Climate warming, plant invasions and plant-enemy interactions

Tim Engelkes

Thesis committee

Thesis supervisor
Prof. dr. ir. Wim H. van der Putten
Professor of Functional Biodiversity (Role of Nematodes in Multitrophic Interactions)
Laboratory of Nematology
Wageningen University

Thesis co-supervisors
Dr. ir. T. Martijn Bezemer
Senior scientist
Netherlands Institute of Ecology, Heteren

Dr. Jeffrey A. Harvey
Senior scientist
Netherlands Institute of Ecology, Heteren

Other members

Prof. dr. Dries Bonte	Ghent University, Belgium
Prof. dr. Marcel Dicke	Wageningen University
Dr. Milena Holmgren	Wageningen University
Prof. dr. Hans de Kroon	Radboud University Nijmegen

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Tim Engelkes

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ABSTRACT

The climate is changing and temperatures are predicted to further increase in the future. Species respond to these changes by either adapting to the local warmer conditions and/or range shifting to higher latitudes. Some of these successful range shifting plants can become invasive in their new range. Therefore, there is a conceptual analogy of successful range shifts and biological invasions originating from other continents. Intra-continental plant species shift their ranges within the same contiguous land mass from which they originate. Inter-continental species originate from other continents from where they have been introduced before expanding in their new range. The aim of this thesis is to gain a better understanding of the plant-insect interactions that may contribute to the success of exotic plants that have expanded their ranges due to climate warming. More specifically I aimed to clarify whether climate warming-induced range expanding exotic plants are less suitable than native congener plants and whether these plants suffer less from aboveground enemies than native congener plants. In addition, I investigated if inter –and intra-continental exotic plant species differed in their suitability, and if they responded differently to potential aboveground enemies.

In the first greenhouse experiment, I tested the hypothesis that inter- and intra-continental exotic plants and phylogenetically related native plants from the same habitat do not respond differently to two aboveground polyphagous herbivores. Further I tested if intra- and inter-continental exotic plants experience less negative soil feedback than related native plants. I grew fifteen plant species with and without naive polyphagous locusts (*Schistocerca gregaria*) and cosmopolitan aphids (*Myzus persicae*) and exposed all plants to soils from their invaded range in order to test the feedback from the soil community to plant biomass production. My results show that that both inter –and intra-continental exotic plants on average were better defended against aboveground and belowground enemies than related native plant species. This suggests that successful range expanding plants may include species with invasive properties.

Exotic plants have been shown to have neutral to positive soil feedbacks, while native plants experience negative effects from their soil biota. Belowground interactions can influence aboveground interactions and may change the relationships between exotic plants and their enemies. I examined how the performance of the two aboveground polyphagous herbivores *S. gregaria* and *M. persicae* species was influenced by feedback interactions between the plants and their soil biota and compared these responses in intra- and inter-continental exotic and related native plants. Locust mass was negatively affected by the plant specific soil community and larger on native than on exotic plants. Locust survival was also higher on native plants, but not affected by soil type. There were no differences

between inter –and intra-continental plants. Aphid population size was not affected by soil type, but was highest on the intra-continental range expander. The body size of *M. persicae* was larger on control than on soils with specific plant communities and not affected by plant origin.

One way of measuring the release of exotic plants from natural enemies is by comparing their herbivore loads with related plants that are native in the invaded range. These loads can be influenced by top down control of insect predators and parasitoids. In the field, I examined herbivore loads and predator pressure on two exotic (inter-continental and intra-continental) and two related native plant species. I found smaller herbivore loads on the exotic plant species than on the related native plants. Moreover, the herbivores on the exotic plants had a higher predator pressure than herbivores on the phylogenetically related native plants. These results imply that both types of exotic plants have a double advantage: enhanced bottom-up and top-down control of herbivores.

Finally, I set up a field experiment to test the effect of herbivory on communities of exotic and native plants. I created ten communities with six exotic plant species and their phylogenetically related native species that co-occur in the same riverine habitat. Half of the communities were exposed to herbivory and the other half was grown in a herbivory-free environment. This study was done in order to test if exotic plants may dominate invaded plant communities exposed to aboveground herbivory and if this advantage of the exotic plants under herbivory would disappear when all plants were free of herbivores. Herbivory reduced aboveground plant biomass by almost half. However, exotic plants did not become the exclusive dominants in these communities, as some native species were well protected against aboveground herbivory as well. Plant species varied considerably in their responses to herbivory resulting in changes in community ranking. Interestingly, the proportional biomass contributions to the community were similar for exotic and native plant species and also not different between inter –and intra-continental plants. I conclude that release from aboveground enemies is not the only factor explaining the invasive success of intra- and inter-continental exotic plant species.

In conclusion, climate warming-induced range expanding plant species originating from the same continent may possess invasive properties comparable to introduced inter-continental exotic plants. In the greenhouse and in the field, both inter- and intra-continental exotic plant species were more resistant against aboveground herbivores than native plants. In the greenhouse, the exotic plants suffered less from herbivory than related natives, although this did not result in their absolute dominance in the field when exposed to herbivory. Therefore, aboveground enemy exposure is not the only factor predicting the invasive success of intra- and inter-continental exotic plant species.

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Chapter 1

GENERAL INTRODUCTION

General introduction

Humans are currently inflicting massive changes on ecosystems across the biosphere through a range of processes including the destruction of natural habitats, various forms of pollution and moving species around the globe (Ehrlich & Mooney, 1983; Vitousek *et al.*, 1997; Pimentel *et al.*, 2000). One of the most serious effects humans are inflicting on nature is through the combustion of fossil fuels and its attendant climate warming. Over the past thirty years, the mean surface temperature of the Earth has increased more rapidly than at any time in at least several thousand years and perhaps much longer (IPCC, 2007). Rapid changes in climate, in combination with other human-induced stresses are challenging species to adapt in ways that many have never experienced in their evolutionary history. This problem is particularly acute for plants, which are often much more constrained in their ability to disperse than animals (Berg *et al.*, 2010).

While some plant species may still be able to adapt to locally warmer conditions in their habitat, others may have to respond by shifting their home ranges pole wards tracking their optimal climatic conditions. A small number of species may additionally gain an advantage in their new habitats by escaping from co-evolved natural enemies of their old range. This may enable them to become invasive pests, exhibiting traits that make them dominant (Mack *et al.*, 2000). Although much attention has been paid to the study of invasive plants, the mechanisms underlying the success of biological invasions under the warming climate remain poorly understood (Levine *et al.*, 2003). In this thesis I focus on exotic plants that are shifting their ranges to the north due to regional climate warming in western Europe and examine how changes in plant-enemy interactions, insects in particular, may contribute to the successful invasion of thermophilic weeds into new plant communities.

Climate warming

The climate is warming over many parts of the world at a significantly faster rate than expected, considering historical records (IPCC, 2007). Over just the past 30 years the consequences of rising temperatures and alterations in precipitation regimes have become visible in many parts of the world. As predicted by circulation models (Keeling & Garcia, 2002), regions farthest from the equator are warming much faster than other regions across the biosphere. The Arctic Ocean, for example, is expected to be free of ice by the middle of the century whereas parts of northern Canada and Alaska have experienced temperature increases of 10 °C or more over the past 100 years (IPCC, 2007). Desert ecosystems, such as the Sahara and Gobi Deserts, are also greatly expanding due to reduced rainfall in these regions (Nicholson, 2001). The mean average temperature of the planet's surface has increased by approximately 0.60 °C, compared with the 1951-1980 average (NASA). However, as described above, regional increases have been much greater. Western

Europe is also warming well above the global average; for example, in the Netherlands, the average temperature has increased by almost 2 °C over the past 50 years (KNMI, 2008).

The consequences of global change, including warming for plants and animals, are not yet fully understood. However, it is clear that climate change may intensify the current extinction spasm that is already underway (Thomas *et al.*, 2004; Lovejoy & Hannah, 2005). Certain species may be able to adapt to current changes within their habitats, for example because they are genetically pre-disposed to do so, however other species may have to move to other, more suitable, habitats in order to survive. A species occupying a large range with many niches may be much less affected than a species with a much smaller range or habitat specialists. Species in the latter category may have to adjust their distributions in order to persist. Approximately one quarter of the flowering plants in the Netherlands originate from more southern regions in Europe (Tamis *et al.*, 2005). Some of these species were accidentally or intentionally introduced in the past few centuries and have become a ubiquitous part of the landscape, whilst others are recent arrivals that have naturally dispersed from the south. The arrival of exotic species into new habitats generates novel interactions amongst the exotics and native species (van der Putten *et al.*, 2004). Ultimately, plant and animal communities may be reshuffled to some extent, creating new communities involving natives and recently arrived exotics. The longer-term consequences of these invasions for community and ecosystem functioning are unknown. In any case, rapid warming is likely to increase the rate at which existing communities are invaded by species responding to the warming (Walther *et al.*, 2009). This will increase the rate at which novel interactions are generated and this, in turn, may also lead to an increase in the rate at which communities become invaded by dominants from warmer regions (Walther *et al.*, 2002; Parmesan & Yohe, 2003; Kelly & Goulden, 2008).

Invasive plants

Global climate change and biological invasions represent two of the greatest anthropogenic threats to the functioning of ecosystems (Vitousek *et al.*, 1996). When they are introduced into new habitats, exotic species can become extremely abundant, thereby displacing local native species. This phenomenon is described as 'invasiveness'. Invasive exotic species not only affect directly competing species, but the effects may cascade to other trophic levels in the food web as well (Simberloff, 2006). Moreover, the invasion process may be affected, positively or negatively, by climate change (Harrington *et al.*, 1999). Only about 0.1% of exotic species become serious pests in their new ranges, (Williamson & Fitter, 1996), and the mechanisms underpinning their success and the consequences arising from this have remained elusive.

Biological invasions have traditionally been interpreted on the basis of human mediated introductions of exotic species between continents. However, climate exerts a dominant control over the distribution of both plants and animals (Woodward & Williams, 1987). If these processes operate together it can lead to two kinds of exotic invasions. One group of exotic plants originates from habitats in other continents whereas another group originates from within the same continent (Fig. 1.1). The two groups may have quite different evolutionary histories when invading a new range because they co-evolved with plants and animals in different geographical realms. On the other hand, because it is so widespread, climate change affects plants on all continents, and thus exotic species in both situations are both forced to respond to warmer conditions by either adapting to local conditions or by shifting their ranges into formerly unsuitable habitats (Hill *et al.*, 1999; Parmesan *et al.*, 1999; Walther *et al.*, 2002).

In order to establish, inter-continental exotic plants generally require similar biotic and abiotic conditions in their new habitat for successful establishment and survival upon introduction. However, these plants are often introduced randomly into new habitats where conditions are not necessarily optimal. For example species from other climatic regions may be introduced into regions where local conditions are optimal, whereas others arrive in habitats that are too warm or too cold. Some species may establish locally and begin randomly spreading from the point of origin until they experience conditions that are physiologically limiting. Alternatively, others in sub-optimal habitats may expand their ranges pole-wards north or south, towards a thermal optimum.

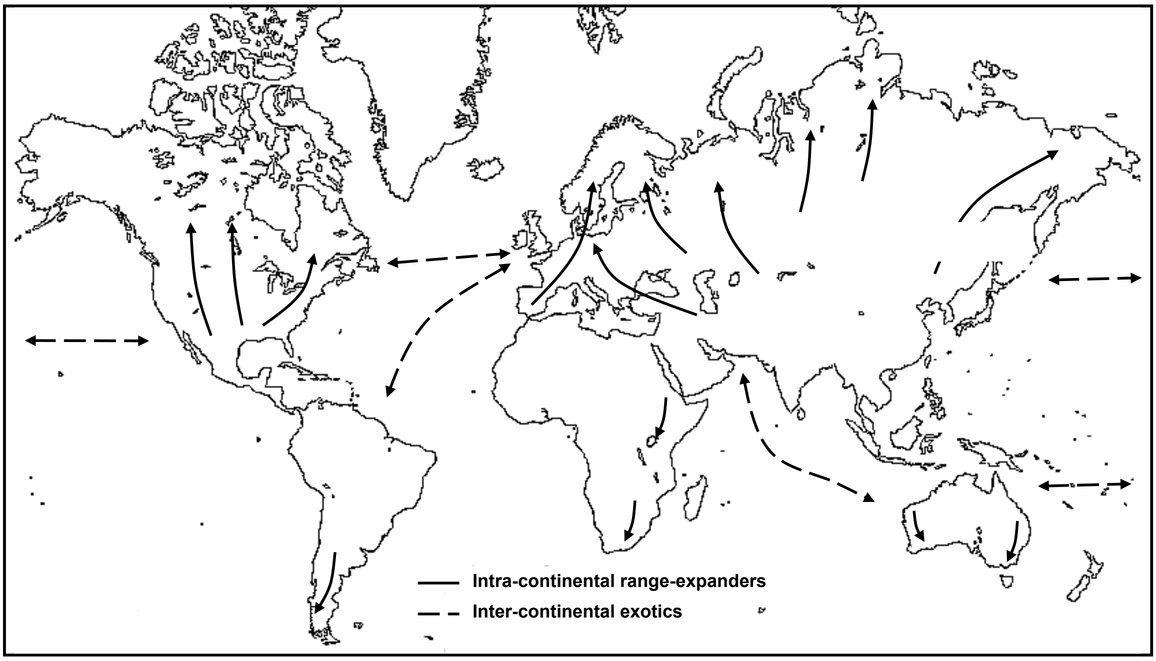

Figure 1.1. Introduction routes for inter- and intra-continental plant species. Both, intra-continental and inter-continental exotic plants that have reached their limiting temperature boundaries expand their ranges pole-wards in all continents due to climate warming. Species in the northern hemisphere disperse northwards and species in southern hemispheres disperse southwards, adapted from (Morriën *et al.*, 2010).

An important difference between invasive plants that originate from the same (intra-continental) or other (inter-continental) continents is that although the effects of climate warming may be similar to both groups, the invasion process for inter-continental species starts in a world not easily accessible by their original enemies. For intra-continental range expanders, the possibilities of their natural enemies to become co-introduced by natural dispersal are much greater (van der Putten, 2010).

Plant-enemy interactions are shaped by reciprocal natural selection (Ehrlich & Raven, 1964). Even small changes in selection pressures experienced by both parties may modify the ecological outcome of their interactions (Thompson, 2005). With the global introduction of species over large distances, species that have evolved in environments that are very different will likely undergo selection pressures that may be completely new (Cadotte *et al.*, 2006). These species establish novel interactions, while old interactions are lost. A comparison between exotic plants with different geographical origins will provide a better understanding of the mechanisms that enable range expanding plants to become invasive.

Plant-enemy interactions

Although over one million insect species are known to exploit plants as their primary food source, the world still appears as a 'green oasis' (Hairston *et al.*, 1960). Interspecific differences in relative levels of damage inflicted by herbivores on plants could reflect concomitant differences in their nutritional quality. The nutritional suitability of a host plant depends on several factors, including the levels of primary (nutrients) and secondary (defensive) metabolites present in plant tissues (Mattson, 1980; Slansky, 1992). Nutrients such as nitrogen and carbon make up an important part of an herbivore's diet. While secondary metabolites may act as repellents or are toxic to poorly adapted herbivores, such as dietary generalists, some insects have become adapted or specialized to certain kinds of allelochemicals whose volatile odours may even serve feeding and oviposition stimulants (Feeny, 1970; Berenbaum *et al.*, 1986; Schoonhoven *et al.*, 1998; Muller-Scharer *et al.*, 2004). These interactions are long evolutionary arms-races consequently making the specialized insect highly dependent on their host species. Hence, specialized insect herbivores that are adapted to particular characteristics of their host plants e.g. by recognizing the shape or odour of their host plant as a suitable oviposition substrate (Renwick & Chew, 1994), may not recognize a novel plant although it may be a suitable host.

Herbivore performance and exotic plants

Herbivores optimize their diet to their nutritional requirements which can be influenced by host plant apparency (Chew & Courtney, 1991) and abundance (Bach, 1988). Hence, the availability of new food resource as a result of invasion by exotic plants may change the performance of herbivores in the invaded plant community.

Different characteristics of the suitability of exotic plants can affect herbivore performance. For example, exotic plants may bring novel defensive compounds into the community they invade which are unknown to the herbivore (Callaway & Ridenour, 2004). Consequently, exotic plants that experience phenotypic 'mismatches' with natural enemies may experience fitness costs or benefits depending on the interaction mechanisms (Verhoeven *et al.*, 2009). The effect of novel allelochemicals in exotic plants on their potential as disruptive invaders has received considerable attention in recent years. Cappuccino and Arnason (Cappuccino & Arnason, 2006) found that invasive plants in North America possessed more novel allelochemistries than did native plants. These novel compounds in exotic plants negatively affect herbivore development compared to native plants (Haribal *et al.*, 2001; Keeler *et al.*, 2006; Cipollini *et al.*, 2008; Keeler & Chew, 2008). Alternatively exotic plants may also exhibit increased levels of defensive compounds when encountering generalist herbivores in their new range. For example, two generalist herbivores, the larvae of *Trichoplusia ni* and *Orgyia vetusts* had decreased performance on the invasive *Eschscholzia californica*, than on the native *E. californica* indicating increased resistance in the invasive type (Leger & Forister, 2005). Similarly, generalist herbivores had lower performance when feeding on invasive seaweed *Fucus evanescens* than on native seaweed (Wikström *et al.*, 2006)

It has also been shown that native herbivores preferred exotic plants over native plants (Parker & Hay, 2005), suggesting that not all exotics may possess invasive characteristics like novel or increased defenses. These results also suggest that some novel defenses can be dealt with by local herbivores. This process is called 'ecological fitting' (Janzen, 1988; Agosta, 2006; Agosta & Klemens, 2008). For example, insects can often complete their development on many more plant species than the range of species that are actually being used in their current habitat (Smiley, 1978; Janz *et al.*, 2001).

When native plants adjust their ranges in response to climate warming, theoretically their co-evolved insects may move with them (Andrew & Hughes, 2004; Agosta, 2006; Merrill *et al.*, 2008). However, in their new range, plants that colonize new areas also may experience changes in the composition of the associated insect community (Lawton & Strong, 1981; Rohde, 1992) where novel interactions with consumers may become established.

Another important point is that plants are exposed to potential enemies in both the above-ground and below-ground 'compartments'. The ability of aboveground and belowground enemies to track their host plant may differ, particularly as the soil biota have much lower, or untargeted dispersal capacity than many aboveground biota (van der Putten *et al.*, 2004; Berg *et al.*, 2010). The consequences for exotic plants to perform under these altered aboveground and belowground community compositions are mostly unknown.

Higher trophic interactions and exotic plants

Higher trophic levels such as predators and parasitoids play and important role in controlling damage to plant tissues by feeding on herbivores (Hairston *et al.*, 1960; Price *et al.*, 1980; Schmitz, 2008). The importance of top-down control by predators and parasitoids is best described from biological control programs in agriculture where these insects are used to naturally control insect pests in crops (Julien & Griffiths, 1996). Importantly, interactions involving exotic plants and native insects will almost certainly work up to the third (or even higher) trophic level (Harvey *et al.*, 2003; Harvey *et al.*, 2009). However, thus far virtually all studies of exotic plants and insects have been based on two-trophic level interactions (Harvey *et al.*, 2010) (e.g. plant and herbivore) but see Cronin & Haynes (2004). Differences in the abundance of herbivores on native and exotic plants may in turn affect the abundance of predators and parasitoids (Cronin & Haynes, 2004). However, differences in the nutritional quality of exotic and native plants may also trickle up the food chain and affect the performance of predators and parasitoids as mediated through the herbivore prey or host (Barbosa *et al.*, 1986; Barbosa *et al.*, 1991; Harvey *et al.*, 2003; Harvey, 2005; Ode, 2006; Gols *et al.*, 2008). For a better understanding how herbivore impact differs between native and exotic plant species we need to include differences in top-down pressures from predators.

Plant soil interactions

Plant performance is influenced by a range of processes, including interactions with biotic and abiotic components of the soil (Wardle *et al.*, 2004). Plants take up resources from the soil and also return resources to the soil. This process interacts with soil organisms that live in or around the root system of plants. Soil organisms may affect plant performance in positive or negative ways. Nematodes, insect herbivores and microbial pathogens can negatively affect plant performance by attacking the root system, although in some situations plants may actually benefit from root herbivory (Agrawal, 2000; Gange, 2001). On the other hand mutualistic organisms, such as nitrogen-fixing bacteria or mycorrhizal fungi, can increase plant performance by making nutrients present in the soil more easily accessible to the plant. In addition, belowground organisms can affect each other in many different ways that depend on the plant species. Therefore, different plant species will develop their own specific soil communities (Bezemer *et al.*, 2010). The ecological outcome of the interplay between the plant and mutualistic and antagonistic organisms in the soil as these affect plant fitness is known as a 'soil feedback' mechanism (Bever *et al.*, 1997; Ehrenfeld *et al.*, 2005).

A mechanism for successful invasion

The enemy release hypothesis implies that plant species can become invasive because of reduced pressure from herbivores in their new range when compared to the original range (Elton, 1958; Keane & Crawley, 2002). The enemy release hypothesis also includes that exotic plants may lose their co-evolved specialized natural enemies from their native range (Wolfe, 2002; Vila *et al.*, 2005). The net result of old enemies lost and new (generalist) enemies gained may be a reduction in herbivore pressure favoring the exotic plant relative to the native plants in the receiving community. However, exotic plants may also be preferred by native herbivores (Parker & Hay, 2005; Parker *et al.*, 2006), a process that may limit the ability of an exotic plant to become established or dominant (Louda *et al.*, 1997). This is called biotic resistance (Keane & Crawley, 2002).

The ultimate test of 'enemy release' is to see whether herbivore densities on a plant in its native range are higher than in the exotic range and whether this results in enhanced performance of the exotic plants in their new range. However, newly encountered enemies may exert stronger selective pressures on the plant than the ones in the native range. The performance of exotic plants thus also depends on its suitability towards newly encountered herbivores. Whether or not exotic plants have lower suitability compared to native plants can be tested by determining herbivore performance. If in general herbivores experience decreased performance on exotic plants as a consequence of lower suitability, this could be a mechanism supporting reduced enemy control leading to invasive plants.

Approaches for testing a mechanism for successful plant invasion

In order to test whether exotic plants experience enemy release, herbivore numbers or herbivore loads could be compared between the native and exotic range or between exotic and native species in the invaded range. Many studies testing the enemy release hypothesis focused on single species comparisons (Jobin *et al.*, 1996; Memmott *et al.*, 2000; Blossey *et al.*, 2001; Agrawal & Kotanen, 2003; DeWalt *et al.*, 2004; Wolfe *et al.*, 2004). However, it is difficult to make general predictions about the efficacy of the Enemy release hypothesis since factors underlying the success of plants in becoming invasive are probably association-specific. This is because different species may respond to novel abiotic and biotic stresses in different ways. Therefore, a better understanding of the different mechanisms underlying the success of invasive exotic plants can be made by including a greater number of exotic species in studies of biotic invasions (Agrawal *et al.*, 2005). Phylogenetically related species are more similar in ecology, biology and chemical and physical properties than phylogenetically more distant or unrelated species. By using phylogenetically related plant species in comparative studies of plants from native and exotic origin, testing for differences in insect performance will more likely reflect actual differences in chemical properties rather than differences in ecology (Felsenstein, 1985; Agrawal & Kotanen, 2003; Strauss *et al.*, 2006).

Belowground interactions between the plant and soil community can be highly species-specific and changes in the abundance of particular soil biota can affect plant performance depending on the nature of the relationship (van der Putten, 2003). Plant root growth affects the belowground community physically and through the production of nutrients and secondary compounds. Antagonists like root herbivores or plant feeding nematodes are expected to negatively influence plant performance, whereas mutualists like mycorrhizal fungi benefit plant performance directly and indirectly via changes in soil nutrient availability. In order to determine the net effect of positive and negative interactions between the plant and its specific soil biota can be compare by plant-soil feedback approach (van der Putten, 2003). In that approach, plants are grown in soil in which they have conditioned biotic soil conditions and their performance is compared with that in a control soil. The difference in plant growth between conditioned and control soil is an indication of whether the plant experienced more negative than positive effects from the soil community. If the soil feedback is neutral, then effects of antagonists are neutralized by those of symbiotic mutualists. By comparing the soil feedback in exotic and related native plant species it is possible to test if exotic plants experience less enemy exposure than native species as indicated by a less negative to positive soil feedback effect.

Thesis outline

The main objective of this thesis is to get insight in whether climate warming induced range expanding exotic plants suffer less from aboveground enemies than native plants and whether exotic plants are less suitable than native plants leading to lower enemy performance. This objective also involved the understanding whether exotic plant species that have expanded their range from warm into previously colder habitats can become invasive via the same reduced enemy exposure mechanism that is assumed to benefit the performance of invasive exotic plants that are introduced from other continents.

In Chapter 2, I start by studying the impact of above and belowground enemy pressure on exotic range-expanding plants and their native congeners. I test the hypothesis that range-expanding plants suffered less from aboveground generalist herbivores and from the soil community.

In Chapter 3, I examine the influence of soil feedback of range-expanding plants and their native congeners on the performance of two aboveground polyphagous herbivores. The variety of soil feedback patterns may result in either enhanced or reduced performance of aboveground insects and could be due to less exposure to soil-borne enemies, or to enhanced exposure to belowground symbionts of other beneficial soil biota. Here we test the null hypothesis that plant-soil feedback interactions do not alter the performance of aboveground insects. To test this I use two herbivores that are known to be able to feed on a wide variety of plant species, one species being a leaf chewing locust and the other species a phloem feeding aphid.

In Chapter 4, I test the validity of the greenhouse results under field conditions. I compare the overall herbivore loads and the loads of four feeding guilds (leaf chewers, sap suckers, gallers and miners) on two exotic species, one from intra –and one from inter-continental origin, and their native congeners. In addition, I also examine differences in predator loads on the invertebrate herbivores. I test the hypothesis that herbivore loads on both exotic species are lower than on their native congeners. Further, I expect that predator loads on the herbivores will not be different. In addition, I hypothesize that herbivore loads, as well as predator loads on herbivores are not different between inter-continental and intra-continental exotic plants.

In Chapter 5, I test the hypothesis that reduced exposure of exotic plants to aboveground herbivores will provide the exotic species with an advantage over related natives. In this test, I include both inter- and intra-continental range expanding exotic plants. This hypothesis is tested in a field experiment using gauze cages to exclude aboveground herbivores (both vertebrate herbivores and insects) from planted communities of exotic and related native plant species.

Finally in Chapter 6, I discuss and synthesize the main findings from this thesis and present ideas for future directions in invasion ecology and in global change research.

Chapter 2

SUCCESSFUL RANGE-EXPANDING PLANTS EXPERIENCE LESS ABOVE-GROUND AND BELOW-GROUND ENEMY IMPACT

T. Engelkes, E. M. Morriën, K. J. F. Verhoeven, T. M. Bezemer, A. Biere,
J. A. Harvey, L. M. McIntyre, W. L. M. Tamis, W. H. van der Putten

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Abstract

Many species are currently moving to higher latitudes and altitudes (Walther *et al.*, 2002; Parmesan & Yohe, 2003; Pearson & Dawson, 2003). However, little is known about the factors that influence the future performance of range expanding species in their new habitats. Here, we show that range expanding plant species from a riverine area were better defended against shoot and root enemies than related native plant species growing in the same area. We grew fifteen plant species with and without non-coevolved polyphagous locusts and cosmopolitan polyphagous aphids. Opposite to our expectations, the locusts performed more poorly on the range expanding than on the congeneric native plant species, whereas the aphids showed no difference. The shoot herbivores reduced biomass of the native plants more than of the congeneric range expanders. Also the range expanding plants developed fewer pathogenic effects (Klironomos, 2002; Van Grunsven *et al.*, 2007) in their root zone soil than the related native species. Current predictions forecast biodiversity loss due to limitations in the ability of species to adjust to climate warming conditions in their range (Warren *et al.*, 2001; Thomas *et al.*, 2004a,b). Our results strongly suggest that the plants that shift ranges towards higher latitudes and altitudes may include potential invaders, as the successful range expanders may experience less control by aboveground or belowground enemies than the natives.

Introduction

Range expansion is a key adaptive feature of species in response to changes in climate, habitat availability and other limiting factors (Walther *et al.*, 2002; Parmesan & Yohe, 2003; Warren *et al.*, 2001; Thomas *et al.*, 2004a,b; Lovejoy & Hannah, 2005; Brinkhuis *et al.*, 2006). Currently, a number of species are showing rapid range expansion from warmer into previously colder biomes (Tamis *et al.*, 2005). As not all species have the same range shift capacity, ecological interactions may become disrupted as the community species pool changes (Lovejoy & Hannah, 2005). Rapid range expansion and the loss of control by natural enemies are key features of invasive species (Levine *et al.*, 2006; Keane & Crawley, 2002). However, very few studies have actually investigated range expansion in relation to enemy exposure (van Grunsven *et al.*, 2007; Menendez *et al.*, 2008). The aim of our study was to examine how rapidly range expanding plant species are defended against above-ground and below-ground natural enemies as compared to related plant species that are native in the expansion zone.

Plants are usually attacked by a wide variety of aboveground and belowground natural enemies (van der Putten *et al.*, 2001). It is well established that invasive exotic plants are less exposed to aboveground and belowground control by natural enemies than related natives in the new range (Klironomos, 2002; Maron & Vila, 2001; Callaway *et al.*, 2004; Reinhart *et al.*, 2003; Mitchell & Power, 2003; van der Putten *et al.*, 2005). However, phylogenetically controlled empirical evidence of exotic plant control by natural enemies is elusive (van Grunsven *et al.*, 2007; Agrawal *et al.*, 2005). Here, we compare range expanding invasive plants of inter-continental origin and intra-continental range expanding species with congeneric native plant species, all co-occurring in a riverine area. Aboveground, we exposed range expanding exotic plants of inter and intra-continental origin and congeneric native species to non-coevolved naïve polyphagous herbivores, as well as to cosmopolitan polyphagous herbivores. In the same experiment, we exposed all plants to a general soil community from the invaded range and compared their plant-soil feedback responses (Bever *et al.*, 1997). We tested the hypothesis that the plants would not differ in their response to the polyphagous shoot herbivores, as all plants had equal familiarity with them, but that both the inter- and intra-continental range expanding species would develop less negative soil feedback than the related natives.

Methods

Floristic data were analyzed to identify exotic plant species in riparian areas in The Netherlands, which all have become well established in the 20th century. We surveyed plants with a strong increase in abundance over the past few decades with congeneric relatives in the same habitat. We obtained seedlings of a selection of three intra-continental range expanders, three species that originated from other continents and naturalized in southern Europe prior to their northward range expansion, and nine natives (Supplementary Table S2.1). Three extra native plant species were included to test the sensitivity of our phylogenetic comparison for species-specific effects. Soil samples were collected from Millingerwaard, inoculated into sterilized sandy loam soil, placed in 4 L pots and planted with 4 individuals of one species pot^{-1}. After 8 weeks in a greenhouse, the plants were harvested and the soils were used for a second growth experiment in order to measure plant-soil feedback effects (Bever *et al.*, 1997; van der Putten *et al.*, 2007). In that second stage, each plant species was grown in own soil (previously containing individuals of the same species) and control soil (a mixture of soil from all other plant species, excluding species from the same genus). After 7 weeks, we placed all pots individually in cages and added aboveground herbivores to half the control soil pots that had been assigned to the herbivory treatment at the start of the experiment (n = 5). We used 5 day-old first instar locust nymphs of the African desert locust, *Schistocerca gregaria* (Forskål) which is highly polyphagous throughout all stages of its development and is non-coevolved with any of the tested plant species. Also *Myzus persicae* (Homoptera; Aphididae), the green peach aphid, a highly polyphagous herbivore, was used which has a cosmopolitan distribution. Three weeks after adding the herbivores, all plants were harvested, dried, weighed, and analysed.

Results

Opposite to our hypothesis, aboveground herbivory influenced plant biomass of range expanding species differently from the natives (plant origin x herbivory interaction: $F_{1,108} = 4.58$; $P = 0.035$; Fig. 2.1a). Herbivores caused significant biomass loss to native plants (the species mean proportional biomass reduction was -38.7% and differed from zero: t = -2.98, d.f. = 8, $P = 0.017$), whereas the effect of herbivory on the range expanding species was much smaller and not significantly different from zero (effect size -17.3%: t = -1.69, d.f. = 5, $P = 0.151$; Fig. 2.2a).

Although the range expanding species overall had more shoot biomass than the native species ($P < 0.0001$), locust survival was significantly lower on the range expanding than on the native species ($F_{2,52} = 9.57$, $P = 0.0003$ after Post-hoc Tukey; Fig 2.3a). Aphid numbers, on the other hand were not significantly affected by host plant origin (n = 15, $H = 0.897$, $P = 0.639$; Fig 2.3b). The negative effect of the range expanding plants on the locusts could not be explained by two general indicators of food quality, C/N-ratio and N content of the foliage ($P = 0.197$ and $P = 0.597$ respectively). Interestingly, the levels of phenolic compounds in the foliage were higher in range expanding plants with herbivory than in range expanding plants without herbivory and in the native plants with and without herbivory (interaction effect $F_{1,103} = 13.07$; $P = 0.0005$; Supplementary Fig. S2.1).

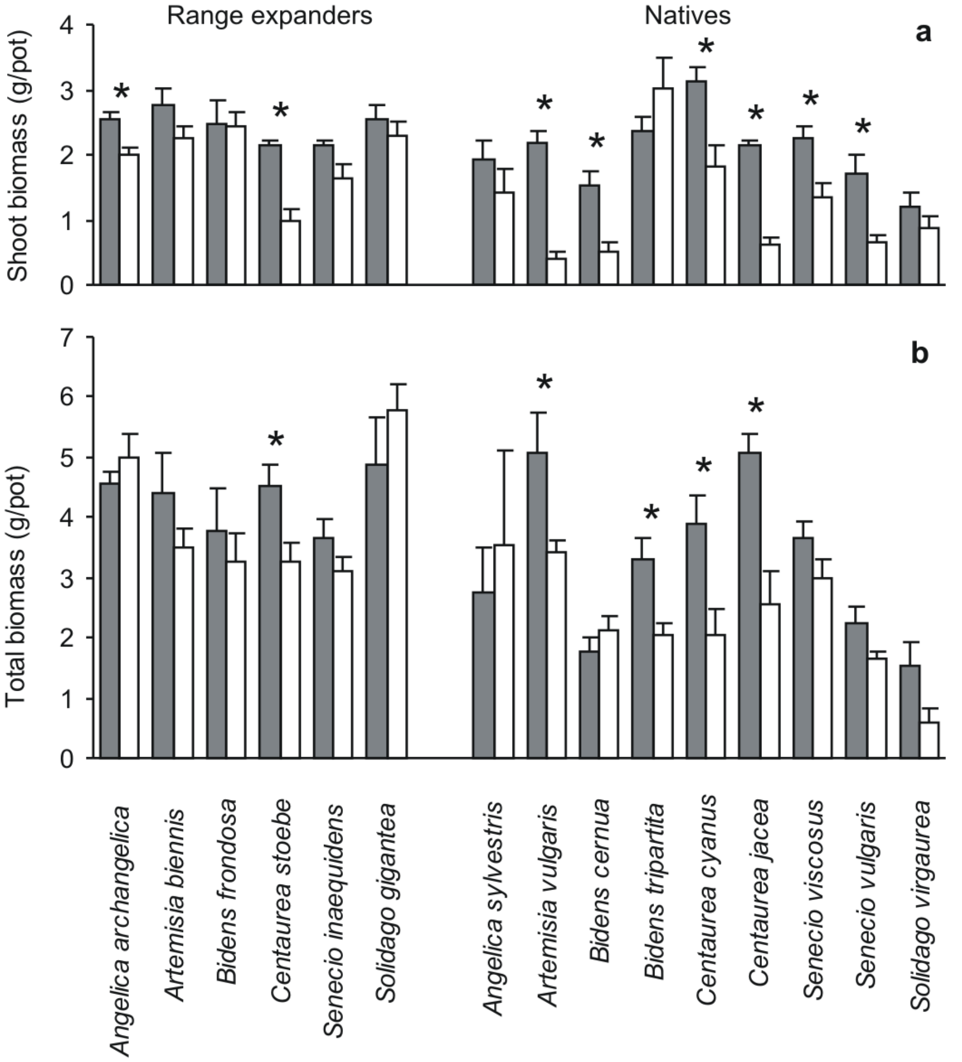

Figure 2.1. Biomass of range expanding exotic and related native plants as influenced by non-coevolved and cosmopolitan polyphagous shoot herbivores and by soil feedback. Upper panel (a): shoot biomass (mean dry weight ± s.e.m.) of range expanding exotic and congeneric native plants without herbivory (grey bars) and plants exposed to aboveground herbivory by the locust *Schistocerca gregaria* and the green peach aphid (*Myzus persicae*) (white bars) show that most plants experienced a significant biomass loss during three weeks of exposure, but that biomass loss due to herbivory was severest on native plants. Lower panel (b): total biomass (mean dry weight ± s.e.m.) on control soil (grey bars) and own soil (white bars) shows that natives are reduced more than range expanding exotic species on own as compared to control soil. Bars show back-transformed means of log-transformed data. In both panels, an asterisk above a pair of bars indicates statistically significant effects of treatment within plant species (t-test, $P < 0.05$).

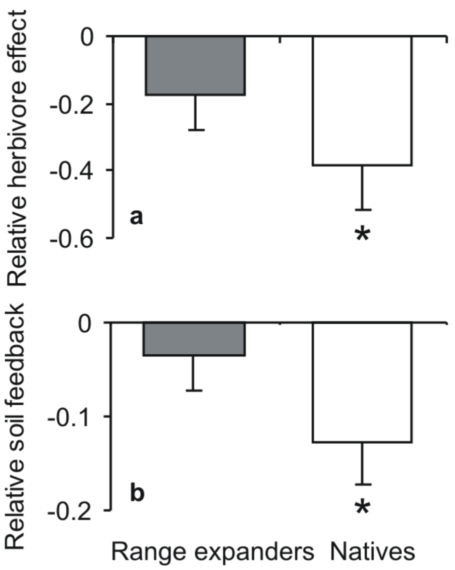

Figure 2.2. Average responses of range expanding exotic plants (grey bars; n=6 species averages) and related native plants (white bars; n=9 species averages) to herbivory by non-coevolved and cosmopolitan polyphagous shoot herbivores and by soil feedback. Upper panel (a): Relative effects of aboveground herbivory by the locust *Schistocerca gregaria* and the green peach aphid (*Myzus persicae*) on shoot biomass (mean dry weight ± s.e.m.). Lower panel (b): The feedback effect of the soil community to total biomass production. Native species on average experienced significant negative soil feedback (indicated by asterisks; $P < 0.05$), whereas exotic range expanding plants did not differ from a neutral response ($P > 0.05$). Panel (b) shows back-transformed means of log-transformed data.

This indicates that range expanding plants were better than natives in inducing general defenses against non-coevolved shoot herbivores. The inter-continental range expanders were slightly less negatively affected by herbivory than the intra-continental range expanders (range expander origin x herbivory: $F_{1,44} = 4.25$, $P = 0.045$; Supplementary Fig. S2.2a). Nevertheless, the three intra-continental range expanders suffered significantly less from shoot herbivory than the congeneric natives (origin x herbivory $F_{1,52} = 6.45$; $P = 0.014$). *Bidens* was the only genus to show contrasting effects between native species within a genus (Supplementary Fig. S2.3a).

Native plant species also suffered more from belowground biotic interactions in their own soil compared to control soil than range expanding plants (plant origin x soil interaction: $F_{1,112} = 4.16$, $P < 0.043$; Fig. 2.1b). The native species experienced significantly negative soil feedback (-12.8%, difference from zero: t = -2.52, d.f. = 8, $P = 0.036$), whereas that of the range expanders was much smaller and not different from a neutral effect (-3.7%, difference from zero: t = -0.96, d.f. = 5, $P = 0.381$; Fig. 2.2b). The performance in own versus control soil did not differ between the intra and inter-continental range expanders (range expander origin, soil and the interaction between range expander origin x soil are: $F_{1,46} = 0.41$, $P = 0.526$; $F_{1,46} = 2.39$, $P = 0.129$ and $F_{1,46} = 0.84$, $P = 0.363$; Supplementary Fig. S2.2b). As observed for aboveground herbivores, a contrasting effect between native species within genus was observed for *Bidens* only (Supplementary Fig. S2.3b).

Across the herbivory and soil feedback treatments, in 14 out of 18 within-genus comparisons the biomass reduction of the natives was stronger than of the range expanders (non-parametric Sign Test M = -5, *P* = 0.031; see Supplementary Information). However, above and belowground biotic interactions did not vary in concert with each other; Spearman's rank order correlation of the shoot herbivore and soil feedback effects on species within sets of native and range expanding plant species were not significant (*P* = 0.865 and *P* = 0.329 respectively; see Supplementary Information), we conclude that although range expanding plants were less sensitive to shoot herbivory and negative soil feedback than natives, the magnitude of the above and belowground effects did not necessarily vary in the same order.

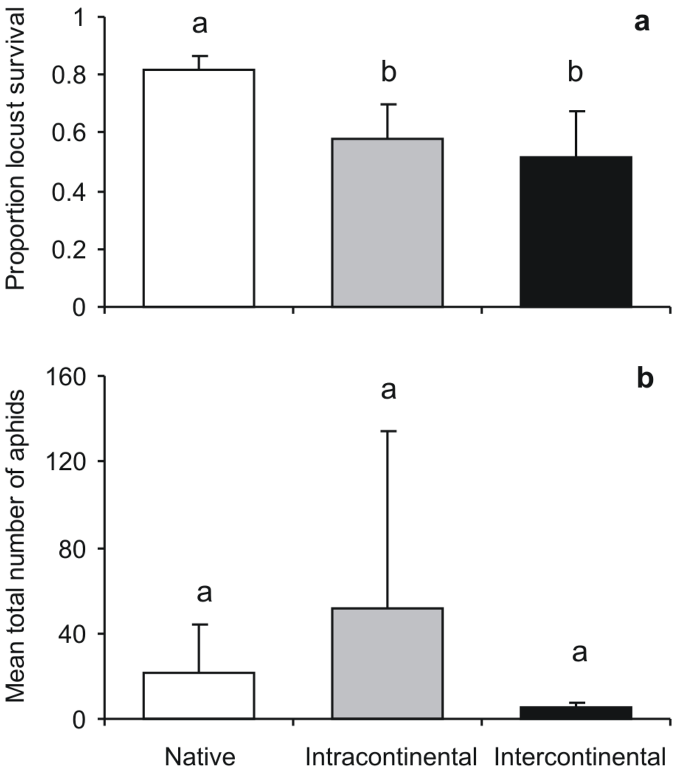

Figure 2.3. Performance of non-coevolved and cosmopolitan polyphagous shoot herbivores on native plant species and range expanding species of intra –and inter-continental exotic origin. Upper panel (a): The proportion of survival (back-transformed means ± s.e.m. from arcsine data) of the naïve generalist herbivore *Schistocerca gregaria*, that did not have any previous experience with any of the plant species used, on native (white bars), intra-continental range expanders (grey bars) and inter-continental range expanders (black bars) shows an on average lower survival on range expanders from both origins relative to native host plant species. Letters indicate significant differences between bars. Lower panel (b): Mean total numbers (± s.d.) of the generalist aphid *Myzus persicae* after 3 weeks feeding assay demonstrate that the on average population increase is independent of the origin of host plants; native (white bars), intra-continental (grey bars) and inter-continental (black bars) respectively.

Discussion

Our results provide new evidence that plants which are successful in range expansion towards higher latitudes interact more differently with shoot herbivores than congeneric plant species that are native to the invaded range. Although all plant species were equally novel to the desert locust, the locusts experienced reduced survival on these successful range expanders, but not on these related native plants. On the other hand, the cosmopolitan aphid was not influenced differentially by plant origin. Our hypothesis predicted no differences; however, the shoot herbivores reduced the biomass of these range expanding plants less than that of these related native plant species. The negative soil feedback of these native plants as compared to these range expanders was more in line with our hypothesis. Thus far, studies on enemy exposure to exotic invasive weeds have usually focused on enemies from the invaded range, or on invasive enemies (Parker *et al.*, 2006). Our results suggest that the plant species successfully expanding their range towards higher latitudinal riparian areas possess superior defense traits when compared to related native species. In this respect, these successful range expanders have similarities with invasive exotic plants (Agrawal *et al.*, 2005), which also are superior in short-term resource acquisition (Funk & Vitousek, 2007), although there was no correlation between the strengths of above and belowground enemy effects.

Thus far, most attention has focused on the uncoupling of food chain interactions due to regional climate warming (Menendez *et al.*, 2008; Davis *et al.*, 1998; Both & Visser, 2001). Here we show that some successful range expanding riparian plant species (Tamis *et al.*, 2005) have less aboveground and belowground enemy impacts, even when exposed to non-coevolved and cosmopolitan polyphagous aboveground herbivores. Thus, these successful range expanders differed in defense trait characteristics from these congeneric natives. Our sampling strategy was focused on successful range expanders into northern riparian habitats. Future studies should also explore other habitats, as well as less successful range expanders in order to test whether, for example, trees and dry land plant species show similar responses. Poor range shift capacity has been predicted to result in a loss of diversity (Warren *et al.*, 2001; Thomas *et al.*, 2004a). However, the prediction of consequences of climate warming and other changes that result in range expansion require inputs from different fields in ecology (Guisan & Thuiller, 2005). Our results suggest that successful range expanding plant species may include species with invasive properties, which is crucial information for the future conservation of biodiversity in temperate and northern latitudes.

Acknowledgements

We thank Staatsbosbeheer Regio Oost for allowing permission to work in Millingerwaard, the late Ruud van der Meijden, Baudewijn Odé, Kees Groen, Roy van Grunsven and Pella Brinkman for discussions, Marco Houtekamer for C and N determination, Tineke Vos, Miranda Vlag, Annemiek Weerheijm and Wiecher Smant for assistance and Leo Koopman for providing the locusts, Linda Young and Evert Jan Bakker for their statistical advice and the anonymous referees for their constructive comments.

Supplementary Information

Plant species, seeds, soil and aboveground herbivores

We set out to compare exotic range expanding and related native species accoding to the following criteria; The range expanding plants have established in the Netherlands in the 20th century, they increased in grid cell abundance in the last decades of the 20th century, they have related native species in the same genus and they all occur in the same habitat. This information was derived from the National Standard List of the Dutch flora (Tamis, 2005) using square kilometer frequency records collected before 1950, between 1975-1987 and between 1988-1999. In order to calculate the national frequency of a plant species, we first calculated the sum of the proportional presence in all the 25 regions of The Netherlands. This proportional presence is calculated by the regional presence of a species (F_E) times its frequency in surveyed kilometer cells (W_E). The national presence of a plant species in The Netherlands (P_{Neth}) is calculated by multiplying the sum in all regions by 1,000 divided by the total number of kilometer cells in the Netherlands (A_{Neth}). This national presence of a species is expressed as a permillage of all kilometer grid cells (Tamis, 2005).

$$P_{Neth} = \frac{1{,}000}{A_{Neth}} \times \sum_{E=1}^{E=25} F_E \times W_E$$

Plants were considered successful range expanders if they were first recorded throughout in the twentieth century and showed a 10-fold increase in frequency in the last decade (nineteen nineties) when compared to the first half of the twentieth century. Based on the above criteria we chose to census range expanders and congeneric natives from the same riverine habitat of the Geldersche Poort region. The floristic database search yielded 17 successful range expanders from this habitat type. From these, we included in the experiment all species (six, from six different genera) that had a native congeneric species occurring in the same habitat and for which we could obtain and successfully germinate seeds. For each exotic range expander we included in the study one (three genera) or two (three genera) native species from the same genus (Supplementary Table S2.1). All species belong to the family *Asteraceae*, except for the genus *Angelica* (*Apiaceae*).

Seeds were collected from the field or, in some cases, purchased through a specialized seed supplier who collects seeds from local plant populations. All seeds were surface sterilized by a 1 % hyperchloride solution and germinated on glass beads supplied with demineralised water at a 10-20 °C, 10-14 hrs night-day regime for early summer species and a 15-25 °C, 8-16 hrs night-day regime for late summer species. In order to synchronize the ontogeny, the seedlings were placed at 4 °C with continuous illumination until transplantation. After transplantation, dead seedlings were replaced until the third week of the experiment.

Supplementary Table S2.1. Origin, climate characteristics and frequency of occurrence of 6 range expanding exotic and 9 related native plant species that have been used in the present study. Range expanding species originate from either Eurasia or other continents; all species entered The Netherlands through range expansion. The original range of distribution of each species (4th column) is matched with the climate conditions (3rd column) within this range according to the modified Köppen-Geiger climate classification (Kottek *et al.*, 2006). The 5th and 6th columns show species frequencies of occurrence in the Netherlands before 1950 and at the end of the 20th century, respectively. These frequencies indicate the amount of grid cells across The Netherlands occupied by that specific plant species before 1950 and between 1988 and 2000, expressed on a per mil basis. In the last column, the percent change in frequency is based on the number of grid cells in The Netherlands where the species have been observed (see Supplementary methods above for calculation) after 1988, when temperatures started to rise (Tamis *et al.*, 2005). When considering species as replicates, the change in frequency is significantly higher for exotic range expanding species than for native species (one-way ANOVA; $F_{1,13}$ = 23.48, $P < 0.001$).

Species	Continental origin	Climate range of origin	Geographical origin	Freq. (‰) < 1950	Freq. (‰) 1988 – 2000	Change (%) 1950 - 2000
Angelica archangelica	*Eurasian*	Humid continental	NE-Europe	9	49	437
Angelica sylvestris	*Eurasian*	Marine west coast	NL	474	460	-2.96
Artemisia biennis	*Eurasian*	Subarctic-Humid continental	N-Asia	0.4	3	669
Artemisia vulgaris	*Eurasian*	Marine west coast	NL	739	792	41.9
Centaurea stoebe	*Eurasian*	Humid continental-Mediterranean	C-Europe	0.05	0.1	82.2
Centaurea cyanus	*Eurasian*	Marine west coast	NL	147	75	-49.3
Centaurea jacea	*Eurasian*	Marine west coast	NL	379	317	-16.5
Bidens frondosa	*non-Eurasian*	Humid continental-Mid latitude steppe	N-America	79	225	186
Bidens cernua	*Eurasian*	Marine west coast	NL	155	175	12.8
Bidens tripartita	*Eurasian*	Marine west coast	NL	360	345	-3.92
Senecio inaequidens	*non-Eurasian*	Humid subtropical	S-Africa	3	119	3634
Senecio viscosus	*Eurasian*	Marine west coast	NL	93	161	73.3
Senecio vulgaris	*Eurasian*	Marine west coast	NL	739	792	7.25
Solidago gigantea	*non-Eurasian*	Subarctic-Mid latitude steppe	N-America	36	193	442
Solidago virgaurea	*Eurasian*	Marine west coast	NL	27	16	-42.9

We collected soil from five randomly chosen sites in the Millingerwaard, (the Netherlands; 51°87′ N, 6°01′ E), a nature reserve in the Geldersche Poort region where all range expanding and related native plant species co-occur. The soil samples were homogenized, as we were not interested in spatial variation in the field, and used as an inoculum and introduced into a sterilized sandy loam soil from Mossel, Planken Wambuis (52°06′ N, 5°75′ E). The soil sterilization was carried out by gamma radiation (25 kGray), which eliminated all soil biota (van der Putten *et al.*, 2007).

As a naïve herbivore, we choose the generalistic African desert locust *Schistocerca gregaria* (Forskål), because this species is highly polyphagous during its gregarious phase. This locust is not native to the Netherlands and is unlikely to share a co-evolutionary history with any of the plant species used, as it occurs in north-central Africa and Asia. The exclusive circumstance of the feeding naïveness of the locust towards all plant species, enabled us to consider all plants, both the range expanding and the native species, as having defenses which are potentially novel to the herbivore. The nymphs were obtained from a gregarious rearing on grasses of the Laboratory of Entomology of Wageningen University, The Netherlands. As a cosmopolitan generalist herbivore, we selected *Myzus persicae* (Homoptera; Aphididae), the green peach aphid, which was obtained from a culture from Wageningen University. It is highly polyphagous and feeds on a wide variety of host plant families.

Experimental setup

Phase I: soil conditioning. One hundred and fifty 4L pots were filled with a 5:1 mixture of sterilized soil and inoculum soil collected from Millingerwaard. We established 10 replicate pots of each plant species (6 range expanders and 9 natives). Each pot received 4 seedlings and the experiment was carried out in a greenhouse under controlled conditions (60 % RH, day: 21 ±2°C; night 16 ±2°C). Additional light was provided by metal halide lamps (225 μmol $^{-1}$ m^{-2} PAR) to ensure a minimum light intensity during 14 hr daytime. Plants were provided with demineralised water every second day to compensate for water uptake and evapotranspiration. Every week, initial soil moisture level was reset by weighing. In order to prevent plants from nutrient depletion, Hoagland solution was added at a rate of 25 ml of 0.5 strength week^{-1}, which is a dosage that does not prevent the establishment of arbuscular mycorrhizal fungi (van der Putten *et al.*, 2007). After 8 weeks of growth, the plants were harvested and the conditioned soils were used for a second growth phase to test the plant-soil feedback effect and the effect of aboveground herbivory.

Phase IIa: soil feedback. The conditioned soil from every pot in phase I of the growth experiment was split into two halves. One half was placed in a 1.3L pot to be called 'own' soil. The other half was used to create a pot with control soil. The control soil of every plant species contained soil conditioned by all other plant species, excluding plants from the same genus. We established five replicates with own and ten with control soils: each replicate was made from a separate replicate from the soil conditioning phase. Five of the ten pots with control soils were assigned randomly to a shoot herbivory treatment (further described below at Phase IIb). We planted 3 seedlings per pot. Water, light and nutrient conditions were supplied as in phase I, except that 10 ml of 0.5 Hoagland solution was added on a weekly basis. This reduced and more concentrated rate was necessary, because the pots were smaller, there were fewer plants and there was less evaporation from the soil surface. After week 10, all roots and shoots were harvested, air-dried at 70 °C for 48 hours and weighed as total root and shoot biomass per pot. Soil-feedback was calculated using total (shoot and root) dry biomass. Soil feedback was calculated for each replicate separately as: (total biomass own soil – total biomass control soil) / (total biomass control soil)^2. A negative feedback indicates net pathogenic activity, whereas a positive feedback indicates net symbiotic activity, whereas a neutral feedback indicates that pathogens and symbionts are either not active, or that they neutralize the effects on each other (van der Putten *et al.*, 2007).

Phase IIb: shoot herbivory. In the 7th week, the five replicates of the control plants that had been assigned randomly to the shoot herbivory treatment at the start of the experiment were exposed to the locusts, which were added at a rate of $3.pot^{-1}$. The African desert locusts (average weight = 0.0858 g, n=79) and the aphids were prevented to escape by placing all pots (including those from the soil feedback experiment) individually in spherical nets (Ø 25 cm, height 1.5 m). Before the start of the treatment the locust nymphs were starved for 24 hours. Subsequently, they were allowed to feed for 3 consecutive weeks until harvest. Once per week, locust survival was determined. The first cohorts of the aphids were reared on white radish (*Raphanus sativus*) in transparent boxes (40 cm • 50 cm • 65 cm) which were stored in a climate room with conditions of 21 °C, a 14-hr light / 10-hr dark period and 60% RH. We started with 8 maternal lines which were mixed in the last growth cohort to ensure sufficient genetic diversity before being transferred to the experiment. From the rearing only apterous adults with similar size were selected. Each replicate from each plant species received 5 individuals. After 3 weeks of feeding we counted total number of aphids per replicate pot.

All phase II shoot herbivory and soil feedback pots were completely randomized in the greenhouse. Plants exposed to herbivory were harvested at the same time as the plants exposed to soil feedback, after week 10 (described above), and we analyzed herbivore effects on shoot biomass. Relative herbivory effects were calculated as (shoot biomass with herbivores – shoot biomass without herbivores) / (shoot biomass without herbivores). We also determined locust survival and aphid population growth (see above).

Chemical analyses

Shoot tissue C and N, as well as levels of phenolic compounds, which are general plant defensive chemicals (Hunter & Forkner, 1999) were determined and insects were counted. Whole dry shoots were used to analyze total phenolic content following a modified Folin-Denis protocol (Waterman & Mole, 1994). Dry plant material was ground and 0.025 g was weighed into a test tube with 5 ml of 1.2 M HCL in 50% aqueous methanol, heated for 2 h. at 90°C and centrifuged at 6000 rpm for 10 min. Folin –Denis reagents (0.2 ml) was mixed with 0.2 ml supernatant and 1.0 ml Na_2CO_3. After 30 min and centrifuging at 14000 rpm for 5 min absorption at 750 nm was measured to determine total phenol content to be calculated as percentage of the dry weight. In addition, total C and N contents of the whole shoots were determined by catalytic oxidation and gas chromatography (Nieuwenhuize *et al.*, 1994).

Statistical analyses

Effects of herbivores or soil pre-treatment on plant biomass were analyzed separately in the fixed effects ANOVA model: $Y_{ijkl}=\mu+O_i+T_j+SO_{k(i)}+ T*O_{ij}+T*SO_{jk(i)}+\varepsilon$;

where Y_{ijkl} is the shoot biomass (herbivory tests) or the log total (ln(biomass+1)-transformation; soil feedback tests) for the l^{th} plant from the k^{th} species in the j^{th} treatment; T is the treatment effect, O is the origin effect and S is the species effect, where species are nested within origins. All variables were considered fixed effects. Species was considered fixed because our approach was to census the available exotics (that meet our criteria for successful range expansion) plus their genus-matched natives from a specific riverine habitat type; our procedure for species selection (see above) did not result in a random sample of natives and exotics. Note that the decision to consider species as a fixed nested effect results in statistical testing of all model terms over the model residual error (Neter *et al.*, 1996) and as a consequence statistical inference is limited to the set of species that are included in the study, although and as argued above, the species represented a selection as complete as possible for such riverine ecosystems.

Of primary interest is the treatment x origin interaction, indicating whether treatment effects differ between native and range expanding species. Similar models were used to test for differences between native and range expanding species in nitrogen content, C/N ratio, levels of phenolic compounds in the herbivore assay. To analyze origin effects on locust survival, aphid numbers were added as a covariate. These analyses were performed in SAS version 9.1 for Windows (proc MIXED, SAS Institute Inc., Cary, USA). Aphid scores did not meet standard assumptions for analysis of variance and we therefore analyzed the effect of plant origin (native, intracontinental and intercontinental) on aphid numbers using a nonparametric Kruskal-Wallis test based on species mean values. Additional t-tests were performed for each species individually to assess significance of the treatment effect at the species level, and we also used t-tests (based on species mean values) to test whether the soil feedback effect and the proportional herbivory effect of the natives and range expanders differed significantly from zero. To improve normality of residuals and homogeneity of variances among groups defined by the statistical models, plant biomass for soil-feedback analysis was natural log-transformed, shoot phenolic content was square root-transformed, shoot N content was square rooted natural log-transformed and locust survival was arcsine transformed prior to analysis. For the analysis on species frequencies the values were square rooted before log transformation. In order to test for a relationship between effects of shoot herbivory and soil feedback a Spearman rank order correlation was performed with the species as replicate units. We did not find a significant correlation, neither for the range expanders ($n = 6$; $R^2 = 0.236$, $P = 0.329$), nor for the natives ($n = 9$; $R^2 = 0.004$, $P = 0.865$). Therefore, we concluded that plants that although range expanding plants were less sensitive to shoot herbivory and negative soil feedback than natives, the magnitude of the above and belowground effects did not necessarily vary in the same order.

Supplementary results

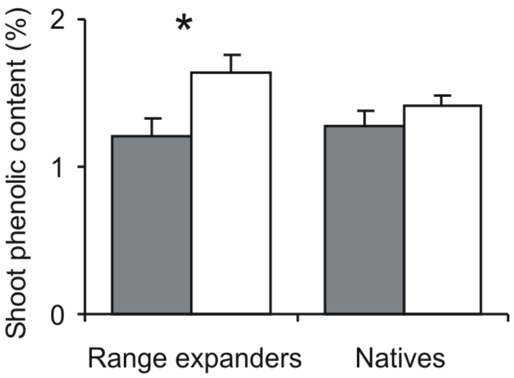

Figure S2.1. Phenolic content of range expanding and native plant species with and without herbivory. Bars show average phenolic content (percentage/g ± s.e.m.) in plant shoot with herbivory (white bars) and controls (grey bars) for range expanding (n=6 species averages) and native (n=9 species averages) plant species. Only range expanding plants exhibit significant (* $P < 0.05$) higher phenolic contents when exposed to herbivory. Back-transformed data of square root transformed data are shown.

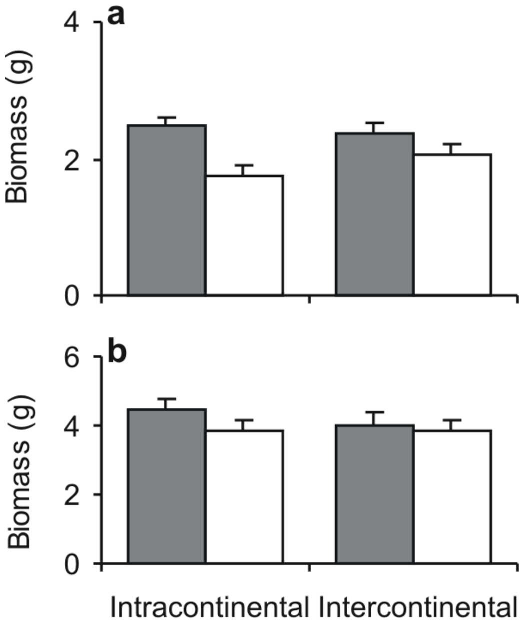

Figure S2.2. Results of soil feedback and herbivory between intra-continental (Eurasian) and inter-continental (non-Eurasian) range expanding plant species. Upper panel (a): The originally inter-continental range expanding species were slightly less negative affected by herbivory than the intra-continental range expanders (range expander origin x herbivory: $F_{1,44} = 4.25$, $P = 0.045$). Lower panel (b): Soil feedback was not different ($P > 0.05$) between inter -and intra-continental range expanders.

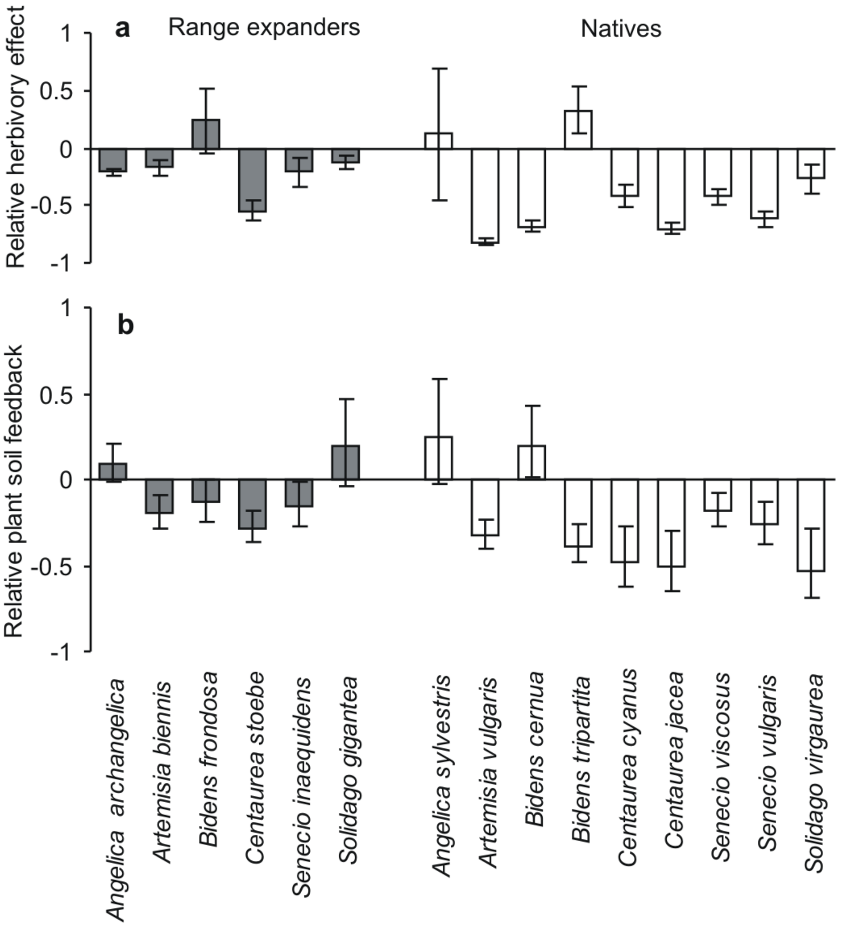

Figure S2.3. Individual proportional responses of the range expanding and native plant species to a naïve aboveground herbivore and soil feedback. Upper panel (a): Relative herbivore effect on range expander (grey bars) and native (white bars) shoot biomass ((shoot biomass with herbivores – shoot biomass without herbivores) / (shoot biomass without herbivores) ± s.e.m.) by *Schistocerca gregaria* and *Myzus persicae* reveals that there was individual variation among plant species, but that the overall impact on range expanding plant species was lower than on native plant species. Lower panel (b): Relative plant soil feedback (back-transformed means of log-transformed data of ((total biomass own soil – total biomass control soil) / (total biomass control soil) ± s.e.m.) of range expanding (grey bars) and native (white bars) species reveals variation among species, but an overall stronger negative impact of soil feedback on native than on range expanding plant species.

Treatment effect sizes for individual species and within-genus comparisons between range expander and native species

The overall analysis of the herbivory dataset and the soil feedback dataset revealed significant treatment-by-origin interactions, with natives suffering more biomass loss than exotics due to the herbivory and the 'own soil' treatments. In order to assess the generality of this pattern across the different species that were included in the study, Supplementary Figures S2.4 and S2.5 show plots of the raw data for each species. For each species the effect size is given as the difference between the means of the two treatment levels, and p values are from t-tests of treatment effect within each species separately. The treatment effect is more often significant in native species than in exotic range expanding species. The effect of herbivory is significant in 6 out of 9 natives versus 2 out of 6 exotics (Supplementary Fig. S2.4) and the effect of soil feedback is significant in 4 out of 9 natives versus 1 out of 6 exotics (Supplementary Fig. S2.5). Within a genus, the biomass reduction caused by the treatment is typically stronger in the native than in the exotic congener. Both herbivory and soil feedback give a stronger biomass reduction (negative effect size) to the native congener in 7 out of 9 within-genus comparisons (Supplementary Figures S2.4 and S2.5).

Over the two experiments, in 14 out of 18 within-genus comparisons the treatment response was stronger in the native species. Using a Sign Test, this is significantly more often than would be expected by chance (M = -5, $P = 0.031$). The non-parametric Sign Test is considered an insensitive, low-power test. Thus, the significant result provides strong evidence that the main results from the overall analysis, namely that natives respond more strongly to the treatments than exotics, hold very generally across the species and the genera that were included in the present study.

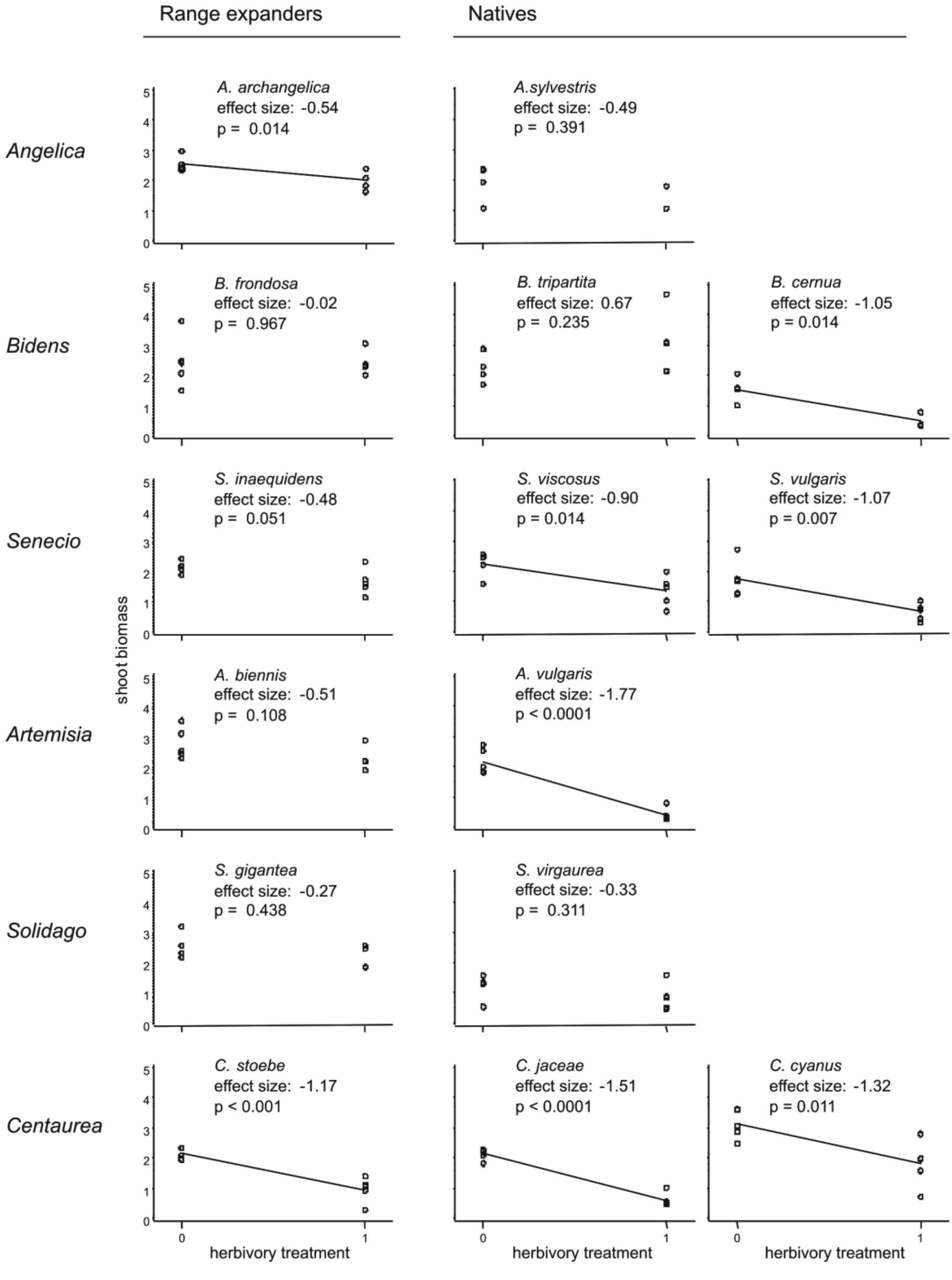

Figure S2.4. Plots of the raw data of the herbivore effects on each plant species. Herbivore effects on each plant species, arranged by genus (rows). Left panels are range expanding species; middle and right panels are congeneric native species (the second native species was added to three genera in order to compare within genus effects between native congeners). Herbivory treatments: 0, without herbivores; 1, with herbivores. For each plant species (left the range expander, middle and right the congeneric native species; in three genera there were two congeneric natives) the effect size is given as the difference between the means of the two treatment levels, and P values are from t-tests of treatment effect within each plant species.

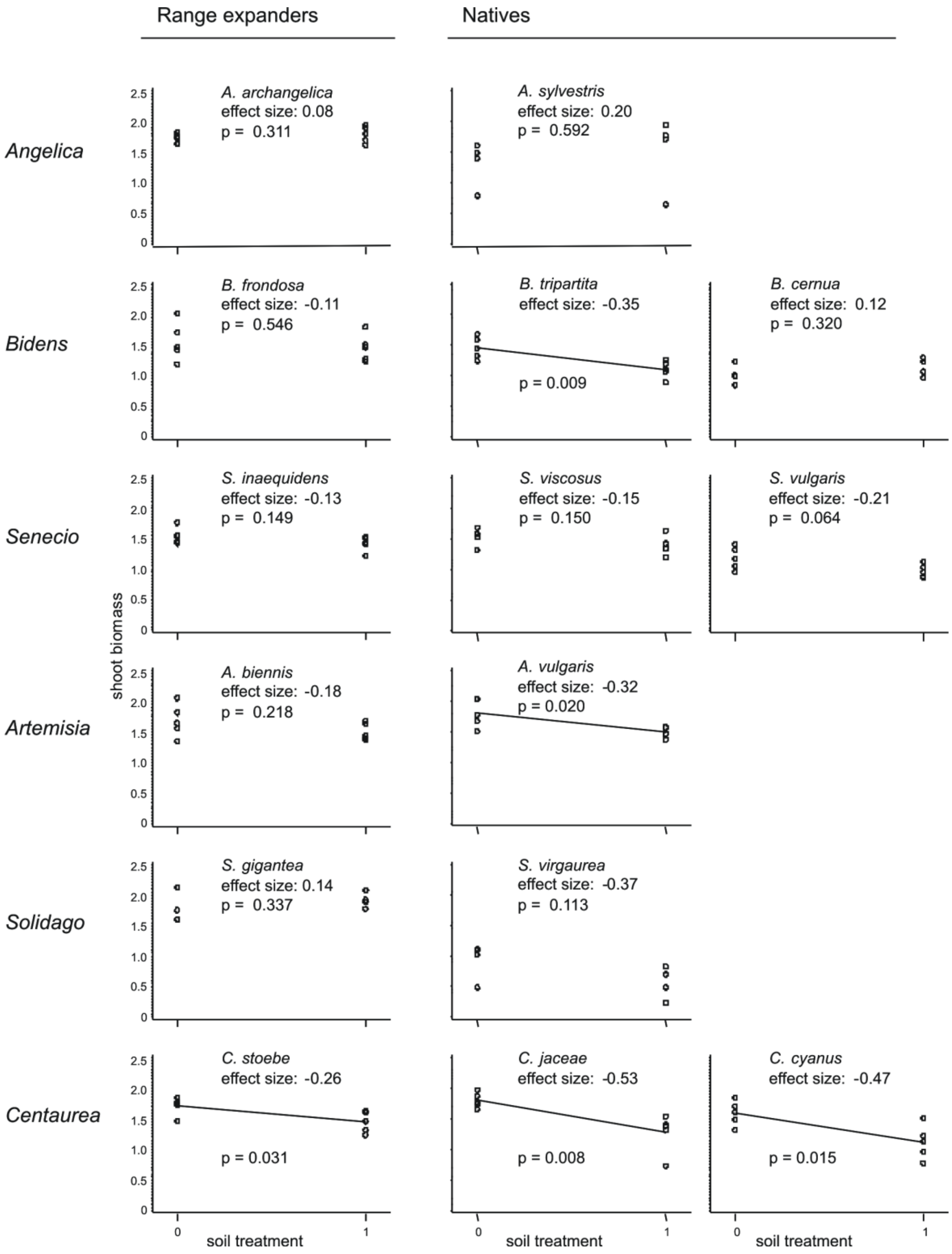

Figure S2.5. Plots of the raw data of the soil feedback effects on each plant species. Soil feedback effects on each plant species, arranged by genus (rows). Left panels are range expanding species; middle and right panels are congeneric native species (the second native species was added to three genera in order to compare within genus effects between native congeners). Soil treatments: 0, control soil; 1, own soil. Total plant biomass scores are after ln(biomass + 1) transformation. For each plant species (left the range expander, middle and right the congeneric native species; in three genera there were two congeneric natives) the effect size is given as the difference between the means of the two treatment levels, and P values are from t-tests of treatment effect within each plant species.

Chapter 3

PERFORMANCE OF POLYPHAGOUS SHOOT-FEEDING INSECTS ON EXOTIC AND NATIVE PLANTS UNDER THE INFLUENCE OF PLANT-SOIL FEEDBACK

T. Engelkes, T. M. Bezemer, J. A. Harvey, W. H. van der Putten

Abstract

Native and exotic plants differ in their interactions with aboveground and belowground organisms, and feedback interactions between plants and their soil communities can play an important role in de success of invasive plant species. There is also some evidence that belowground organisms can influence interactions between plants and shoot feeders and that these effects differ between native and exotic plant species. However, whether and how plant soil-feedback effects influence the performance of shoot-feeding insects in native and exotic plant species is unknown. We examined the performance of polyphagous shoot-feeding insects on native and exotic plants growing in soil previously conditioned by the same (own soil) or by other plant species (control soil). In addition, we tested whether the effects of the soil community on herbivore performance were different between exotic plants originating from other continents (inter-continental exotics) and exotic plants that originate from the same continent (intra-continental exotics). We exposed the plants on own and control soil to two polyphagous invertebrate herbivore species that differ in their feeding strategy. We measure adult size, individual mass and survival of the leaf chewing desert locust *Schistocerca gregaria* and population size and individual body size of the phloem feeding green peach aphid *Myzus persicae*. Locust mass was on average lower on exotic plants than on native plants and also lower on plants grown in own soil than in control soil. Survival of the locusts was higher on native plants and not influenced by soil type. The locust mass and survival was significantly lower on both intra-continental and inter-continental exotic plants than on native plants; there were no differences between the inter- and intra-continental exotic plants. The population size and body size of the aphids did on average not significantly differ between exotic and native plants, although aphids attained higher population sizes on intra-continental than on inter-continental exotic plants. Aphid body size was not different between the range expanders. This could be due to a faster rate of population growth on exotic compared to native plants in two of the six plant genera that were used in this study: *Angelica* and *Artemisia*. The results show that the performance of two polyphagous aboveground herbivores can be influenced by plant specific soil communities and that herbivore performance was low on exotic plants on both soils. Our results suggest that exotic plants not only differ from related native plants in their soil feed-back effects and in their effects on aboveground herbivores, but also on aboveground herbivores via the effect of the plant specific soil community.

Keywords: Range expansion, exotic, climate warming, polyphagous herbivore, phenolic content, plant defense, nutritional quality, enemy release

Introduction

Invasions by exotic plants can have highly deleterious effects on the functioning, diversity and stability of the invaded ecosystems. Therefore, plant invasions are of great concern worldwide (Vitousek *et al.*, 1996). A recent meta-analysis showed that on average invasive exotic plants contained more novel plant defense compounds and are therefore potentially better defended than native plant species (Cappuccino & Arnason, 2006). The growth and reproduction of insect herbivores depends on the nutritional quality of the host plant, which is determined by the concentrations of primary compounds such as carbon and nitrogen, as well as the types and concentrations of secondary, or plant defense compounds (Mattson, 1980; Slansky, 1992; Awmack & Leather, 2002). Consequently, insect herbivores will perform less well on exotic than native plants (Renwick, 2002; Keeler & Chew, 2008; Harvey *et al.*, 2010). Reduced impact from herbivory in the introduced range has even been proposed as one of the major mechanisms explaining the invasive success of exotic plant species in new ranges (Keane & Crawley, 2002).

The effectiveness of a plant's anti-herbivore defenses can be greatly influenced the herbivores' mode of feeding (Tallamy, 1986). For example, defensive compounds that are effective against leaf chewing herbivores such as caterpillars or locusts may not be effective against phloem feeders like aphids that seal damaged cell walls directly after penetration of the leaf surface with their stylet (Walling, 2008). Likewise, leaf chewing insects exhibit adaptations that enable them to avoid the negative effects of plant chemical defenses. For example, some herbivores feed on older leaves with lower concentrations of allelochemicals, whilst others may chew trenches in leaf veins that prevent the delivery of allelochemicals to outer leaf tissues (Schultz, 1983; Bezemer *et al.*, 2004).

Although the majority of studies on invasive plants and their associated herbivores focus on aboveground herbivory, plants are also exposed to belowground enemies such as pathogens and root herbivores. Exotic plants can greatly impact their soil community (Kourtev *et al.*, 2002; Callaway *et al.*, 2004; van der Putten *et al.*, 2007a). Moreover, similar to what has been reported from aboveground studies, native plants typically suffer more from negative feedback interactions between plants and soil communities than exotic plants (Ehrenfeld *et al.*, 2001; Klironomos, 2002; Callaway *et al.*, 2004; Reinhart & Callaway, 2004; Agrawal *et al.*, 2005; van Grunsven *et al.*, 2007; Engelkes *et al.*, 2008). Interactions between plants and their associated soil organisms can influence aboveground plant quality and foliar herbivore performance (Gange & Brown, 1989; Masters *et al.*, 1993; Bezemer *et al.*, 2003; Bezemer & van Dam, 2005; Soler *et al.*, 2005). However, while the separate effects of invasive plants on their associated aboveground and belowground antagonists have been investigated extensively (Willis *et al.*, 1999; Siemann & Rogers, 2001; Bossdorf *et al.*, 2004; Lankau *et al.*, 2004; Leger & Forister, 2005), very little is known about how the different impact of plant-soil effects on native and exotic plant species affect aboveground herbivore.

Most studied biological invasions are intentional or unintentional introductions of exotic species from one continent to another due to human activities. Another type of exotic invader is due to the current rapid climate warming, which enables species from lower latitudes to shift their range to higher latitudes (Parmesan *et al.*, 1999; Walther *et al.*, 2002; Tamis *et al.*, 2005). As a result, within continents exotic plants may expand their range, creating new interactions with newly encountered herbivores (Agosta & Klemens, 2008). Most studies investigating novel interactions such as herbivore performance on exotic plant species have focused on plants that originate from overseas (inter-continental origin). While the effects of the soil community on growth of range expanding plants has recently been investigated (van Grunsven *et al.*, 2007; Engelkes *et al.*, 2008; MacKay & Kotanen, 2008; van Grunsven *et al.*, 2009), the effects of their soil communities on aboveground herbivore performance is still unknown.

It has been shown that exotic plants experience lower impact from negative soil biota in their new range, resulting in a less negative to a positive soil feedback compared to natives (Klironomos, 2002; Reinhart & Callaway, 2004; Agrawal *et al.*, 2005; Andrew *et al.*, 2006; van der Putten *et al.*, 2007b; van Grunsven *et al.*, 2007; Engelkes *et al.*, 2008). These feedback patterns may be due to less exposure to soil-borne enemies, or to enhanced exposure to belowground symbionts of other beneficial soil biota (Colautti *et al.*, 2004). This variety of plant-soil feedback effects may result in either enhanced or reduced performance of aboveground insects. Therefore we will test the null hypothesis that plant-soil feedback interactions do not alter the performance of aboveground insects. If the null hypothesis is rejected, we will examine whether aboveground insect performance is enhanced or reduced compared to insects feeding on plants in control soil. We used two aboveground polyphagous herbivores, the desert locust *S. gregaria* and the green peach aphid *M. persicae* and compared their performances on exotic and related native plant species between plants in own soil and in control soil. The own soil was conditioned by a conspecific plant, whereas the control soil was conditioned by other plant species that did not belong to the same genus. We also compared the influence of soil biota on the herbivore performance between inter –and intra-continental exotic plants.

We performed our tests, using exotic plant species and phylogenetically related natives from six different genera. While we did our test using two polyphageous herbivore species with a very broad host plant range, it is inevitable that their performance will greatly differ between plant species and genera due to phylogenetically conserved traits (Winkler & Mitter, 2007) such as anti-herbivore defenses (Agrawal & Kotanen, 2003; Strauss *et al.*, 2006). Within genera, however, differences in insect performance between exotic and native species more likely will be due to differences in species traits, since we selected closely related plant species that are ecologically and biologically alike (Felsenstein, 1985; Futuyma & Mitter, 1996; Jermy & Szentesi, 2003; Lewinsohn *et al.*, 2005). In order to determine to what extent differences between genera may be due to plant-species specificity, for three different genera, we included two native species in the comparison with the exotic species.

Methods

Herbivore selection and bioassay

Locusts. As a leaf chewing generalist herbivore we selected the African desert locust *Schistocerca gregaria* (Forskål). This locust species originates from semi-arid areas in north-central Africa to Asia. The nymphs were obtained from a gregarious rearing of the Laboratory of Entomology at Wageningen University. They were reared on rye (*Secale cereale*) and rye-grass (*Lolium perenne*). At the beginning of the experiment five day-old first instars were starved for 12 hours. The initial mass of these instars was 85.8 ± 2.3 mg (mean ± SE, based on 78 randomly selected five day-old instars). 10 replicates of each plant species received 3 instars. The locusts were prevented from escaping by spherical nets (Ø 25 cm, height 1.5 m) that were placed over the plants. The insects were allowed to feed for 3 consecutive weeks. Once a week, surviving locusts were checked and their fresh mass was determined on a Mettler-Toledo MT5 Microbalance. For locusts that did not survive the full 3 weeks, we used their final measured mass instead in order to maintain replicates which would otherwise be lost if the locusts that did not survive the first week were excluded. In case none of the locusts in a cage survived the first week, the initial mass was extrapolated from the mass of the surviving locusts of the other replicates. If there were no surviving locusts on all replicates for a plant species the initial mass was used.

Aphids. As a phloem-feeding herbivore, we selected *Myzus persicae* (Homoptera; Aphididae), the green peach aphid. We obtained nymphs from a culture from Wageningen University. They had been reared on white radish (*Raphanus sativus*). We started with 8 maternal lines that were mixed before the start of the experiment to ensure sufficient genetic diversity before transferring them to the experimental plants. From the rearing only apterous adults with similar size were selected. All replicates from each plant species received 5 individuals. After 3 weeks of feeding all aphids were collected and stored in 70% ethanol. Aphid population size was recorded and the body size (length x width) (mm) of each individual aphid was determined using the software WinSeedle Pro (Version 2006a, Regent Instruments Inc. 1992-2006).

Plant species, seeds and soil

We used the National Standard List of the Dutch flora TAMIS2005 to select 6 exotic plant species (*Angelica archangelica, Artemisia biennis, Bidens frondosa, Centaurea stoebe, Senecio inaequidens* and *Solidago gigantea*), which have become established in the Netherlands in the last half of the 20th century and are potentially invasive. We paired these exotic plants with 9 native congeneric species using two native congeners for 3 genera (*Angelica sylvestris, Artemisia vulgaris, Bidens cernua/Bidens tripartita, Centaurea cyanus/Centaurea jaceae, Senecio viscosus/Senecio vulgaris, Solidago virgaurea*). The Angelica species pair belonged to the family *Apiaceae*, whereas all other plants were *Asteraceae*. Seeds from all species were collected from the Geldersche Poort region in eastern Netherlands in the stream area of the Rhine (51°87′ N, 6°01′ E), where all species co-occur. Plants were grown in sterilized soil (25 kGray gamma radiation), mixed with one fifth non-sterilized inoculum soil (5-15 cm depth) collected from the Gelderse Poort region (51°87′ N, 6°01′ E). Seeds were germinated on sterile glass beads with demineralized water. Early summer or late summer species were given specific germination regimes; 20°C:14 hrs day / 10°C:10 hrs night and 25°C:16 hrs day/ 15°C:8 hrs night respectively. There were ten replicate 1.3 L pots for each plant species in which four seedlings of the same species were planted and placed in the greenhouse with an additional light source (225 μmol^{-1} m^{-2} PAR) and 21±2 / 16±2 day/night temperature with 60% RH to create favorable growth conditions. Dead individuals within the first 2 weeks were replaced with new seedlings. Plants received deminerialized water every second day and an additional nutrient solution once a week of 25 ml 0.5 Hoagland (Hoagland 1950). After seven weeks of growth plants were harvested and roots were removed from the soil. Plants were then grown again in own and control soil in 1.3 L pots. To obtain own and control soil, the soil of the ten replicate pots per species was first mixed and then split into two equal parts. One part was used as 'own' soil, and three seedlings from the same species were planted into this soil. To obtain 'control' soil, soil mixtures were made in which soil was homogenized from all replicates of the native and exotic plant species,excluding one particular genus. All species of the genus that was not included in the particular mixture were then grown in this soil. Three seedlings were planted into each pot. Growth and light conditions were as described above, with the exception that 10 ml 0.5 strength Hoagland was added. After 7 weeks each pot was caged and locusts and aphids were introduced into each cage.

At the end of the experiment, all aboveground plant biomass was dried and ground. Total phenolic content of shoot material of each species was analyzed following a modified Folin-Denis protocol standardized to gram plant (Waterman & Mole, 1994). Nitrogen content was also determined by catalytic oxidation and gas chromatography (Nieuwenhuize *et al.*, 1994).

Statistical analyses

Locust and aphid performance were analyzed separately. Data were analyzed in a factorial nested ANOVA with as factors species nested in origin (native or exotic), soil (own or control) and their interactions. The same model was used when we analysed differences between native, intra-continental and inter-continental exotic plants, but then origin had 3 levels. Differences in locust fresh mass (averaged per replicate), shoot nitrogen and shoot phenolic content were analysed using ANOVA with a similar model as described above. Locust survival was analysed with a general linear model with log link and binomial error distribution from which χ^2 values were calculated. Locust fresh mass was square-root transformed and nitrogen and phenolic data both needed a log transformation to meet assumptions for ANOVA. Aphid population size was also tested with ANOVA but needed a double cubic root transformation to meet the assumption of homogeneity of variance. Separate factorial ANOVA's were also carried out for each native/exotic species combination within each genus with origin and soil as factors. The genus *Solidago* was excluded from the within genus comparisons, because there were no surviving aphids in the genus *Solidago*. Differences in aphid body size were tested with a mixed model with fixed factors similar to the model described above and cage as random factor using residual analysis (REML). This analysis produces Wald-type *F*–statistics and has the ability to use different sources of variation. For tests of ANOVA, assumptions were checked using a Levene test and normality of residuals were visually inspected. Relationships between herbivore performance, phenolic and nitrogen concentrations were tested with a Pearson correlation test. Analyses were performed using R version 7.2.1 (R Development Core Team 2008) and the mixed model procedure in Genstat 11.1 (VSN International Ltd. 2008).

Results

Locust performance

The biomass of the *Schistocerca gregaria* individuals was on average larger on native than on exotic plants ($F_{1,106}$ = 167.601, P < 0.0001) and larger on plants growing in control soil than on own soil ($F_{1,106}$ = 14.611, P < 0.001). There was a marginally significant interaction between soil treatment and plant origin ($F_{1,106}$ = 3.698, P = 0.057), because the difference in mass between own and control soil was larger for the native plants than for the exotic plants (data not shown). Locust mass was not different between inter-continental and intra-continental exotic plants (Posthoc Tukey; P = 0.1244). Within plant genera locust mass was larger on native than on exotic plants in the genera *Artemisia*, *Bidens*, and *Senecio* (Fig. 3.1). Locust mass was higher on plants in control soil than in own soil in the genera *Artemisia* and *Centaurea* ($F_{1,16}$ = 7.805, P = 0.013 and $F_{1,16}$ = 11.346, P = 0.004, respectively. See also Fig. 3.1). There was one significant origin x soil interaction in the genus *Centaurea* ($F_{1,16}$ = 8.144, P = 0.012).

The proportion of surviving *S. gregaria* after three weeks was on average higher on native than on exotic plants ($\chi2$ = 8.658, df = 1, P = 0.004; Fig. 3.1). The proportion of survival was not different between soil types ($\chi2$ = 1.075, df = 1, P = 0.301; Fig. 3.1). Therefore, survival was less sensitive to plant-soil feedback than biomass. As expected, survival of *S. gregaria* on intra-continental and inter-continental did not differ ($\chi2$ = 0.169, df = 1, P = 0.681. In two genera, the proportion of survival was lower on exotic than on native plants ($\chi2$ = 46.305, df = 1, P < 0.0001 for *Artemisia* and $\chi2$ = 7.884, df = 1, P = 0.005 for *B. frondosa* vs. *B. cernua*). However, survival was not different between *B. frondosa* and the native *B. tripartita* ($\chi2$ = 1.741, df = 1, $P = 0.187$). On native *C. jacea*, survival was significantly lower than on the exotic *C. stoebe* ($\chi2 = 13.959$, df = 1, $P < 0.001$),whereas survival was not different between the exotic *C. stoebe* and the native *C. cyanus* ($\chi2 = 0.582$, df = 1, $P = 0.455$). Survival was not different between the two *Angelica* species, the two *Solidago* species, or between the exotic and native *Senecio* species. In none of these comparisons soil type interacted with plant origin.

Aphid performance

The average population size of *Myzus persicae* was not different between exotic and native plants ($F_{1,106} = 1.655$, $P = 0.201$) and was not affected by the soil community ($F_{1,106} = 0.949$, P = 0.273; Fig. 3.2). When inter -and intra-continental exotics and native plants were compared as separate entities, aphid population sizes were significantly different ($F_{1,106} = 23.126$, P < 0.0001). Population size was highest on intra-continental plants and lowest on inter-continental plants. Within genera, population size was higher on the native than on the exotic *Senecio*, but lower on the native plants in the genera *Angelica*, *Artemisia* and one of the natives in the genus *Bidens* ($P < 0.05$; Fig. 3.2).

The body size of *M. persicae* was not different between exotic and native plants ($F_{1,28.3} = 1.30$, $P = 0.264$). Body sizes were significantly larger on control than on own soil ($F_{1,37.7} = 8.69$, $P = 0.005$), but there was no interaction with plant origin ($F_{1,38.4} = 0.82$, $P = 0.369$). There was also no difference in body size between intra-continental and inter-continental plants ($F_{1,31.4} = 3.03$, $P = 0.063$). There was no relationship between aphid population growth and locust weight, suggesting that the phloem feeders and the leaf feeders were affected differentially by the plant species characteristics ($R^2 = 0.009$, $P = 0.601$).

Origin and soil effects on nitrogen and phenolics

There was no significant origin effect, but phenolic content was higher in plants on control soil than on own soil ($F_{1,88} = 5.927$, $P = 0.017$), and there was a significant interaction between soil and origin ($F_{1,88} = 28.981$, $P < 0.0001$), because exotics both had lower phenolic concentrations in own soil than in control soil. There was variation between genera and between native-exotic comparisons within genera (see Table 3.1). For nitrogen concentrations in the shoot we found an interaction between plant origin and the soil treatment ($F_{1,92} = 4.930$, $P = 0.029$). Nitrogen concentrations were higher in native plants on own soil than on control soil, but there were no such differences in exotic plants. For the genera *Angelica*, *Solidago* and *Bidens*, nitrogen was lower in the exotic than in the native species independent of soil, but the exotic *S. inaequidens* had higher nitrogen than *S. vulgaris* and lower than *S. viscosus* (Table 3.1). In the genera *Artemisia* and *Senecio* nitrogen was overall higher on control than on own soil.

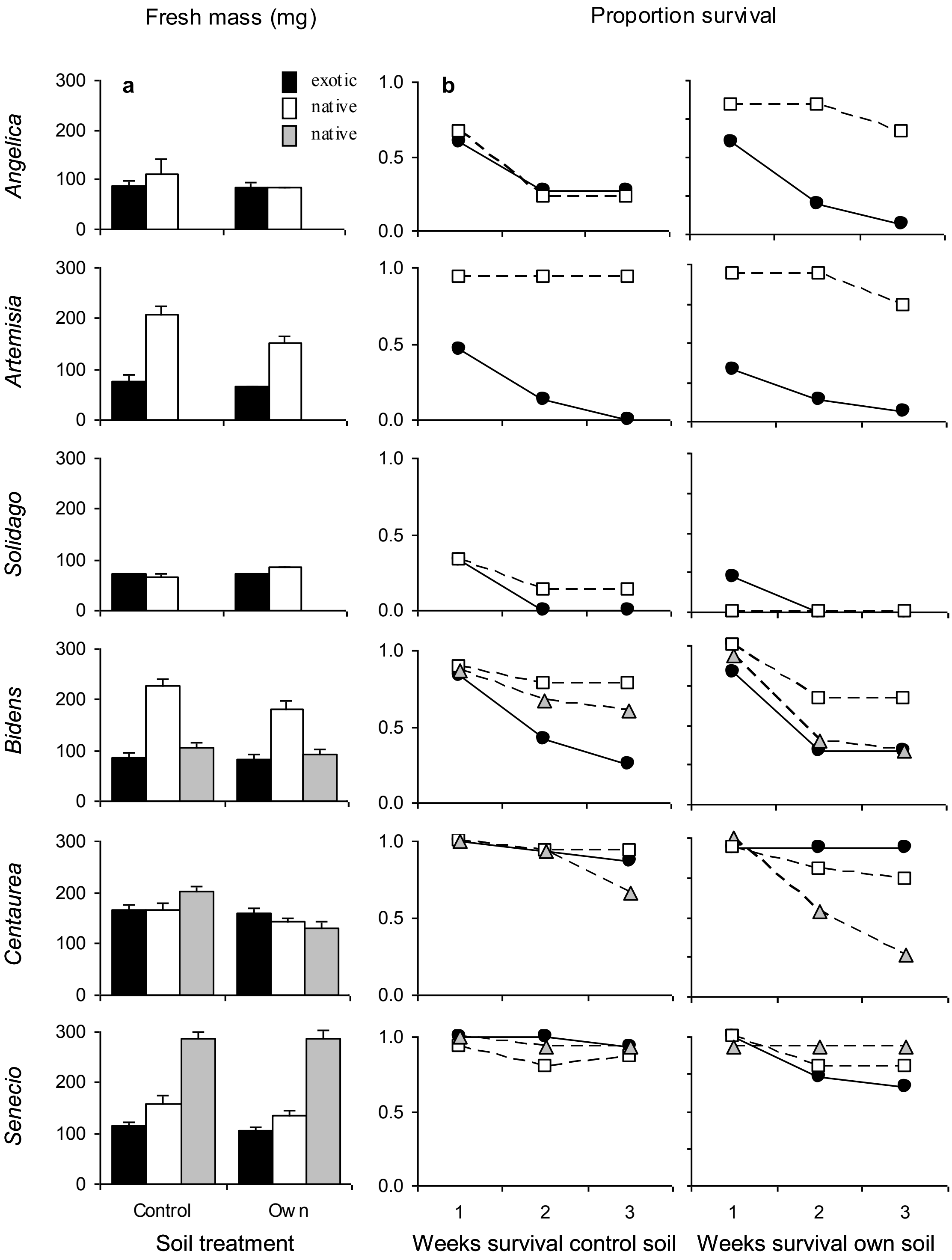

Figure 3.1. Performance of the the locust *Schistocerca gregaria* on exotic and native plants in 6 genera. (**a**) Fresh mass of *S. gregaria* (mg) on exotic plants (black bars) and native plants (grey and white bars) grown on foreign and own soil. Data shown are the means (± SE). (**b**) Proportion of survival of *S. gregaria* on foreign soil (left panel) and own soil (right panel) on exotic plants (black dots, solid lines) and native plants (white squares and grey triangles, broken lines). Data shown is the survival over a period of three weeks (x-axis) for the genera *Angelica*, *Artemisia*, *Solidago*, *Bidens*, *Centaurea* and *Senecio*. In the genera with 2 native plants *Bidens cernua*, *Centaurea cyanus* and *Senecio viscosus* are shown in white and *Bidens tripartita*, *Centaurea jacea* and *Senecio vulgaris* are shown in grey.

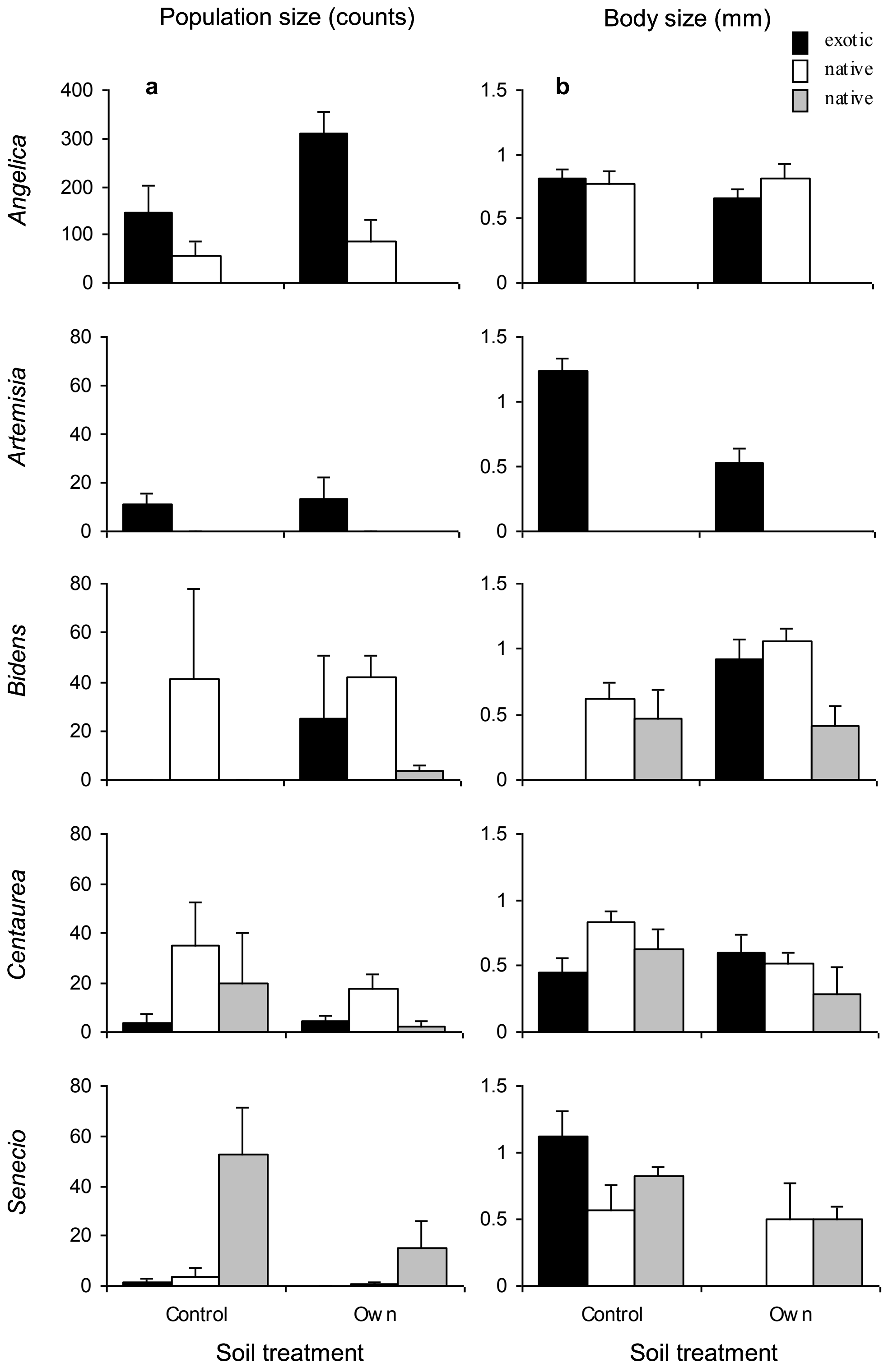

Figure 3.2. Performance of the aphid *Myzus persicae* on exotic and native plants in 5 genera. Population size (**a**) and body size (length x width in mm) (**b**) after three weeks on exotic plants (black bars) and native plants (grey and white bars) grown on foreign and own soil. The genus *Solidago* is not shown, because aphid survival was zero. In the genera with 2 native plants *Bidens cernua*, *Centaurea cyanus* and *Senecio viscosus* are shown in white and *Bidens tripartita*, *Centaurea jacea* and *Senecio vulgaris* are shown in grey. Means (± SE) are presented.

Table 3.1. Mean (± s.e.m.) shoot nitrogen content and shoot phenolic content in plants grown in control soil and own soil. Here after in each column the *F*-values of ANOVA for independent variables origin (O) and soil type (S) on shoot nitrogen content and shoot phenolic content for pairwise exotic and native species comparison. Different genera are separated as blocks. Exotic species are shown in bold.

	Shoot N content (%)					Shoot phenolic content (%)				
	Control	Own	O	S	OxS	Control	Own	O	S	OxS
Ang arc	1.34 ± 0.18	1.81 ± 0.06	8.26*	1.55	2.96	1.28 ± 0.09	1.02 ± 0.07	1.63	2.71	2.03
Ang syl	2.37 ± 0.45	2.07 ± 0.35				1.23 ± 0.00	1.35 ± 0.22			
Art bie	1.31 ± 0.12	1.37 ± 0.18	0.66	5.04*	4.15	1.44 ± 0.12	0.98 ± 0.08	2.72	26.55***	2.42
Art vul	1.15 ± 0.18	1.87 ± 0.16				1.78 ± 0.17	0.95 ± 0.06			
Sol gig	1.67 ± 0.26	1.54 ± 0.22	7.76*	0.50	0.96	2.86 ± 0.71	1.77 ± 0.42	15.17**	1.31	2.25
Sol vir	2.75 ± 0.57	3.66 ± 0.26				1.07 ± 0.17	1.05 ± 0.11			
Bid fro	0.76 ± 0.05	0.70 ± 0.11				2.39 ± 0.15	1.16 ± 0.08			
Bid cer	2.21 ± 0.45	2.49 ± 0.18	48.62***	0.09	0.56	1.10 ± 0.12	1.43 ± 0.23	11.65**	14.41**	27.44***
Bid tri	0.69 ± 0.03	0.93 ± 0.09	1.28	1.31	3.33	2.18 ± 0.03	2.09 ± 0.27	4.51	12.48**	11.09**
Cen sto	2.07 ± 0.25	2.22 ± 0.12				1.24 ± 0.14	0.76 ± 0.20			
Cen cya	1.19 ± 0.34	1.79 ± 0.37	0.77	1.19	0.45	1.37 ± 0.07	1.22 ± 0.11	4.18	5.00*	1.96
Cen jac	2.23 ± 0.31	2.75 ± 0.05	4.48	1.66	0.54	2.05 ± 0.05	2.42 ± 0.12	94.11***	0.08	12.58**
Sen ina	1.04 ± 0.09	1.37 ± 0.12				1.54 ± 0.07	1.21 ± 0.18			
Sen vis	1.30 ± 0.19	2.06 ± 0.19	9.38**	12.38**	1.89	1.09 ± 0.05	1.91 ± 0.39	0.27	1.67	5.66*
Sen vul	0.47 ± 0.06	0.69 ± 0.11	37.51***	7.56*	0.34	0.88 ± 0.05	2.28 ± 0.16	4.42	19.31***	42.18***

Asterisks denote significant effects. $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

Irrespective of soil and origin there was no significant relationship between locust mass and the concentration of phenolics in the plant shoots. Similarly, the relationship between aphid performance and phenolics was not significant. There was also no significant relationship between nitrogen concentrations and either of the herbivores. However, there was a significant positive relationship between nitrogen and locust mass when only native plants were considered ($R^2 = 0.2451$, $P = 0.0367$).

Discussion

In this study we explored the influence of plant-soil feedback effects of exotic and native plant species on the performance of two polyphagous herbivores, a locust and an aphid. Locust mass was not only larger on native than exotic plants, but also larger on plants in control soil than in own soil. Locust survival was higher on native than on exotic plants, but there were no differences in locust survival when own and control soil was compared. Moreover, there was no difference in locust mass or survival between intra- and inter-continental range expanders. Aphid responses were somewhat different from those of the locusts. Aphid population size was highest on intra-continental exotic plants. Furthermore, aphid body size was larger on plants growing in control compared to own soil, but it there was no significant difference between exotic and native plants or between range expanders.

Our results show that some aboveground herbivores generally perform better on native plants than on exotic plants, possibly because exotic plants are less suitable than native plants. This result was mainly true for the locust, which had reduced body mass and reduced survival on exotic plants. With respect to the aphid species, population sizes were higher on the intra-continental exotic, but lower on the inter-continental exotic, than on native plants. However, body sizes were not different between the exotic and native plants. This supports findings of other studies where insect herbivores performed better on native than on exotic plant species (Cipollini *et al.*, 2008; Keeler & Chew, 2008; Harvey *et al.*, 2010).

Our results also reveal that the performance of both herbivores was better on plants growing in control soil than in own soil. The fact that plants on control soil provide a better food source than on own soil may have different explanations: (i) plants having negative effects from their own soil, e.g. by accumulation of soil pathogens, may be induced to produce more, or other defense compounds (Joosten *et al.*, 2009). Such defenses induced by soil biota may reduce aboveground plant suitability, thereby affecting aboveground herbivore performance; (ii) plant attack by below ground pathogens might result in reallocation of resources, which could reduce resource availability to the above ground herbivores; (iii) stressed plants may become more susceptible to herbivores, because increased nitrogen in leaf tissue, as our results showed for native plants, and stressed plants are less likely to synthesize defensive compounds (Rhoades, 1979; Mattson & Haack, 1987).

Phenolic concentrations in the shoots were lowest in exotic plants, although this was manifested only when grown in their own soil, in spite the minor effects that own soil has on the performance of the exotic plants (Engelkes et al. 2008). In general, exotic plants typically have neutral to positive soil feedbacks (Klironomos, 2002; Callaway *et al.*, 2004; Reinhart & Callaway, 2004). Our results show that under these conditions soil biota still can have an indirect effect on aboveground plant defensive compound levels, although locusts on the exotic plants were not affected by these differences in defensive compounds. Interestingly, induced levels of phenolic defenses in exotic plants seemed to have a negative effect on aboveground herbivory (Engelkes *et al.*, 2008). However, these levels were based on the difference between plants with and without aboveground herbivores, while in our study all plants received herbivory and the difference in defensive quantities is directly related to soil type. In addition, we showed that there was no significant relationship between phenolic shoot content and locust or aphid performance. This further suggests that differences in the performance of locusts on exotic and native plants may be driven by other factors, or that other allelochemicals maybe involved, because soil organisms can influence quantity and quality of defenses of aboveground plant tissues affecting aboveground interactions (Bezemer *et al.*, 2005; Bezemer & van Dam, 2005; Joosten *et al.*, 2009). Further research is needed to determine how plant-soil feedback influences these defense levels and what the consequences are for plant-insect interactions in the field.

The soil pre-treatment did not affect locust survival, but it affected locust mass. Soil effects may have been masked by the mortality of locusts earlier in the experiment. The biomass may be a more sensitive measure to detect plant-soil feedback effects. Similarly, aphid population size was not different between control and own soil, but plants on control soil may still have been a better food source since aphid body sizes were larger on control than on own soil. In the field, such soil biota-mediated aphid body sizes may influence top-down control by aphid parasitoids, which respond to body size (Bezemer *et al.*, 2005). Although we have only used two aboveground herbivore species, our results confirm the conclusion that aboveground-belowground interactions are species-specific (Wurst & van der Putten, 2007).

Studies comparing the performance of native insects on native and exotic plants have produced mixed results (Keeler & Chew, 2008; Harvey *et al.*, 2010) and in those studies different types of chewing herbivores were used. Some herbivores are able to sequester defensive compounds and to use them for their own benefit, whereas others excrete plant toxins in their excrements (Schoonhoven *et al.*, 1998). Leaf chewing insects posses specific enzymes to break down plant defensive compounds like glucosides, but *S. gregaria* can only compensate for the negative effects over a short period of time (Mainguet *et al.*, 2000). Alternatively, in natural conditions the locust avoids long exposure to detrimental compounds by feeding on a mixed diet of plants (Bernays & Minkenberg, 1997). For *Myzus persicae* it is known that this species is not affected by glucosinolates, because it can sequester these intact compounds (Weber *et al.*, 1986) and this species can also successfully develop on *Lupinus angustifolius* despite high alkaloid levels in this plant species (Cardoza *et al.*, 2006). This indicates that this phloem feeding insect is either able to better cope with defensive compounds or is able to avoid their most negative effects, for example by sealing damaged cell walls directly after penetration of the leaf surface (Walling, 2008).

The performance of the two herbivores was not different between inter-continental and intra-continental exotic plants and not differently affected by their soil communities. This shows that climate warming induced range expanders influence aboveground herbivores similarly negative as intercontinental invaders and that the effects of their plant-soil feedback is also comparable. Thus, our results suggest that invasive exotic plants not only differ from related native plants in their feed-back effects with the soil communities and in their effects on (at least some) aboveground herbivores, but also in the effect that soil feedback has, through the plant, on (some) aboveground herbivores. If and how these effects of plant-soil feedback on aboveground plant-herbivore interactions influence their invasiveness through altering aboveground plant-herbivore interactions requires further studies.

Thus far, most published studies assume generally negative effects of exotic plants on aboveground herbivores, because they are thought to have qualitatively and quantitatively different levels of allelochemicals than native plants (Callaway & Ridenour, 2004; Leger & Forister, 2005). However, other studies show that herbivores can feed well on exotic plants (Parker & Hay, 2005). In our study, we show that these apparently contrasting findings depend strongly on plant species-specific differences, or on differences between plant genera. For example, *M. persicae* performed better on the exotic *A. biennis* but perished on the native *A. vulgaris*. This emphasizes two points: that some exotic plants might be under control by generalist herbivores in the invaded range, but also that general conclusions require multiple species comparisons.

In this study we have shown that the performance of two polyphagous aboveground herbivores can be influenced by plant specific soil communities. In particular, herbivore performance was low on exotic plants on both soils. If and how this relates to other mechanisms contributing to the success of exotic plants should be addressed in future studies.

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Chapter 4

SMALLER HERBIVORE LOADS, BUT LARGER PREDATOR PRESSURE ON AN EXOTIC PLANT AND AN INTRA-CONTINENTAL RANGE EXPANDER THAN ON RELATED NATIVE PLANTS

T. Engelkes, B. Wouters, T. M. Bezemer, J. A. Harvey, W. H. van der Putten

Abstract

Invasive exotic plant species generally have fewer herbivorous insects than related native plant species. However, little is known on how herbivorous insects on exotic plants are exposed to carnivorous insects, and even less is known on how insect exposure of invasive exotic plants compares with plants that expand their range within continents, for example due to climate warming. Here, we examine the herbivore load and predator pressure on an exotic plant that has crossed continental barriers and one that has expanded its distribution range pole-wards within Eurasia. We compare insect loads on both exotic plant species with two related natives from riparian habitats in north-western Europe. The insects were classified to carnivores and four guilds of herbivores (leaf miners, sap suckers, leaf chewers and gallers), counted and weighed. We tested the hypothesis that herbivore and predator loads are smaller on both the exotic plant species than on the native congeners. Indeed, total herbivore loads were smaller on exotic plants than on native plants, but the differences depended on the period in the growing season, as well as on the feeding guild of the herbivore. The predator load on exotic plants was not larger than on natives; however, both exotic plants had greater predator pressure on the herbivores than the natives. Our results, therefore, suggest that the exotic plants may have better bottom-up, as well as top-down control of herbivores. Moreover, we show that exposure to invertebrate herbivores of an intra-continental range expanding plant does not necessarily differ from an exotic weed originating from another continent. Our results imply that intra-continental range expansion of plants, for example due to climate warming, proceed faster than of aboveground insects, or that the insects may shift range as well, but the original feeding relationships may not become re-assembled in the new range.

Keywords: climate warming, community structure, guild, herbivory, plant invasion, predation, prey, range expansion, *Rorippa austriaca*, *Senecio inaequidens*

Introduction

One of the most important hypotheses explaining the success of invasive exotic plant species in their new range is their release from former enemies, such as herbivores and pathogens, with which they had a long co-evolutionary history (Elton, 1958; Keane & Crawley, 2002). Release from the enemies of the native range coupled with reduced enemy pressure from the local herbivore community in the new range has been studied for a range of invasive plant species (Wolfe, 2002; Agrawal *et al.*, 2005; Vila *et al.*, 2005). These studies included oligophagous and polyphagous herbivores such as seed predators, folivores and phloem feeders (Jobin *et al.*, 1996; Fenner & Lee, 2001). But also entire insect communities on exotic plants in their new ranges have been studied (Memmott *et al.*, 2000; Degomez & Wagner, 2001; Imura, 2003; Liu & Stiling, 2006; Zuefle *et al.*, 2008; Wu *et al.*, 2009). However, almost all these studies have considered exotic plant species originating from other continents.

The distribution and abundance of many species is affected by a range of natural and anthropogenic-induced processes. A prominent example is the response of species to the recent climate warming (Warren *et al.*, 2001; Bale *et al.*, 2002; Thomas *et al.*, 2004; Ward & Masters, 2007). However, plants may also shift their range within the same continent, for example due to recent climate warming (Walther et al. 2002, Tamis et al. 2005; Parmesan & Yohe, 2003; Kelly & Goulden, 2008). Little is known about communities of insects associated with exotic plants that have shifted their range within the same continent. If the ability of exotic plant species to become ecologically disruptive pests in their new range involves release from co-evolved natural enemies, such as pathogens and herbivores which limit the abundance of the plant in its native range, such range shifts could enable some species to become disproportionately more abundant than native species in their new ranges (van Grunsven *et al.*, 2007; Engelkes *et al.*, 2008). Here, we compare the aboveground insect community of two exotic plants with related natives. One of the exotic plant species originates from another continent, whereas the other exotic plant is an intra-continental range expander.

It has been long acknowledged that climate exerts a dominant control over the distribution of plant species (Woodward & Williams, 1987). Exotic plants that have been introduced from other continents into new but suitable environmental conditions are predicted to expand their ranges until they meet their climatic constraints. Plants in their original geographic range have presumably already reached their geographical climatic limits, unless these limits are changing. In that case, plants may shift range and can be considered exotic in the expanded range. The main difference is that the inter-continental exotic plants have a greater chance of being released from their natural enemies than intra-continental exotic plants, because range expansion is not exclusively limited to plants alone.

The structure and composition of insect communities is known to vary in different biogeographical realms and in north-south gradients (Rohde, 1992; Pennings & Silliman, 2005). Estimates of dispersal rates predict that aboveground insects should be able to keep track of their host plants (Berg *et al.*, 2009). However, consumers from different trophic levels like herbivores, carnivores and parasitoids, and of different plants may expand their range at variable rates when responding to climate change (Davis *et al.*, 1998; van der Putten *et al.*, 2004), as has also been shown for insect responses to habitat fragmentation (Kruess & Tscharntke 1995). Therefore, in their new range, exotic plants of both inter- and intra-continental origin may interact with novel assemblages of herbivores and carnivores, whereas native species encounter familiar herbivores and predators with which they may have a long co-evolutionary history. Reduced herbivore loads (herbivore biomass per plant biomass) and enhanced pressure from natural enemies (predator biomass per herbivore biomass) would relax the plant's exposure to top-down control, thus enhancing the ability of exotic invaders to become dominant (Keane & Crawley, 2002; MacKay & Kotanen, 2008). However, herbivore loads have not yet been compared exotic plants of inter-continental and intra-continental origin, also including related natives.

In a seminal paper, Price and colleagues (1980) argued that a better understanding of the factors that shape plant life-histories and productivity requires the incorporation of natural enemies of herbivores. The importance of higher trophic interactions for plant performance has been shown for a variety of natural plant systems in the field (Schmitz *et al.*, 2000), as well as in the biological control of insect pests of a range of different crops (Julien & Griffiths, 1996). However, thus far studies on invasive plants have focused almost exclusively on plant-herbivore interactions, whereas the impact of predators and parasitoids of the herbivores has been virtually ignored. Since predators of the herbivores are also present, top-down control is to be expected on herbivores on invasive plants as well. Although reduced susceptibility to novel herbivores may explain the invasive success of exotic plants in their new range to some extent, differences in top-down pressure from predators could further reduce, or alternatively enhance herbivory on the exotic plants.

In an earlier greenhouse study, exotic plants from both intra- and inter-continental origin suffered less shoot damage from polyphagous herbivores than their native congeners (Engelkes *et al.*, 2008), probably because the exotic plants contained higher levels of secondary plant compounds than the related native species. Extrapolating from this greenhouse experiment, we hypothesize that in the field, exotic plant species will have a smaller herbivore load than related native species. Density-dependent predator prey models (Hassell, 1976) suggest that there will be higher predator pressure on plants harboring larger herbivore loads. Therefore, we assume that fewer herbivorous insects on the exotic plants will lead to a reduced predator load and predator pressure on exotic plants when compared to related natives.

We tested two hypotheses. The first is that exotic plants have fewer herbivores than native plants, but that the difference will be greater for an inter-continental exotic plant than for an intra-continental range expander. The second hypothesis is that the exotic plants have a smaller predator load and pressure than related native plant species. In order to test these two hypotheses, we compared insect communities on two pairs of phylogenetically related native and exotic plants that co-occurred in the same riverine habitat. This approach enabled us, to some extent, to control for plant chemistry, thereby excluding differences in insect loads due to variation in secondary plant compounds among plant genera (Jermy, 1984). One of the exotic plant species, *Senecio inaequidens*, originates from South Africa and the other, *Rorippa austriaca*, from central and south-eastern Europe. In the past few decades, both exotic species have become increasingly abundant in the Netherlands (Tamis *et al.*, 2005). Since both exotic species are novel to the herbivorous insects in their new range, we expected patterns of herbivore loads, as well as predator loads on herbivore loads, on *R. austriaca* and *S. inaequidens* to be smaller compared to *S. sylvestris* and *S. jacobaea*. We separated the total insect assemblage into feeding guilds and collected samples during the growing season to account for seasonal variability in herbivore loads. We also quantified predatory insects in order to assess their potential effects on the phytophagous community.

Methods

Site description

All selected plant species co-occur in the same local riverine habitat in east-central Netherlands. We studied each species pair at three locations: two locations in the Millingerwaard (51°52′ N, 6°00′ E) and one location at Ewijkse Plaat (51°52′ N, 5°45′ E). The three locations were all situated along the same river (Waal), varying from sandy soil close to the river to sandy loam further away from the river. At each location and sampling date, we studied the insect communities on adjacent pairs of native and exotic plants.

Species pairs

We used the National Standard List of the Dutch Flora (Tamis *et al.*, 2005) to select 2 exotic and 2 related native plant species. One exotic species originates from the same continent as where its range has expanded, whereas the other exotic species was first introduced in Europe before range expansion.

Rorippa austriaca (Crantz) Besser (*Brassicaceae*) is a short-lived perennial species originating from south-eastern Europe and predominantly occurs along riversides and in other moist habitats such as wetlands. This species has expanded its range northwards and westwards within the Eurasian continent (Tutin *et al.*, 1993). Although it has the potential to reproduce sexually, it has the ability of vegetative propagation of the lateral roots (Dietz *et al.*, 2005). Root fragments are easily being transported via river systems. The species occurs in dense patches with stems up to 1 m tall. After its first discovery in the Netherlands around the 1920′s it steadily colonized nutrient rich river banks but strongly increased in abundance the last decades. *Rorippa sylvestris* (L.) Besser (*Brassicaceae*) was chosen as the native related species, as it is native to the Netherlands and occurs on wet to moist, often disturbed agricultural land and flood plains. The stem of *R. sylvestris* is 30 to 40 cm tall. Also *R. sylvestris* has the ability to regenerate vegetatively by root fragments and rhizomes.

Senecio inaequidens (DC.) (*Asteraceae*) is a perennial herbaceous species that originates from South Africa. It was accidentally introduced into southern and central Europe by human transport of wool (Werner *et al.*, 1991) and increased dramatically in abundance, particularly during the second half of the 20th century (Ernst, 1997). *S. inaequidens* colonizes sandy and gravelly banks to ruderal dry areas. It can grow in a variety of climatic conditions ranging from Mediterranean, coping with summer temperatures up to +35 °C, to north-western Europe while tolerating winter temperatures as low as -15 °C. The plant can reach a height of approximately 1 meter and the woody remains of the stems stay until the next growing season. *Senecio jacobaea* (L.) (*Asteraceae*) (syn. *Jacobaea vulgaris*) was chosen as the native relative. It is native in the Netherlands, has a biennial life history and occurs on open dry, sandy soils, where it can be locally abundant. It may reach approximately 1 to 1.5 m in height.

Insect collection and determination

Each population of plant species was visited 3 times during the growing season of 2007: in May, June, and late July/early August. Within each population 10 randomly chosen plants were sampled with a minimal distance of 5 m in between each individual. At the first visit the plants were mainly in a vegetative state with developing flower buds. During the second and third visits all plants were flowering. All insect and plant samples were collected pair-wise, so that variation in daily conditions averaged out between the two plant species within each genus. For each plant during a period of 10 minutes, all insects that were on the plant were collected using an aspirator, a small net or a pair of forceps. All insects were stored in 70% ethanol until further examination. After these 10 min, plant height was measured, the plant was clipped 2 cm above the soil surface and stored in a sealed paper bag. In the laboratory the harvested plants were examined for a second time and the remaining insects were also collected. Subsequently, all plants were oven-dried at 70 ºC for 72 h. We sampled 90 plants per species for the *Senecio* pair. For the *Rorippa* pair a total of 82 plants per species were sampled due to a smaller number of plants available in one of the populations.

All collected insects were classified to order and then divided into feeding guilds (Table 4.1). The group of transients includes all collected arthropods that are not herbivorous or predaceous; most of the transients feed mainly on fungi, dead plant material or nectar. Parasitoid wasps (Hymenoptera: Braconidae, Ichneumonidae) and parasitoid and predatory flies (Diptera: Tachinidae, Asilidae) were included in the trophic guild of predators. After identification, total dry mass of each guild was determined (oven-dried, 50°C, 48 h) using a microbalance (Mettler MT5). To obtain an indication of the herbivore to plant load on each plant, we determined the chewer, miner, sucker, and galler load, expressed as herbivore guild biomass per g aboveground plant biomass. The total herbivore load was the sum of all herbivore guild loads. Predator pressure was determined as the predator biomass per g herbivore biomass. This is a measure of potential pressure on the herbivore community, although predators may also attack each other, because aboveground food webs can include five or more trophic levels (Harrington *et al.*, 1999; Harvey *et al.*, 2009). The predator load (predator biomass per g aboveground plant biomass) was also determined. Species in the order Collembola, which feed on detritus and occur in the leaf litter, were excluded from all analyses.

Plant nutrient analysis

Nitrogen levels and carbon/nitrogen ratios of aboveground plant material were determined for a subset of the sampled plants. For each species we randomly selected 3 individual plants from each of the 3 populations for each period in the growing season, resulting in 27 samples per species. For each plant the entire shoot dry mass was ground and a subsample was used to analyze C and N levels using gas chromatography with retention times 106 and 214 s respectively.

Table 4.1. Classes of feeding guilds and associated orders (families) used in the study. Insects were identified to order or family level to allow guild determination. The guild 'Transient' includes non-herbivorous arthropods.

Guild	Order (Family)
Chewer	Hymenoptera larvae, Lepidoptera, Orthoptera Coleoptera (Oedemeridae, Carabidae, Scarabaeidae, Cerambycidae, Chrysomelidae, Curculionidae)
Sap sucker	Hemiptera, Thysanoptera
Miner	Diptera larvae (Agromyzidae)
Galler	Diptera larvae (Cecidomyiidae)
Transient	Acarina, Dermaptera, Diptera, Ephemeroptera, Hymenoptera, Isopoda, Mecoptera, Psocoptera, Trichoptera
Predator	Araneae, Coleoptera (Cantharidae, Coccinellidae, Syrphidae larvae), Neuroptera, Hymenoptera (Braconidae, Ichneumonidae), Diptera (Tachinidae, Asilidae)

Data analysis

The two plant pairs were analyzed separately. For each individual plant, the Shannon diversity and Shannon evenness indices (Shannon & Weaver, 1949) were calculated based on the total numbers of individuals in the different orders. Herbivore and predator diversity were analyzed separately. Homogeneity of variance and normality was checked for all data sets, and values were transformed when appropriate. Inspection of the residuals indicated that predator diversity violated the assumptions of normality even after transformation; therefore, predator diversity was analyzed using a Wilcoxon rank test. Plant biomass and nitrogen levels for both groups were ln-transformed and total herbivore load, predator pressure and predator load on *Senecio* were ln(x+1)-transformed to improve normality. For the predator load on *Rorippa* and the C/N ratios for both plant pairs a quadratic ln(x+1)-transformation was needed to meet assumptions for ANOVA.

Analyses on all plant pair comparisons were performed using linear mixed models with origin (exotic or native) and period (3 periods of sampling) as fixed effects and populations as random effect. Due to the small number of miners on only a few plants, the miner load for the *Rorippa* species was analyzed using a Wilcoxon rank test. In the mixed models the significances of the fixed effects were calculated by model simplification and comparing the deviances (Crawley, 2007). Comparison of total herbivore loads, predator loads and predator pressure between each of the plant origins was done by Posthoc Tukey tests after one-way ANOVA with origin as fixed factor. Total herbivore load, predator load and predator pressure were ln(x+1) transformed to meet assumptions for ANOVA. Analyses were performed using R version 7.2.1 (R Development Core Team 2008). The package lme4 (Venables & Ripley, 2002) was implemented for lmer.

Results

Plant biomass and total herbivore load

The two exotic species, on average, had significantly more shoot biomass than their native congeners (Fig. 4.1). *R. austriaca* had more shoot biomass than *R. sylvestris* throughout the season (Origin: $\chi^2 = 66.13$; df = 1, $P < 0.0001$), whereas *S. inaequidens* had more shoot biomass than *S. jacobaea* in the early and mid summer period only, resulting in a significant interaction between origin and period ($\chi^2 = 17.25$, df = 2, $P < 0.001$). For both species pairs, the total herbivore loads were significantly larger on the native than on the exotic species resulting in a significant origin effect (*Rorippa*: $\chi^2 = 15.10$, df = 1, $P < 0.001$; *Senecio*: $\chi^2 = 52.08$, df = 1, $P < 0.001$; Fig. 4.2).

The herbivore load on *S. inaequidens* was larger during early summer than during mid and late summer (origin x period interaction: $\chi^2 = 11.95$, df = 2, $P = 0.002$; Fig. 4.2b). When total herbivore mass was used as a dependent variable there was no difference between the two *Rorippa* species and the interpretation of the result remained unchanged in the *Senecio* species pair (Supporting Information: Fig. S4.1). The total herbivore load on each native species was larger than on each of the exotic species (Posthoc Tukey after one-way Anova, $P < 0.01$). However, the total herbivore load did not differ between the exotic species (Posthoc Tukey $P = 0.95$), showing that the intra-continental range expander had a herbivore load as little as the exotic plant originating from South Africa.

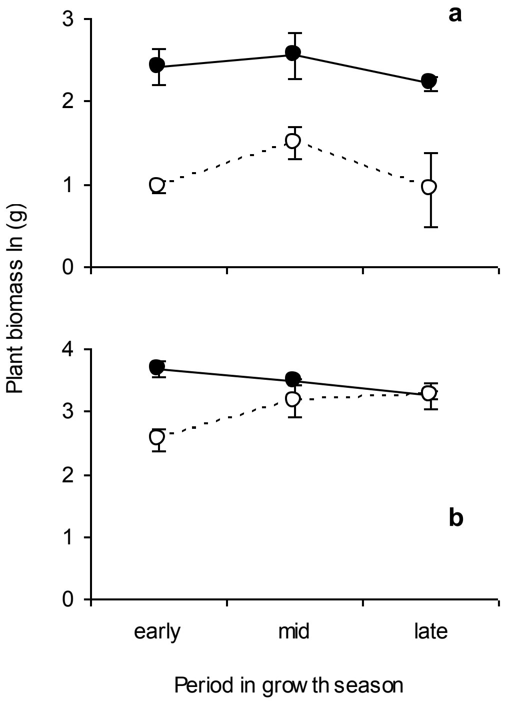

Figure 4.1. Intra-seasonality of plant dry biomass per species origin. Shown is the natural logarithm of plant dry biomass (g) in early, mid and late summer for *Rorippa austriaca* and *Rorippa sylvestris* (a) and *Senecio inaequidens* and *Senecio jacobaea* (b). Exotic species are represented by solid dots and lines and native species by open dots and dashed lines. Means (± SE) are presented.

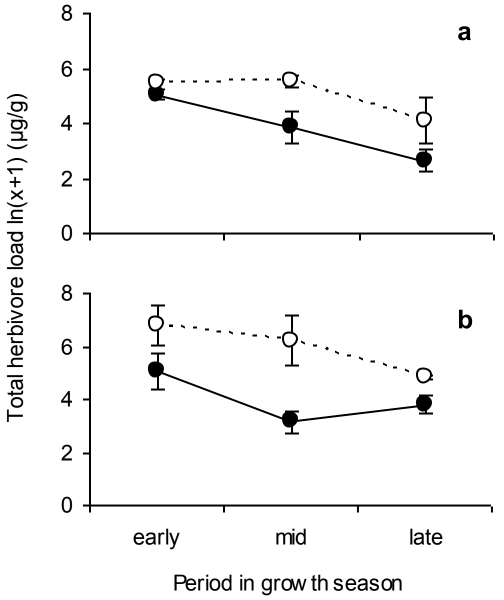

Figure 4.2. Intra-seasonality of total herbivore load per species origin. Shown is the total herbivore load as the natural logarithm of (µg herbivore biomass / g plant biomass) on (a) the *Rorippa* and (b) *Senecio* species in early, mid and late summer. Exotic species are represented by solid dots and lines and native species by open dots and dashed lines. Means (± SE) are presented.

Herbivore guild load

On *Rorippa*, galler load was 5 times greater on the native species than on the exotic species ($\chi^2 = 75.16$, df = 1, $P < 0.0001$; Fig. 4.3a). There was also a significant interaction between period and origin for galler load due to the relatively high load on native plants in mid-summer ($\chi^2 = 8.50$, df = 2, $P = 0.014$). The sapsucker load on average was significantly larger on the native species ($\chi^2 = 4.41$, df = 1, $P = 0.035$; Fig. 4.3a). The chewer and leaf miner loads on *Rorippa* did not significantly differ between the native and exotic species. When total herbivore biomass was analyzed instead of herbivore load (herbivore biomass per unit plant biomass), the significance of the effect for sapsuckers was the only one to disappear (Supporting Information: Fig. S4.2a).

The chewer and sapsucker loads on *Senecio* species differed between periods and origin resulting in significant period x origin interactions (Chewer: $\chi^2 = 8.61$, df = 2, $P = 0.013$; Sapsucker: $\chi^2 = 6.60$, df = 2, $P = 0.037$). On average, the chewer ($\chi^2 = 15.58$, df = 1, $P < 0.0001$) and sapsucker ($\chi^2 = 35.72$, df = 1, $P < 0.0001$) loads were significantly larger on the native species than on the exotic species (Fig. 4.3a). Leaf miner load on the native and exotic *Senecio* species did not differ throughout the growth season (Wilcoxon test for each period; $P > 0.05$). The use of total guild biomass instead of guild load in the same analyses did not change the outcome of the main effects and their interactions (see Supporting Information: Fig. S4.2b).

Transient load

Transient load did not differ between the two *Rorippa* species. In early summer, the native *S. jacobaea* had a larger load of transient insects than its exotic congener (Fig. 4.3b). During the mid and late summer the transient load did not differ anymore between the two *Senecio* species, resulting in a significant interaction between period and origin ($\chi^2 = 8.48$, df = 2, $P = 0.014$). Total transient biomass was greater on the exotic *R. austriaca* than on the native *R. sylvestris* (Fig. S4.2a).

Predator load and predator pressure

The predator load on *R. austriaca* did not differ from that on *R. sylvestris* ($\chi^2 = 0.15$, df = 1, $P = 0.69$; Fig. 4.4a). The predator load on the native *S. jacobaea* was overall larger than on *S. inaequidens* ($\chi^2 = 9.76$, df = 1, $P = 0.002$; Fig. 4.4b). If we used predator biomass as the dependent variable in the analysis, there was a significant origin x period interaction for the *Rorippa* species ($\chi^2 = 6.86$, df = 2, $P = 0.032$), whilst the origin effect remained significantly greater on the native *Senecio* species (Supporting Information: Fig. S4.3). Interestingly, the predator pressure was significantly greater on the exotic *Rorippa* than on the native species (Fig. 4.5a; $\chi^2 = 7.49$, df = 1, $P = 0.006$). Also *S. inaequidens* had on average a greater predator pressure than its native congener *S. jacobaea*,(Fig 4.5b; $\chi^2 = 6.94$, df = 1, $P = 0.008$). The predator loads and predator pressure were neither different between the two exotic species, nor between the two native species (Posthoc Tukey after one-way Anova $P > 0.05$).

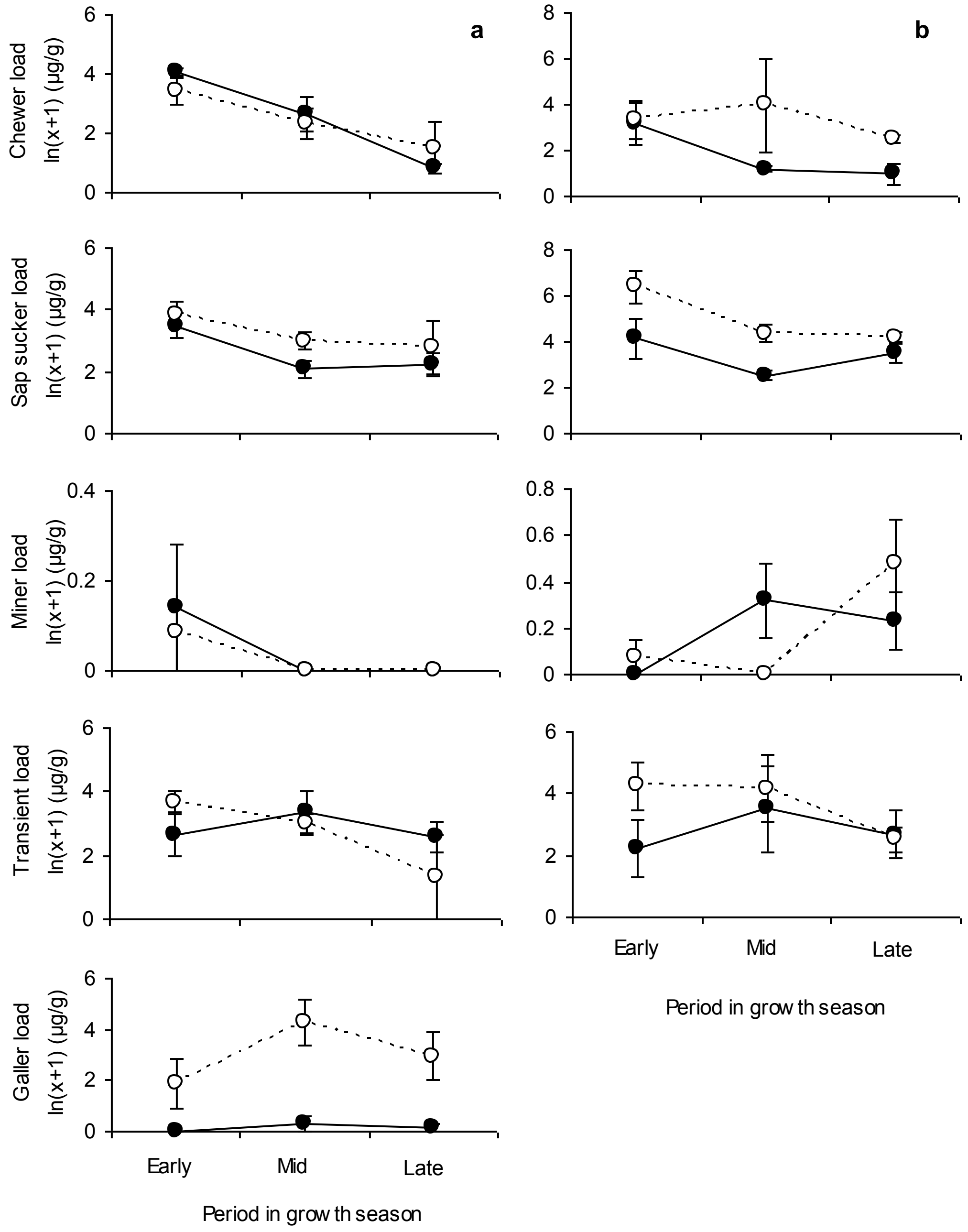

Figure 4.3. Intra-seasonality of insect guild load on the exotic and native plants. Panels show differences of insect specific guild load as the natural logarithm of (µg guild biomass / g plant biomass) between (a) the exotic (solid dots and solid line) and the native plants (open dots and dashed lines) during early, mid and late summer. Means (± SE) are presented.

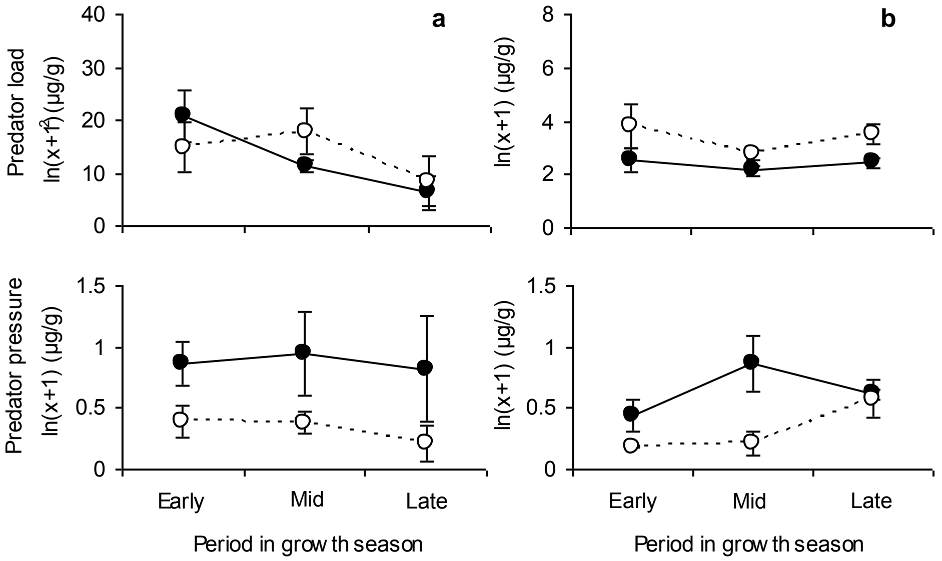

Figure 4.4. Intra-seasonality of predator load (upper panel) and predator pressure (lower panel) on (a) *Rorippa* and (b) *Senecio* species in early mid and late summer. Means (± SE) are presented. The exotic and native species are represented by solid and open/dashed dots and lines respectively.

Herbivore and predator diversity

Herbivore taxonomic richness on *R. austriaca* was greater than on *R. sylvestris* in early summer, but smaller in late summer (origin x period interaction: $\chi^2 = 12.03$, df = 2, $P = 0.002$). Evenness ($\chi^2 = 0.13$, df = 1, $P = 0.71$) and predator richness did not differ between the two *Rorippa* species (W = 3565.5, $P = 0.60$). Herbivore richness on *S. inaequidens* was significantly greater than on *S. jacobaea* in early summer, whereas it was smaller in later summer (origin x period interaction: $\chi^2 = 19.38$, df = 2, $P < 0.0001$). The herbivore evenness on *S. jacobaea* increased during the growth season. However, on average, herbivore evenness on the native *S. jacobaea* was lower than for the exotic *S. inaequidens* ($\chi^2 = 10.82$, df = 1, $P = 0.004$). Richness of predators was significantly lower on the exotic *S. inaequidens* in mid summer (W = 301.5, $P = 0.008$); on other sampling dates there was no difference between the native species and the exotic species. Predator evenness was higher on *S. inaequidens* than on *S. jacobaea* in mid summer only (W = 307.5, $P = 0.011$).

Plant nutritional quality

The carbon/nitrogen ratio was on average higher in *R. austriaca* (25.1 ± 1.59) than in *R. sylvestris* (20.3 ± 1.26) ($\chi^2 = 8.40$, df = 1, $P = 0.004$) and nitrogen content was lower in the exotic plant (*R. austriaca*: 1.77 ± 0.08; *R. sylvestris*: 2.12 ± 0.05) ($\chi^2 = 4.86$, df = 1, $P = 0.027$). The carbon/nitrogen ratio between *S. inaequidens* (29.41 ± 3.96) and *S. jacobaea* (27.49 ± 4.56), as well as nitrogen levels in the two plants (*S. inaequidens*: 1.63 ± 0.23; *S. jacobaea*: 1.72 ± 0.28) did not significantly differ (C/N ratio: $\chi^2 = 1.93$, df = 1, $P = 0.16$; N content: $\chi^2 = 1.70$, df = 1, $P = 0.19$).

Discussion

In support of the enemy release hypothesis and also of our first hypothesis, we found smaller total herbivore loads on the exotics *Senecio inaequidens* and *Rorippa austriaca* than on their native congeners *Senecio jacobaea* and *Rorippa sylvestris*, respectively. This result is in accordance with other field observations of insect abundance on successful inter-continental invasive exotic plants (Memmott *et al.*, 2000; Wolfe *et al.*, 2004). Our results on *Senecio* support this finding, whereas the results on *Rorippa* show that such patterns can also apply to species that have expanded their range within Europe. However, that result contradicts the second part of our first hypothesis, assuming that an intra-continental range expander may have an intermediary herbivore load when compared with the inter-continental exotic and a related native. Escape from herbivore damage may contribute to an increase in the local abundance of an exotic weed (MacKay & Kotanen, 2008). This, in turn, can contribute to rapid range expansion when abundance enhances propagule availability and when propagule availability is related positively to dispersal (Jongejans *et al.*, 2007). Therefore, our results suggest that intra-continental exotics would have similar escape from herbivory as inter-continental exotics.

We interpreted herbivore load as a proxy for enemy pressure on the plants, whereas predator pressure was considered as a proxy for potential top down control on the herbivores. Thus far, most studies assessing herbivore pressure on inter-continental invasive exotic plants have used insect counts expressed as 'herbivore load' (Jobin *et al.*, 1996; Cripps *et al.*, 2006). Using herbivore biomass may provide a closer estimate of herbivore activity, as plants can be attacked by both micro-and macro insect herbivores that would clearly inflict different levels of damage to plant tissues (Southwood & Henderson, 2000). Other studies have used plant damage as a measure of herbivore effectiveness (Liu & Stiling, 2006). This approach results in a net effect, which does not provide information about the potential role of predators in controlling levels of herbivory on native and exotic plants. Ultimately, a combination of these methods needs to be applied in order to provide quantitative effects of plant- and herbivore consumers on their resources.

Studies on invasive exotic plants typically examine whether generalist or specialist agents are likely to control exotic plants, rather than which feeding strategies may provide the strongest control. For most herbivore feeding guilds, loads were consistently largest on the natives throughout the growing season. On *R. austriaca* and *S. inaequidens*, half the herbivore guilds had significantly less biomass per gram dry plant tissue than on the native congeners. In the case of *Rorippa*, the sapsucker and galler loads were smallest on the exotic species, whereas in the case of *Senecio* the chewer and sapsucker loads were smallest on the exotic species. A reduction in specific enemy guilds attacking vital plant parts could partly benefit the competitive ability of the invasive plant because the natives receive stronger control from their own suite of co-evolved enemies (Agrawal *et al.*, 2005). On the other hand, preference for exotic plants by native herbivores have also been shown (Parker & Hay, 2005). For example, the gall midge *Dasineura sisymbrii* (Shrank), which forms galls in the flower heads of *R. sylvestris*, is associated with many *Rorippa* species including *R. austriaca* (Nijveldt, 1969). Although exotic plants are likely faster exploited by chewing and sucking insects than by leaf-miners and gallers (Strong *et al.*, 1984), a longer temporal exposure of the exotic plant to this insect community may lead to increased use by the flower galler and control of *R. austriaca*. The miner loads did not differ between the exotic and native species. However, it is possible that these leaf miners still play a significant role, e.g. via interactions with insects of other guilds. Other studies have also shown that low abundant species can still be key players in the functioning of ecosystem (Lyons *et al.*, 2005)

The smaller total herbivore - and guild loads on the exotic plants could have resulted from lower plant quality in early, mid, or late summer for some insects. Differences in resource usage exert variable pressures on the plant (Simberloff & Dayan, 1991). We observed in different periods of the growth season that chewer, sapsucker and miner loads on the exotic *Senecio* species were greater or smaller than, or not different from the native *Senecio* species. Plant quality also changes over the course of a season, and this can account for variation in herbivore and predator performance (Feeny, 1976; Awmack & Leather, 2002). The lower nitrogen content in the exotic *R. austriaca* may account for the smaller sapsucker – and galler loads observed on this species. Although the *Senecio* species did not differ in nutrient levels, higher levels of secondary plant compounds in *S. inaequidens* compared to *S. jacobaea* may be responsible for lower plant quality resulting in smaller herbivore loads (Caño *et al.*, 2009). Phenological differences in plant quality and the life-cycles of insects occupying various guilds may also account for variable herbivore loads over the course of a growing season. The herbivore load could have been larger on native plants which were smaller than exotic plants, however, total herbivore biomass was not higher on the exotic species (Fig. S4.1).

In contrast with our second hypothesis, predator pressure was higher on the exotic than on the native species. This is counterintuitive to density dependent predator-prey dynamics as proposed by Nicholson-Bailey models (Hassell, 1976), which state that predator abundance will increase if prey abundance increases. On the other hand, we observed that predator loads were not different between the *Rorippa* species and smaller on *S. inaequidens* than on *S. jacobaea*, while total herbivore loads were larger on the native plants. In support of our results, a study on invertebrate assemblages in *Fallopia*-invaded habitats found that spider abundance did not differ between invaded and uninvaded habitats although herbivore abundance was two times lower in invaded plots (Gerber *et al.*, 2008). In contrast, greater herbivore biomass was associated with greater predator biomass on invasive *Cytisus scoparius*, but this pattern did not differ between native and exotic continents (Memmott *et al.*, 2000). Recently, Heleno *et al.* (2009) used a food web approach to investigate effects of exotic plants on insects and observed that herbivore biomass did decrease, but top predators were absent in heavily invaded plots. However, only parasitoids were considered as top predators and intermediate predators were excluded, whereas these may become important and increase when top predation is relaxed.

The relative contribution of top-down or bottom-up control of the herbivore community and its consequences for plant productivity are both acknowledged to be important (Walker & Jones, 2001; Gripenberg & Roslin, 2007). From a plant's point of view, the evolutionary novelty of exotic plants in their new environment makes their susceptibility to novel herbivores hard to predict. When the herbivores do not recognize the plant as a suitable host, or when they are not tolerant to the plant's potential novel defense compounds, the plant may be released from enemies. However, when the enemies of the herbivores do not recognize the cues emitted upon herbivory, top-down control may become less efficient and the exotic plants may then be more vulnerable to herbivory (Verhoeven *et al.*, 2009). Hence, the incorporation of higher trophic level organisms in studies on enemy pressure on exotic plants can enhance our insight into the bottom-up and top-down controls that are operating in nature.

Transient insects which do not damage the plant directly may still influence effects of herbivory in an indirect way. For example, transients can make up part of the predatory diet, which could influence predator pressure through apparent competition (Holt, 1977). In our study, the transient load did not differ between the two *Rorippa* species, suggesting that the effects of transient insects on predator pressure experienced by herbivorous insects, does not differ between the native and exotic plant species. On the native *S. jacobaea* transient biomass was lower than on the exotic *S. inaequidens* in early and mid summer, suggesting that for these species transients could cause enhanced predator pressure on herbivores on the range expanding species.

We observed higher herbivore richness on both exotic plant species in early season but lower than their native congeners in late season. High herbivore richness in the native plant community could lead to biotic resistance (Elton, 1958) and may hinder the ability of exotic plants to proliferate (Frenzel & Brandl, 2003). For example, there is an increased chance that the herbivore community includes species that can recognize, or deal with the defensive chemicals of the exotic plants. Earlier growth and therefore higher abundance of exotics may attract arthropods that later in the season preferably feed on qualitative better natives. A high richness of herbivores may support a high richness of predators controlling the ecological impact of this higher herbivore diversity. In our study, the higher herbivore richness on both native species coincided with no difference (on *Rorippa* species), and a lower (on *S. jacobaea*) predator richness, suggesting reduced control of herbivory on native plants or increased control of herbivory on exotic plants. Also, in a study on the range expanding butterfly *Aricia agestis,* the larvae experienced reduced enemy impact in the new range despite similar parasitoid richness in the native and exotic range (Menendez *et al.*, 2008).

In conclusion, we show that exotic plant species from inter- and intra-continental origin both have smaller herbivore loads than their closely related native plants. For the exotic plant that is expanding its range but originates from the same continent, the herbivore community might move along with the plants. We speculate that two factors may be at play. First, insects may not track their food plants when expanding their range, if the local conditions in the native range are optimal. Second, the same insect species associated with the exotic plant in its native range may also occur in the new range, but these insects may be locally adapted to other related plants species and do not recognize (at least in the short term) the new invader. The higher predator pressure on the herbivore community of the exotic range expanders might contribute to herbivore control, however, experimental predator removal in the native and expanded range is needed in order to further study if the predator pressure is driving, or following herbivore loads on these plants. Whether or not such reduced herbivore loads may promote invasiveness of intra-continental range expanders requires experimental herbivore exclusion studies. Nevertheless, our results suggest that if regional climatic warming continues at the current rate, plants from lower latitude regions with effective dispersal strategies may become dominant in plant communities from higher latitude regions. Rapid climate change represents an enormous challenge for ecosystems across much of the biosphere and we can expect ecological communities to become re-assembled as new antagonistic and mutualistic interactions are formed between plants and consumers, involving (temporary) risks of species outbreaks.

Acknowledgements

We are grateful to Staatsbosbeheer Regio Oost for giving us permission to work in Millingerwaard and the Ewijkse Plaat. We thank Wiecher Smant for analyzing shoot carbon and nitrogen levels. We thank Koen Verhoeven for useful discussion on statistical approaches. Annelein Meisner and Ingrid Nieuwenhuis both helped with sampling.

Supporting Information

Analyses of total herbivore biomass and guild biomass on the exotic plants *Rorippa austriaca* and *Senecio inaequidens* and their native congeners *Rorippa sylvestris* and *Senecio jacobaea*

Here we present the results of analyses on the total herbivore biomass and the herbivore biomass according to feeding guilds, all per plant, to provide insight in the difference between the analysis approaches of biomass alone and biomass corrected for plant size. While some guild specific results differed from the approach taken in the main article, the main conclusion, based on these results, did not change.

Analyses were done separately for the two species pairs. Total herbivore biomass for *Rorippa* was ln(x+1)-transformed to improve normality. For the separate guilds predator biomass for *Rorippa*, and chewer, predator and transient biomass for *Senecio* we used $\ln(x+1)^2$-transformed data to meet assumptions. All analyses on total herbivore biomass and herbivore guild biomass were performed using linear mixed models with origin (exotic or native) and period (3 periods of sampling) as fixed effects and populations as random effect. The leaf miner biomass for the *Rorippa* and *Senecio* species was analyzed by Wilcoxon rank test. The analyses were performed using R version 7.2.1 (R Development Core Team 2008) with the package lme4 (Venables & Ripley 2002) implemented for lmer.

Results

Total herbivore biomass

Total herbivore biomass per plant did not differ between the *Rorippa* species (χ^2 = 0.47, df = 1, P = 0.49; Fig. S4.1a). In the case of *Senecio*, the native species had significantly more herbivore biomass in early and late summer (χ^2 = 12.82, df = 2, P = 0.002; Fig. S4.1b).

Herbivore guild biomass

The galler biomass was significantly higher greater on the native *R. sylvestris* (χ^2 = 76.04, df = 1, $P < 0.0001$) and varied over time (χ^2 = 10.38, df = 2, P = 0.006; Fig. S4.2a). The biomass for chewers, sapsuckers and leaf miners were not different between origins. The biomass of the chewers on *S. inaequidens* was less than on *S. jacobaea* in mid and late summer (χ^2 = 14.61, df = 2, P = 0.0006; Fig. S4.2b). On average the sapsucker biomass was higher on *S. jacobaea* (χ^2 = 20.37, df = 1, $P < 0.0001$). Miner biomass was not different between the *Senecio* species.

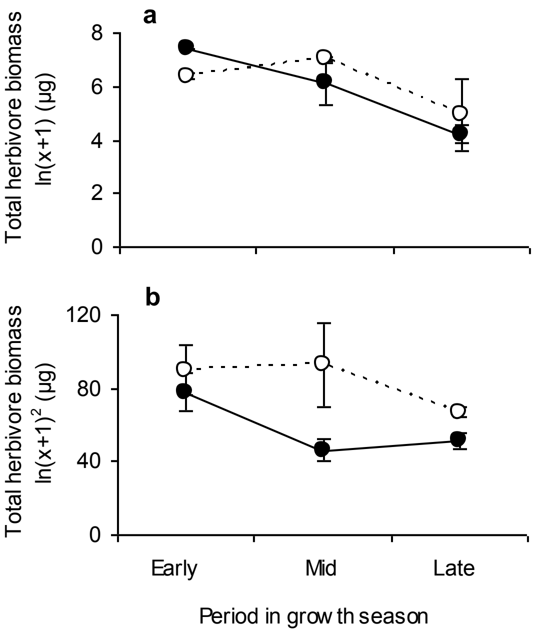

Figure S4.1. Total herbivore biomass on (a) the *Rorippa* and (b) *Senecio* species in early, mid and late summer. Exotic species are represented by solid dots and lines and native species by open dots and dashed lines. Means (± SE) are presented.

Transient biomass

Transient biomass was on the exotic *R. austriaca* than on *R. sylvestris*. (χ^2 = 4.24, df = 1, *P* = 0.039; Fig. S4.2a), but did not differ between *S. inaequidens* and *S. Jacobaea*.

Predator guild biomass

R austriaca had a lower predator biomass than *R. sylvestris* (χ^2 = 6.86, df = 2, *P* = 0.032; Fig. S4.3a), however, only in early summer. The predator biomass on the native *S. jacobaea* was overall higher than on exotic *S. inaequidens* (χ^2 = 4.03, df = 1, *P* = 0.045; Fig. S4.3b).

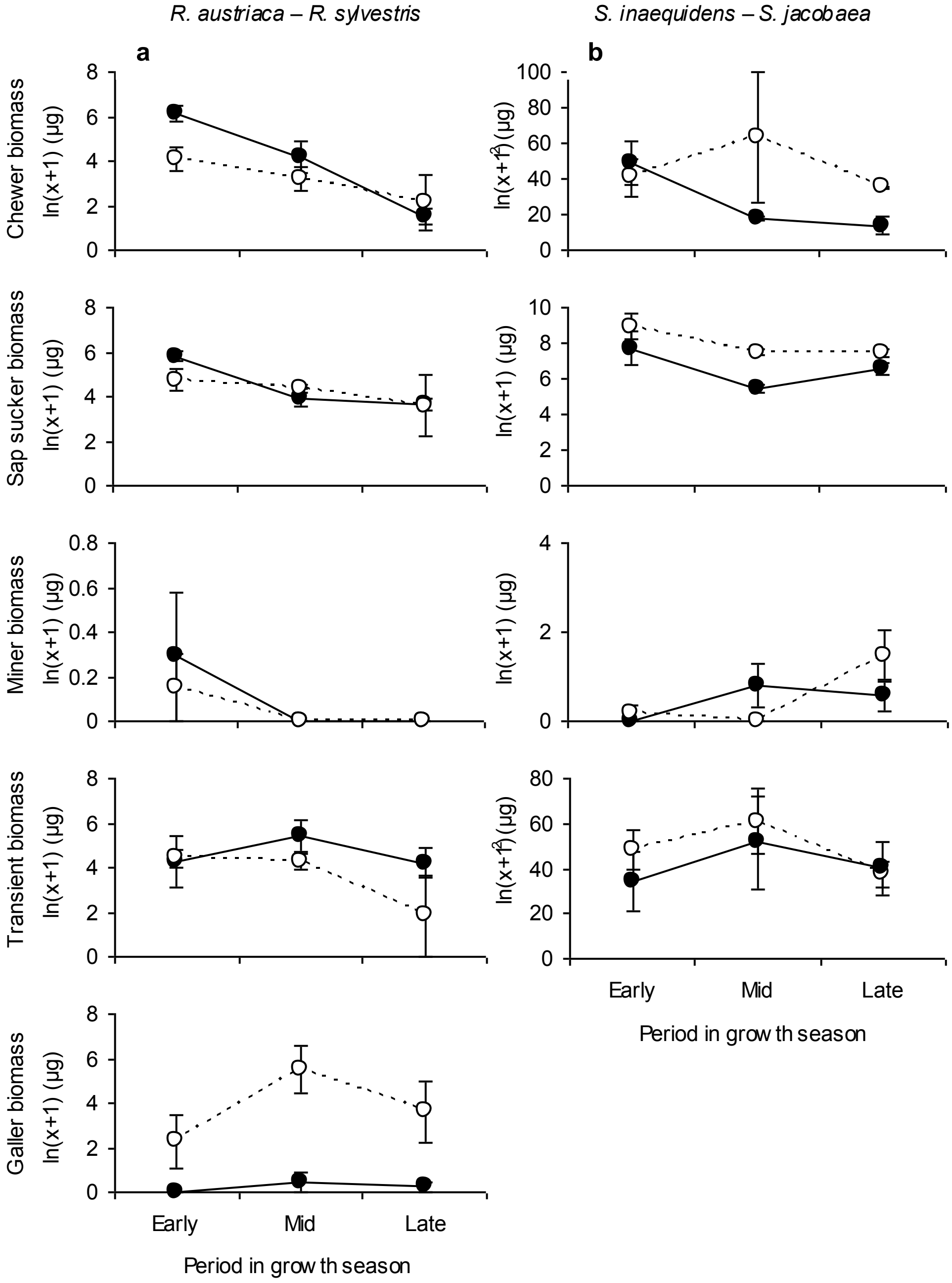

Figure S4.2. Insect guild biomass of (a) the exotic *R. austriaca* (solid dots and lines) and the native *R. sylvestris* (open dots and dashed lines) and (b) the exotic *S. inaequidens* (solid dots) and the native *S. jacobaea* during early, mid and late summer. Means (± SE) are presented.

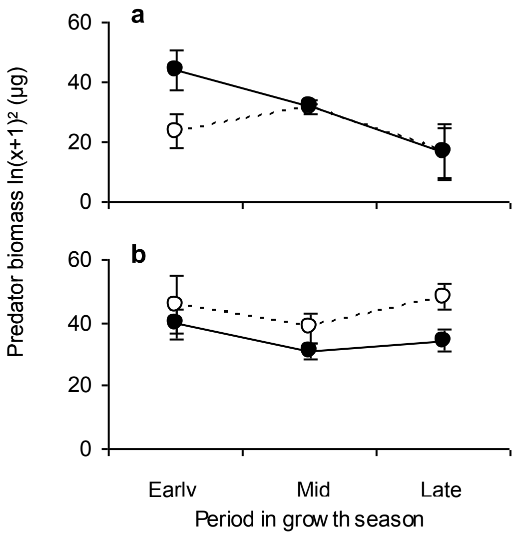

Figure S4.3. Predator biomass on (a) the exotic *R. austriaca* and the native *R. sylvestris* and (b) the exotic *S. inaequidens* and the native *S. jacobaea* in early, mid and late summer. The exotic and native species are represented by solid and open/dashed dots and lines respectively and the means (± SE) are presented.

Chapter 5

HERBIVORY DOES NOT NECESSARILY RESULT IN COMMUNITY DOMINANCE IN INVADED PLANT COMMUNITIES

T. Engelkes, A. Meisner, E. M. Morriën, W. H. van der Putten, M. Macel

Abstract

Plant species that are introduced to new ranges due to such anthropogenic processes as climate change and habitat loss may become dominant community members that pose a threat to local biodiversity. The ability of exotic species to become dominant in their new habitats has frequently been explained by the enemy release hypothesis. By contrast, here we show that, using a community-level approach, herbivory does not exclusively promote exotic dominance, and that community composition is reshuffled, whereas evenness does not change. Under natural field conditions we established mixed communities of exotic and native plant species in which we allowed half of the communities to be exposed to aboveground herbivory while the other half was excluded. Herbivory reduced almost half the species biomass, but we found that this did not lead to dominance by the exotics. Species varied considerably in their response to herbivory through changes in community ranking, but on average native and exotic species substituted biomass production equally. We suggest that there is more that one key mechanism, for instance enemy release that explains the dominance of exotic plants in invaded communities.

Keywords: Aboveground herbivory, climate change, community evenness, enemy release, range expanding.

Introduction

Exotic plants pose a major threat to biodiversity and the functioning of ecosystems worldwide, because some exotic plants may become very dominant, displacing local species and changing the cycling of elements (Lodge, 1993; Mack *et al.*, 2000). The enemy-release hypothesis proposes that introduced exotic plants may become invasive because they are no longer controlled by their specialized, co-evolved natural enemies (Keane & Crawley, 2002). Support for enemy release has emerged from a large number of studies. However, there are relatively few experimental tests of enemy release using invaded communities in the field (Stohlgren *et al.*, 1999; Hejda & Pysek, 2006; Emery, 2007; Emery & Gross, 2007; Hejda *et al.*, 2009). In invaded communities, enemy release will promote invasiveness only when the exotic plants are also able to outcompete native species (Colautti *et al.*, 2004; Blumenthal *et al.*, 2009). Here, we test the hypothesis that invaded plant communities will be dominated more by exotic plants when the plant communities are exposed to aboveground herbivory than when the aboveground herbivores have been excluded.

The validity of the enemy release hypothesis has been tested by different approaches such as database analyses (Mitchell & Power, 2003; Cappuccino & Arnason, 2006; van Kleunen *et al.*, 2010), food web analyses (Memmott *et al.*, 2000), herbivore numbers in controlled community conditions (Agrawal & Kotanen, 2003), single exotic-native species comparisons (Leger & Forister, 2005; Keeler & Chew, 2008), and reciprocal transplantations (Wolfe, 2002; Genton *et al.*, 2005; Vila *et al.*, 2005). Both within and between these approaches, results differ and they are not always unequivocal (Mitchell & Power, 2003; Agrawal *et al.*, 2005; Parker & Hay, 2005; Parker *et al.*, 2006; van Kleunen *et al.*, 2010). These varying results between some studies may be due to the use of different exotic plant species. Not all exotic plants will experience the same degree of enemy release and even if they do, the interaction strengths amongst species interacting with the exotic plant could be different. Also, test conditions may be different which will affect the outcome of plant-environment and plant-plant interactions. Comparing multiple exotic and native species in a community context makes it possible to detect how enemy release of exotic plants may contribute to changes in plant community composition under natural conditions.

Some exotic species in our study are from inter-continental origin whereas others originated from lower latitude regions of the same continent. Inter-continental plants have been intentionally or unintentionally introduced by humans after which time they started spreading naturally in their new range. Intra-continental exotics have naturally expanded their range polewards, most likely due to changed environmental conditions, such as habitat change and recent climate warming (Walther *et al.*, 2002; Parmesan & Yohe, 2003; Tamis *et al.*, 2005; Kelly & Goulden, 2008). Intra-continental range expansion may lead to enemy release, although it is possible that their natural enemies co-disperse. However, as species may move beyond their existing range boundaries at different speeds (Berg *et al.*, 2010; van

Grunsven *et al.*, 2010), or host-enemy interactions may not necessarily re-establish in the new range (Menendez *et al.*, 2008), release from natural enemies is well possible. Moreover, both inter -and intra-continental range expanding plant species can be better defended against novel enemies than related natives (Engelkes *et al.*, 2008), which may give them an advantage over native plants as well.

In order to test our hypothesis, we created experimental field plots with plant communities consisting of native and exotic species. The plant communities consisted of 6 exotic species that have increased strongly in abundance during the last decades and 6 phylogenetically related native species. This phylogenetically controlled comparison may create a condition where differences in dominance more likely will be the consequence of enemy release, rather than of differences in the ecology or biology of the species involved (Agrawal *et al.*, 2005; Strauss *et al.*, 2006; Funk & Vitousek, 2007). In half of the communities we excluded aboveground vertebrate and invertebrate herbivores, while the other half was exposed to herbivory. Based on our previous finding that intra-continental range expanding exotic plants had similar invasive properties as inter-continental range expanders (Engelkes *et al.*, 2008), we expected no differences between these groups of exotics.

Methods

Study design

The field site was situated in a riverine habitat in the nature reserve De Afferdense en Deestse Waarden, The Netherlands (51°89′ N, 5°64′ E). We removed the vegetation by soil tillage and created 10 plots of 3x3 m at 5 m distances from each other. In each plot, we established plant communities with a similar composition (6 exotic and 6 congeneric native plant species). Of each plant species we established 3 individuals, resulting in a total of 36 plants. In every plot, the positions of the plant individuals were randomized for each plot over a 6x6 grid pattern 30 cm apart from each other. Other plants were kept out by covering the soil with root cloth and hand weeding of the planting holes.

Each of these ten plant communities was enclosed by a 3 x 3 x 2 m (l x w x h) tent of fine nylon mesh (0.6 mm^2) in order to keep out insects and vertebrate herbivores. The mesh removed maximally about 30-48 % of the ambient light. In order to allow access of herbivores, of five tents, the east side away from the most frequent wind direction was opened, which changed the microclimate as little as possible. The closed tents were considered as control treatment and the open tents as herbivory treatment. We had two rows of five tents each. Both within and between rows, open and closed tents were alternated. Therefore, between rows, of two adjacent tents one was always open and one was closed. Distances between neighboring tents were equal both within and between rows. In order to control for spatial correlation between close and distant tents, tent was nested in treatment to test the effect of herbivory while accounting for differences between tents. The plant communities in the open tents were exposed to vertebrate herbivory by rabbits at the start and continuous insect herbivory throughout the entire course of the experiment. The regular checks of the closed tents confirmed that the plant communities in them were not exposed to aboveground herbivory.

Plant species selection and seedling growth

The National Standard List of the Dutch flora was used to select the plant species that made up our experimental communities. Selection criteria were that (1) all exotic and native plant species co-occurred in riverine areas along the rivers Rhine-Waal, (2) that the exotic plants increased in frequency in the second half of the 20th century and (3) that each exotic plant had a closely related native (within the same genus, except one species of which we took a relative at the family level with a comparable ecology) (Table 5.1).

Seeds were collected from the region where the field site was situated or, in an exceptional case, purchased through a specialized seed supplier who collected the seeds locally. Before germination, all seeds were surface sterilized by a 1 % hypochlorite solution. Seeds were planted in trays with homogenized sterilized soil (γ-radiation 25 kGray) that was collected from the same region. The trays with seedlings were placed in a growth chamber at 15-25 °C, 10-14 hrs night-day regime for early summer species and a 15-25 °C, 8-16 hrs night-day regime for late summer species. Seedlings were 6 weeks old when planted in the field and seedlings that had died due to factors other than herbivory were replanted until the 4th week of the experiment. The experiment ran from June 1st until September 30th.

Plant biomass and cover

Plant cover was determined at the end of the experiment as the total cover of individual plant biomass projected as surface area. Since the seedlings were planted 30 cm from each other, a cover of 1 would indicate that the plant biomass covers an area of 30 x 30 cm^2. The cover per plant species was an average the cover of the 3 individuals of that species in the community. As plants may overlap, their total cover can be > 1. After 4 months all shoots were clipped to 1 cm above the soil surface. The shoots were dried at 70°C for 72 h before determining dry weight.

Analyses

The Shannon-Wiener diversity and evenness were based on aboveground plant biomass. The diversity index was calculated as $H = -\Sigma p_i \cdot \ln p_i$ (p_i is the proportional contribution of the i^{th} species to the total aboveground biomass). The evenness equals 1 if all species contribute the same proportion of biomass. The lower the evenness the more the community biomass is dominated by a few plant species.

Table 5.1. Plant species selected for the experiment. Exotic species are presented in bold and differ in continental origin (3rd column). All species co-occur in riverine habitats but differ in family and life history. The 4th and 5th column show the frequency of square kilometers (log-value) for 2 monitored periods in the last century.

Species	Family	Origin	Life history	$Log_{10}(km^2$ frequency) 1975-1988	1988-2000
Artemisia biennis	Asteraceae	intracontinental	biennial	2	5
Bidens frondosa	Asteraceae	intercontinental	annual	8	8
Bunias orientalis	Brassicaceae	intracontinental	perennial	3	4
Rorippa austriaca	Brassicaceae	intracontinental	perennial	5	6
Senecio inaequidens	Asteraceae	intercontinental	perennial	6	8
Tragopogon dubius	Asteraceae	intracontinental	biennial/perennial	2	4
Artemisia vulgaris	Asteraceae	native	perennial	9	9
Bidens tripartita	Asteraceae	native	annual	9	9
Sinapis arvensis	Brassicaceae	native	annual	8	9
Rorippa sylvestris	Brassicaceae	native	perennial	8	9
Senecio jacobaea *	Asteraceae	native	biennial/perennial	8	9
Tragopogon pratensis	Asteraceae	native	biennial/perennial	8	8

∗recently the name of *Senecio jacobaea* has changed to *Jacobaea vulgaris*

Data were analysed using R version 7.2.1 (R Development Core Team 2008). Plant biomass and biomass proportion were analysed using ANOVA with the factors origin (exotic, native), treatment (control, herbivory), their interaction, species nested in origin and the interaction of treatment x species nested in origin. The difference in proportional biomass between range expanding plants from inter-continental and intra-continental origin was analysed with an almost identical ANOVA model, except that the factor origin was substituted with the factor continent (inter-continental vs intra-continental origin). In order to correct for effects of spatial correlation between communities we included block as a factor nested within treatment. Shannon Evenness of the plant biomass was calculated for the entire plant community using ANOVA with factor treatment (control, herbivory), as well as for exotic and native plants separately using ANOVA with the factor origin (exotic, native), treatment (control, herbivory) and the interaction between origin and treatment. Homogeneity of variance was checked with Levene's test and normality of residuals with Shapiro-Wilk test. Biomass data and biomass proportions were square root-transformed, while for biomass proportion in the analysis with the factor continent a quadratic transformation was needed, whereas the evenness indices were transformed by the exponential function e^x to meet assumptions for ANOVA. Testing for similarity of ranking between treatments (control, herbivory) was done with a Kendall W for concordance test. The effect of herbivory on the community composition was tested using the proportional biomass for each species and each community in a Principal Component Analysis (PCA) and Redundancy Analysis

(RDA) (499 unrestricted permutations, CANOCO, V. 4.55, (Ter Braak & Šmilauer, 1997-2006).

Results

Communities exposed to herbivory had on average significantly less aboveground biomass than the control communities without herbivory ($F_{1,88} = 40.155$, $P < 0.001$; data not shown). Aboveground biomass was on average not different between native plant species and exotic plant species ($F_{1,88} = 0.057$, $P = 0.812$) and in communities exposed to herbivory, exotic plants did not have more aboveground biomass than native plants (Fig. 5.1). Herbivory severely reduced biomass of all species, except for the species *Bunias orientalis* and *Senecio jacobaea* ($F_{10,88} = 11.706$, $P < 0.001$; but see Fig. S5.1 for all individual plant species data per community). Opposite to our hypothesis, there was no interaction between herbivory treatment x plant origin ($F_{1,88} = 1.158$, $P = 0.285$ Fig. 5.1) indicating that exotics are not favored by enemy release when grown in communities with native species.

Herbivory changed the ranking in proportional biomass per plant species (Kendall's W of concordance, W = 0.71, $\chi^2 = 15.6$, df = 11, $P = 0.156$; Fig. 5.2). Kendall' W tests for similarity, so that with $P > 0.05$ the rankings differ significantly. However, exposure to herbivory did not make exotic plants on average proportionally more abundant than native plants ($F_{1,} = 0.929$, $P = 0.338$; Fig. 5.2).

There were clear species-specific effects of herbivory. Without herbivory the exotic and native *Bidens* produced 48% of the aboveground community biomass, whereas they contributed less than 10% to the aboveground biomass in the plant communities that were exposed to herbivory. The exotic *Senecio inaequidens* produced more than 20% of the aboveground biomass in communities with herbivory and became one of the most dominant species in the plant communities exposed to herbivory (Fig. 5.2).

The biomass of the native *Artemisia vulgaris* and *Senecio jacobaea*, as well as the exotic *Rorippa austriaca* and *Bunias orientalis* had 10% more biomass in the communities exposed to herbivory than in communities where herbivory was excluded. Figure 5.2 shows that also in this group of subordinate plant species exotics were not more dominant than the natives (see also Fig. S5.1). Therefore, we have to reject our hypothesis that exotic species will dominate in communities with herbivory.

Comparing the proportional biomass of inter-continental and intra-continental exotic plants resulted in a significant interaction of treatment x continent ($F_{1,40} = 5.098$, $P = 0.029$) indicating that the proportional biomass of inter-continental exotics was more decreased due to herbivory than intra-continental exotics. But, the proportional biomass was not different between communities with and without herbivory for inter-continental exotics, nor for intra-continental exotics (TukeyHSD: inter-continental, $P = 0.249$; intra-continental, $P = 0.605$) suggesting that relative importance in the community of inter –and intra-continental plants was not changed.

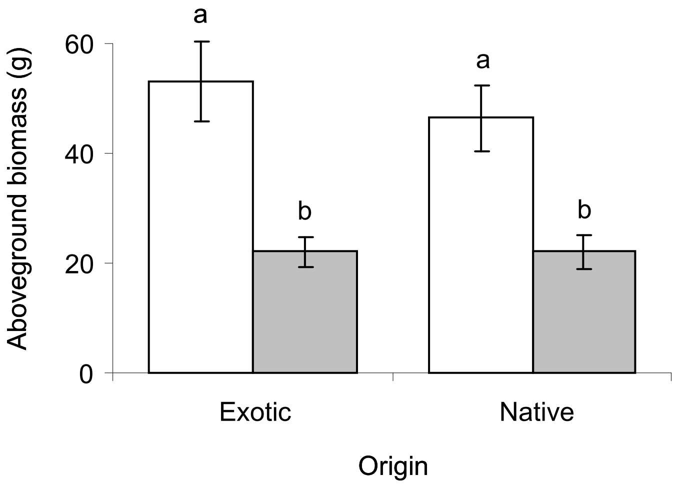

Figure 5.1. Average aboveground biomass production of the plant community per origin. Data shown is aboveground biomass (g) averaged over 5 communities for exotic species and native species (n=12) in control treatments (open bars) and herbivory treatment (solid bars). Data shown are the means (± SE). Letters indicate significant differences according to posthoc Tukey HSD at $p < 0.05$.

Herbivory did not change the evenness of the total plant community when compared to the control treatment ($F_{1,8} = 0.038$, $P = 0.849$; Fig. 5.3a). Moreover, when the evenness of native and exotic plant species was considered separately, they did not differ ($F_{1,8} = 0.038$, $P = 0.995$) and there was no significant interaction between plant origin and herbivory ($F_{1,8} = 2.549$, $P = 0.149$; Fig. 5.3b). Thus, herbivory did not change the evenness of the aboveground biomass and this effect did not depend on whether plants were of exotic or of native origin. Instead, herbivory increased the variation in species proportional biomass indicating increased spatial heterogeneity between communities exposed to herbivory (PC-axis 2, 26.2%; Fig. S5.2). Redundancy analysis (RDA) showed that herbivory significantly shifted community composition in that the species that increased their proportional biomass were replaced by species that decreased in their proportional biomass ($F = 7.131$, $P = 0.014$). There was a significantly positive correlation between proportional biomass and cover ($r^2 = 0.68$, $P < 0.001$) suggesting that plants with large cover may overgrow and inhibit the growth of small individuals thereby increasing variation in proportional biomass.

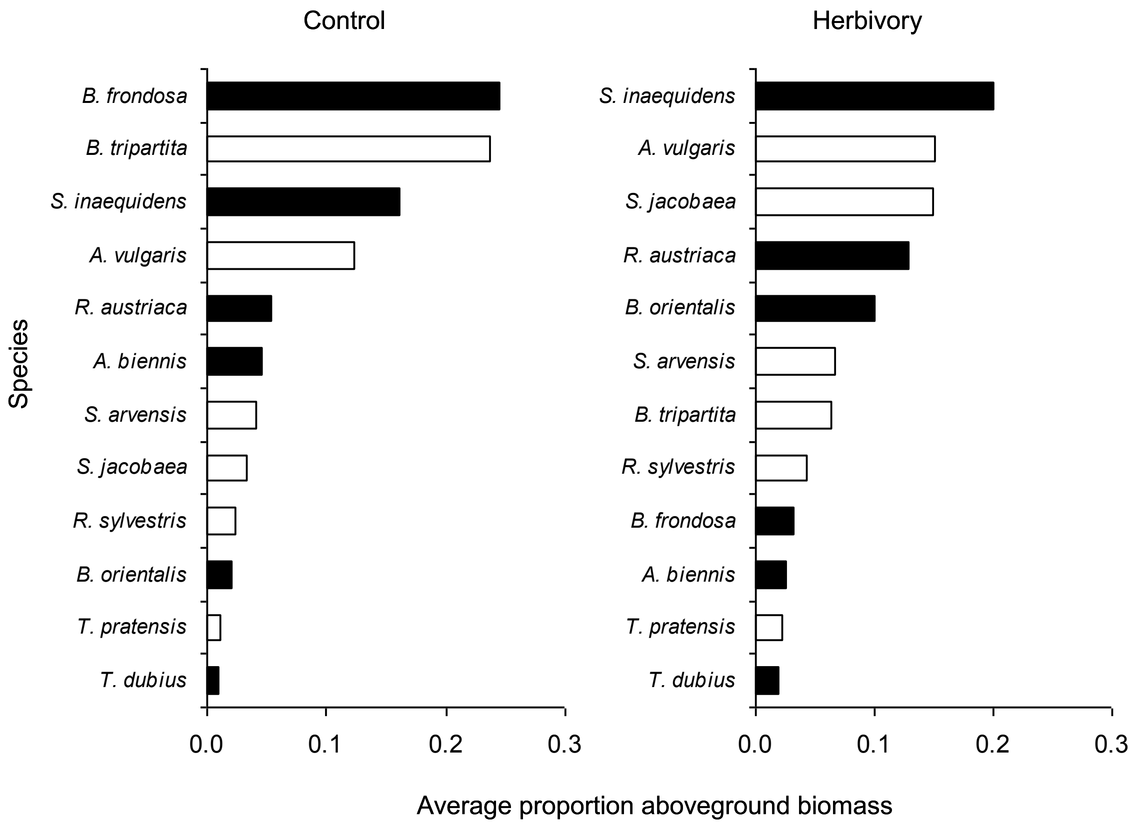

Figure 5.2. Relative contribution of the individual plant species to the total aboveground plant community biomass ranked according to the size of the contribution (n = 5). Exotic plant species are indicated by solid bars and native plant species by open bars. Left panel are control communities without aboveground herbivory and the right panel are communities exposed to aboveground herbivory.

Discussion

We show that aboveground herbivory may promote the dominance of some invading exotic plant species, but that herbivory also counteracts potential dominance by other invaders. In a field study we created mixed communities of native and exotic plants and compared composition of communities exposed to aboveground vertebrate and insect herbivores with unexposed plant communities. Opposite to what we expected, plant communities exposed to aboveground herbivores did not become completely dominated by exotic plants. Instead, some well defended native plant species also benefited from exposure to herbivory. Exposure to herbivory resulted in a major shift in plant community composition, but it did not alter plant community evenness. Thus, plant communities exposed to herbivory did not become more exclusively dominated by a few exotic plant species than unexposed plant communities. Moreover, herbivory also did not influence evenness of the native plants differently from that of the exotics.

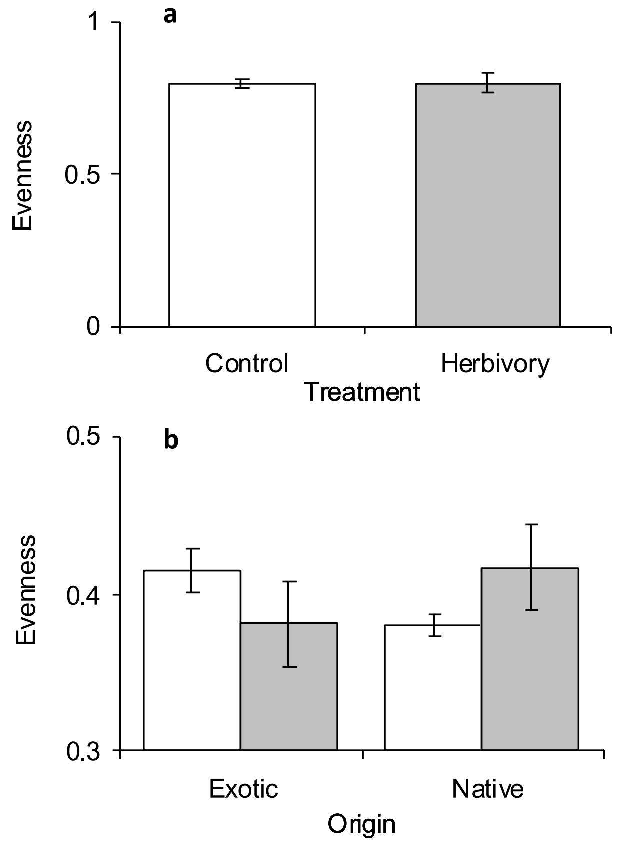

Figure 5.3. Shannon Evenness of the aboveground biomass (a) of the total community per treatment and; (b) for range expanding exotic species and native species separately, in the control (open bars) and herbivory (solid bars) treatment. Means (± SE) are presented.

Herbivory reduced aboveground plant biomass almost to half the biomass of communities without herbivory. However, in contrast to our expectation, there was no difference in biomass reduction between native and exotic species. This could be the result of non-selective grazing at the species level by vertebrate herbivores (Olff & Ritchie, 1998), but herbivores may also prefer exotic species over native species (Parker & Hay, 2005; Parker *et al.*, 2006), so that not all exotic plants may benefit from being released from their native enemies. Exposure to aboveground herbivory changed the rank order of the different plant species according to their proportional contribution to total biomass. However, the aboveground biomass of plant communities exposed to herbivory was almost equally made up by exotic and native plant species. One possibility is that by using phylogenetically related plant species, related exotic and native species have overlapping defenses allowing local herbivores to incorporate them into their diet (Cappuccino & Arnason, 2006; Strauss *et al.*, 2006). On the other hand, exposure of a quite similar set of exotic and native plant species to polyphagous aboveground herbivores in a greenhouse showed that the exotic species were better defended against non-coevolved herbivores than the natives (Engelkes *et al.*, 2008). That would make the possibility of overlapping defenses less likely.

We expected inter-continental exotic plants to have a higher chance to have lost natural enemies than intra-continental range expanders, since for example herbivorous insects may also shift their ranges (Parmesan & Yohe, 2003). However, this expectation was not confirmed, because the proportional biomass of both inter – and intra-continental exotic species did not differ between communities with and without herbivory, suggesting that climate induced range expanding plants may experience similar enemy release as classic invaders from other continents.

Evenness did not differ between communities with and without herbivory. Thus, the proportional biomass of individual plant species changed after herbivory, but the distribution of biomass over plant species in the community was not changed. Similar observations were made in grazed and ungrazed sites in Rocky Mountain grasslands (Stohlgren *et al.*, 1999). In that study, herbivory may have had a mediating effect on the biomass production of plants in the community, decreasing dominant herbaceous species but increasing competitively subordinate species performance (Olff & Ritchie, 1998; Carson & Root, 2000). Although exotic species were not considered in the latter studies, similar processes appear to have been going on in our study.

Our data suggest that native species with particular traits (Emery & Gross, 2007), for example being chemically well defended against native aboveground herbivores, may prevent exclusive domination of invaded plant communities by exotic species. This mechanism has been suggested by Levine *et al.* (Levine *et al.*, 2004). In our study, one such plant species is *S. jacobaea,* which is well defended by pyrrolizidine alkaloids against a range of herbivorous insects (Macel *et al.*, 2005), may be better adapted to native herbivory than naïve exotic plants (Verhoeven *et al.*, 2009). Our data may also point at conflicts among range expanding species in that they are controlling each others spatial habitat occupancy, rather than facilitating each others the invasion (Simberloff & Von Holle, 1999). Indeed, although spatial heterogeneity increased with herbivory, both exotic and native species did not exclusively take advantage of the disturbance.

In conclusion, we show that aboveground herbivory reduced the aboveground biomass of both native and exotic plant species equally well. Herbivory caused a shift in dominance among both exotic and native plant species, but the plant communities did not become dominated by exotics only. Exotic plants from inter-continental origin did not become more dominant than intra-continental range expanders in communities with herbivory. Therefore, if herbivores from lower latitudes have expanded their range as well, they did not exert proportionally more control on the plants from lower latitudes. Finally, the shifts in plant community composition did not change the plant community evenness and native plants were not less even than exotics. Our study emphasizes the need for examining invasive exotic plants at the community level, because enemy release is only one of the mechanisms involved, besides competition and other factors that structure plant community composition influencing the success of exotic plants. A community approach may help to further understand the implication of exotic plant invasions on the vegetation composition in the invaded range. Finally, our data suggests that, although climate warming may lead to range expansion of plant species with invasive properties (van Grunsven *et al.*, 2007; Engelkes *et al.*, 2008), not all of these range-expanding plant species will become dominant members of plant communities in the exotic range.

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Supporting Information

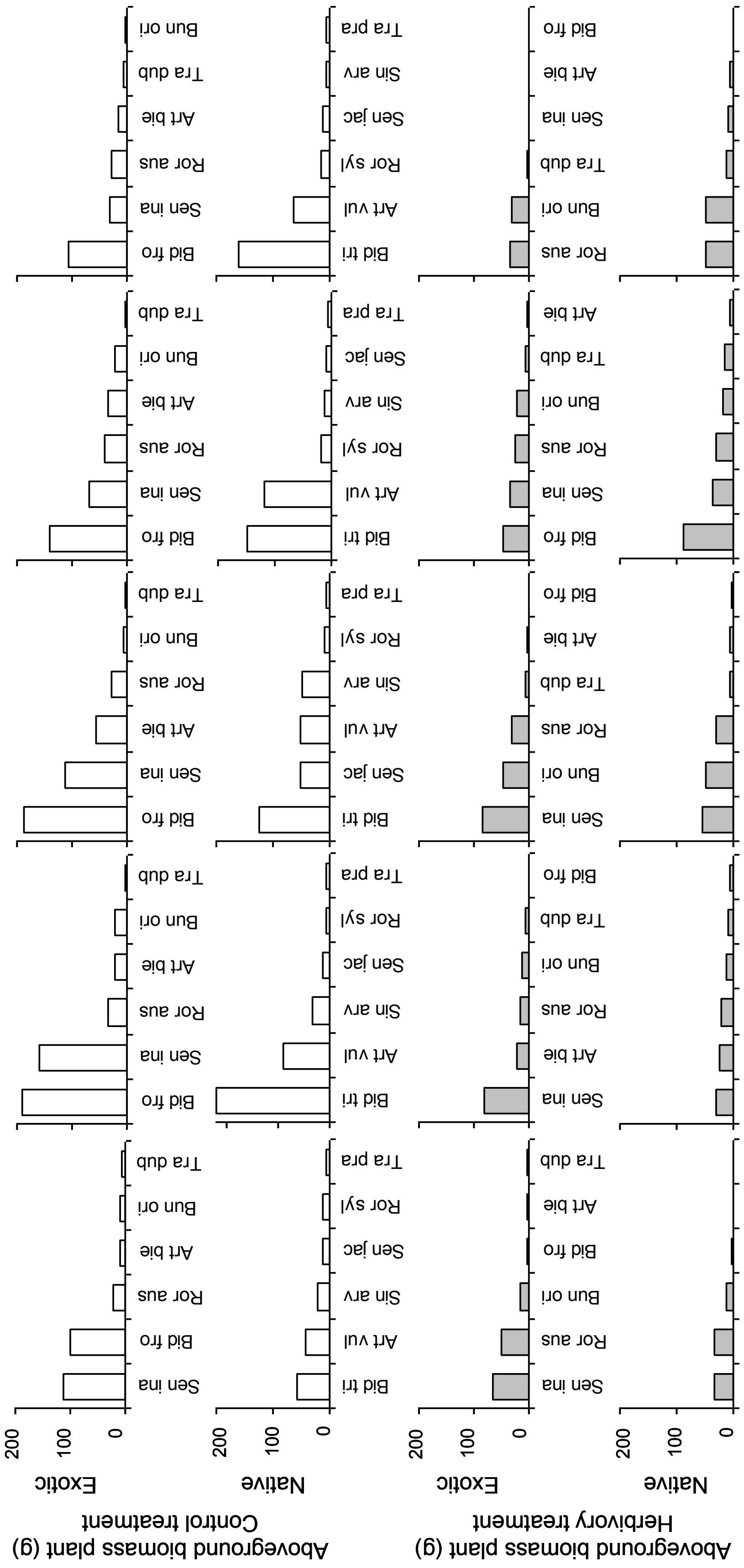

Figure S5.1. Average aboveground plant biomass (g) per origin per treatment and per community. The aboveground biomass production within each community is shown and ranked from the largest first to the smallest last according to Berger-Parker index. Control and herbivory treatments are shown in white and grey bars, respectively.

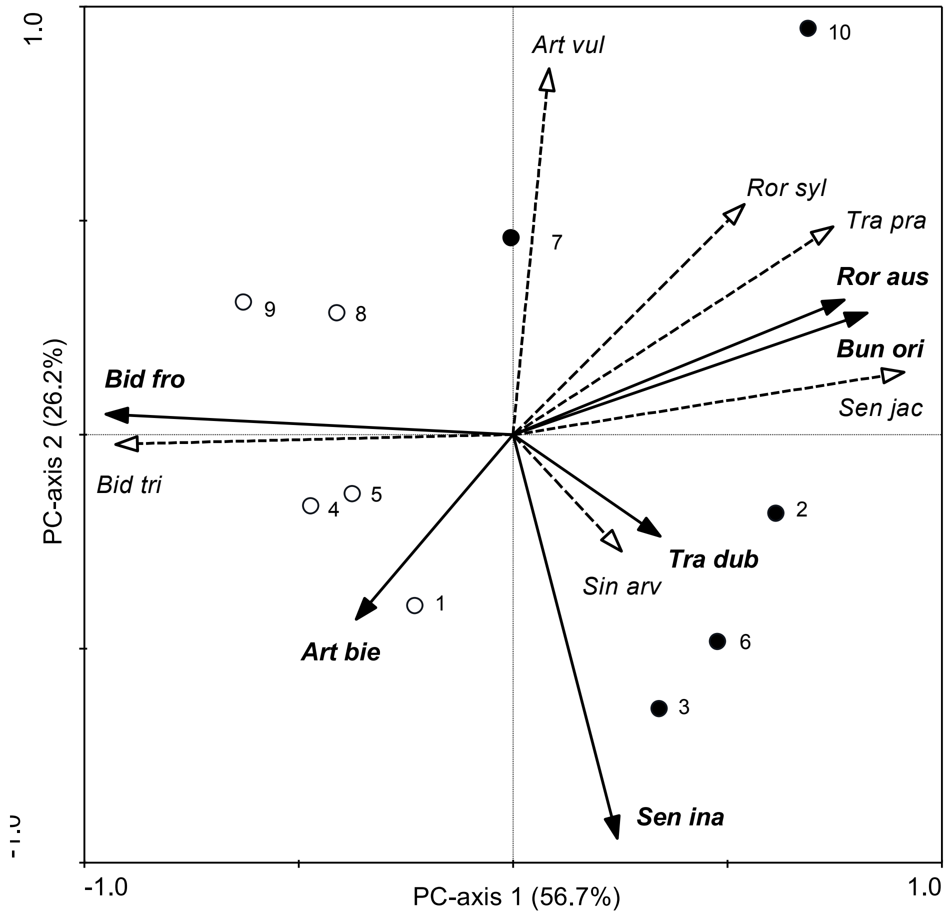

Figure S5.2. PCA ordination of proportion of aboveground biomass in control treatments (open dots) and treatments with herbivory (closed dots) for range expanding plant species (bold, solid arrows), and native plant species (dashed arrows). PC-axis 1 and PC-axis 2 explain 56.7% and 26.2% of the variation in aboveground biomass production by range expanding and native plant species in the community. The directions of the arrows indicate the variation explained by the proportion of biomass of individual species between control and herbivory treatment. For example, the two *Bidens* species (*Bid fro*, *Bid tri*) explain most variation in proportional biomass in control treatments (open dots) compared to the herbivory treatment (closed dots), but have low explanation for variation within the treatments. Numbers indicate the identity of the treatment replicates. Abbreviations next to the arrows are the first three letters of the plant genus and species name (for full names see Table 5.1).

Chapter 6

SYNTHESIS

Synthesis

The general aim of my thesis was to elucidate if differences in aboveground plant-insect interactions may explain why exotic plants are more successful than native plants in their new habitats. I hypothesized that exotic plants are more successful than related natives, because they suffer less from aboveground herbivorous insects. These results will be discussed in the first part of this synthesis (6.1). Exotic plants can be broken down arbitrarily into two categories based on their origin: inter-continental exotics that have been introduced intentionally or unintentionally from overseas and intra-continental exotics that are expanding their range pole-wards in response to recent climate warming. In this thesis I examined whether there was a difference between inter-continental exotic plants and intra-continental range expanding plants. In the second part (6.2), I discuss the main findings in which I examine differences between these two types of exotic invaders. Finally, I discuss some ideas and directions for future research (6.3).

6.1 Influence of plant-insect interactions on exotic and native plant performance

Under some conditions, the amount of biomass produced by plants can be controlled by insect herbivores (Strong *et al.*, 1984; Crawley, 1989; Huntly, 1991). Exotic plants may affect control by herbivorous insects differently than related natives, because they possess different properties that make them less or more suitable for the herbivorous insects that they encounter in the new range (Levine *et al.*, 2003; Wolfe & Klironomos, 2005; Cappuccino & Arnason, 2006). In this research, I consider both aspects.

Exotic and native plant performance under herbivory

In Chapter 2, I compared the performance of exotic and native plants under herbivory in the greenhouse. The selected plant species all had been collected from the same natural habitat, a riverine area in Gelderland Province in the east of the Netherlands. By studying plant performance I measured biomass loss due to aboveground herbivory due to polyphagous leaf chewers and sap suckers, which are the two largest insect feeding guilds regarding species number and biomass (Strong *et al.*, 1984). I used the locust *Schistocerca gregaria* as a leaf chewing herbivore and the aphid *Myzus persicae* as a sap feeding herbivore. The locust, *S. gregaria*, which originates in central Africa and has been fed in the lab mmainly on grasses, presumably did not have an evolutionary history with either of the plant species (hence it was a 'double-blind test' situation) included in the experiment and therefore allowed an objective measure of plant resistance to herbivory. *M. persicae*, on the other hand is a cosmopolitan polyphagous species that may have had some kind of history, however diffuse, with the various plant species used in the test, although this was impossible to ascertain. I tested the hypothesis that the polyphagous herbivores would not show different responses to the plants, irrespective of any history (or not) with any of the plants under investigation. In contrast to our expectations, the locust performed more poorly on the exotic plants than on congeneric native plants. These results suggest that the exotic plant species possess superior defense traits in comparison with related native species.

I also considered differences in plant performance between exotic and native plants in the field. We planted exotic and native plants in mixed communities in a similar area from which they had been collected, e.g. a riverine nature reserve in the east of the Netherlands. The communities were planted in cages; half of the cages were closed to protect the plant communities from herbivory whereas half of the cages were kept open at the lee side to allow herbivores to enter, while providing comparable light, wind and temperature conditions as in the closed cages. I hypothesized that exotic plants would be less attacked by herbivores than native plants, because they are not controlled by their specialized, co-evolved natural enemies (Keane & Crawley, 2002). Thus, even over a short time span, I expected the exotic plants to become dominant in the plant communities in the open tents at the expense of the native plants. After one growing season, under herbivory both exotic and native plants showed a large reduction in biomass. The hierarchy in plant biomass among plant species between the communities in the closed and open cages had also been reshuffled. However, in contrast to my hypothesis not all exotic plant species did become dominant in the plant communities in the presence of herbivory. Exotics and natives were quite well distributed in their rank order from most to lowest total biomass in the plant community. This was because some well defended native plant species, for example *Senecio jacobaea* did well in the plant communities exposed to herbivory showing that some native plants can be better defended against local herbivores than some exotic plants. This may also partly explain why many exotic plants never become invasive pests while only a small number actually do. At the same time, it shows that native plants, under the right conditions, can become 'outbreaking' species, effectively exhibiting the same characteristics as invasive exotic species.

Herbivore performance on exotic and native plants

In the greenhouse experiment (Chapter 2) the locust *Schistocerca gregaria* survived better and produced more biomass on native plants, while the aphids were unaffected by plant origin. This poor performance of the locusts on the exotic plants may be due to a number of factors, for example variations in nutrient content and defensive chemical compounds, plant architecture, or morphological defenses such as the presence of surface waxes, leaf toughness and trichomes (Speight *et al.*, 1999). Levels of phenolic compounds increased in presence of herbivory for the exotic plants, and were higher than in native plants with and without herbivory. The levels in exotic plants with herbivory were also higher than in native plants with and without herbivory. This result is in line with research showing that exotic plants may be less suitable for herbivores than natives, as a consequence of higher levels of defensive secondary compounds (Leger & Forister, 2005; Wikström *et al.*, 2006). The locust and the aphid responded differently to exotic and native plants. This could be the consequence of chewing insects eating whole parts of the leaf and thereby being exposed to all defenses present in the leaf tissues (Speight *et al.*, 1999). Phloem feeders, on the other hand, only use particular plant parts using specific feeding strategies by which effective defenses can be avoided (Walling, 2008).

In the field I examined herbivore and predator loads on exotic and related native plant species. I hypothesized that exotic plants would have smaller herbivore and predator loads, because they are released from their co-evolved natural enemies. In line with this assumption, we found smaller herbivore loads on the exotic plant species compared to native plants. We also examined potential predator pressure in the field, because top down control by insect predators and parasitoids can influence herbivore damage on plants (Price *et al.*, 1980). It appeared that predator pressure was higher on exotic plants than on native plants (Chapter 4). This suggests a potentially stronger top-down control on herbivore loads on exotic plants. However, further research is needed, for example by experimental predator removal in the native and invaded range, in order to test to what extent predators are controlling the herbivores on the exotic plants, or whether the control is mainly due to bottom-up (plant-mediated) effects.

Plant-insect interactions contribute to explain success of exotic plants

Enemy release is considered an important mechanism explaining the abundance and invasiveness of exotic plants in the new range (Keane & Crawley, 2002). In this thesis I have found evidence supporting some predictions of the enemy release hypothesis, but there were also results that are not in support of the enemy release hypothesis. The field observations described in Chapter 4 suggested that exotic plants have lower herbivore loads than related native plants. This may have been due to exotics resisting local insect herbivores through bottom-up effects, but the role of top-down control by carnivores could not be excluded due to the relatively high predator numbers also found on the exotic plants. Top-down control effects from members of the third trophic level have not been considered in the enemy release hypothesis and are generally overlooked in studies on invasive exotic plants (Harvey *et al.*, 2010).

Interestingly, in the field experiment described in Chapter 5, I did not find a difference in biomass between exotic and native plants under herbivory, while this would be predicted by the enemy release hypothesis. In this experiment vertebrate herbivores were present which are known to graze less selectively than insects (Olff & Ritchie, 1998). However, the results of Chapter 4, revealed that there were smaller herbivore loads on the exotic than native plants, supporting enemy release, while also here vertebrate herbivores were potentially present. This indicates that other factors can explain exotic success, for example indirect effects on plant competition. I am aware of the fact that the observational study of Chapter 4 was exclusively done in grazed field. Following, species that were used in the closed tents may have been present in the field, but absent because they were consumed completely by herbivores. In order to directly compare the communities in the open tents with the field, plant biomass of the exotic and native plants should be measured and compared in both systems. It is also possible that specific conditions in the cages, such as competition for lower light levels or competition for belowground resources, could have affected total plant biomass production (Reader *et al.*, 1994; Blumenthal, 2006). However, the conditions in the cages with and without herbivore exposure were quite comparable, so I believe that these effects will not have had a profound effect on the overall conclusions. Therefore, my research shows that plants can be released from their natural enemies, but that this does not necessarily mean that they will become dominant in an invaded plant community.

6.2 Plant-insect interactions between inter –and intra-continental exotic plants

Most studies have focused on inter-continental plants, while it is unknown whether differences exist in plant-insect interactions between inter –and intra-continental range expanders. It could be that the release from enemies is less strong for intra-continental range expanders, since co-evolved enemies might disperse along with the plants, tracking them to new habitats (Berg *et al.*, 2010). In this thesis in all experiments I tested, whether the involved responses by plants to herbivory (biomass loss) or herbivore performance (suitability), were different between inter-continental and intra-continental range expanding species. In the greenhouse experiment (Chapter 2) we expected no difference in herbivore performance on inter –and intra-continental exotic plants, because the herbivores were a naïve locust and a cosmopolitan aphid. Indeed, the performance was not different between the two range expanders, and on both range expanders lower than on their native congeners. Examining insect loads in the field (Chapter 4) revealed that both the intra-continental and the inter-continental exotics had smaller herbivorous insect loads than their native congeners. As there was only one inter- and one intra-continental exotic plant involved in the field study, I cannot make generalizations with respect to comparisons of inter- and intra-continental exotics, but the pattern clearly points in the same direction. Including more plant species into the research was logistically not possible as it would have greatly increased the work load per sampling date. Instead, I preferred to include several sampling dates for a limited set of plant species. Insect herbivore abundance changes over the course of a growing season and there are population peaks for different species and/orfeeding guilds at different times in summer. Furthermore, early season herbivory can affect the performance and acceptance of later season herbivores by changing plant quality through what is known as ‘priming’ (Agrawal, 2000; Kessler & Baldwin, 2004). This can have consequences for the abundance of herbivores later in the season (Faeth, 1986; Poelman *et al.*, 2008). Multiple sampling dates provide a good representation of herbivore pressure throughout the season, and generate information on seasonal differences in herbivore attack between exotic and native plants.

Also in the cage experiment (Chapter 5) the intra –and inter-continental plants did not perform differently under herbivory. However, in that experiment it was evident that it was not only the exotic plants that became dominant, but also well defended native plants that performed well under herbivory, as discussed above.

Overall, the intra-continental range expanding plants and the inter-continental exotic plants both increased defense against herbivores, and showed some similar trends pointing at release from their co-evolved enemies. Of course these results have to be interpreted with caution, because there were many other factors that were not explored and in the end the sample, size was still comparatively small. However, there is some evidence that the mechanisms that might explain the success of classical, inter-continental invading plants might also apply to intra-continental exotics. This makes some species of intra-continental plants as potentially invasive as inter-continental exotic weeds in the medium term. It also suggests that the aboveground enemies of range-expanding intra-continental plant species may not (yet) have dispersed with the plant to The Netherlands, although this further studies are required along north-south gradients tracing back the intra-continental range expanders to their native range in order to determine if this argument is valid or not.

The proportion of plant species in the Netherlands that originates from more southern regions in Europe has increased in recent decades (Tamis *et al.*, 2005) suggesting that exotic range expanders may become more dominant in native vegetation assemblages. Climate warming is predicted to persist well into this century and perhaps longer (IPCC, 2007). It is stated that climate warming could even exacerbate plant invasions (Willis *et al.*, 2010). Since warming-induced range expanding plant species can become invasive by the same mechanism as has been shown for introduced exotic plants (this thesis), there may be concomitant effects on biodiversity, ecological communities and the functioning of ecosystems. How and to what extent this happens needs further research.

Soil feedbacks, defined as the net effect on plants of pathogenic soil biota and symbiotic and mutualistic organisms, are mostly negative for plant growth (Kulmatiski *et al.*, 2008). Inter-continental plants are expected to be released from pathogenic soil biota (a positive feedback) in particular, whereas soil pathogens of intra-continental range expanding plants might be dispersed more easily than of intercontinental exotic plants. However, the success of intra-continental range expanders in their new ranges shows that negative effects from the soil are reduced as well. In Chapter 3, I tested if the soil feedback of exotic range expanding plants was less negative than that of their phylogentically related natives. Plants were grown on control soil that had been preconditioned by all other plants in the experiment, and on their own soil, that had been preconditioned by conspecifics. A reduction of plant biomass on own soil is an indication that plants experienced negative effects from their soil community. The results from this study demonstrated that both exotic plants experienced neutral to positive soil feedbacks, while the native species experienced negative soil feedbacks. Above all, the soil feedbacks of inter –and intra-continental exotics were not different, suggesting that release from negative soil feedback also promotes climate warming-induced invasions by intra-continental range expanding plants.

It has been well established that belowground processes can affect aboveground interactions (Gange & Brown, 1989; Masters *et al.*, 1993; Bezemer *et al.*, 2003; Soler *et al.*, 2005). However, there has rarely been a synthesis in above- and below ground systems in studies with invasive plants. Instead, the soil and above-ground compartments have been studied independently (Willis *et al.*, 1999; Siemann & Rogers, 2001; Lankau *et al.*, 2004). In Chapter 3, I examined the influence of soil feedback on the performance of two aboveground polyphagous herbivores. My experiments revealed that aboveground herbivore performance was reduced on plants growing in their own soil community compared to control soil. The effect of soil-feedbacks on aboveground herbivores was stronger on native than on exotic plants. Although I cannot make any conclusions on the mechanism behind this finding, the results suggests that, besides neutral to positive soil feedbacks promoting exotic success, additional factors may enhance the invasibility of both inter –and intra-continental range expanders.

6.3 Future directions

In the majority of the experiments we used a broad selection of plant genera and even tested within genera effects against more native plants. In general, I found that exotics were more successful than natives, but some exceptions that were found within genera did not support my hypotheses or even showed effects in the opposite direction. Testing for general patterns demands a large species pool, because results that rely on small datasets might be biased by the identity of a certain specific species. Also, comparisons with phylogenetically related native and exotic plants, is recommended for future studies. In Chapter 2, there were opposing results in two genera where I used two congeneric native species in comparisons with the exotic species. The responses of aboveground herbivores were similar and they performed better on the two native *Centaurea* species than on the exotic species. However, in the genus *Bidens*, the response of the herbivores to the native species went in the opposite direction in that herbivore performance of one species was better and the other species worse when reared on the exotic species. If the main interest is to compare intra- or inter generic effects, then no conclusions can be drawn from these data and consistency should be checked with broader selections of native species, if they are available.

Many studies have focused on the enemy release hypothesis (Keane & Crawley, 2002), but most of these are based on plants in their invaded range (Liu & Stiling, 2006). In order to know if exotic plants are released from their natural enemies, herbivore numbers and identities should be compared with those in their native range. Climate warming-induced range expanders have may be less susceptible to herbivores in their exotic than in their native range (this thesis).

Whether or not herbivory is indeed less in the exotic range, levels of herbivory should be compared between the two ranges. Importantly, there may be changes in the susceptibility of plants to herbivores along their range expansion gradients. For example, during range expansion populations may encounter variable and novel herbivore pressures against which they have to defend, which could lead to decreased suitability. In order to study the mechanisms that contribute to enemy reduction, future research is needed in which herbivory is compared along with genetic differences in certain plant traits, such as allelochemistry, along a transect covering terrain well into the invaded range back to the native range.

In Chapter 4, I showed that the predator loads were higher on herbivores developing on range expanding plants. If future plant communities exhibit a larger proportion of exotic plants, the relationships between predators, herbivores and plants are also likely to change. Considering changes in insect biomass and abundance, even higher trophic level predators, e.g. hyper parasitoids, but also vertebrates such as insectivorous birds, may experience changes in food abundance and or quality (Harvey *et al.*, 2003; Tallamy, 2004; Heleno *et al.*, 2009). Whether predators do contribute to the increased control of herbivores on exotic plants, needs further experiments in which predators should be excluded from the plants in both their exotic and native ranges.

Climate warming and plant range shifts within geographic regions induce natural enemies to shift their ranges as well (Thomas *et al.*, 2004). Consequently, insect communities in the exotic range comprise a mixture of native and exotic herbivores and predators. However, the extent to which interactions reassemble in the expanded range and the consequences for exotic plant performance needs further investigation. This information is necessary in order to make predictions about the effects of exotic species on a range of ecosystem functions.

REFERENCES

Agosta SJ (2006) On ecological fitting, plant-insect associations, herbivore host shifts, and host plant selection. *Oikos*, **114**, 556-565.

Agosta SJ, Klemens JA (2008) Ecological fitting by phenotypically flexible genotypes: implications for species associations, community assembly and evolution. *Ecology Letters*, **11**, 1123-1134.

Agrawal AA (2000) Specificity of induced resistance in wild radish: causes and consequences for two specialist and two generalist caterpillars. *Oikos*, **89**, 493-500.

Agrawal AA, Kotanen PM (2003) Herbivores and the success of exotic plants: a phylogenetically controlled experiment. *Ecology Letters*, **6**, 712-715.

Agrawal AA, Kotanen PM, Mitchell CE, Power AG, Godsoe W, Klironomos J (2005) Enemy release? An experiment with congeneric plant pairs and diverse above- and belowground enemies. *Ecology*, **86**, 2979-2989.

Andow DA, Imura O (1994) Specialization of phytophagous arthropod communities on introduced plants. *Ecology*, **75**, 296-300.

Andrew K, Karen H B, John M S (2006) Soil history as a primary control on plant invasion in abandoned agricultural fields. *Journal of Applied Ecology*, **43**, 868-876.

Andrew NR, Hughes L (2004) Species diversity and structure of phytophagous beetle assemblages along a latitudinal gradient: predicting the potential impacts of climate change. *Ecological Entomology*, **29**, 527-542.

Awmack CS, Leather SR (2002) Host plant quality and fecundity in herbivorous insects. *Annual Review of Entomology*, **47**, 817-844.

Bach CE (1988) Effects of host plant patch size on herbivore density patterns. *Ecology*, **69**, 1090-1102.

Bale JS, Masters GJ, Hodkinson ID *et al.* (2002) Herbivory in global climate change research: direct effects of rising temperature on insect herbivores. *Global Change Biology*, **8**, 1-16.

Barbosa P, Gross P, Kemper J (1991) Influence of plant allelochemical on the tobacco hornworm and its parasitoid, *Cotesia congregata*. *Ecology*, **72**, 1567-1575.

Barbosa P, Saunders JA, Kemper J, Trumbule R, Olechno J, Martinat P (1986) Plant allelochemical and insect parasitoids effects of nicotine on *Cotesia congregata* (Say) (Hymenoptera, Braconidae) and *Hyposoter annupiles* (Cresson) (Hymenoptera, Ichneumonidae) *Journal of Chemical Ecology*, **12**, 1319-1328.

Berenbaum MR, Zangerl AR, Nitao JK (1986) Constraints on chemical coevolution - wild parsnips and the parsnip webworm. *Evolution*, **40**, 1215-1228.

Berg MP, Kiers ET, Driessen G *et al.* (2010) Adapt or disperse: understanding species persistence in a changing world. *Global Change Biology*, **16**, 587-598.

Bernays EA, Minkenberg O (1997) Insect herbivores: Different reasons for being a generalist. *Ecology*, **78**, 1157-1169.

Bever JD, Westover KM, Antonovics J (1997) Incorporating the soil community into plant population dynamics: the utility of the feedback approach. *Journal of Ecology*, **85**, 561-573.

Bezemer TM, De Deyn GB, Bossinga TM, van Dam NM, Harvey JA, Van der Putten WH (2005) Soil community composition drives aboveground plant-herbivore-parasitoid interactions. *Ecology Letters*, **8**, 652-661.

Bezemer TM, Fountain M, Barea J *et al.* (2010) Divergent composition but similar function of soil food webs beneath individual plants: plant species and community effects. *Ecology*, **In press**.

Bezemer TM, van Dam NM (2005) Linking aboveground and belowground interactions via induced plant defenses. *Trends in Ecology & Evolution*, **20**, 617-624.

Bezemer TM, Wagenaar R, Van Dam NM, Van Der Putten WH, Wackers FL (2004) Above- and below-ground terpenoid aldehyde induction in cotton, *Gossypium herbaceum*, following root and leaf injury. *Journal of Chemical Ecology*, **30**, 53-67.

Bezemer TM, Wagenaar R, Van Dam NM, Wackers FL (2003) Interactions between above- and belowground insect herbivores as mediated by the plant defense system. *Oikos*, **101**, 555-562.

Blossey B, Nuzzo V, Hinz H, Gerber E (2001) Developing biological control of *Alliaria petiolata* (M. Bieb.) Cavara and Grande (garlic mustard). *Natural Areas Journal*, **21**, 357-367.

Blumenthal D, Mitchell CE, Pysek P, Jarosik V (2009) Synergy between pathogen release and resource availability in plant invasion. *Proceedings of the National Academy of Sciences of the United States of America*, **106**, 7899-7904.

Blumenthal DM (2006) Interactions between resource availability and enemy release in plant invasion. *Ecology Letters*, **9**, 887-895.

Bossdorf O, Schroder S, Prati D, Auge H (2004) Palatability and tolerance to simulated herbivory in native and introduced populations of *Alliaria petiolata* (Brassicaceae). *American Journal of Botany*, **91**, 856-862.

Both C, Visser ME (2001) Adjustment to climate change is constrained by arrival date in a long-distance migrant bird. *Nature*, **411**, 296-298.

Brändle M, Kuhn I, Klotz S, Belle C, Brandl R (2008) Species richness of herbivores on exotic host plants increases with time since introduction of the host. *Diversity and Distributions*, **14**, 905-912.

Brinkhuis H, Schouten S, Collinson ME *et al.* (2006) Episodic fresh surface waters in the Eocene Arctic Ocean. *Nature*, **441**, 606-609.

Cadotte MW, McMahon SM, Fukami T (2006) *Conceptual ecology and invasion biology: approaches to nature.* Springer, Netherlands, 505 pp.

Callaway RM, Aschehoug ET (2000) Invasive plants versus their new and old neighbors: A mechanism for exotic invasion. *Science*, **290**, 521-523.

Callaway RM, Ridenour WM (2004) Novel weapons: invasive success and the evolution of increased competitive ability. *Frontiers in Ecology and the Environment*, **2**, 436-443.

Callaway RM, Thelen GC, Rodriguez A, Holben WE (2004) Soil biota and exotic plant invasion. *Nature*, **427**, 731-733.

Caño L, Escarré J, Vrieling K, Sans F (2009) Palatability to a generalist herbivore, defence and growth of invasive and native *Senecio* species: testing the evolution of increased competitive ability hypothesis. *Oecologia*, **159**, 95-106.

Cappuccino N, Arnason JT (2006) Novel chemistry of invasive exotic plants. *Biology Letters*, **2**, 189-193.

Cardoza YJ, Wang SF, Reidy-Crofts J, Edwards OR (2006) Phloem alkaloid tolerance allows feeding on resistant *Lupinus angustifolius* by the aphid *Myzus persicae*. *Journal of Chemical Ecology*, **32**, 1965-1976.

Carson WP, Root RB (2000) Herbivory and plant species coexistence: Community regulation by an outbreaking phytophagous insect. *Ecological Monographs*, **70**, 73-99.

Chew FS, Courtney SP (1991) Plant apparency and evolutionary escape from insect herbivory. *American Naturalist*, **138**, 729-750.

Cipollini D, Stevenson R, Enright S, Eyles A, Bonello P (2008) Phenolic Metabolites in Leaves of the Invasive Shrub, *Lonicera maackii* , and Their Potential Phytotoxic and Anti-Herbivore Effects. *Journal of Chemical Ecology*, **34**, 144-152.

Colautti RI, Ricciardi A, Grigorovich IA, MacIsaac HJ (2004) Is invasion success explained by the enemy release hypothesis? *Ecology Letters*, **7**, 721-733.

Crawley MJ (1989) Insect herbivores and plant-population dynamics. *Annual Review of Entomology*, **34**, 531-564.

Crawley MJ (2007) *The R Book*. John Wiley & Sons Ltd, Chichester, 950 pp.

Cripps MG, Hinz HL, McKenney JL, Harmon BL, Merickel FW, Schwarzlaender M (2006) Comparative survey of the phytophagous arthropod faunas associated with *Lepidium draba* in Europe and the western United States, and the potential for biological weed control. *Biocontrol Science and Technology*, **16**, 1007-1030.

Cronin JT, Haynes KJ (2004) An invasive plant promotes unstable host-parasitoid patch dynamics. *Ecology*, **85**, 2772-2782.

Davis AJ, Jenkinson LS, Lawton JH, Shorrocks B, Wood S (1998) Making mistakes when predicting shifts in species range in response to global warming. *Nature*, **391**, 783-786.

Degomez T, Wagner MR (2001) Arthropod diversity of exotic vs. native *Robinia* species in northern Arizona. *Agricultural and Forest Entomology*, **3**, 19-27.

DeWalt SJ, Denslow JS, Ickes K (2004) Natural-enemy release facilitates habitat expansion of the invasive tropical shrub *Clidemia hirta*. *Ecology*, **85**, 471-483.

Dietz H, Wirth LR, Buschmann H (2005) Variation in herbivore damage to invasive and native woody plant species in open forest vegetation on Mahe, Seychelles (vol 4, pg 511, 2004). *Biological Invasions*, **7**, 351-351.

Ehrenfeld JG, Kourtev P, Huang WZ (2001) Changes in soil functions following invasions of exotic understory plants in deciduous forests. *Ecological Applications*, **11**, 1287-1300.

Ehrenfeld JG, Ravit B, Elgersma K (2005) Feedback in the plant-soil system. *Annual Review of Environment and Resources*, **30**, 75-115.

Ehrlich PR, Mooney HA (1983) Extinction, substitution, and ecosystem services. *Bioscience*, **33**, 248-254.

Ehrlich PR, Raven PH (1964) Butterflies and plants - a study in coevolution. *Evolution*, **18**, 586-608.

Elton CS (1958) *The ecology of invasions by plants and animals.* Meuthuen and company, Ltd, London, pp.

Emery SM (2007) Limiting similarity between invaders and dominant species in herbaceous plant communities? *Journal of Ecology*, **95**, 1027-1035.

Emery SM, Gross KL (2007) Dominant species identity, not community evenness, regulates invasion in experimental grassland plant communities. *Ecology*, **88**, 954-964.

Engelkes T, Morrien E, Verhoeven KJF *et al.* (2008) Successful range-expanding plants experience less above-ground and below-ground enemy impact. *Nature*, **456**, 946-948.

Ernst WHO (1997) Invasion, dispersal and ecology of the South African neophyte Senecio inaequidens in The Netherlands: from wool alien to railway and road alien. *Meeting on Re-Introduction of Endangered Plant Species*, Netherlands.

Faeth SH (1986) Indirect interactions between temporally separated herbivores mediated by the host plant. *Ecology*, **67**, 479-494.

Feeny P (1970) Seasonal changes in oak leaf tannins and nutrients as a cause of spring feeding by winter moth caterpillars *Ecology*, **51**, 565-581.

Feeny P (1976) *Plant appearance and chemical defence, p. 1-40. In J.W. Wallace& R.L. Mansell (eds.), Biochemical interactions between plants and insects.* Plenum Press, New York.

Feeny P, Rosenberry L (1982) Seasonal-variation in the glucosinolate content of North-American *Brassica nigra* and *Dentaria* species *Biochemical Systematics and Ecology*, **10**, 23-32.

Felsenstein J (1985) Phylogenies and the Comparative Method. *American Naturalist*, **125**, 1-15.

Fenner M, Lee WG (2001) Lack of pre-dispersal seed predators in introduced Asteraceae in New Zealand. *New Zealand Journal of Ecology*, **25**, 95-99.

Finke DL, Denno RF (2004) Predator diversity dampens trophic cascades. *Nature*, **429**, 407-410.

Frenzel M, Brandl R (2003) Diversity and abundance patterns of phytophagous insect communities on alien and native host plants in the Brassicaceae. *Ecography*, **26**, 723-730.

Funk JL, Vitousek PM (2007) Resource-use efficiency and plant invasion in low-resource systems. *Nature*, **446**, 1079-1081.

Futuyma DJ, Mitter C (1996) Insect-plant interactions: The evolution of component communities. *Philosophical Transactions of the Royal Society B-Biological Sciences*, **351**, 1361-1366.

Gange AC (2001) Species-specific responses of a root- and shoot-feeding insect to arbuscular mycorrhizal colonization of its host plant. *New Phytologist*, **150**, 611-618.

Gange AC, Brown VK (1989) Effects of root herbivory by an insect on a foliar-feeding species, mediated through changes in the host plant. *Oecologia*, **81**, 38-42.

Genton BJ, Kotanen PM, Cheptou PO, Adolphe C, Shykoff JA (2005) Enemy release but no evolutionary loss of defence in a plant invasion: an inter-continental reciprocal transplant experiment. *Oecologia*, **146**, 404-414.

Gerber E, Krebs C, Murrell C, Moretti M, Rocklin R, Schaffner U (2008) Exotic invasive knotweeds (*Fallopia* spp.) negatively affect native plant and invertebrate assemblages in European riparian habitats. *Biological Conservation*, **141**, 646-654.

Gols R, Bukovinszky T, van Dam NM, Dicke M, Bullock JM, Harvey JA (2008) Performance of generalist and specialist herbivores and their endoparasitoids differs on cultivated and wild Brassica populations. *Journal of Chemical Ecology*, **34**, 132-143.

Gols R, Raaijmakers CE, van Dam NM, Dicke M, Bukovinszky T, Harvey JA (2007) Temporal changes affect plant chemistry and tritrophic interactions. *Basic and Applied Ecology*, **8**, 421-433.

Gripenberg S, Roslin T (2007) Up or down in space? Uniting the bottom-up versus top-down paradigm and spatial ecology. *Oikos*, **116**, 181-188.

Guisan A, Thuiller W (2005) Predicting species distribution: offering more than simple habitat models. *Ecology Letters*, **8**, 993-1009.

Hairston NG, Smith FE, Slobodkin LB (1960) Community structure, population control, and competition. *American Naturalist*, **94**, 421-425.

Haribal M, Yang ZC, Attygalle AB, Renwick JAA, Meinwald J (2001) A cyanoallyl glucoside from *Alliaria petiolata*, as a feeding deterrent for larvae of *Pieris napi oleracea*. *Journal of Natural Products*, **64**, 440-443.

Harrington R, Woiwod I, Sparks T (1999) Climate change and trophic interactions. *Trends in Ecology & Evolution*, **14**, 146-150.

Harvey J, Biere A, Fortuna T *et al.* (2010a) Ecological fits, mis-fits and lotteries involving insect herbivores on the invasive plant, Bunias orientalis. *Biological Invasions*,

Harvey JA (2005) Factors affecting the evolution of development strategies in parasitoid wasps: the importance of functional constraints and incorporating complexity. *Entomologia Experimentalis Et Applicata*, **117**, 1-13.

Harvey JA, Bukovinsky T, Van der Putten WH (2010b) Mechanisms of plant-insect ecology that influence invasion success in exotic plants: a plea for a multitrophic perspective. *Biological Conservation*, **In Press**.

Harvey JA, van Dam NM, Gols R (2003) Interactions over four trophic levels: foodplant quality affects development of a hyperparasitoid as mediated through a herbivore and its primary parasitoid. *Journal of Animal Ecology*, **72**, 520-531.

Harvey JA, Wagenaar R, Bezemer TM (2009) Interactions to the fifth trophic level: secondary and tertiary parasitoid wasps show extraordinary efficiency in utilizing host resources. *Journal of Animal Ecology*, **78**, 686-692.

Hassell MP (1976) *The dynamics of competition and predation*. Edward Arnold Ltd, London, 68 pp.

Hejda M, Pysek P (2006) What is the impact of *Impatiens glandulifera* on species diversity of invaded riparian vegetation? *Biological Conservation*, **132**, 143-152.

Hejda M, Pysek P, Jarosik V (2009) Impact of invasive plants on the species richness, diversity and composition of invaded communities. *Journal of Ecology*, **97**, 393-403.

Heleno RH, Ceia RS, Ramos JA, Memmott J (2009) Effects of alien plants on insect abundance and biomass: a food-web approach. *Conservation Biology*, **23**, 410-419.

Hill JK, Thomas CD, Huntley B (1999) Climate and habitat availability determine 20th century changes in a butterfly's range margin. *Proceedings of the Royal Society of London Series B-Biological Sciences*, **266**, 1197-1206.

Hobbs RJ, Arico S, Aronson J *et al.* (2006) Novel ecosystems: theoretical and management aspects of the new ecological world order. *Global Ecology and Biogeography*, **15**, 1-7.

Holt RD (1977) Predation, apparent competition, and structure of prey communities. *Theoretical Population Biology*, **12**, 197-229.

Hunter MD, Forkner RE (1999) Hurricane damage influences foliar polyphenolics and subsequent herbivory on surviving trees. *Ecology*, **80**, 2676-2682.

Huntley B, Green RE, Collingham YC *et al.* (2004) The performance of models relating species geographical distributions to climate is independent of trophic level. *Ecology Letters*, **7**, 417-426.

Huntly N (1991) Herbivores and the dynamics of communities and ecosystems. *Annual Review of Ecology and Systematics*, **22**, 477-503.

Imura O (2003) Herbivorous arthropod community of an alien weed *Solanum carolinense* L. *Applied Entomology and Zoology*, **38**, 293-300.

IPCC (2007) *Climate Change 2007: Synthesis Report. Contribution of Working Groups I, II and III to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change* Geneva, Switzerland, 104 pp.

Janz N, Nyblom K, Nylin S (2001) Evolutionary dynamics of host-plant specialization: A case study of the tribe Nymphalini. *Evolution*, **55**, 783-796.

Janzen DH (1988) On the broadening of insect-plant research. *Ecology*, **69**, 915.

Jermy T (1984) Evolution of insect/host plant relationships. *The American Naturalist*, **124**, 609-630.

Jermy T, Szentesi R (2003) Evolutionary aspects of host plant specialisation - a study on *bruchids* (Coleoptera : Bruchidae). *Oikos*, **101**, 196-204.

Jobin A, Schaffner U, Nentwig W (1996) The structure of the phytophagous insect fauna on the introduced weed *Solidago altissima* in Switzerland. *Entomologia Experimentalis et Applicata*, **79**, 33-42.

Jongejans E, Skarpaas O, Tipping PW, Shea K (2007) Establishment and spread of founding populations of an invasive thistle: the role of competition and seed limitation. *Biological Invasions*, **9**, 317-325.

Joosten L, Mulder PPJ, Klinkhamer PGL, van Veen JA (2009) Soil-borne microorganisms and soil-type affect pyrrolizidine alkaloids in Jacobaea vulgaris. *Plant and Soil*, **325**, 133-143.

Julien MH, Griffiths MW (1996) *Biological control of weeds : a world catalogue of agents and their target weeds* CABI Publishers, New York, pp.

Keane RM, Crawley MJ (2002) Exotic plant invasions and the enemy release hypothesis. *Trends in Ecology & Evolution*, **17**, 164-170.

Keeler M, Chew F (2008a) Escaping an evolutionary trap: preference and performance of a native insect on an exotic invasive host. *Oecologia*, **156**, 559-568.

Keeler MS, Chew FS (2008b) Escaping an evolutionary trap: preference and performance of a native insect on an exotic invasive host. *Oecologia*, **156**, 559-568.

Keeler MS, Chew FS, Goodale BC, Reed JM (2006) Modelling the impacts of two exotic invasive species on a native butterfly: top-down vs. bottom-up effects. *Journal of Animal Ecology*, **75**, 777-788.

Keeling RF, Garcia HE (2002) The change in oceanic O-2 inventory associated with recent global warming. *Proceedings of the National Academy of Sciences of the United States of America*, **99**, 7848-7853.

Kelly AE, Goulden ML (2008) Rapid shifts in plant distribution with recent climate change. *Proceedings of the National Academy of Sciences of the United States of America*, **105**, 11823-11826.

Kessler A, Baldwin IT (2004) Herbivore-induced plant vaccination. Part I. The orchestration of plant defenses in nature and their fitness consequences in the wild tobacco Nicotiana attenuata. *Plant Journal*, **38**, 639-649.

Klironomos JN (2002) Feedback with soil biota contributes to plant rarity and invasiveness in communities. *Nature*, **417**, 67-70.

KNMI (2008): De toestand van het klimaat in Nederland. KNMI, De Bilt, 47 pp.

Kottek M, Grieser J, Beck C, Rudolf B, Rubel F (2006) World map of the Koppen-Geiger climate classification updated. *Meteorologische Zeitschrift*, **15**, 259-263.

Kourtev PS, Ehrenfeld JG, Huang WZ (2002) Enzyme activities during litter decomposition of two exotic and two native plant species in hardwood forests of New Jersey. *Soil Biology & Biochemistry*, **34**, 1207-1218.

Kruess A, Tscharntke T (1994) Habitat fragmentation, species loss, and biological-control. *Science*, **264**, 1581-1584.

Kulmatiski A, Beard KH, Stevens JR, Cobbold SM (2008) Plant-soil feedbacks: a meta-analytical review. *Ecology Letters*, **11**, 980-992.

Lankau RA, Rogers WE, Siemann E (2004) Constraints on the utilisation of the invasive Chinese tallow tree *Sapium sebiferum* by generalist native herbivores in coastal prairies. *Ecological Entomology*, **29**, 66-75.

Lau JA, Strauss SY (2005) Insect herbivores drive important indirect effects of exotic plants on native communities. *Ecology*, **86**, 2990-2997.

Lawton JH, Strong DR (1981) Community patterns and competition in folivorous insects. *American Naturalist*, **118**, 317-338.

Leger EA, Forister ML (2005) Increased resistance to generalist herbivores in invasive populations of the California poppy (*Eschscholzia californica*). *Diversity and Distributions*, **11**, 311-317.

Levine JM, Adler PB, Yelenik SG (2004) A meta-analysis of biotic resistance to exotic plant invasions. *Ecology Letters*, **7**, 975-989.

Levine JM, Pachepsky E, Kendall BE, Yelenik SG, Lambers JHR (2006) Plant-soil feedbacks and invasive spread. *Ecology Letters*, **9**, 1005-1014.

Levine JM, Vila M, D'Antonio CM, Dukes JS, Grigulis K, Lavorel S (2003) Mechanisms underlying the impacts of exotic plant invasions. *Proceedings of the Royal Society of London Series B-Biological Sciences*, **270**, 775-781.

Lewinsohn TM, Novotny V, Basset Y (2005) Insects on plants: Diversity of herbivore assemblages revisited. *Annual Review of Ecology Evolution and Systematics*, **36**, 597-620.

Liu H, Stiling P (2006) Testing the enemy release hypothesis: a review and meta-analysis. *Biological Invasions*, **8**, 1535-1545.

Lodge DM (1993) Biological invasions - lessons for ecology. *Trends in Ecology & Evolution*, **8**, 133-137.

Louda SM, Kendall D, Connor J, Simberloff D (1997) Ecological effects of an insect introduced for the biological control of weeds. *Science*, **277**, 1088-1090.

Lovejoy TE, Hannah L (2005) *Climate Change and Biodiversity*. Yale University Press, New Haven, 418 pp.

Lyons KG, Brigham CA, Traut BH, Schwartz MW (2005) Rare species and ecosystem functioning. *Conservation Biology*, **19**, 1019-1024.

Macel M, Bruinsma M, Dijkstra SM, Ooijendijk T, Niemeyer HM, Klinkhamer PGL (2005) Differences in effects of pyrrolizidine alkaloids on five generalist insect herbivore species. *Journal of Chemical Ecology*, **31**, 1493-1508.

Mack RN, Simberloff D, Lonsdale WM, Evans H, Clout M, Bazzaz FA (2000) Biotic invasions: Causes, epidemiology, global consequences, and control. *Ecological Applications*, **10**, 689-710.

MacKay J, Kotanen PM (2008) Local escape of an invasive plant, common ragweed (*Ambrosia artemisiifolia* L.), from above-ground and below-ground enemies in its native area. *Journal of Ecology*, **96**, 1152-1161.

Mainguet AM, Louveaux A, El Sayed G, Rollin P (2000) Ability of a generalist insect, *Schistocerca gregaria*, to overcome thioglucoside defense in desert plants: tolerance or adaptation? *Entomologia Experimentalis Et Applicata*, **94**, 309-317.

Maron JL, Vila M (2001) When do herbivores affect plant invasion? Evidence for the natural enemies and biotic resistance hypotheses. *Oikos*, **95**, 361-373.

Masters GJ, Brown VK, Gange AC (1993) Plant mediated interactions between aboveground and belowground insect herbivores. *Oikos*, **66**, 148-151.

Mattson WJ (1980) Herbivory in relation to plant nitrogen-content. *Annual Review of Ecology and Systematics*, **11**, 119-161.

Mattson WJ, Haack RA (1987) The role of drought in outbreaks of plant-eating insects. *Bioscience*, **37**, 110-118.

Memmott J, Fowler SV, Paynter Q, Sheppard AW, Syrett P (2000) The invertebrate fauna on broom, *Cytisus scoparius*, in two native and two exotic habitats. *Acta Oecologica*, **21**, 213-222.

Menendez R, Gonzalez-Megias A, Lewis OT, Shaw MR, Thomas CD (2008) Escape from natural enemies during climate-driven range expansion: a case study. *Ecological Entomology*, **33**, 413-421.

Merrill RM, Gutierrez D, Lewis OT, Gutierrez J, Diez SB, Wilson RJ (2008) Combined effects of climate and biotic interactions on the elevational range of a phytophagous insect. *Journal of Animal Ecology*, **77**, 145-155.

Mitchell CE, Power AG (2003) Release of invasive plants from fungal and viral pathogens. *Nature*, **421**, 625-627.

Morriën EM, Engelkes T, Meisner A, Macel M, van der Putten WH (2010) Climate change and invasion by intracontinental range-expanding exotic plants: the role of biotic interactions. *Annals of Botany*, **In press**.

Muller-Scharer H, Schaffner U, Steinger T (2004) Evolution in invasive plants: implications for biological control. *Trends in Ecology & Evolution*, **19**, 417-422.

NASA Goddard Institute for Space Studies. http://data.giss.nasa.gov/gistemp/. New York.

Neter J, Kutner MH, Nachtsheim CJ, Wasserman W (1996) *Applied Linear Statistical Models*. WCB/McGraw-Hill USA, 1408 pp.

Nicholson SE (2001) Climatic and environmental change in Africa during the last two centuries. *Climate Research*, **17**, 123-144.

Nieuwenhuize J, Maas YEM, Middelburg JJ (1994) Rapid analysis of organic-carbon and nitrogen in particulate materials. *Marine Chemistry*, **45**, 217-224.

Nijveldt W (1969) *Gall midges of economic importance. Vol. 8. Gall midges-miscellaneous (zoophagous, fungivorous and those that attack weeds)*. London, pp.

Ode PJ (2006) Plant chemistry and natural enemy fitness: Effects on herbivore and natural enemy interactions. *Annual Review of Entomology*, **51**, 163-185.

Olff H, Ritchie ME (1998) Effects of herbivores on grassland plant diversity. *Trends in Ecology & Evolution*, **13**, 261-265.

Parker JD, Burkepile DE, Hay ME (2006) Opposing effects of native and exotic herbivores on plant invasions. *Science*, **311**, 1459-1461.

Parker JD, Hay ME (2005) Biotic resistance to plant invasions? Native herbivores prefer non-native plants. *Ecology Letters*, **8**, 959-967.

Parmesan C, Ryrholm N, Stefanescu C *et al.* (1999) Poleward shifts in geographical ranges of butterfly species associated with regional warming. *Nature*, **399**, 579-583.

Parmesan C, Yohe G (2003) A globally coherent fingerprint of climate change impacts across natural systems. *Nature*, **421**, 37-42.

Pearson RG, Dawson TP (2003) Predicting the impacts of climate change on the distribution of species: are bioclimate envelope models useful? *Global Ecology and Biogeography*, **12**, 361-371.

Pennings SC, Silliman BR (2005) Linking biogeography and community ecology: Latitudinal variation in plant-herbivore interaction strength. *Ecology*, **86**, 2310-2319.

Pimentel D, Lach L, Zuniga R, Morrison D (2000) Environmental and economic costs of nonindigenous species in the United States. *Bioscience*, **50**, 53-65.

Poelman EH, Broekgaarden C, Van Loon JJA, Dicke M (2008) Early season herbivore differentially affects plant defence responses to subsequently colonizing herbivores and their abundance in the field. *Molecular Ecology*, **17**, 3352-3365.

Price PW, Bouton CE, Gross P, McPheron BA, Thompson JN, Weis AE (1980) Interactions among 3 trophic levels - influence of plants on interaction between insect herbivores and natural enemies. *Annual Review of Ecology and Systematics*, **11**, 41-65.

Reader RJ, Wilson SD, Belcher JW *et al.* (1994) Plant competition in relation to neighbor biomass - an intercontinental study with Poa pratensis. *Ecology*, **75**, 1753-1760.

Reinhart KO, Callaway RM (2004) Soil biota facilitate exotic Acer invasions in Europe and North America. *Ecological Applications*, **14**, 1737-1745.

Reinhart KO, Callaway RM (2006) Soil biota and invasive plants. *New Phytologist*, **170**, 445-457.

Reinhart KO, Packer A, Van der Putten WH, Clay K (2003) Plant-soil biota interactions and spatial distribution of black cherry in its native and invasive ranges. *Ecology Letters*, **6**, 1046-1050.

Renwick JAA (2002) The chemical world of crucivores: lures, treats and traps. *Entomologia Experimentalis Et Applicata*, **104**, 35-42.

Renwick JAA, Chew FS (1994) Oviposition behavior in lepidoptera. *Annual Review of Entomology*, **39**, 377-400.

Rhoades DF (1979) Evolution of plant chemical defense against herbivores. In: *Herbivores: their interaction with secondary plant metabolites* (eds Rosenthal GA, Janzen DH), pp. 3-54. Academic Press, New York.

Rohde K (1992) Latitudinal gradients in species-diversity - the search for the primary cause. *Oikos*, **65**, 514-527.

Root RB (1973) Organization of a plant-arthropod association in simple and diverse habitats: the fauna of collards (*Brassica oleracea*). *Ecological Monographs*, **43**, 95-120.

Schmitz OJ (2008) Herbivory from Individuals to Ecosystems. *Annual Review of Ecology Evolution and Systematics*, **39**, 133-152.

Schmitz OJ, Hamback PA, Beckerman AP (2000) Trophic cascades in terrestrial systems: A review of the effects of carnivore removals on plants. *American Naturalist*, **155**, 141-153.

Schoonhoven LM, Jermy T, Loon JJH (1998) *Insect-plant biology*. Chapman and Hall, London, 409 pp.

Schultz JC (1983) Impact of variable plant defencive chemistry on susceptibility of insects to natural enemies. *Acs Symposium Series*, **208**, 37-54.

Shannon CE, Weaver W (1949) *The mathematical theory of communication*. Urbana, pp.

Siemann E (1998) Experimental tests of effects of plant productivity and diversity on grassland arthropod diversity. *Ecology*, **79**, 2057-2070.

Siemann E, Rogers WE (2001) Genetic differences in growth of an invasive tree species. *Ecology Letters*, **4**, 514-518.

Simberloff D (2006) Invasional meltdown 6 years later: important phenomenon, unfortunate metaphor, or both? *Ecology Letters*, **9**, 912-919.

Simberloff D, Dayan T (1991) The guild concept and the structure of ecological communities. *Annual Review of Ecology and Systematics*, **22**, 115-143.

Simberloff D, Von Holle B (1999) Positive Interactions of Nonindigenous Species: Invasional Meltdown? *Biological Invasions*, **1**, 21-32.

Slansky F (1992) Allelochemical-nutrient interactions in herbivore nutritional ecology. In: *Ecological and evolutionary processes* (eds Rosenthal GA, Berenbaum MR), pp. 135-174. Academic Press, San Diego.

Smiley J (1978) Plant chemistry and evolution of host specificity - new evidence from heliconius and passiflora *Science*, **201**, 745-747.

Soler R, Bezemer TM, Van der Putten WH, Vet LEM, Harvey JA (2005) Root herbivore effects on above-ground herbivore, parasitoid and hyperparasitoid performance via changes in plant quality. *Journal of Animal Ecology*, **74**, 1121-1130.

Southwood TRE, Henderson PA (2000) *Ecological methods*. Blackwell Science, Oxford, pp.

Speight MR, Hunter MD, Watt AD (1999) *Ecology of insects. Concepts and applications.* Blackwell Science Ltd, Oxford, 350 pp.

Stinson CSA, Brown VK (1983) Seasonal-changes in the architecture of natural plant-communites and its relevance to insect herbivores. *Oecologia*, **56**, 67-69.

Stohlgren TJ, Schell LD, Vanden Heuvel B (1999) How grazing and soil quality affect native and exotic plant diversity in rocky mountain grasslands. *Ecological Applications*, **9**, 45-64.

Strauss SY, Lau JA, Carroll SP (2006a) Evolutionary responses of natives to introduced species: what do introductions tell us about natural communities? *Ecology Letters*, **9**, 357-374.

Strauss SY, Webb CO, Salamin N (2006b) Exotic taxa less related to native species are more invasive. *Proceedings of the National Academy of Sciences of the United States of America*, **103**, 5841-5845.

Strong DR, Lawton JH, Southwood TRE (1984) *Insects on plants*. Blackwell Scientific Publications, Oxford, pp.

Tallamy DW (1986) Behavioral adaptations in insects to plant allelochemicals. In: *Molecular Mechanisms in Insect/Plant Associations* (eds Brattsten LB, Ahmad S), pp. 273-289. Plenum Press, New York.

Tallamy DW (2004) Do alien plants reduce insect biomass? *Conservation Biology*, **18**, 1689-1692.

Tamis WLM (2005) Changes in the flora of the Netherlands in the 20th century. *Gorteria*, **Supplement 6**, 154-218.

Tamis WLM, Van't Zelfde M, Van der Meijden R, De Haes HAU (2005) Changes in vascular plant biodiversity in the Netherlands in the 20th century explained by their climatic and other environmental characteristics. *Climatic Change*, **72**, 37-56.

Ter Braak CJF, Šmilauer P (1997-2006): Canoco for Windows. Wageningen, Biometris, Wageningen-UR.

Thomas CD, Cameron A, Green RE *et al.* (2004a) Extinction risk from climate change. *Nature*, **427**, 145-148.

Thomas JA, Telfer MG, Roy DB *et al.* (2004b) Comparative losses of British butterflies, birds, and plants and the global extinction crisis. *Science*, **303**, 1879-1881.

Thompson JN (2005) *The geographic mosaic of coevolution*. University of Chicago Press., Chicago, 456 pp.

Tutin TG, Heywood VH, Burgess NA, Moore DM, Valentine DH, Walters SM, Webb DA (1993) *Flora europaea (F Eur)*. Cambridge University Press, Cambridge, pp.

van der Putten WH (2003) Plant defense belowground and spatiotemporal processes in natural vegetation. *Ecology*, **84**, 2269-2280.

van der Putten WH, Macel M, Visser ME (2010) Predicting species distribution and abundance responses to climate change: why it is essential to include biotic interactions across trophic levels. *Philosophical Transactions of the Royal Society of London, Series B, Biological Sciences*, **In press**.

van der Putten WH, de Ruiter PC, Bezemer TM, Harvey JA, Wassen M, Wolters V (2004) Trophic interactions in a changing world. *Basic and Applied Ecology*, **5**, 487-494.

van der Putten WH, Klironomos JN, Wardle DA (2007a) Microbial ecology of biological invasions. *Isme Journal*, **1**, 28-37.

van der Putten WH, Kowalchuk GA, Brinkman EP *et al.* (2007b) Soil feedback of exotic savanna grass relates to pathogen absence and mycorrhizal selectivity. *Ecology*, **88**, 978-988.

van der Putten WH, Vet LEM, Harvey JA, Wackers FL (2001) Linking above- and belowground multitrophic interactions of plants, herbivores, pathogens, and their antagonists. *Trends in Ecology & Evolution*, **16**, 547-554.

van der Putten WH, Yeates GW, Duyts H, Reis CS, Karssen G (2005) Invasive plants and their escape from root herbivory: a worldwide comparison of the root-feeding nematode communities of the dune grass Ammophila arenaria in natural and introduced ranges. *Biological Invasions*, **7**, 733-746.

van Grunsven RHA, Bos F, Ripley BS, Suehs CM, Veenendaal EM (2009) Release from soil pathogens plays an important role in the success of invasive Carpobrotus in the Mediterranean. *South African Journal of Botany*, **75**, 172-175.

van Grunsven RHA, van der Putten WH, Bezemer TM, Berendse F, Veenendaal EM (2010) Plant-soil interactions in the expansion and native range of a poleward shifting plant species. *Global Change Biology*, **16**, 380-385.

van Grunsven RHA, Van Der Putten WH, Bezemer TM, Tamis WLM, Berendse F, Veenendaal EM (2007) Reduced plant-soil feedback of plant species expanding their range as compared to natives. *Journal of Ecology*, **95**, 1050-1057.

van Kleunen M, Weber E, Fischer M (2010) A meta-analysis of trait differences between invasive and non-invasive plant species. *Ecology Letters*, **13**, 235-245.

Venables WN, Ripley BD (2002) *Modern applied statistics with S*. Springer-Verlag, Berlin, DE, 518 pp.

Verhoeven KJF, Biere A, Harvey JA, van der Putten WH (2009) Plant invaders and their novel natural enemies: who is naive? *Ecology Letters*, **12**, 107-117.

Vila M, Maron JL, Marco L (2005) Evidence for the enemy release hypothesis in Hypericum perforatum. *Oecologia*, **142**, 474-479.

Vitousek PM, Dantonio CM, Loope LL, Westbrooks R (1996) Biological invasions as global environmental change. *American Scientist*, **84**, 468-478.

Vitousek PM, Mooney HA, Lubchenco J, Melillo JM (1997) Human domination of Earth's ecosystems. *Science*, **277**, 494-499.

Walker M, Jones TH (2001) Relative roles of top-down and bottom-up forces in terrestrial tritrophic plant-insect herbivore-natural enemy systems. *Oikos*, **93**, 177-187.

Walling LL (2008) Avoiding Effective Defenses: Strategies Employed by Phloem-Feeding Insects. *Plant Physiology*, **146**, 859-866.

Walther GR, Post E, Convey P *et al.* (2002) Ecological responses to recent climate change. *Nature*, **416**, 389-395.

Walther GR, Roques A, Hulme PE *et al.* (2009) Alien species in a warmer world: risks and opportunities. *Trends in Ecology & Evolution*, **24**, 686-693.

Ward NL, Masters GJ (2007) Linking climate change and species invasion: an illustration using insect herbivores. *Global Change Biology*, **13**, 1605-1615.

Wardle DA, Bardgett RD, Klironomos JN, Setala H, van der Putten WH, Wall DH (2004) Ecological linkages between aboveground and belowground biota. *Science*, **304**, 1629-1633.

Warren MS, Hill JK, Thomas JA *et al.* (2001) Rapid responses of British butterflies to opposing forces of climate and habitat change. *Nature*, **414**, 65-69.

Waterman PG, Mole S (1994) Methods in Ecology: Analysis of Phenolic Plant Metabolites (first ed.). *Blackwell Scientific Publication, Oxford*, 74–145.

Weber G, Oswald S, Zollner U (1986) Suitability of rape cultivars with a different glucosinolate content for *Brevicoryne brassicae* (L.) and *Myzus persicae* (Sulzer) (Hemiptera, Aphididae) *Zeitschrift für Pflanzenkrankheiten und Pflanzenschutz* **93**, 113-124.

Werner DJ, Rockenbach T, Hölscher ML (1991) Herkunft, Ausbreitung, Vergesellschaftung und Ökologie von *Senecio inaequidens* DC. unter besonder Berücksichtigung des Köln-Aachener Raumes. *Tuexenia*, **11**, 73-107.

Wikström S, Steinarsdóttir M, Kautsky L, Pavia H (2006) Increased chemical resistance explains low herbivore colonization of introduced seaweed. *Oecologia*, **148**, 593-601.

Williamson M, Fitter A (1996) The varying success of invaders. *Ecology*, **77**, 1661-1666.

Willis AJ, Thomas MB, Lawton JH (1999) Is the increased vigour of invasive weeds explained by a trade-off between growth and herbivore resistance? *Oecologia*, **120**, 632-640.

Willis CG, Ruhfel BR, Primack RB, Miller-Rushing AJ, Losos JB, Davis CC (2010) Favorable Climate Change Response Explains Non-Native Species' Success in Thoreau's Woods. *Plos One*, **5**, 5.

Winkler IS, Mitter C (2007) The phylogenetic dimension of insect-plant interactions: a review of recent evidence. In: *Specialization, speciation, and radiation: the evolutionary biology of herbivorous insects* (eds Tilmon KJ), pp. 240–263. Berkeley University of California Press., Berkeley.

Wolfe BE, Klironomos JN (2005) Breaking new ground: Soil communities and exotic plant invasion. *Bioscience*, **55**, 477-487.

Wolfe LM (2002) Why alien invaders succeed: Support for the escape-from-enemy hypothesis. *American Naturalist*, **160**, 705-711.

Wolfe LM, Elzinga JA, Biere A (2004) Increased susceptibility to enemies following introduction in the invasive plant *Silene latifolia*. *Ecology Letters*, **7**, 813-820.

Woodward FI, Williams BG (1987) Climate and plant-distribution at global and local scales. *Vegetatio*, **69**, 189-197.

Wu YT, Wang CH, Zhang XD, Zhao B, Jiang LF, Chen JK, Li B (2009) Effects of saltmarsh invasion by *Spartina alterniflora* on arthropod community structure and diets. *Biological Invasions*, **11**, 635-649.

Wurst S, van der Putten WH (2007) Root herbivore identity matters in plant-mediated interactions between root and shoot herbivores. *Basic and Applied Ecology*, **8**, 491-499.

Zuefle M, Brown W, Tallamy D (2008) Effects of non-native plants on the native insect community of Delaware. *Biological Invasions*, **10**, 1159-1169.

SAMENVATTING

Het klimaat verandert en gemiddelde jaar temperaturen zullen naar verwachting hoger zijn in de toekomst. Planten en dieren reageren op deze veranderingen door zich aan te passen aan de lokaal warmere omstandigheden en of door hun leefgebied op te schuiven richting de polen. Sommige planten die succesvol hun areaal hebben uitgebreid ontwikkelen zich tot pest soort, net zoals sommige nieuw geintroduceerde soorten dat doen. Er is dus een conceptuele analogie tussen planten soorten die hun leef gebied succesvol verschuiven en invasieve planten met een oorsprong in andere continenten. Intra-continentale plantensoorten verschuiven hun areaal binnen het continent waar ze van oorsprong voorkomen. Inter-continentale soorten hebben hun oorsprong in andere continenten van waar ze zijn geintroduceerd, alvorens ze hun areaal uitbreiden in hun nieuwe omgeving. Het doel van dit proefschrift is, om meer inzicht te krijgen hoe interacties tussen planten en insecten kunnen bijdragen aan het succes van uitheemse planten die hun areaal richting de polen uitbreiden als gevolg van een opwarmend klimaat. In het bijzonder had ik tot doel om te onderzoeken of uitheemse planten die geïnduceerd door een warmer klimaat hun leefgebied verschuiven, minder voedzaam zijn dan verwante inheemse soorten en of deze uitheemse planten minder last hebben van bovengrondse vijanden dan verwante inheemse planten. Bovendien heb ik onderzocht of inter –en intra-continentale uitheemse planten verschillend zijn in hun kwaliteit als voedselplant en of ze verschilden in hun respons op bovengrondse vraat. In het eerste experiment heb ik the hypothese getest dat inter –en intra-continentale uitheemse planten en hun inheemse verwante soorten, allen afkomstig van hetzelfe habitat, niet verschillend reageerden op twee bovengrondse polyfage herbivoren. Daarnaast heb ik getest of de plant-bodem interactie minder negatief was voor de biomassa van intra –en inter-continentale uitheemse planten dan voor inheemse verwanten. In dit experiment liet ik 15 planten soorten groeien, met en zonder naive polyfage sprinkhanen (*Schistocerca gregaria*) en cosmopolitische bladluizen (*Myzus persicae*), en heb alle planten vervolgens ook laten groeien op bodem afkomstig van hun nieuw gekoloniseerde leefgebied om het effect van biota in deze bodem op de productie van plant biomassa te testen. Mijn resultaten laten zien dat zowel inter, als intra-continentale uitheemse planten gemiddeld beter waren verdedigd tegen bovengrondse en ondergrondse vijanden, dan verwante inheemse planten soorten. Dit duidt erop dat uitheemse planten die hun leefgebied succesvol uitbreiden mogelijk invasieve eigenschappen bezitten.

De effecten van plant-bodem interacties voor uitheemse planten kunnen neutraal tot postief zijn, terwijl inheemse planten negatieve effecten van hun bodem biota ondervinden. Ondergrondse interacties kunnen bovengrondse interacties beinvloeden waardoor relaties tussen uitheemse planten en hun bovengrondse vijanden kunnen veranderen. Ik heb onderzocht hoe de prestaties van de twee bovengrondse polyfage herbivoren *S. gregaria* en *M. persicae* werd beïnvloed door interacties tussen planten en hun bodem biota en deze effecten vergeleken tussen intra –en inter-continentale uitheemse planten en verwante inheemse planten. Het bleek dat het gewicht van de sprinkhanen groter was op planten die groeiden op bodem door henzelf geconditioneerd, maar kleiner op inheemse dan op uitheemse planten. De overleving van sprinkhanen was ook hoger op inheemse planten, maar was niet beinvloed door plant specifieke bodem biota. Het sprinkhanen gewicht, noch de overleving verschilde tussen inter –en intra-continentale planten. De populatie grootte van de bladluis was niet beinvloed door plant specifieke bodem biota, maar het grootste op de intra-continentale uitheemse soorten. Echter, de lichaamsgrootte van *M. persicae* was niet verschillend tussen de planten met verschillende oorsprong, maar wel groter op controle planten dan op planten die groeiden met hun soort specifieke bodem gemeenschap.

Of uitheemse planten minder natuurlijke vijanden hebben in hun nieuwe omgeving kan worden gemeten door de potentiele herbivore druk te vergelijken tussen uitheems planten en hun inheemse verwanten. Deze potentiele druk kan worden beinvloed door predatoren en parasitoiden uit hogere trofische niveaus. Ik heb in het veld de potentiele druk van zowel herbivoren op planten, als predatoren en parasitoiden op de herbivoren vergeleken tussen twee uitheemse (inter-continentaal en intra-continentaal) –en twee verwante inheemse planten soorten. Ik heb gevonden dat de potentiele herbivore druk lager was op uitheemse dan op verwante inheemse planten en bovendien dat de predatie druk op herbivoren op uitheemse planten ook hoger was. Deze resultaten impliceren that beide typen uitheemse planten een dubbel voordeel hebben, namelijk toegenomen verdediging jegens herbivoren en controle van herbivoren door insecten uit hogere trophische niveaus.

Tot slot heb ik een veld experiment opgezet om het effect the testen van herbivorie op gemeenschappen bestaande uit uitheemse en inheemse planten soorten. Dit experiment bestond uit 10 gemeenschappen met elk zes uitheemse soorten en zes verwante inheemse soorten die voorkomen in hetzelfde rivieren gebied. We lieten herbivorie toe in de helft van de gemeenschappen. De andere helft werd vrijgehouden van herbivorie. Op deze manier konden we bepalen of uitheemse planten de gemeenschap domineren wanneer de gemeenschap is blootgesteld aan herbivorie en of dit voordeel voor uitheemse planten verdwijnt wanneer er geen herbivore druk aanwezig is. We vonden dat herbivorie de totale biomassa van de gemeenschap met bijna de helft reduceerde, echter, deze gemeenschappen werden niet gedomineerd door uitheemse planten. Er was grote variatie in het effect van herbivorie op de verschillende planten soorten waardoor de hierarchy in dominantie veranderde. Interessant is dat de relatieve bijdrage van biomassa aan de gemeenschap niet verschillend was tussen uitheemse en inheemse planten en ook niet verschillend tussen inter-continentale en intra-continentale uitheemse planten. Hiermee concludeer ik dat het ontsnappen aan bovengrondse vijanden niet de enige verklarende factor is voor het succes van invasieve inter –en intra-continentale uitheemse planten.

Tot slot, planten soorten die van oorsprong voorkomen op het continent waar zij, geïnduceerd door klimaat opwarming, hun areaal uitbreiden, hebben mogelijk vergelijkbaare invasieve eigenschappen als inter-continentale uitheemse planten.

Zowel in gecontroleerde omstandigheden als in het veld waren inter –en intra-continentale uitheemse planten beter bestand tegen bovegrondse herbivorie dan inheemse planten. Uitheemse planten hadden minder last van herbivoren onder gecontroleerde omstandigheden, echter dit was niet terug te zien in een onvermengde dominantie in het veld. Verschillen in het effect van bovengrondse herbivorie is dus niet de enige voorspellende factor voor het succes van uitheemse planten van inter –en intra-continentale origine.

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Ybo, je bent klein, maar de grootste lessen leer ik van jou!

CURRICULUM VITAE

Tim Engelkes was born on June 14 1975, in Zwolle, in the Netherlands. He moved already at the age of 2 to Hoogkarspel where he was raised most of his youth. After 8 years he completed secondary education (VWO) in 1995 at the Martinus College in Grootebroek. From 1996 until 1998 he studied Cultural anthropology and Social pedagogy focusing on human interactions. A small side step in these two years was a 10 month work holiday in Australia. Then in 1998, he started the study Biology at the University of Amsterdam and specialized in population ecology and biodiversity. One of his internships was in the research group of palaeo-ecology at the University of Amsterdam where he investigated climate change events by reconstruction of historical vegetation by using pollen records. In the second internship he moved to the population ecology group at the Copenhagen University. Here he worked with an invasive ant species and examined the genetic population structure of this species in its exotic range by using molecular tools. In 2003 he got the Master degree in Ecology and Biodiversity at the University of Amsterdam. Subsequently Tim worked many months for Greenpeace and realized after his experience with social matters that he was attracted to the more profound approach of fundamental science. Therefore he decided to apply for a PhD project in the department of Multitrophic interactions, that recently changed into the department of Terrestrial ecology at the Netherlands Institute of Ecology (NIOO-CTE) in Heteren. For 4 years he focused on climate warming, plant invasions and plant-enemy interactions. In August 2010 he will start a Postdoc position at the Univeristy of California in Berkeley to work on the population ecology and the aspects of biological control of an exotic moth species.

PUBLICATIONS

Morriën EM, **Engelkes T**, Van Der Putten WH. Additive effects of aboveground generalists herbivores and soil feedback in native and range expanding exotic plants. Submitted to Ecology.

Bezemer TM, Harvey JA, Kamp AFD, Wagenaar R, Gols R, Kostenko O, Fortuna T, **Engelkes T**, Vet LEM, Van der Putten WH, Soler R (2010). Behaviour of male and female parasitoids in the field: influence of host density, patch size and habitat complexity. Ecological Entomology 35: 341-351.

Morriën EM, **Engelkes T**, Meisner A, Macel M, Van der Putten WH (2010). Climate change and invasion by intra-continental range-expanding exotic plants: the role of biotic interactions. Annals of Botany, in press.

Harvey JA, Biere A, Fortuna T, Vet LEM, **Engelkes T**, Morriën EM, Gols R, Verhoeven KJF, Vogel H, Macel M, Heidel-Fischer HM, Schramm K, Van der Putten WH (2010). Ecological fits, mis-fits and lotteries involving insect herbivores on the invasive plant, *Bunias orientalis*. Biological Invasions, in press.

Engelkes T, Morriën EM, Verhoeven KJF, Bezemer TM, Biere A, Harvey JA, McIntyre LM, Tamis WLM, Van der Putten WH (2008). Successful range-expanding plants experience less above-ground and below-ground enemy impact. Nature 456, 946-948.

Mauquoy D, **Engelkes T**, Groot MHM, Markesteijn F, Oudejans MG, Van der Plicht J, Van Geel B (2002). High-resolution records of late Holocene climate change and carbon accumulation in two north-west European ombrotrophic peat bogs. Palaeogeography, Palaeoclimatology, Palaeoecology 186: 275-310.

PE&RC PhD Education Certificate

With the educational activities listed below the PhD candidate has complied with the educational requirements set by the C.T. de Wit Graduate School for Production Ecology and Resource Conservation (PE&RC) which comprises of a minimum total of 32 ECTS (= 22 weeks of activities)

Review of literature (4.5 ECTS)

- Plant invasions and plant-insect interactions

Post-graduate courses (5 ECTS)

- Community ecology: processes, models and applications (2005)
- Consumer resource interactions: adaptive foraging, adaptive defenses and ecosystem engineering (2006)
- Soil ecology: crossing the frontier between below- and above-ground (2007)
- Mixed linear models (2009)

Laboratory training and working visits (1.5 ECTS)

- Plant invasions; Plant Protection Institute, Bulgaria (2008)

Competence strengthening / skills courses (4.2 ECTS)

- Time management (2006)
- Database management; VLIS, Belgium (2008)
- Writing grant proposals (2009)
- Scientific writing (2009)

PE&RC Annual meetings, seminars and the PE&RC weekend (1.5 ECTS)

- PE&RC Day (2006)
- Workshop Plant-Insect-Interactions; Wageningen (2006)
- Workshop Plant-Insect-Interactions; Amsterdam (2007)
- NIOO Days (2007)
- Workshop Plant-Insect-Interactions; Leiden (2008)
- PE&RC Day (2009)

Discussion groups / local seminars / other scientific meetings (6 ECTS)

- Entomologendag 2005; NEV (2005)
- Current themes in ecology; NIOO, RUN & WUR (2005)
- Multi trophic interactions (2005-2009)
- NIOO Days (2006)
- Current themes in ecology; NIOO, RUN & WUR (2006)
- Entomologendag 2006; NEV (2006)
- Entomologendag 2007; NEV (2007)
- Current themes in ecology; NIOO, RUN & WUR (2008)
- Entomologendag 2009; NEV (2009)

International symposia, workshops and conferences (20.3 ECTS)

- Workshop: Multitrophic Interactions; Göttingen, Germany (2006)
- NEOBIOTA. 4th European Conference on Biological Invasions: from ecology to conservation; Vienna, Austria (2006)
- 150 years Park Grass celebration; Rothamsted, UK (2006)
- Opportunities in Global Change Research; Amsterdam, the Netherlands (2006)
- Colonization versus Invasion: do the same traits matter; Ascona, Switzerland (2007)
- Workshop: Multitrophic Interactions; Göttingen, Germany (2008)
- GFOE; Leipzig, Germany (2008)
- ESA; Albuquerque, USA (2009)
- Netherlands Annual Ecology Meetings (2009)
- Netherlands Annual Ecology Meetings (2010)

Supervision of MSc students: 40 days (9 ECTS)

- Comparison of aboveground herbivore communities on three closely related pairs of native and non-native plant species (9 months)
- Experienced herbivory on phylogenetically related exotic and native plants in the Netherlands (7 months)

The research presented in this thesis was conducted at the Department of Terrestrial Ecology at the Centre for Terrestrial Ecology of the Netherlands Institute of Ecology (NIOO-KNAW) in Heteren. This is NIOO thesis 78.

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