	79.32^{c}	8.55^{bc}	1.48^{b}	1.88^{b}	1.29^{b}	2.03^{a}	4.96^{b}	0.08^{a}
CC	4.75^{c}	0.78^{ab}	0.99^{a}	2.12^{a}	2.63^{c}	7.58^{a}	57.97^{d}	17.76^{a}	1.50^{a}	1.98^{b}	4.00^{a}	0.56^{a}	4.85^{b}	0.02^{a}
FE	4.91	0.55	0.25	1.18	8.25	3.73	62.53	5.83	1.48	1.48	1.21	0.00	4.53	0.00
C.V.*	10.5	74.5	134.0	108.7	66.8	71.7	50.4	55.5	1.5	45.6	88.3	199.0	39.8	196.8

*Coefficient of variation for each variable, across sites, excluding HMF2 and FE

Table 2.3 Population plant traits (medians reported). Differences in letter superscripts next to mean values indicate significant differences (at $P<0.05$, Kruskall-Wallis rank sums test, Wilcoxon post-hoc pairwise comparisons), beginning with the largest site value for the soil variable. The coefficient of variation (denoted as C.V.) is provided for each variable, and was calculated across sites. Analyses performed using JMP.

Field site	Rosette biomass (g)	Bolting biomass (g)	Average siliques/plant
HMF	0.40^{b}	3.99^{b}	5^{bc}
FF	0.75^{a}	2.30^{c}	4^{c}
CB	0.89^{a}	5.18^{ab}	17^{a}
AA	0.74^{a}	6.84^{a}	6^{b}
CC	0.43^{b}	0.93^{d}	3^{c}
C.V.*	55.8	118.1	129.2

*Coefficient of variation for each variable, across sites

Table 2.4 Correlations and regressions of rosette biomass and soil variables. Bold values indicate significance at P<0.05. Analyses performed using JMP.

Rosette biomass vs.	Correlation coefficient	R^2 (adjusted)	P-value
Soil pH	**0.39**	**0.14**	**<0.0001**
Ammonium	-0.09	-0.00	0.3909
Nitrate	-0.18	0.02	0.0799
Sulfur	-0.20	0.03	0.0513
Calcium	**0.30**	**0.08**	**0.0027**
Magnesium	**-0.26**	**0.06**	**0.0082**
Sodium	0.10	-0.00	0.3311
Iron	-0.09	-0.00	0.3615
Manganese	**-0.26**	**0.06**	**0.0080**
Potassium	**0.35**	**0.11**	**0.0003**
Aluminum	**-0.26**	**0.06**	**0.0085**
Phosphorus	-0.17	0.02	0.0931
CEC	**0.24**	**0.05**	**0.0164**
SAR	0.11	0.00	0.2582

Table 2.5 Correlated soil variables. Only variables positively correlated with rosette biomass (Table 2.4) considered. Bold correlation scores indicate significance at P<0.05. Analyses performed using JMP.

	Soil pH	**Calcium**	**Magnesium**	**Manganese**	**Potassium**	**Aluminum**	**CEC**
Soil pH		**0.82**	-0.19	**-0.39**	**0.34**	**-0.56**	**0.77**
Calcium	**0.82**		-0.04	**-0.24**	0.03	**-0.44**	**0.98**
Magnesium	-0.19	-0.04		0.028	**-0.20**	**0.22**	0.17
Manganese	**-0.39**	**-0.24**	0.028		**-0.43**	0.07	**-0.24**
Potassium	**0.34**	0.03	**-0.20**	**-0.43**		-0.19	0.01
Aluminum	**-0.56**	**-0.44**	**0.22**	0.07	-0.19		**-0.33**
CEC	**0.77**	**0.98**	0.17	**-0.24**	0.01	**-0.33**	

Table 2.6 Multiple regression models of soil variables (predictors) on rosette biomass (response variable). All possible models, containing both single and two-way interaction terms, were considered. The sign of the parameter vector for each predictor is given in parentheses. Models were evaluated using AICc scores, and only competing models are reported. Models reported have some nested variables, however none are completely nested so as to be eliminated. The model weight, *w*, is also reported. All analyses were performed using JMP.

Model	Predictors	R^2 adjusted	ΔAICc	*w*
1	soil pH (-), potassium (+), magnesium(-), aluminum (-), CEC (+), SAR (+), potassium*SAR (-), soil pH*magnesium (-), SAR*aluminum (-)	0.38	0	0.42
2	soil pH (-), potassium (+), magnesium (-), aluminum (-), manganese (-), CEC (+), SAR (+), potassium*SAR (-), soil pH*magnesium (-), SAR*aluminum (-), potassium*manganese (-)	0.39	1.13	0.24
3	potassium (+), magnesium (-), manganese (-), sodium (+), sulfur (-), CEC (-), SAR (-), SAR*CEC (-), magnesium*ammonium (+), aluminum*sulfur (+), potassium*manganese (-)	0.38	1.68	0.18
4	soil pH (-), potassium (+), nitrate (-), aluminum (-), potassium*SAR (-), soil pH*magnesium (-), SAR*CEC (-), SAR*aluminum (-)	0.35	1.95	0.16

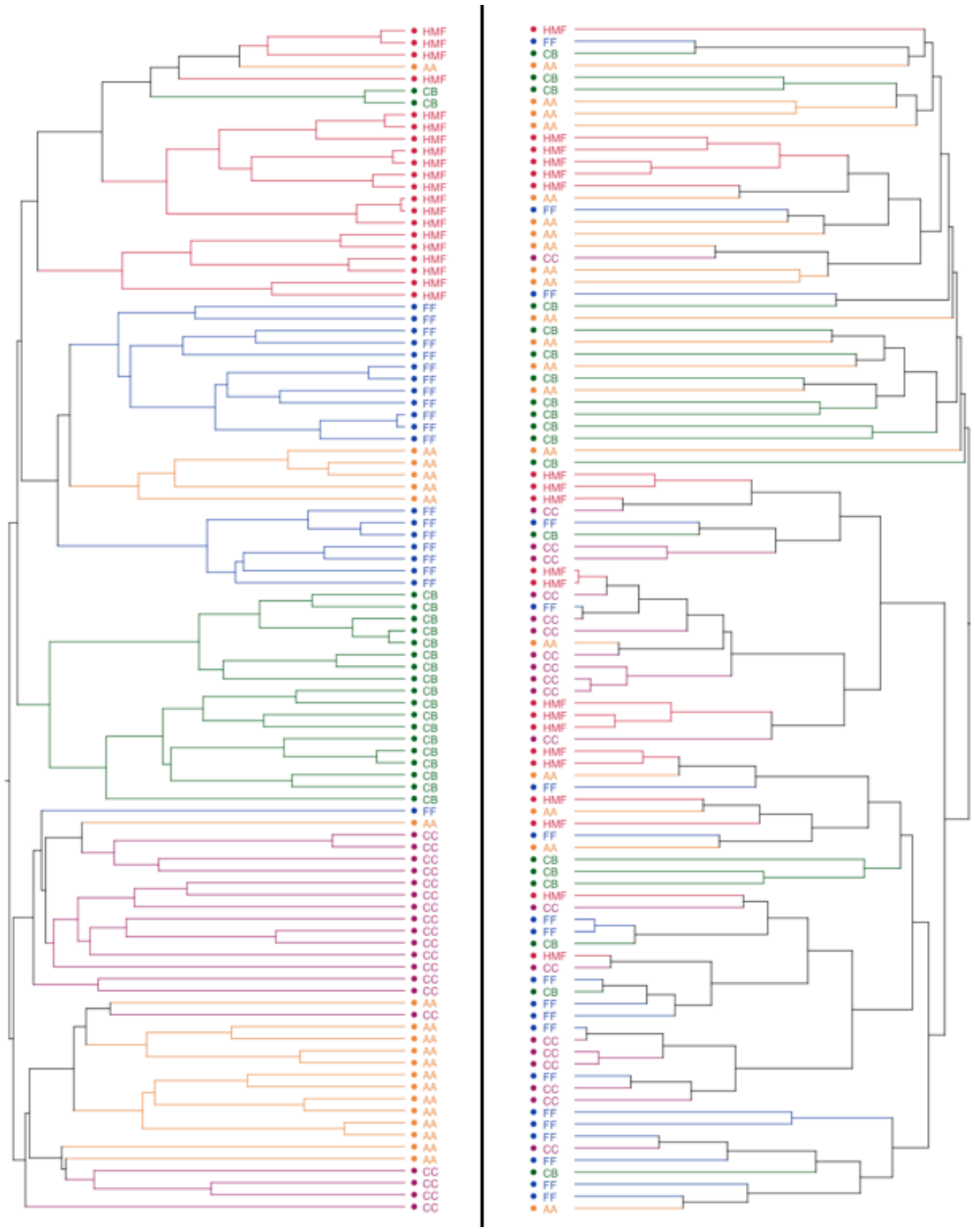

Figure 2.1 Cluster analysis of transect intervals/individuals for soil (left) and plant (right) population variables. Colors indicate separate field sites. Hierarchical cluster analysis performed using JMP.

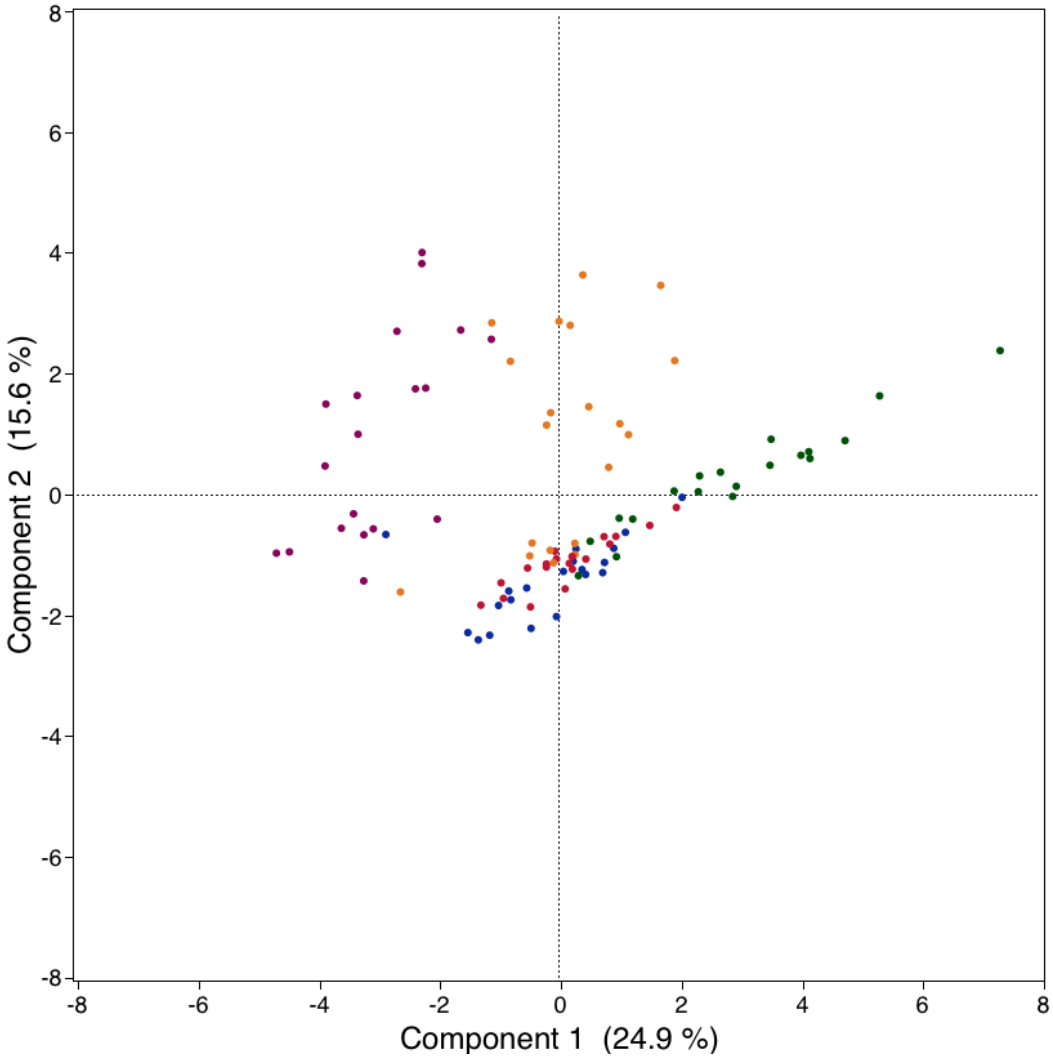

Figure 2.2 Principal component analysis showing derived components for each field site. Distinct clustering of colored points, particularly CC (purple) and AA (yellow) is evident in the PCA, and this confirms some of the conclusions drawn based on cluster analysis (Figure 2.1). Color coding of sites follows conventions set by cluster analysis (Figure 2.1).

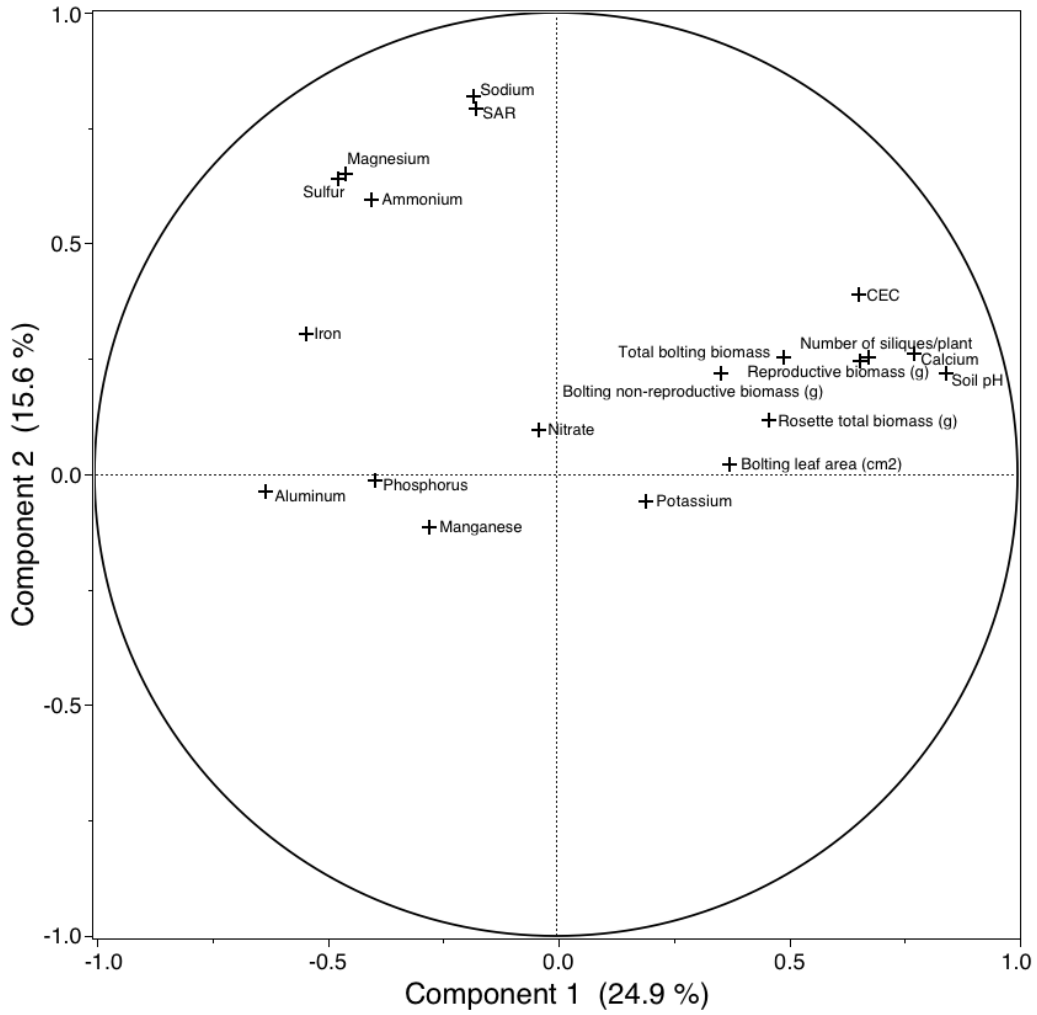

Figure 2.3 Ordination of variables in principal component analysis. PC1 describes plant traits and the soil variables of pH, calcium, potassium and CEC. PC2 describes plant traits and several soil variables, excluding potassium phosphorus, aluminum and manganese.

CHAPTER THREE

Evolutionary traps and enemy release: evolution of a native butterfly following serial invasions by exotic species

Introduction

One mechanism of native species extinctions following invasion of alien species occurs when previously adaptive behaviors (of the native) are maladaptive in the face of this new selective pressure, so-called evolutionary traps (Schlaepfer et al. 2002). Garlic mustard (*Alliaria petiolata* [Bieb.] Cavara & Grande), an invasive plant first documented in North America in 1868 (Nuzzo 1993), is an evolutionary trap for the native butterfly *Pieris napi oleracea* Harris (Lepidoptera: Pieridae) (Courant et al. 1994, Keeler and Chew 2008). The trap is due to the presence of aliphatic glucosinolates in garlic mustard (Huang et al., 1995), which are used as cues for oviposition by *P.n. oleracea* (Chew and Renwick 1995), and are similar to those of a native host plant *Cardamine* (=*Dentaria*) *diphylla* (Feeny and Rosenberry 1982). However, when feeding on garlic mustard, *P. n. oleracea* larvae develop slowly and most do not survive (Courant et al. 1994, Keeler and Chew 2008) at least in part due to feeding deterrents (Haribal and Renwick 1998, Haribal et al. 2001). *Pieris napi oleracea* has been decreasing in abundance and range since the late 1800s (Scudder 1889, Longstaff 1912, Klots 1951, Opler and Krizek 1984, unpubl. data from Massachusetts Natural Heritage and Endangered Species Program), possibly due to decreased native food plant availability caused by habitat fragmentation and loss (Chew 1981, Keeler et al. 2006) and to attack

by *Cotesia glomerata* (L.), an exotic parasitoid that was introduced as biological control for the exotic pest *Pieris rapae* (Clausen 1978, Benson et al. 2003). Invasion by garlic mustard following habitat disturbance is likely exacerbating the decline (Courant et al. 1994, cf. Casagrande and Dacey 2007). Results from a population simulation model by Keeler et al. (2006) supported the hypothesis that severe *P. n. oleracea* population decline and a high extinction probability could be associated with increased cover of garlic mustard.

If sufficient genetic variation exists in the native butterfly population, however, we would expect natural selection to ameliorate this evolutionary trap in two possible ways: (1) evolution of oviposition avoidance of garlic mustard by adult females, and/or (2) improved larval performance on garlic mustard (Keeler 2005). Variation in larval performance on garlic mustard has been documented for a population of *P. n. oleracea*, where the small fraction of caterpillars that complete development to pupation on garlic mustard have mothers with strong oviposition preference for garlic mustard (Keeler and Chew 2008). Hybridization experiments with resistant and susceptible *Pieris* suggest that the ability of larvae to develop on these exotic host plants is inherited as an autosomal dominant. Specifically, F1 hybrids of English *P. napi napi* L. with either North American *P. virginiensis* Edwards or *P. napi oleracea* are able to use garlic mustard successfully (Bowden 1971). Similar results were observed for F1 hybrids of Swedish *P. napi* and North American *P. napi macdunnoughii* from the Rocky Mountains in response to a different exotic crucifer, *Thlaspi arvense* L. (Brassicaceae) (C. Boggs, Stanford Univ., pers. comm.), on which the North

American caterpillars fail to complete development (Chew 1975, 1977, Rodman and Chew 1980). The North American *Pieris napi* species complex is an example of dissected polymorphism (Bowden 1979, Chew and Watt 2006): isolation in North America of Holarctic populations of *P. napi* into refugia occurred during glaciation, followed by fixation of trait alleles due to drift. However, residual polymorphism for the ability to use garlic mustard from prior to glaciation appears to remain in some North American populations, and could be favored following garlic mustard invasion.

Further complicating our expectations about the potential proliferation of a *P. n. oleracea* genotype that can develop on garlic mustard is the recent introduction of another parasitoid wasp, *Cotesia rubecula* (Marshall), which was introduced in the 1980s as another biocontrol agent for *P. rapae* (Van Driesche and Nunn 2002). This parasitoid does not parasitize *P. n. oleracea* (Brodeur et al. 1996, Van Driesche et al. 2003). Interestingly, *C. rubecula* is competitively dominant to *C. glomerata* (Laing and Corrigan 1987), greatly lowering the abundance of *C. glomerata* from habitats in Massachusetts (USA) where it was previously abundant (Van Driesche 2008), and potentially creating enemy free space for the native butterfly.

Community composition changes, and differences in top-down versus bottom-up effects, could allow for adaptive evolution. The serial invasions described above, in tandem with the heritable variation in the ability to use garlic mustard that has been documented in the laboratory, could potentially lead to adaptive evolution by *P. n. oleracea* to expand its host range to include garlic

mustard. We used a stochastic simulation model to investigate the conditions under which an allele conferring positive larval development on garlic mustard could arise and spread in a population. Different scenarios of two basic possibilities were examined: (1) a spontaneous mutation producing heterozygotes in the population; and (2) residual polymorphism remaining due to dissected polymorphism in the Holarctic species complex *P. napi*. In addition, we investigated (3) whether top-down regulation in the form of the exotic, generalist parasitoid *C. glomerata* could limit proliferation of an allele introduced through either of the scenarios above, and (4) whether release from parasitism via introduction of the competitively dominant, specialist parasitoid *C. rubecula* could allow for allele proliferation.

Methods

MODEL OVERVIEW AND PARAMETERS

For our analyses we modified the published stochastic population simulation model of Keeler et al. (2006) (Figure 3.1). This model simulates females only. All parameter values are specified below, and, unless otherwise noted, are the same as those used in the original model (Keeler et al. 2006; see therein for references). The model was built and modified using STELLA software (version 8, isee systems, Inc., New Hampshire, USA). The original model included top-down regulation by the exotic braconid parasitoid, *C. glomerata*. Although in the original model, Keeler et al. (2006) concluded that parasitism by *C. glomerata* alone could not cause butterfly extinction, they did show that parasitism could exacerbate the damaging effects of garlic mustard invasion on *P. n. oleracea*

(Keeler et al. 2006). The original model did not, however, include the potential effects of the second parasitoid, *C. rubecula*, so we included this in some scenarios of our model. Thus, the first significant modifications to the existing model were to capture the effect of decreased *C. glomerata* parasitism as *C. rubecula* invades (Brodeur et al. 1996). We increased the number of instars that are separately modeled in the second generation (Figure 3.1) and included decreasing risk with increasing larval instar because susceptibility is size-dependent (Brodeur et al. 1996). Since larvae develop at a slower pace on garlic mustard than on native crucifers (Keeler and Chew 2008), we also simulated an increased parasitism risk for those larvae on garlic mustard as a function of exposure time within a generation (see equations 7-10).

The second major modification we made to the model was to simulate genetic polymorphism for resistance to garlic mustard, as determined by the alleles R (dominant, allowing caterpillar development) and r (recessive, wild-type non-resistant North American allele). Based on hybridization studies by Bowden (1971) on *P.n. oleracea* and similar findings from on the closely related *P. n. macdunnoughii* Remington (C. Boggs, Stanford Univ., pers. comm.), we assumed an autosomal dominant allele for resistance to garlic mustard that allowed larvae to complete development to pupation on garlic mustard. Within each generation we simulated changes in the number of individuals of each of the three genotypes (RR, Rr, and rr). We simulated random mating among genotypes at the end of each generation using allele frequencies calculated according to Hardy-Weinberg equilibrium to determine the number of individuals of each genotype in the

following generation. Survivorship on garlic mustard was varied among the three genotypes, as described below.

In each model run, population size was recorded at the start of each year of simulated time, and our time horizon was 50 years. Each scenario was run at each of 11 levels of garlic mustard ground cover (from 0% to 100% at 10% intervals). Each combination of conditions and garlic mustard cover was run 1,000 times. The 1,000 runs were used to generate a probability of persistence for each genotype; i.e., the percentage of the 1,000 runs with an extant population at the end of 50 years.

MODEL SCENARIOS

In all scenarios (Fig. 2), survival of larvae with a dominant allele on garlic mustard ($S_{GM, R}$) was equal to the probability of survival on native crucifers (S_{NC}). Survival on garlic mustard was equal to zero for homozygous recessive individuals ($S_{GM, rr}$).

A series of scenarios (Scenarios 1-5, Figure 3.2) were run varying the initial population sizes of the three genotypes. All scenarios, unless explicitly stated below, began with 3,000 homozygous recessive individuals. To generate a comparison useful for indicating the upper bounds of the possible probability of proliferation and persistence of the allele, an extra scenario was run beginning with 3,000 individuals of each genotype; however this is not depicted in the scenario schema (Figure 3.2).

To simulate spontaneous mutation of a resistance allele, simulations began with 1 (Scenario 1, Figure 3.2) or 5 heterozygotes (Scenario 2, Figure 3.2).

Additionally, a similar scenario was run in which 1 heterozygote was reintroduced into the population every year so as to simulate immigration of resistance individuals (Scenario 3, Figure 3.2). Stochastic reintroductions of mutants were simulated at annual probabilities of 1/3001, 1/1000 and 1/100 (Online Appendix A). To model a residual polymorphism, simulations began with heterozygotes comprising 1% (30 individuals; Scenario 4, Figure 3.2) or 5% (150 individuals; Scenario 5, Figure 3.2) of the total population of 3,000 individuals; the remaining individuals were homozygous recessive.

In scenarios simulating spontaneous mutation (1 or 5 heterozygotes, Scenarios 1-2) and residual polymorphism (1% or 5% heterozygotes, Scenario 4-5), parasitism in the second generation was added (Scenarios 1a-5a, Scenario 2). Finally, to simulate the invasion of the specialist parasitoid *C. rubecula*, a set of scenarios decreased parasitism over time to zero halfway through the simulated 50 years (Scenarios 1b-5b, Figure 3.2).

MODEL STRUCTURE

Each simulated year begins with the emergence of females in the first generation, and there were two butterfly generations per year (Figure 1). The number of eggs produced each generation was calculated separately for each genotype. The numbers of female heterozygote eggs (E_{Rr}), homozygous dominant eggs (E_{RR}), and homozygous recessive eggs (E_{rr}) were calculated, respectively, as:

$$E_{Rr} = \left(\frac{E_f * N_{i,Rr} * F_{Rr} * D}{2} \right) + \left(E_f * N_{i,RR} * F_{RR} * D * q_i \right) + \left(E_f * N_{i,rr} * F_{rr} * D * p_i \right) + R$$

Eqn. 1

$$E_{RR} = \left(E_f * N_{i,RR} * F_{RR} * D * p_i\right) + \left(E_f * N_{i,Rr} * F_{Rr} * D * \left(\frac{p_i}{2}\right)\right) \quad \text{Eqn. 2}$$

$$E_{rr} = \left(E_f * N_{i,rr} * F_{rr} * D * q_i\right) + \left(E_f * N_{i,Rr} * F_{Rr} * D * \left(\frac{q_i}{2}\right)\right) \quad \text{Eqn. 3}$$

Here E_f is the lifetime number of female eggs produced per female (determined from empirical data as 114 $\pm$ 42 SD, see Keeler et al. 2006), and we assumed an equal primary sex ratio; F_g is the proportion of females of genotype *g* not emigrating from the population; *D* is the proportion of days suitable for oviposition (based on weather; see Keeler et al. 2006 for description); $N_{i,g}$ is the number of females in generation *i* of genotype *g*; *R* is the number of heterozygotes added to the genotype population due to spontaneous mutation; and p_i is the frequency of the dominant alleles, calculated as:

$$p_i = \frac{\left[\left(2 * N_{i,RR}\right) + N_{i,Rr}\right]}{\left[\left(2 * N_{i,RR}\right) + \left(2 * N_{i,Rr}\right) + \left(2 * N_{i,rr}\right)\right]} \quad \text{Eqn. 4}$$

The proportion of the recessive allele, q_i , is $q_i = 1 - p_i$.

As with the original model (Keeler et al. 2006), we included density dependence of emigration (their equation 2), and we used the same process for the distribution of larvae on plants (their equations 4 and 5). The number of first instar larvae in the first generation ($I_{I,1}$) is the lifetime fecundity of females, times the hatching success of the first generation (H_1 = 0.73 $\pm$ 0.073):

$$I_{I,1} = E_f * H_1 \quad \text{Eqn. 5}$$

The number of fifth instar larvae produced in the first generation ($I_{V,1}$) is modified from Keeler et al. (2006) to allow survival on garlic mustard:

$$I_{V,1} = I_{I,1} - \left[I_{I,1} * GM * \left(1 - S_{GM}\right)\right] - \left[I_{I,1} * (1 - GM) * (1 - S_{NC})\right] - \left(I_{I,1} * GM * S_{GM} * \Omega\right) -$$
$$\left[I_{I,1} * \left(1 - GM\right) * S_{NC} * \Omega\right] + \left[I_{I,1} * GM * S_{GM} * \Omega * \left(1 - GM) * e^{-\mu}\right)\right] +$$
$$\left[I_{I,1} * \left(1 - GM\right) * S_{NC} * \left(\Omega * \left(1 - GM\right)\right) * e^{-\mu}\right] + \left[I_{I,1} * GM * S_{GM} * \left(\Omega * GM\right) * e^{-\mu}\right] +$$
$$\left[I_{I,1} * \left(1 - GM\right) * S_{NC} * \left(\Omega * GM\right) * e^{-\mu}\right]$$

Eqn. 6

That is, the number of fifth instar larvae is the number of first instar larvae ($I_{I,1}$); minus the number of larvae on garlic mustard that die; minus the number of larvae on native host plants that die; minus the number of larvae on garlic mustard that leave due to co-occupancy; minus those larvae on native host plants that leave due to co-occupancy; plus those larvae that survive on garlic mustard, leave due to co-occupancy, and find an empty native host plant; plus those larvae that survive on native crucifers, and then leave the initial plant and find an empty native host plant; plus those larvae that survive and leave the initial garlic mustard plant and find an empty garlic mustard plant; plus the number of larvae on native host plants that survive and then leave to find an empty garlic mustard plant. Here, *GM* is the proportion of larvae on garlic mustard, and S_{GM} and S_{NC} are survival rates on garlic mustard and native plants, respectively, calculated by genotype. A value of $S_{NC} = 0.388 \pm 0.167$ was used, identical to that in the original model. All stochastic variables were modeled based on a normal distribution about the mean (as did Keeler et al. 2006). The survival rate on garlic mustard for resistant genotypes ($S_{GM, RR, Rr}$) was equal to that of survival on native crucifers. Survival on garlic mustard of the wild-type ($S_{GM, rr}$) was always equal to zero. Ω is the

probability of a plant having more than one larva, and *m* is the mean number of larvae on a plant (see Keeler et al. 2006 for calculations).

The number of first instar larvae produced in the second generation ($I_{I,2}$) is:

$$I_{I,2} = \left[E_f * H_2 * GM * \left(1 - C_I\right)^2\right] + \left[E_f * H_2 * \left(1 - GM\right) * \left(1 - C_I\right)\right] \quad \text{Eqn. 7}$$

That is, the number of first instar larvae is a function of the number of eggs in the second generation on garlic mustard; times hatching success ($H_2 = 0.53 \pm 0.053$); times the proportion of larvae on garlic mustard that escape death due to parasitism ($1\text{-}C_I$); plus the number of eggs in the second generation laid on native host plants; times the hatching success; times the proportion of larvae that escape death due to parasitism. Larvae develop more slowly on garlic mustard than on native crucifers (Keeler and Chew 2008) and in our model we included this longer exposure time to parasitism for larvae feeding on garlic mustard. Thus, the proportion of larvae on garlic mustard that escape parasitism is squared to represent the smaller fraction that escaped parasitism on garlic mustard. The values for parasitism rate were derived from Brodeur et al. (1996), who reported *C. glomerata* parasitism rates in European *P. napi* for early and late larval instars. We assumed each instar stage lasted three days and plotted the data, followed by creating a linear fit to describe the decline in parasitism rate with increasing instar. We then calculated a parasitism rate for each instar by using the line of best fit to calculate the predicted parasitism rate at 1.5 and 4.5 days of development for the first and second instars, respectively. Although documented in the laboratory for European *P. napi* (Brodeur et al. 1996), *C. glomerata* in the field do not

appear to parasitize larvae after the second instar in the North American subspecies (R. Van Driesche, pers. obs.) so the parasitism in the third instar (C_{III}) was set to zero in all scenarios. The estimated parasitism rates for first two instars was calculated as $C_I = 0.6425$, and $C_{II} = 0.4433$, respectively.

The number of second and third instar larvae in the second generation ($I_{II,2}$ and $I_{III,2}$, respectively) is the number of previous instar larvae on garlic mustard and on native host plants that escape death due to parasitism:

$$I_{II,2} = \left[I_{I,2} * GM * \left(1 - C_{II}\right)^2\right] + \left[I_{I,2} * \left(1 - GM\right) * \left(1 - C_{II}\right)\right] \quad \text{Eqn. 8}$$

$$I_{III,2} = \left[I_{II,2} * GM * \left(1 - C_{III}\right)^2\right] + \left[II_{I,2} * \left(1 - GM\right) * \left(1 - C_{III}\right)\right] \quad \text{Eqn. 9}$$

In scenarios that included the competitive exclusion of *C. glomerata* by *C. rubecula* over time (t, in years), we modeled caterpillar mortality as:

$$C = C_i \; - \left[0.5 + \left(\frac{1}{p}\right)\arctan\left(0.05pt - p\right)\right] \quad \text{Eqn. 10}$$

when $C \geq 0$, else $C = 0$; C_i is equal to the calculated parasitism value for the instar, i.

The number of fifth instar larvae in the second generation ($I_{V,2}$) is:

$$I_{V,2} = I_{III,2} - \left[I_{III,2} * GM * \left(1 - S_{GM}\right)\right] - \left[I_{III,2} * \left(1 - GM\right) * \left(1 - S_{NC}\right)\right] - \left(I_{III,2} * GM * S_{GM} * \Omega\right) -$$
$$\left[I_{III,2} * \left(1 - GM\right) * S_{NC} * \Omega\right] + \left[I_{III,2} * GM * S_{GM} * \Omega * \left(1 - GM\right) * \left(\frac{k}{k+m}\right)^k\right] +$$
$$\left[I_{III,2} * \left(1 - GM\right) * S_{NC} * \Omega * \left(1 - GM\right) * \left(\frac{k}{k+m}\right)^k\right] + \left[I_{III,2} * GM * S_{GM} * \left(\Omega * GM\right) * \left(\frac{k}{k+m}\right)^k\right] +$$
$$\left[I_{III,2} * \left(1 - GM\right) * S_{NC} * \left(\Omega * GM\right) * \left(\frac{k}{k+m}\right)^k\right]$$

Eqn. 11

So, fifth instar larvae abundance is a function of the number of third instar larvae; minus the number of larvae that die on garlic mustard; minus the number of larvae on native host plants that die; minus those larvae that survive on garlic mustard and leave due to plant co-occupancy; minus those larvae that survive on native host plants and leave due to co-occupancy; plus those larvae that survive on garlic mustard and leave to find an empty native host plant; plus those larvae that survive on native crucifers and leave and find an empty native host plant; plus the number of larvae that survive on garlic mustard and leave to find an empty garlic mustard plant; plus those larvae that survive on native crucifers and leave to find an empty garlic mustard plant. The final four bracketed terms of equation 11 model the negative binomial distribution of larvae on host plants, where *k* is a clumping factor; see Keeler et al. (2006) for details.

The number of pupae (*Pu*) in each generation is the number of fifth instar larvae times the pupation success rate ($U = 0.83 \pm 0.083$):

$$Pu = I_{V,i} * U \qquad \text{Eqn. 12}$$

The number of emerging females in the second generation (N_2) is determined by the number of pupae not entering diapause (O) times emergence success ($M = 0.41 \pm 0.041$):

$$N_2 = Pu * (1 - O) * M \qquad \text{Eqn. 13}$$

Surviving pupae from the first generation that do not develop directly enter diapause and overwinter. All pupae of the second generation overwinter in diapause and emerge the following spring. Thus, the number of emerging females

the following year is the number of pupae from each generation emerging from diapause, times the over-winter survival rate (S_W = 0.169 $\pm$ 0.0169):

$$N_{1,t+1} = S_W * \left[\left(P_{u1,t} * O_{1,t}\right) + \left(Pu_{2,t} * O_{2,t}\right)\right] \quad \text{Eqn. 14}$$

Results

In all scenarios, once garlic mustard cover reached approximately 50%, the wild-type (rr) butterflies disappeared from the population (identical to results reported by Keeler et al. 2006). Consequently, the heterozygous population crashes soon after the homozygous recessive (wild-type), because the proportion of heterozygote genotypes declines as the frequency of the wild-type r allele declines, and the (dominant) resistant allele approaches fixation in the population. Because our interest is in the persistence of the (dominant) resistant allele, we show only the results of the homozygous dominant subpopulation (i.e., a self-sustaining resistant population) to illustrate the important trends.

Scenarios beginning with 3,000 individuals of each genotype show the population dynamics of persistence rather than establishment, as with low beginning allele frequencies, and are useful for comparison to document what specific variables contribute to the decline of garlic mustard-adapted individuals. For all scenarios except those beginning with 3,000 heterozygous individuals, there was no proliferation or persistence of homozygous dominant individuals at 0% garlic mustard cover. Furthermore, simulations beginning with 3,000 individuals of each genotype can be considered the upper bounds of what would be expected for persistence.

Simulations beginning with one mutant individual (Scenario 1) had a low (<10%) probability of persistence for the homozygous dominant genotype under all scenarios of garlic mustard cover (Figure 3.3). Increasing the initial number of heterozygotes to 5 individuals (Scenario 3) significantly increased resistant population persistence once garlic mustard cover reached 20%; persistence peaked at intermediate garlic mustard cover values (Figure 3.3). Reintroduction of the mutation as one heterozygote per year (Scenario 2) increased persistence to levels virtually indistinguishable from the scenario starting with 3,000 resistant, heterozygous individuals, once garlic mustard cover reached 20% (Figure 3.3). Subsequent scenarios with the stochastic annual introduction of the mutants to the population at an annual probability of 1/3001, 1/1000 or 1/100 (purposefully unrealistically high mutation rates) did not produce results different from simulations beginning with one mutant and no subsequent introductions (data not shown).

Parasitism substantially decreased the probability of persistence for populations starting with 1 and 5 mutants (Figure 3.4). Parasitism amelioration (creation of enemy-free space) by the introduction of *Cotesia rubecula* had little effect on persistence for simulations beginning with a single mutant (Figure 3.4). The effect was stronger for simulations beginning with five mutants, with persistence >20% when garlic mustard cover was 30-70% (Figure 3.4). However, this persistence was not increased by more than approximately 5% compared to persistence without amelioration (Figure 3.4).

Scenarios simulating residual polymorphism (Scenarios 4-5) show persistence of the resistant populations higher than that exhibited by mutation, but lower than that modeled for annual immigration. When beginning with 1% (30 individuals) of the population heterozygous for the resistant allele, homozygous dominant individuals persist at levels close to the upper expected bound (i.e., when beginning with 3,000 heterozygous individuals) when garlic mustard cover is 20% or higher (Figure 3.4). Constant parasitism substantially decreases the probability of persistence, although a decline in parasitism over time (due to enemy release) increases the probability of persistence by approximately 10% at every level of garlic mustard ground cover above 0% (Figure 3.4). We found similar patterns for scenarios beginning with 5% of the population polymorphic for garlic mustard resistance, although the absolute values of probability of persistence are consistently higher (Figure 3.4).

Discussion

Exotic, invasive species can impose novel selective pressures upon members of their recipient communities and thus provide insights into community dynamics and evolution (Strauss et al. 2006). We used simulation modeling to investigate the potential persistence of a native butterfly *P. n. oleracea* via a rare, extant but previously selectively neutral genotype, in the presence of the serial invasions of three exotic species, two of which exert negative effects on the native, and the third has contributed to amelioration. The predicted effects of a suite of tritrophic interactions under various scenarios of resistance prevalence show a wide range of possible outcomes, ranging from extinction to a high

likelihood of a persistent population of native butterflies resistant to the exotic, invasive host plant

The genotype that allows *P. n. oleracea* development on garlic mustard is of interest because it allows garlic mustard to be used as a host plant. When we evaluated scenarios in which one mutant (a heterozygous resistant individual) arises, we found that a resistant population failed to establish and persist. Increasing the initial number of mutants to five individuals is sufficient to cause a substantial increase in the probability of persistence of a homozygous resistant population. Based on these results, we conclude that a larger number of heterozygotes are required for a greater than 50% likelihood of persistence at moderate cover of garlic mustard; with very high and very low garlic mustard cover the resistant population had much lower proliferation. Larval performance on garlic mustard varies widely among maternal families (Keeler and Chew 2008), which suggests that several resistant offspring could arise in one generation from one mother. Consequently, we envision that such small groups of offspring arising in a single generation could cause establishment and persistence of a garlic mustard-resistant population in the presence of the favorable selective pressure caused by moderate levels of garlic mustard cover.

One alternative hypothesis we evaluated is whether establishment and persistence of a resistant genotype would be more likely if it were a residual polymorphism following differentiation of populations in refugia of the Holarctic species complex *Pieris napi* (Bowden 1979, Chew and Watt 2006). Simulations beginning with a polymorphism of 1% or 5% of the total population carrying the

resistant allele showed substantial probabilities of persistence close to the predicted upper bounds estimated by simulations beginning with 3,000 heterozygotes. This is a higher persistence likelihood than occurred in our mutation scenarios, suggesting that any documented evolution resulting in populations with dominant resistant types is more likely to be the result of residual polymorphism than of spontaneous mutation. The possibility that there is residual resistance in *P. n. oleracea* populations is supported by field evidence from populations in Vermont that are unexposed to garlic mustard, but where a sampling of maternal lines has yielded resistant offspring (Keeler and Chew 2008, R.A. Steward and F.S. Chew, unpubl. data).

Top-down pressure on *P. n. oleracea* from *C. glomerata* parasitism substantially decreases the probability of persistence of resistant populations. The probability of resistant-population persistence for modeled scenarios beginning with five mutants never exceeded 20% in our models, even in cases where parasitism is reduced significantly and eliminated over time to simulate *C. rubecula* displacement of *C. glomerata*. In contrast, for scenarios of residual polymorphism, parasitism does not cause complete extinction of resistant populations (i.e., probability of persistence >0%); however the probability of persistence is substantially reduced, especially at high levels of garlic mustard cover. Contrary to expectations, a decrease to elimination in parasitism over time did not increase persistence likelihood to no-parasitism levels, although the likelihood of persistence of the homozygous resistant genotype increased approximately 10-15%.

Our model results therefore lead us to predict that if a resistant allele arises in a *P. n. oleracea* population via mutation under parasitism pressure, even if that pressure decreases over time, it is unlikely to allow proliferation of resistant individuals. This is true even in the presence of strong favorable selective pressure in the form of extensive garlic mustard cover. In our model we account for parasitism only in the second generation, consistent with published data on emergence (Benson et al. 2003). However, there is an anecdotal observation of *C. glomerata* being present earlier in the season than is typically reported, successfully attacking first generation larvae of *P. n. oleracea* in northern Vermont (F.S. Chew, pers. obs.). If we were to include parasitism in the first generation of our model, then the importance and negative effects of *C. glomerata* on *P. n. oleracea* persistence and the spread of the resistance allele would be greater.

Pieris napi oleracea populations declined during the 19th and 20th century because of habitat loss (Scudder, 1889, Chew 1981), but residual populations continue to be vulnerable to extirpation by the butterfly's now maladaptive attraction to an exotic, invasive host plant (Keeler et al. 2006). Similar evolutionary traps have been documented for other Lepidoptera, including the silkmoth *Hemileuca* sp. and the wetland plant *Lythrum salicaria* (Gratton 2006), as well as for the monarch butterfly (*Danaus plexippus*) and swallow-worts (*Vincetoxicum nigrum*, Casagrande and Dacey 2007). Our assessments demonstrate a possible escape route from this type of evolutionary trap. Specifically, proliferation of a previously neutral allele allowing larval

development on a novel host plant, followed by release of parasitism pressure from an introduced biocontrol agent by a second biocontrol agent that outcompetes the first but does not parasitize the native butterfly.

Top-down regulation in the form of parasitism would be predicted to decrease the probability of adaptation due to the "challenge" (sensu Cox 2004) it presents to population growth. Population growth is important in directional selection to off-set the population size reduction from hard selection and to buffer the population against drift (Reznick and Ghalamboor 2001). Enemy release removes this challenge to population growth and could allow adaptation. This result is particularly intriguing and important with regards to the possibility of native herbivore adaptation to exotic weeds because as noted by Harvey et al. (2010) the third trophic level is rarely accounted for in studies of community responses to exotic weeds. Furthermore, top-down regulation is considered a potentially important evolutionary force in phytophagous insect diet breadth (Dyer and Floyd 1993, Dyer 1995). Based on studies of native Japanese *Pieris* butterflies, Ohsaki and Sato (1994, 1999) suggest that parasitism pressure can significantly shape host plant usage at the population level. Enemy free space (Jeffries and Lawton 1984) has been reported to drive host plant shifts and expansions to novel host plants in other Lepidoptera as well, as reported in Baltimore checkerspot (*Euphydryas phaeton*, Bowers et al. 1992), diamondback moth (*Plutella xylostella*, Fox and Eisenbach 1992), buckeye butterfly (*Junonia coenia*, Camara 1997), potato tuber moth (*Phthorimaea operculella*, Mulatu et al.

2004), and Alaskan swallowtail butterfly (*Papilio machaon aliaska*, Murphy 2004).

In our study system, there are two types of geographic variation that might be encountered by *P. n. oleracea* that could affect adaptation and expansion of host plant breadth: the existence of residual genetic variation in butterfly populations that are fragmented in space, and the degree of enemy release. Both factors of variation occur in the *P. n. oleracea* range (Benson et al. 2003, Keeler and Chew 2008, Van Driesche 2008, R.A. Steward and F.S. Chew, unpubl. data) suggesting the possibility of a geographic mosaic of evolution (cf. Thompson 1999). We would expect in populations lacking variation or with strong top-down regulation, *P. n. oleracea* decline following garlic mustard invasion. However, populations with residual variation and enemy release, or enemy free space due to other landscape factors, would be expected to be evolutionary hotspots (cf. Thompson 1999). Gene flow among populations and mobility should magnify the adoption of garlic mustard as a novel host plant, as has been reported for several British butterfly species (Hardy and Dennis 2008) and further investigations of the degree of isolation among populations would inform future predictions of community effects.

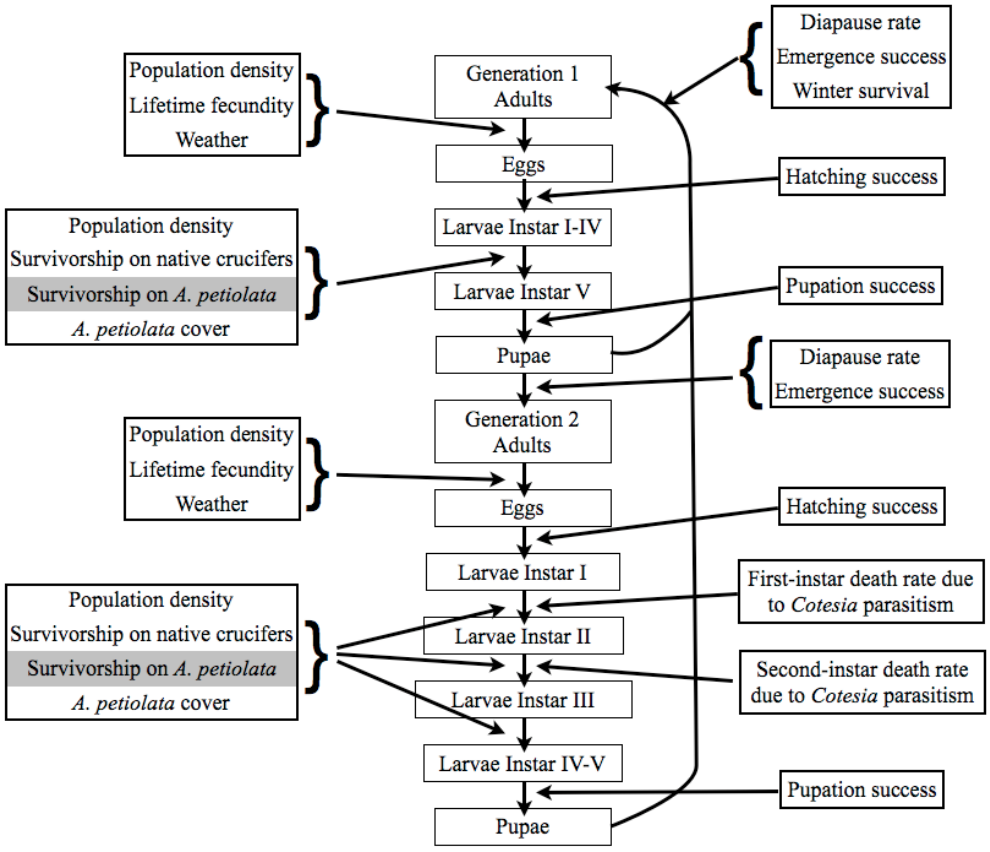

Figure 3.1 A graphical representation of the life-cycle of *P. n. oleracea* modified from Keeler et al. (2006). Life-history and community parameters affecting abundance of each lifecycle stage are depicted on each side. Survivorship on garlic mustard (shaded) differs among genotypes.

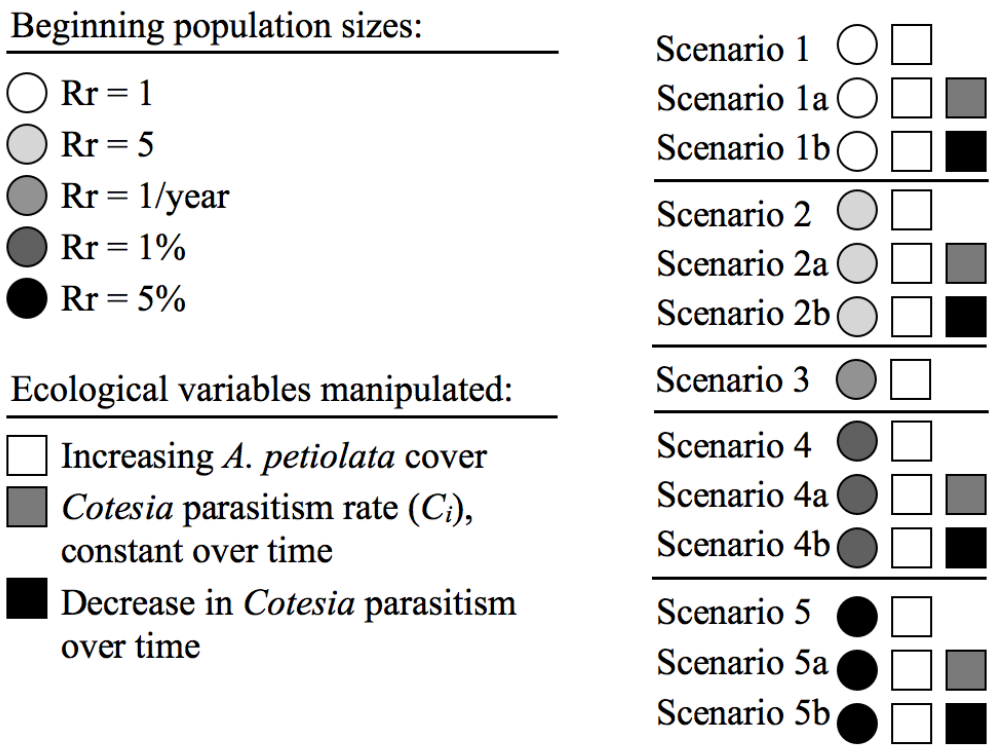

Figure 3.2 List of model scenarios, 1 through 5b in the right hand column, that we evaluated. Circles and squares adjacent to scenario numbers show the starting conditions and ecological variables manipulated in each. Shaded circles indicate the method of introduction of the dominant allele via heterozygotes. An absolute number of heterozygotes represents scenarios simulating spontaneous mutation, whereas percentages indicated scenarios simulation residual polymorphism in the trait. Shaded squares show which variables were manipulated in each scenario. Specifics on values changed in relevant scenarios are given in the text.

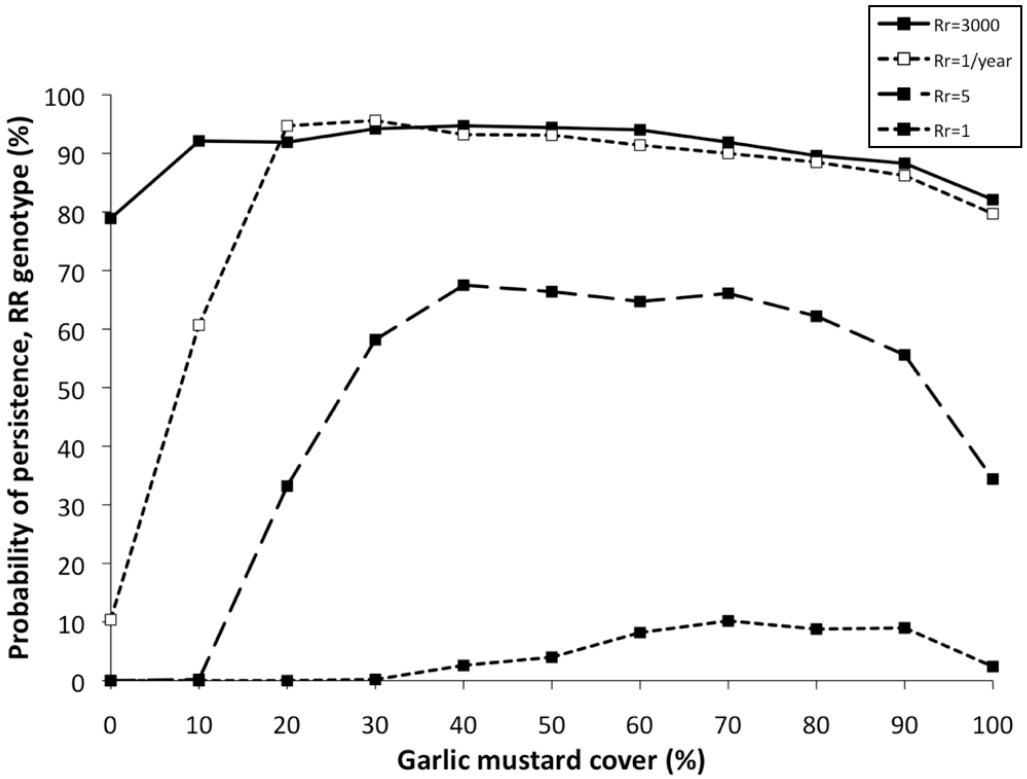

Figure 3.3 Probability of persistence of the homozygous dominant (RR) genotype for scenarios simulating spontaneous mutation of the resistant allele within a population at variable numbers and frequencies. A simulation beginning with 3,000 mutant individuals is used for comparison to the upper-bounds of persistence and establishment that could be expected. These scenarios depict the likelihood of resistant individuals becoming established in simulations beginning with 1 heterozygote (Rr=1), 5 heterozygotes (Rr=5), and when the mutation occurs each year (Rr=1/year), or a continual reintroduction of the mutation. We assumed survival on garlic mustard was equal to that on native hosts for individuals with one copy of the dominant allele. Each data point represents the probability of persistence, which is defined as the proportion of 1000 runs with an extant population at the end of the 50 years of the simulation.

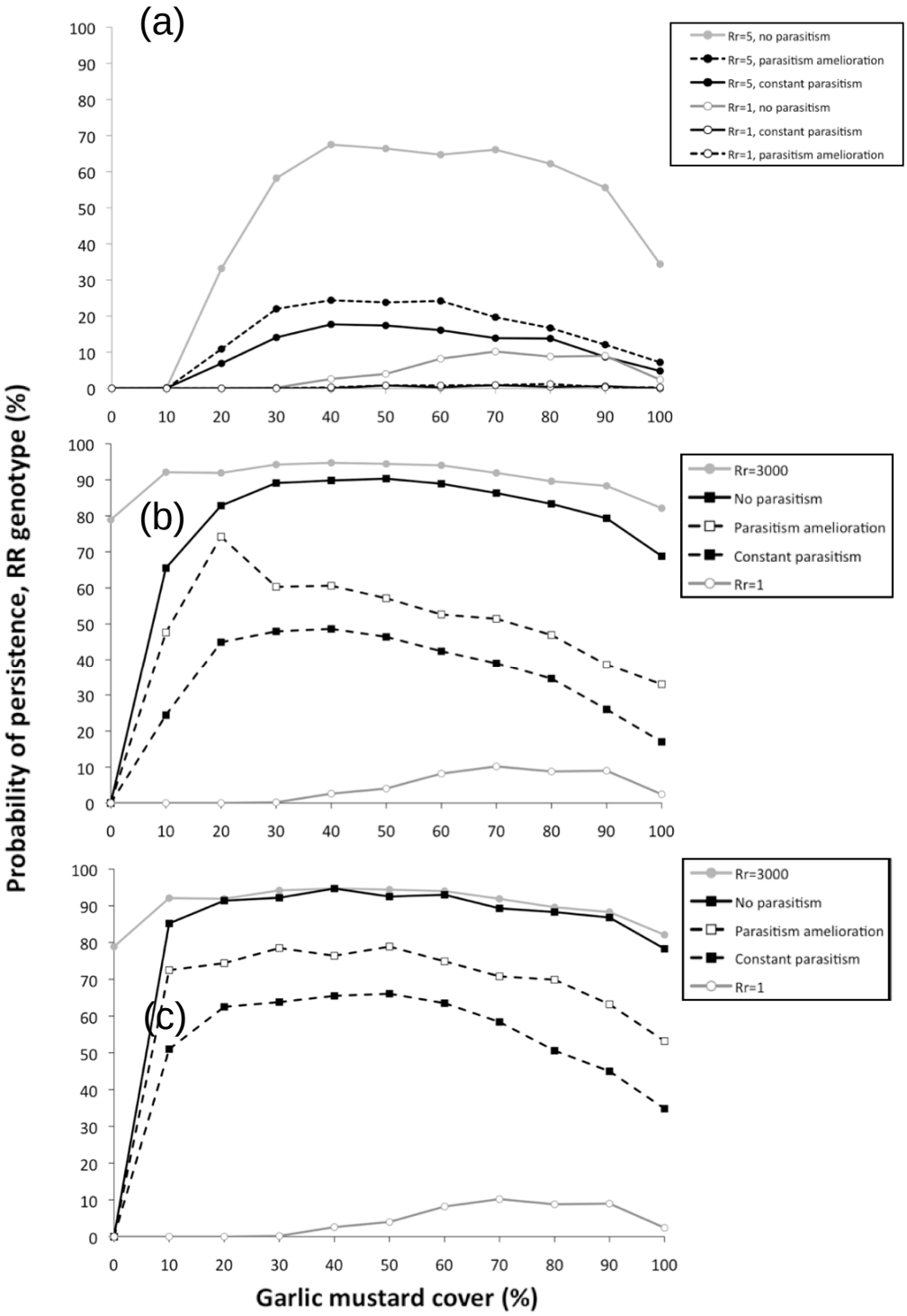

Figure 3.4. Probability of persistence of the homozygous dominant (RR) genotype. (a) Scenarios in which parasitism was absent (gray lines) or present in varying levels of treatment (constant over time, or decreased to zero halfway

through the simulation time) combined with spontaneous mutation levels. Scenarios depict the likelihood of resistant individuals becoming established under conditions of the resistant allele conferring survival on garlic mustard equal to that on native host plants. Lines represent simulations beginning with 1 or 5 mutants for each of the parasitism treatments. (b) For scenarios examining a residual polymorphism of 1% (30 heterozygotes) of the total population of 3,000 individuals, gray lines represent the upper- and lower-bounds of probability of establishment and persistence expected based on simulations with high (3,000) and low (1) number of heterozygotes at the beginning of the population for comparison (also depicted in Figure 1). Black lines indicate the probability of resistant individuals becoming established and persisting under conditions of no parasitism, constant parasitism, and a decline in parasitism over time. (c) Scenarios examining a residual polymorphism of 5% (150 heterozygotes) of the total population of 3,000 individuals. Each data point represents the probability of persistence, which is defined as the proportion of 1000 runs with an extant population at the end of the 50 years of the simulation. Gray lines represent the upper- and lower bounds of probability of establishment and persistence expected based on simulations with high (3,000) and low (1) number of heterozygotes at the beginning of the population for comparison. Black lines indicate the probability of resistant individuals becoming established and persisting under conditions of no parasitism, constant parasitism, and a decline in parasitism over time.

CHAPTER 4

Conclusion

The purpose of my research, presented in this thesis, was two-fold. First, I aimed to understand how and to what extent limitations present in the soil environment, particularly nutrient abundance, limited performance of the exotic, invasive plant garlic mustard (*Alliaria petiolata*). Second, I assessed the community conditions likely to allow adaptation by a native butterfly to increase larval diet breadth through adaptation to garlic mustard, a previously documented evolutionary trap for this species.

As outlined in my second chapter, I concluded based on the results of my first aim that the soil nutrient environment, or both the abundance and availability of nutrients, has limited explanatory and predictive power for plant performance. Both principal component analysis and regression suggest association with potassium and soil pH. Contrary to expectations, phosphorus was not a significant predictor of plant performance, possibly due to high rates of root phosphatase activity (Elk 2010). My study did not evaluate soil moisture, which is suggested to be a greater limitation to plant growth than nutrients (Meekins & McCarthy 2001, Hillstrom & Cipollini 2011).

Hillstrom and Cipollini (2011) evaluated the plastic response of garlic mustard to soil nutrient availability and found large degrees of phenotypic plasticity. Furthermore, it has been established previously that garlic mustard has a high degree of photosynthetic plasticity (Myers et al. 2005). Cumulatively, this

suggests that garlic mustard is tolerant of a variety of nutrient availability conditions, though there is some suggestion that soil moisture can be limiting and impose high mortality to rosettes (Meekins & McCarthy 2001). My study did not evaluate rosette mortality, but since this factor is one of the most important controlling population demography, effects of soil nutrients on summer and overwinter persistence should be evaluated. Any understanding of limitations to population growth, due to rosette mortality, can inform biocontrol and other management efforts.

With regards to my second research goal, I found that the community conditions significantly alter population growth and persistence dynamics for the native herbivore *Pieris napi oleracea*. I evaluated first the likely source of genetic variation for the adaptive trait, and my results suggest that residual variation of the *P. napi* species complex following colonization of and subsequent glaciation in North America are the most likely source explaining presence of the trait in studied populations. The effect of top-down regulation in the form of parasitism from an exotic parasitoid substantially decreased the likelihood of adaptation of the butterfly population. A decrease in parasitism over time did not substantially increase the likelihood of adaptation, though this is possibly an artifact of the model not reintroducing mutants past the first generation. Simulations of enemy free space were the only scenarios to see reasonable levels of population persistence, and thus adaptation. We therefore predict that only with relative enemy free space, and a moderate degree of residual variation in population, will *P. n. oleracea* adapt to include garlic mustard in the larval diet.

This work emphasizes the important of evaluating community conditions, particularly that of the third trophic level, when evaluating native species adaptation to exotic, invasive species. Further work should evaluate the extent to which *P. n. oleracea* has been released from parasitoid pressure due to *Cotesia rubecula* introduction. Previous research has documented that both populations exposed and unexposed to garlic mustard have families positive for the trait of interest (Keeler & Chew 2008, R.A. Steward & Chew unpubl. data). Our model evaluated residual variation at levels of 1 and 5% of the population, however it is unclear whether these are reasonable approximations. Further work should produce realistic estimations of the degree of residual variation, improving predictions for adaptation.

Cumulatively, the work presented in this thesis shows that invasion of New England forests by the exotic plant garlic mustard is in part possible due to a lack of substantial effects of the soil environment on performance. Garlic mustard is relatively free of herbivores in the invasive range (Szentesi 1991, Nuzzo 2000, Blossey et al. 2001, Renwick et al. 2001), and my work shows that possible adaptation of a native herbivore to include this plant in the larval diet has been inhibited by top-down regulation due to an exotic, invasive parasitoid.

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