Multitrophic Interactions on a Range-expanding Plant Species

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Thesis

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The path of a PhD is like an insect metamorphosis.
A young caterpillar emerged from the egg of knowledge
passes through several phases in the quest for results,
reaches a pupal phase while writing and writing and writing
And in the end, it emerges as a butterfly
with wings to fly..
(Taia 2013)

À minha mãe, guerreira e sonhadora..

Abstract

Studies on the ecological impacts of exotic invasive plants have mainly focused on inter-continental invasions. However due to global environmental changes, a rapid increase in intra-continental range-expanding plants has been observed. In this context, multitrophic interactions between exotic plants, native herbivores and their natural enemies have been largely ignored. This thesis aimed at examining how an exotic range-expanding plant interacts with aboveground insect herbivores and their natural enemies and how it can contribute to the successful establishment of the exotic plant. In addition, it examines how resistance traits of different populations of the range-expander affect the behaviour and performance of herbivores and their natural enemies in the new habitat. Bunias orientalis (Capparales: Brassicaceae) is perennial plant from extreme south-eastern Europe and Asia that has recently expanded its range and become invasive in northern and central parts of Europe. In the Netherlands, it is considered naturalized but non-invasive.

Firstly, using a community approach, I found that Bu. orientalis suffered less herbivore damage and harboured smaller invertebrate communities than sympatric native Brassicaceae in the Netherlands. The exotic plant has been found of low quality for the larval growth of the specialist herbivore (Pieris brassicae). Furthermore, two of its gregarious parasitoids were differentially affected by the quality of the exotic plant. The pupal parasitoid (Pteromalus puparum) survived better than the larval parasitoid (Cotesia glomerata), and the latter parasitized less hosts on the exotic than on native plants. Therefore, the herbivore can be selected to adapt to the new plant by conferring an enemy free space to the herbivore. In this case, a plant shift by the specialist herbivore might occur and thus preventing the further spread of the exotic plant. Conversely, in the field I found greater carnivore pressure on Bu. orientalis compared to other native Brassicaceae, particularly in the peak of arthropod abundance. Hence, top-down forces exerted by herbivore natural enemies may act in concert with bottom-up control of plant resistance traits to counteract herbivore plant shift and promote the successful range expansion of the exotic plant.

Secondly, using a biogeographical approach, I found a considerable intraspecific variation in defence traits (trichomes, glucosinolates, metabolic fingerprints) of Bu. orientalis populations from the native and the exotic range. Plants collected in the native range were better defended than their exotic conspecifics. This variation matched with the performance of a generalist herbivore (Mamestra brassicae) and its parasitoid (Microplitis mediator), which developed poorly in plants from the native range. The results suggest that the defensive mechanisms of Bu. orientalis might have been counter-selected during the range expansion of the exotic plant. Further studies, however, need to examine if enemy release in the new range is followed by an increase in performance of the exotic plant. Finally, a comparative study of multitrophic interactions, both above- and belowground, in the plant native range and along the transect of its range expansion can help to clarify the mechanisms underlying the invasive success of Bu. orientalis.

Contents

CHAPTER 1

General Introduction

Taiadjana M. Fortuna

Introduction

Due to current global changes driven in part by anthropogenic processes, many plant species are being intentionally or accidentally moved from their native ranges, transported across the world or within land masses and introduced into novel environments (Vitousek et al. 1997). Some of these species find favourable abiotic and biotic conditions to establish in the new range. Exotic species may also establish in an environment where biotic factors, such as absence of their co-evolved enemies, can give them competitive advantages in the new community. This process can contribute to the rapid increase in abundance of the exotic species, which can become invasive and disruptive pest in the new range (Mack et al. 2000). Although a considerable body of research has reported the ecological impacts of invasive plant species (Vila et al. 2011 for review), most of these studies have, thus far, focused on inter-continental invasions, whereas there is relatively little known about mechanisms of invasions within the same continent, known as intra-continental range expansions. This is important, because some plant species have been rapidly increasing their range in recent decades due to climate change and other environmental changes (van der Putten 2012). Therefore, the main aim of this thesis was to study how such an exotic range-expanding plant species interacts with biotic conditions in the new range and how plant-herbivore-carnivore interactions are affected by the novel plant species.

Process of plant invasion

Exotic plant species can become established and increase in abundance in new habitats via a phased process, in which the exotic species has to overcome various abiotic and biotic barriers or filters in order to persist in the invaded environment. First, the exotic plant (or its propagule) is introduced accidentally by humans through long-distance transport (e.g. via water bodies, rails, roads) or intentionally for ornamental or horticultural proposes, passing a major geographical barrier (Vitousek et al. 1997; Mack et al. 2000; Richardson et al. 2000a). Upon introduction, exotic plants have to colonize and establish in a given habitat. In the colonization phase, exotic species often do not form self-replacing populations and instead rely on repeated introductions for their persistence (Richardson et al. 2000a). The survival of propagules is determined by propagule pressure (also termed introduction effort) and abiotic filters, such as the climate of the new region (Theoharides and Dukes 2007). The more propagules that are introduced in the new area, the greater the chance that an exotic plant will survive during the next steps of invasion (Lockwood et al. 2005; Colautti et al. 2006; but see Nunez et al. 2011). In the establishment or naturalized phase, exotic populations reproduce consistently and are sustained over many life cycles with freely recruited offspring usually close to adult plants (Richardson et al. 2000a). Several processes can create biotic resistance to the establishment and reproduction of the exotic plant (Theoharides and Dukes 2007). For example, exotic populations may be inhibited by competing with native plant species, interacting with enemies (e.g. herbivores, pathogens) or by being unable to establish important mutualistic associations (e.g. mycorrhiza, pollinators) (Richardson et al. 2000b; Levine et al. 2004). Nevertheless, although biotic resistance in the native community significantly reduce establishment of exotic plants, species interactions are unlikely to completely repel invasions (Levine et al. 2004). A lag-phase often takes place between establishment and spread, when small populations of established exotic plants adapt to their new community (Kowarik 1995; Crooks and Soulé 1999; Theoharides and Dukes 2007). This phase can occur in response to a lack of genetic variation, which prevents rapid adaptation to novel conditions, or the time necessary for the population to reach a critical size that allows it to spread (Theoharides and Dukes 2007). Lag time may also be a result of a lack of suitable local habitats and/or the presence of harsh environmental conditions (Pysek and Hulme 2005; Richardson and Pysek 2012). When exotic plant species are able to sustain self-replacing populations over several life cycles, to produce reproductive offspring, and have the potential to spread over long distances, they can be defined as invasive plant species (Richardson et al. 2000a). At this phase the exotic species can overcome the dispersal barriers and cope with biotic and abiotic constraints within the new region (Richardson et al. 2000a). This definition is irrespective of the environmental or economic impact the species has in the new range, since the impact is influenced by human perception (Ricciardi and Cohen 2007).

Nevertheless, in Europe only about 1% of all the introduced species become naturalized, and about 0.1% of the introduced species will have an ecological and economic impact in the new range (Williamson and Fitter 1996; Pimentel 2002; Pysek et al. 2004). Although these numbers appear miniscule, a number of exotic invasive species are recognized as a fundamental component of human-caused global changes and an important driver in ecosystems change with global losses of biological diversity (Millenium Ecosystem Assessment 2005; Wardle et al. 2011).

Lag-phase in plant invasion

Most studies of plant invasion have focused on invasive species with the greatest impacts on native communities. However, as suggested recently by Richardson and Pysek (2012), in order to better understand the mechanisms leading to successful invasion more studies of species that are naturalized but that are not or not yet invasive are needed. Knowledge of the factors that determine naturalization is important because, in addition to being a crucial phase in the invasion process, some of the species in lag-phase have the potential to become problematic invaders, so called ‘sleeper weeds’ (Kowarik 1995; Hallett 2006). Additionally, most widely distributed invasive species have naturalized populations in most of the regions where they occur, but are invasive in only a small subset of regions (Weber 2003; Richardson and Pysek 2012). One of the reasons why it is difficult to understand the factors underlying the duration of the lag-phase is because this period may vary greatly among different regions of the globe and can vary between a few years to several centuries. In Germany for example, Kowarik (1995) has estimated the average length of the lag-phase, between introduction and the beginning of the invasive spread, to be between 131 and 170 years for shrubs and trees respectively. Ultimately, a better understanding of why and under which conditions species become naturalized may improve weed management, by allowing managers to target potentially invasive species before their negative ecological impacts on the ecosystems.

Plant invasion and range expansion

Most research on plant invasions has been focused on species that are introduced over long distances, for example across continents, and that are called inter-continental invaders. However, due to recent climate change many plant species are expanding their ranges to higher latitudes (Walther et al. 2002). Data on a wide variety of plants and animals in the northern hemisphere has revealed large-scale range shifts (6.1 km per decade) towards the pole in the past decade (Parmesan and Yohe 2003). These range expansions occur within continents by plant species of inter-continental and intra-continental origins (Morrien et al. 2010). Although a considerable number of inter-continental range-expanding plant species is known to be highly invasive in their new range (Pysek et al. 2008 and references therein), little is still known on the invasive abilities of many species of intra-continental range expanders.

In Europe, approximately 51% of all exotic plant species come from another parts in the same continent where plants are native (Lambdon et al. 2008). In the Netherlands, one quarter of the exotic flowering plant species originate from more southerly regions of Europe (Tamis et al. 2005). Some of these species have been intentionally and accidentally introduced, but many have arrived by natural dispersal from the south due to climate warming. These novel exotic plant species are expected to generate novel interactions among local plant communities, as well as novel interactions with food web networks in the invaded area (van der Putten et al. 2004). This thesis focuses on the biotic interactions of an intra-continental range-expanding plant that has recently become invasive in many parts of its new range.

Range-expanding plants

Intra-continental plants are often able to expand their ranges in disturbed habitats such as roadsides, railways, and river banks. Therefore, species with traits related to rapid dispersal have an advantage in colonizing new habitats. Many of these species establish along the dispersal corridors, from where some of them may spread into natural areas (Richardson et al. 2000a). Intra-continental range expanders may have the same invasive traits as inter-continental invaders, since they most likely are facing biotic interactions that differ from those in their native range. Novel biotic interactions in the new range of these range expanders can result from the different migration rate of their associated aboveground and belowground species (Berg et al. 2010). Recently, it was shown that biotic interactions of intra-continental range expanders with aboveground polyphagous herbivores and soil communities were only slightly different from the corresponding interactions of inter-continental invasive plant species (Engelkes et al. 2008). In addition, some inter-continental invaders may, as well, exhibit range expansion in their native range and can escape both aboveground and belowground enemies by dispersing to new sites, linking possible mechanisms of local spread with long-distance invasion (MacKay and Kotanen 2008). Therefore, and since much of the ecology of range expanders in their news habitats is unknown, I will begin by introducing the basic concepts developed for inter-continental plant invasions.

Several theories have been proposed to explain the success of exotic invasive plants based on the novel biotic conditions they experience in the new range. One commonly accepted mechanism is the enemy release hypothesis (ERH), which states that plant species introduced to an exotic region benefit from escaping from their co-evolved enemies (e.g. herbivores and pathogens), resulting in a rapid increase in distribution and abundance (Keane and Crawley 2002; Clay 2003; Mitchell and Power 2003). Although a great body of research has demonstrated the importance of enemies in regulations of exotic plant populations, studies of ERH are equivocal (Colautti et al. 2004 for revision). This is mainly because enemy release is only one of several explanations for invasiveness (Mitchell et al. 2006). For instance, exotic plants may not only lose their co-evolved enemies, but also gain non-coevolved biotic interactions with herbivores and pathogens in the new range (Hallett 2006; Agosta and Klemens 2008). This idea is inherent to the biotic resistance hypothesis (BR), which proposes that plant enemies, as well as competing plant species, may prevent establishment and spread of an invader in novel communities (Elton 1968; Maron and Vila 2001; Levine et al. 2004; Parker and Hay 2005). This hypothesis suggests that certain habitats are more or less prone to be invaded, but does not predict why certain species are more likely to invade that habitat. Although it has been suggested that the success of exotic plant species is the net effect of losing old enemies and acquiring new ones, the mechanisms that determine the outcome of interactions with native communities and exotic plant species in the new range are still poorly understood (Mitchell et al. 2006; Verhoeven et al. 2009).

Exotic invasive plants may also possess novel phytochemicals (e.g. plant secondary metabolites) that evolved in their native range against their enemies but to which plants and other biota in the new range have no tolerance or defences against (Cappuccino and Arnason 2006). Many of these novel compounds have been reported to have multiple activities, including anti-herbivore, anti-fungal, anti-microbial and allelopathic (phytotoxic) effects, which may provide several advantages to the exotic plants in their new environments (Callaway and Ridenour 2004; Vivanco et al. 2004; Cappuccino and Arnason 2006). Exotic plants may also manipulate soil biota by altering nutrient cycling, enhancing pathogen levels or disrupting symbiont communities, which affects neighboring native plants more negatively than the exotics (van Grunsven et al. 2007; Inderjit and van der Putten 2010; Meisner et al. 2012). Finally, selection on plant traits may differ between exotic species and native species in the invaded region (Blossey and Nötzold 1995; Müller-Schärer et al. 2004; Joshi and Vrieling 2005). For example, invasive plants can allocate more resources to vegetative and reproductive biomass in absence of specialized enemies, which may give them a competitive advantage in the new range (Wolfe et al. 2004; Joshi and Vrieling 2005; Oduor et al. 2011). All these mechanisms have been studied for inter-continental invasive species, whereas knowledge on novel biotic interactions of intra-continental range expanding plant species is still scarce (van der Putten 2012).

Plant-enemy interactions

Plant enemies, pathogens and herbivores, can greatly affect plant communities, with serious impacts on vegetation structure, community composition and plant abundance (Strong et al. 1984; Crawley 1989; Marquis 2004). Most plant species have evolved, therefore, strategies to minimize the impact of the enemies, either by producing chemicals (e.g. secondary metabolites) or structures (e.g. trichomes, thick cuticle) to deter enemy attack or by developing ways to tolerate biomass loss by their enemies. On the other hand, enemies have also been important in evolutionary time, developing mechanisms to deal with plants defences and selecting therefore for better-defended genotypes (Ehrlich and Raven 1964; Schoonhoven et al. 2005). However, when an exotic plant is introduced into a new habitat, the outcome of novel relationships with non co-evolved enemies may depend on the match or mismatch of phenotypes that evolved elsewhere (Janzen 1985; Agosta and Klemens 2008).

To what extent the exotic plant experiences enemy release depends on the kind of release, and the nature of novel biotic interactions in the new range. Specialist enemies, which are often engaged in arms race with their host plant, can exert strong top-down control in the plant’s native range. Therefore, the loss of specialized enemies in the new range may release the exotic plant from top-down pressure and facilitate the invasion process (Memmott et al. 2000; Wolfe 2002; Mitchell and Power 2003; van der Putten et al. 2005). However, specialist enemies can be found on exotic plants through two main mechanisms: host shifting and via the co-introduction of enemies. Host shifting can occur if the exotic plant is introduced to a region that contains closely related native congeners from which specialist enemies might switch to attack the exotic species (Auerbach and Simberloff 1988; Blaney and Kotanen 2001; Louda and Rand 2003; Parker and Gilbert 2007). However, herbivores of native plants may also shift to novel hosts that are phytochemically similar to, but not necessarily related to, their traditional hosts (Strong et al. 1984; Cappuccino and Arnason 2006). Moreover, exotic invasive plants are typically more often released from their specialist than generalist enemies (Hinz and Schwarzlaender 2004; Torchin and Mitchell 2004; van der Putten et al. 2005; Morrison and Hay 2011). This is the case, because generalist herbivores are present in all habitats and colonize often new hosts faster than specialists (Strong et al. 1984). Additionally, in the new range, native plants may also be better defended against the native generalists than exotic plants, thereby leading to higher enemy attack of exotic species (Parker and Hay 2005; Parker et al. 2006). Recent studies suggest that, in response, introduced populations of exotic species evolve lower allocation to defences against specialists, but not against generalists (Müller-Schärer et al. 2004; Joshi and Vrieling 2005). Finally, native enemies may also evolve to attack exotic plant species (Graves and Shapiro 2003; Torchin and Mitchell 2004; Carroll et al. 2005; Siemann et al. 2006; Keeler and Chew 2008). Moreover, in the new region, exotic plants may not escape their enemies when these have also been introduced into the same area (Zangerl et al. 2008). Thus, enemy impact experienced by exotic plants will be the net effect between the impact of the enemies lost in their native range and the enemies gained in the novel range. Finally, climate barriers may differ for plants and insects and changes in climatic conditions can also exert different effects in insect species (Bale et al. 2002). Consequently, as with range-expanding plants, their enemies may also encounter novel conditions and range expansion of plants and their enemies may not occur at the same rate (Berg et al. 2010).

Interactions between plants and their enemies are controlled by mechanisms of recognition, host selection and preference, as well as by offensive and defensive strategies. While these mechanisms have been well studied in co-evolved systems, little is still known about the consequences of these mechanisms for novel interactions during plant invasions (Cappuccino and Arnason 2006; Hallett 2006; Mitchell et al. 2006; Verhoeven et al. 2009). Novel interactions between herbivores and exotic plants involve several steps that if successful may lead to herbivore colonization of new food plants (Pearse et al. 2013). The exotic plant and an insect must first overlap in both space (habitat level) and time (phenology). Then after plant location, the herbivore must recognize appropriate plants cues (e.g. attractants) in order to oviposit on it (Figure 1f). The plant cues involved in herbivore attraction can be visual, tactile or olfactory. Plant volatile profiles, among the most well-studied mechanisms, are often complex and can include hundreds of individual metabolites, which act as attractants or deterrents of herbivores, as well as attractants of herbivore natural enemies, such as parasitoids (Vet and Dicke 1992; Dicke and van Loon 2000; van Wijk et al. 2011). Finally, the herbivore has to receive the proper cues to initiate feeding and must be able to digest the plant resources, whilst avoiding its own suite of enemies, until successful pupation (Figure 1h) and adult eclosion. Failure of the herbivore to complete one of these steps means that the insect has no realized fitness on the plant, creating a mis-match (Harvey et al. 2010a). The novel plant may, therefore, become an ecological and evolutionary trap if it is attractive to insects, but on which their offspring perform very poorly or die precociously (Renwick 2002; Keeler and Chew 2008; Kühnle and Müller 2009). Conversely, novel plants may also present evolutionary opportunities if herbivores can adapt to them (Pearse et al. 2013).

Plants have evolved a wide variety of physical and chemical defence mechanisms that inhibit herbivores' ability to exploit their tissues. Hence, because exotic plants may undergo thorough changes in herbivore pressure in the new region, they also provide a good opportunity to explore the evolution of defence allocation in plants (Orians and Ward 2010). Plant defences are often costly to produce, and therefore allocation to defence depends on the risk of attack, the value of specific tissues, and the availability of resources (Strauss et al. 2002). Under lower herbivore pressure exotic plants may sometimes evolve reduced plant defences (Wolfe 2002), or re-allocate resources to less costly defence traits (e.g. toxic allelochemicals) when herbivore attack shift from specialist to generalist herbivores in the new range (Müller-Schärer et al. 2004; Joshi and Vrieling 2005; Doorduin and Vrieling 2011). Moreover, plant defences can be constitutive, meaning that they are always expressed in a plant, and/or inducible, meaning that levels are increased in response to herbivore damage. Plants may evolve inducible defences if the cost of defence is high and the frequency of herbivore attack is low or unpredictable (Cipollini et al. 2003). Thus, exotic populations may exhibit lower levels of constitutive defences, but exhibit greater levels of inducible defences (e.g. secondary metabolites) compared to populations from its native range (Cipollini et al. 2005). Although it has been suggested that effects of inducible defences should be considered in comparisons of invasive plant species, thus far very few studies have examined induced defences in exotic plants (Cipollini et al. 2005; Eigenbrode et al. 2008) and still even less in range-expanding plant species (Engelkes et al. 2008). These defence mechanisms should be considered in studies exploring novel biotic interactions of exotic species in order to understand which possible mismatches are more intrinsically beneficial to introduced plants or to their enemies (Verhoeven et al. 2009).

Plant-herbivore-carnivore interactions

Natural enemies of herbivorous insects, such as predators and parasitoids, can play an important role in regulating the structure and function of terrestrial communities (Hairston et al. 1960; Price et al. 1980; Cronin and Reeve 2005). However, thus far most research with exotic plants have been restricted to interactions between the first trophic level (the plant) and the second trophic level, such as potential enemies (e.g. insect herbivores), while interactions with higher trophic levels (e.g. parasitoids) have been largely ignored. Consequently, the relative importance of top-down control of herbivores by their natural enemies on exotic plants is far from clear (Harvey et al. 2010b). A recent study by Engelkes et al. (2012) showed that exotic plants had greater potential predator pressure on herbivores than their native congeners in the new range. In this scenario, if the community of herbivore natural enemies switches to exert greater top-down control on herbivores attacking exotic plants (Figure 1g), this may limit the effectiveness of the herbivores that attack these plants and at least partially explain their invasive success through a new form of enemy release.

Many plants interact with carnivores as an ‘indirect defence’ against herbivores. For instance, it is well established that plants attract, nourish or house other organisms and that this can reduce enemy pressure (Price et al. 1980; Dicke and Sabelis 1988; Vet and Dicke 1992). The plant traits involved in this context can be volatile organic compounds (VOCs), extrafloral nectar, food bodies and structures used as refuges or nesting space (Heil 2008). Plants can synthesise and release many VOCs in response to egg deposition and herbivore damage from damaged and undamaged tissues (Vet and Dicke 1992; Hilker and Meiners 2006). Thus, the quantity and quality of VOCs that are released by damaged plants can change dramatically (Turlings et al. 1995; Pare and Tumlinson 1999; Bukovinszky et al. 2005). These herbivore-induced plant volatiles (HIPV) can be used by carnivores as important host and prey-location cues and have been recognised to play a crucial role in their foraging behaviour (Vet and Dicke 1992; Takabayashi and Dicke 1996).

Carnivore-searching behaviour and development relies on the same steps used by herbivores to select their food plants, and includes plant location, host location, host selection, acceptance and suitability (Vinson 1998). Since host-related cues are less conspicuous at the habitat level, natural enemies such as parasitoids rely on visual and volatile cues associated to the food plant to locate their hosts, while on the plant they rely on host-contact cues to detect and select their hosts (Vet et al. 1991; Vet and Dicke 1992; Vinson 1998). If chemical cues, used by parasitoids to locate suitable plants, differ between exotic and native plant species, parasitoids may fail to respond to novel odours associated with exotic plants, leading to the possibility of locally unstable host-parasitoid patch dynamics and possible extinctions (Cronin and Haynes 2004). On the other hand, herbivore-induced plant volatiles can be relatively specific in some plant species, differing in response to damage by particular herbivore species, and thus being reliable indicators of the identity of the feeding herbivores for their natural enemies (De Moraes et al. 1998; Dicke 1999; Vet 1999). Parasitoids for instance, often specialised on one or few host species and host stages, have evolved complex mechanisms that allow them to efficiently find their hosts even when they are found in chemically and structurally complex habitats (Godfray 1994; Vet 1999). Parasitic wasps can exhibit associative learning by responding to stimuli which are newly acquired or enhanced by linking them to a reinforcing stimulus (Vet and Dicke 1992; Vet et al. 1995). Hence, in habitats invaded by exotic plants, specialised natural enemies such as parasitoids may learn novel plant cues during their host finding behaviour, once they gain a positive experience with their hosts on these plants, which ultimately can affect host plant shift by native herbivores (Vet and Dicke 1992).

The performance of carnivorous arthropods can also be indirectly affected by the host plant as mediated through their host or prey (Hare 1992). Several studies have reported that the effects of plant quality can have repercussions in the third and fourth trophic levels (Barbosa et al. 1986; Harvey et al. 2003; Soler et al. 2005; Ode 2006; Gols et al. 2008a). These effects can be due to direct toxicity of plant allelochemicals on the development of natural enemies (Campbell and Duffey 1979; Barbosa et al. 1986; Barbosa et al. 1991) or because their host or prey performs badly on certain plant types (Harvey 2005; Harvey et al. 2007). In contrast, the development and survival of specialized natural enemies can be less affected by differences in phytotoxins in the food source of the herbivores (Harvey 2005; Gols et al. 2008a,b). Plant quality effects on higher trophic level organisms, such as parasitoids, may also vary according to their host exploitation strategies. Amongst parasitoids, host resources are exploited in two different ways. Idiobiont parasitoids are species that develop in non-growing hosts, such as eggs, paralyzed larvae or pupae, while koinobiont parasitoids attack hosts that continue to grow after parasitism, such as larvae (Askew and Shaw 1986; Harvey 2005). Based on the models proposed by Mackauer and Sequeira (1993), plant quality can affect more negatively the fitness of koinobiont parasitoids, because they rely on a dynamic condition of the host which might change during parasitoid development, whereas idiobionts rely on static host traits. This means that the nutritional quality of an exotic plant species can also affect the growth and development of their parasitoids through the quality of their insect herbivore hosts that consequently influence fitness-related traits of the emerging parasitoids. However, few studies have thus far examined interactions of higher trophic level organisms with inter-continental invaders

(Cronin and Haynes 2004; Pearson 2009), and still fewer with intra-continental range expanders (Harvey and Gols 2011).

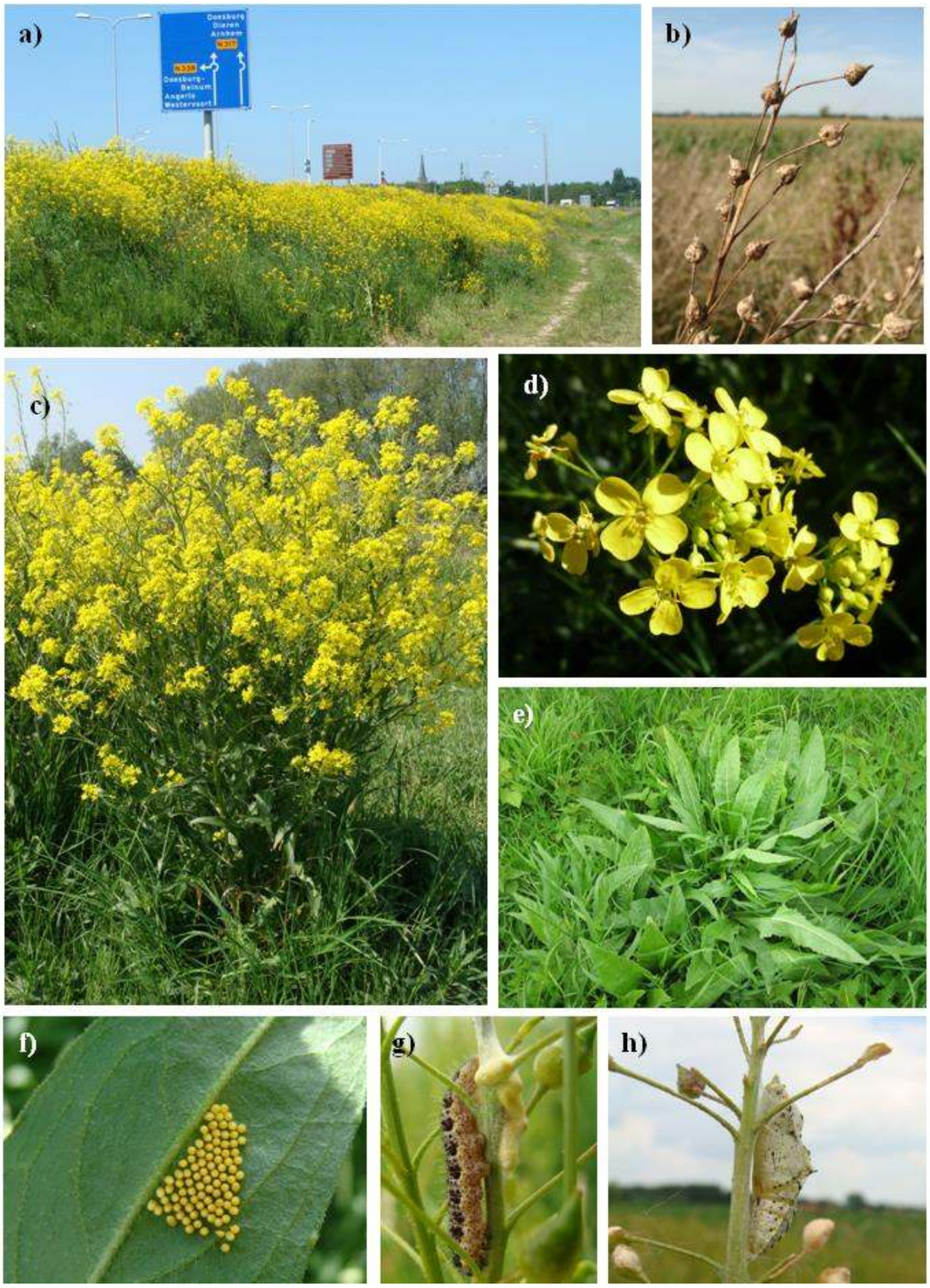

Figure 1 Phenology and biotic interactions of the exotic plant Bunias orientalis in natural populations in the Netherlands (non-native range) in the growing season 2010 and 2011. a) roadside population of Bu. orientalis in Drempt; b) plant seeds; c) adult plant; d) plant flowers; e) first plant rosette; f) eggs of the specialist herbivore Pieris brassicae on the abaxial leaf surface of Bu. orientalis; g) cocoons of the endoparasitoid Cotesia glomerata that emerged from the parasitized P. brassicae caterpillar; h) P. brassicae pupa hanging on the stem of a Bu. orientalis plant. Reproduced with permission from Hedwig Ens (all photos except d).

The model-system

To approach the process of range expansion, I explore multitrophic interactions between invertebrates, mainly insects, and a range-expanding plant species, Bunias orientalis L. (Brassicales: Brassicaceae) (Figure 1). For studies at the community-level, I look at the performance of local herbivores and their natural enemies and the plant defence mechanisms of the range-expander and three other related native plant species in the new range. For biogeographical comparisons, I compare the effect of plant defence traits on the performance of local specialist and generalist herbivores and a parasitoid between different populations of the range expander from its native, exotic invasive and exotic non-invasive range. For these comparisons, I use three related wild plant species which are native to the Paleartic region: Brassica rapa L., Sinapis arvensis L., and Brassica nigra L. Koch (all Brassicales: Brassicaceae). These plant species were selected because they are closely related to Bu. orientalis, exhibit phenotypic similarity and all grow in ruderal habitats. Moreover, wild brassicaceous species possess potent inducible direct defences via the production of glucosinolates (GS) and their breakdown products, and their insect communities are well known, since they include several important crop plants. On this basis, they provide good model systems for the study of multitrophic interactions (van Dam et al. 2003). Furthermore, many native cruciferous species are abundant in the Netherlands, whereas several other exotic crucifer species have become more common in recent years (Harvey et al. 2010a).

Bunias orientalis

Bunias orientalis, commonly known as warty cabbage or Turkish rocket, is a perennial herbaceous plant that is native to extreme parts of south-eastern Europe and Asia (Tutin et al. 1993). This plant was introduced to central Europe in the 18th century. Over the next 200 years the species slowly spread through central Europe to the continental parts of western and northern Europe, exhibiting the typical 'lag-phase' reported for most alien plant species (Steinlein et al. 1996 and references therein). During recent decades Bu. orientalis expanded its range rapidly into large parts of Europe and North America (Schurkens and Chittka 2001; Birnbaum 2006; Harvey et al. 2010a). It is now naturalized in many countries in Europe, being quite common, for instance, in Germany, Switzerland, Poland, Latvia, Norway, and Sweden. At the same time, the plant is less common in other parts of western Europe, such in the Netherlands, France and Britain (DAISIE ; Birnbaum 2006; Harvey et al. 2010a). In the new range, Bu. orientalis grows in fertile and disturbed habitats, occurring in roadsides

(Figure 1a), grass fields, along railways and fallow lands, and from there it can invade meadows, dry grasslands and orchards (Steinlein et al. 1996; Birnbaum 2006).

As suggested by Woitke and Dietz (2002), Bu. orientalis possesses certain traits which may promote (re-)establishment of populations in nutrient-rich habitats: (i) it is a relatively long-lived plant (10 to 12 years) with an extensive growing season (April to October); (ii) it develops a permanent deep tap root that can produce offspring through root fragments; (iii) in early spring leaves grow in rosette (Figure 1e) that can be very plastic in size and occasionally a second rosette is formed later in the year; (iv) reproductive individuals, after the 2nd year, produce tall stems with copious hermaphroditic yellow flowers in May and June (Figure 1c,d); (v) it has a high fecundity, and individual plants may produce up to several thousand fruits (oval and hard pods containing one or two seeds) that ripen by late July and form persistent seed banks during the winter (Figure 1b). Seeds, which often germinate in early spring, are the major propagules for stand regeneration and the colonization of new sites (Dietz and Steinlein 1998; Dietz et al. 1999). Moderate anthropogenic disturbance, such as mowing, has been shown to contribute to high genetic variability of Bu. orientalis populations by increasing gene flow and thereby promoting the adaptability of the exotic species to the often unpredictable conditions at disturbed sites (Dietz et al. 1999). Considering the immense amount of human disturbance in the Netherlands and over much of Western Europe, it is likely that the abundance of Bu. orientalis will increase in areas where it is still comparatively rare. Additionally, observations suggest that the increased abundance of many established exotic plant species in the Netherlands from warmer climate regions, such as Bu. orientalis, coincides with the current climate warming (Tamis et al. 2005). Thus, increased temperatures may also increase the dominance of this exotic plant in future plant communities.

Brassicaceous species are known to have important defences against enemies, which include qualitative defences through the production of secondary metabolites, such as glucosinolates (Renwick 2002; Halkier and Gershenzon 2006), and quantitative defences based on digestibility reducers, such as cellulose, tannins, proteinase inhibitors, and trichomes (Handley et al. 2005; Travers-Martin and Müller 2008; Traw and Feeny 2008).

Glucosinolates are a class of naturally occurring thioglucosides that upon hydrolysis by myrosinases are converted into biologically active products such as isothiocyanates, nitriles and thiocyanates (Halkier and Gershenzon 2006). The hydrolysis of glucosinolates occurs immediately upon plant tissue damage and these compounds are known to act as insect deterrents and attractants. For instance, glucosinolate breakdown products are toxic to many generalist herbivores, whereas specialist herbivores use these toxins to find their host plants and can even sequester them for the defence against their own natural enemies (Halkier and Gershenzon 2006 and references therein). However, at higher dosages these toxic compounds can also have a negative effect on the development of specialist herbivores (Agrawal and Kurashige 2003; van Dam and Raaijmakers 2006). Some hydrolyzed products of glucosinolates, such as nitriles and isothiocyanates, have volatile properties which can provide cues to parasitoids, and even hyperparasitoids, to locate their hosts from a distance (Scascighini et al. 2005; Gols et al. 2011; Poelman et al. 2012). In contrast, quantitative defences protect the plant against specialist herbivores and those generalist herbivores that are adapted to the plant toxins (Müller-Schärer et al. 2004). Glucosinolates and their breakdown compounds, as well as quantitative defences, such as trichomes have been shown to play an important role in the invasive success of some Brassicaceae (Traw and Dawson 2002a,b; Müller 2009 and references therein; Oduor et al. 2011).

The major glucosinolate thus far described in Bu. orientalis leaves is sinalbin (p-Hydroxybenzyl), which is an aromatic glucosinolate, but other aliphatic glucosinolates are also present, though in smaller quantities (Harvey et al. 2010a). Sinalbin makes up 85-95% of the total glucosinolate content found in the plant leaves (Harvey et al. 2010a). The role of sinalbin in the defence of Bu. orientalis remains unknown and possibly controversial. Indeed, some studies have found that this compound stimulates the feeding behaviour of specialist herbivores (Kühnle and Müller 2009), whereas others have shown that specialist herbivores performed worse on Bu. orientalis than on S. arvensis plants, which also produce high concentrations of sinalbin (Harvey et al. 2010a). Other non-glucosinolate allelochemicals may be involved in the Bu. orientalis resistance, such as flavonoids and hydroxycinnamic acid derivatives (Dietz and Winterhalter 1996; Kühnle and Müller 2009), as well as other mechanical defences, such high trichome densities (Travers-Martin and Müller 2008).

Research objectives and thesis outline

The main aims of this thesis are to study how an exotic range-expanding plant species interacts with aboveground insect herbivores and their natural enemies, how these novel multitrophic interactions could contribute to the successful establishment of the exotic plant in the new range, and to better understand the mechanisms that mediate interactions between the three trophic levels.

Chapter 2

In Chapter 2, using a community approach, I compare the vegetation composition and invertebrate assemblages between the intra-continental range-expanding plant and the three common related native species throughout a growing season in the Netherlands. Most empirical evidence of enemy release on exotic plants has been based on bi-trophic relationships (plant-herbivore) without considering top-down carnivore pressure, which can play an important role in regulating function and structure of natural communities (Price et al. 1980; Harvey et al. 2010b). In this study, I test the enemy-release hypothesis to examine whether herbivore loads, foliar damage and carnivore pressure differ between the exotic and the native plants.

Chapter 3

In Chapter 3 we discuss hypotheses on how structural and chemical traits of exotic invasive plant species can affect plant-finding abilities of herbivores and host/prey-finding behaviour of parasitoids and predators in the invaded communities. The incursion of invasive plants into native communities can create structural barriers that impede dispersal and host plant-finding abilities of herbivores and their natural enemies. Invasive plants may also be attractive to native herbivores, but be toxic to their offspring, thus acting as an ecological ‘trap’. On the other hand, herbivores and their natural enemies may benefit from the establishment of exotic plants because they locally increase the amount of available resources to exploit. We discuss implications of exotic species for pest control in agro-ecosystems and for the conservation of native arthropod communities.

Chapter 4

A major challenge in plant invasion is to understand how novel plants, particularly intra-continental range-expanding species, affect trophic interactions in their new range, and how native herbivores and their natural enemies respond to these species. To examine this and test some of the hypotheses formulated in the previous I performed Chapter 4 and 5.

In Chapter 4, I evaluate the influence of plant quality of the exotic species via host quality on the performance of the specialist herbivore, Pieris brassicae, and two of its gregarious parasitoids, the larval parasitoid Cotesia glomerata and the pupal parasitoid Pteromalus puparum. Emphasis was placed on parasitoid host-resource use strategies and how these may be differently affected by the quality of the exotic plant.

Chapter 5

In this chapter, I compared the oviposition preference and offspring performance of a brassicaceous leaf-chewing specialist, P. brassicae, on the exotic plant and on a related native plant, Sinapis arvensis. I also studied the response of a P. brassicae larval parasitoid, Cotesia glomerata, to herbivore-induced plant volatiles emitted by the exotic and the native plant, and determined the volatile blend composition to elucidate which compound(s) can be involved in parasitoid attraction. Many studies in wind-tunnel and flight-tents only examine where female wasps alight, but they do not necessarily look at final acceptance of the host, which is a crucial step for the realized fitness of the parasitoid. In this study, I also studied the host acceptance behaviour of C. glomerata females mediated by host-quality by comparing the parasitism rate of P. brassicae larvae feeding on the exotic and the native species. Finally, I discuss the potential for dietary breadth expansion of the herbivore to include the exotic plant and the role of top-down pressure by parasitoids in shaping herbivore host shifts.

Chapter 6

As they are expanding the range in the same land mass, intra-continental range-expanding plants may or may not encounter the same insect species that co-occur with them in their native range. In Chapter 6, I examine the effects of intraspecific variation in resistance traits of Bu. orientalis on the performance of the specialist herbivore, P. brassicae, the generalist herbivore, Mamestra brassicae and the larval parasitoid, Microplitis mediator. Nine populations of the range-expanding plant that originated from the native range (Turkey), the exotic invasive range (Germany, Czech Republic), and the exotic non-invasive range (The Netherlands) were used to study their effects on the parasitoid and herbivores from the Netherlands. As plant resistance traits, I examined chemical defences by looking at the whole metabolomic profile of plants, and more specifically to the glucosinolate profiles. I also compared mechanical defences by measuring leaf trichome densities in plants from the different origins and populations.

Chapter 7

In this chapter, I discuss and synthesise the main findings of this thesis and discuss future research directions in the study of plant invasions by range-expanding plant species and their effects on native communities of plants and consumers at different trophic levels.

CHAPTER 2

Comparing vegetation diversity and invertebrate communities on an exotic range-expanding plant and related natives

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Louise E. M. Vet, Jeffrey A. Harvey

Abstract

Global changes mediated by human activities, including climate warming are contributing to rapid range expansion of some invasive plants. It becomes increasingly important to understand the mechanisms facilitating invasion success. The decreased enemy regulation experienced by exotic species in the new area, known as the ‘enemy release hypothesis’ (ERH), is a commonly accepted mechanism proposed to explain the invasive success of many of these species. However, most of the empirical evidence of ERH has been based on studies with inter-continental invaders excluding intra-continental range-expanding plant species and on bi-trophic relationships (plant-herbivore) without considering top-down predator pressures. Here, we compared invertebrate assemblages and vegetation composition to examine whether herbivore loads, foliar damage and carnivore pressure differ between the exotic intra-continental range-expander, Bunias orientalis, and three related native species, Brassica rapa, Sinapis arvensis and Brassica nigra. Invertebrates were collected in different periods of the growing season to assess natural variation in communities, and subdivided into feeding guilds. Both herbivore load and leaf damage were significantly smaller on the exotic than on the native species. Carnivore pressure on the herbivores was only greater between Bu. orientalis and the native Br. nigra. Plant communities around the exotic and the native species differed in species identity and abundance, but no differences were found in plant species richness. The exotic also exhibited tougher leaves than the native species, which can act as a mechanical defence against herbivores. We conclude that the ERH, through both top-down and bottom-up control of herbivores, can be an important ecological mechanism underlying the successful range expansion of Bu. orientalis. However, over time plant shifts by native herbivores may occur, particularly during mid-late period of the growing season.

Introduction

Worldwide trade and climate change are leading to rapid shifts in the distributions of many species (Walther et al. 2002). Some of the most invasive species in many parts of the world are plants, which, because of their position on the basis of the food chain, can have disproportionate effects on higher trophic levels and ultimately on the structure and function of ecological communities (Strong et al. 1984). Although the number of introduced plant species is difficult to predict accurately, in most of the countries at least 10% of the plant species are non-native or exotic, and in some areas, such as Hawaii, this figure approaches 100% (Meijer et al. 2012; Richardson and Pysek 2012). Although most introduced plants fail to establish in their new ranges or else remain rare, others spread and a few even become invasive pests, disrupting the integrity of natural ecosystems and generating huge economic costs (Pimentel 2002; Vila et al. 2011).

Many studies have focused on the role that interspecific interactions may play in facilitating or impeding invasions (Maron and Vila 2001; Keane and Crawley 2002; Agrawal et al. 2005; Engelkes et al. 2008). One important hypothesis that has been proposed to explain the success of exotic invasive plants in their new ranges is the ‘enemy release hypothesis’ (ERH). The ERH predicts that introduced species are successful invaders in novel habitats because their co-evolved enemies are absent, whereas competing native plant species experience attack from their co-evolved enemies (Elton 1968; Maron and Vila 2001; Keane and Crawley 2002). Although the ERH has been widely tested, a considerable number of studies have failed to provide strong support for it (see Colautti et al. 2004; Liu and Stiling 2006 for a review). One reason why the ERH often fails is because exotic plants can rapidly accumulate enemies in the invaded regions, replacing those lost from their native ranges (Parker and Hay 2005; Siemann et al. 2006; Verhoeven et al. 2009). For instance, plants that have expanded their range within the same continent (intra-continental range expanders) are more likely to encounter a similar invertebrate community associated to native counterparts, than species that have been introduced from other continents (inter-continental invasions). However, thus far little is known about communities of invertebrates associated with intra-continental range-expanding plants (van der Putten 2012).

Different approaches have been used to test the ERH; biogeographical studies have examined biotic interactions of exotic plants in their native and introduced range, while community studies compared interspecific relationships between native and exotic species co-occurring in the introduced range. Although biogeographical studies have found strong support for the ERH (Wolfe 2002; Mitchell and Power 2003; Genton et al. 2005), at the community-level the support for ERH is more equivocal. Some of these studies showed that invasive species experience less leaf damage and/or harbour smaller herbivore loads than native congeners (Agrawal et al. 2005; Carpenter and Cappuccino 2005; Liu et al. 2007; Jogesh et al. 2008; Engelkes et al. 2012), while others found no difference in herbivore pressure between exotic and native species (Ernst and Cappuccino 2005; Liu et al. 2006). Additionally, some studies even reported higher levels of damage on exotic plants than on their native counterparts (Agrawal and Kotanen 2003; Parker and Gilbert 2007). Therefore, little is known about whether the loss of enemies translates to reduced levels of damage and vice-versa relative to native counterparts in the same environment (community), making it difficult to draw robust conclusions on validity of the ERH.

Most of the studies testing the ERH have excluded, however, important aspects of the dynamics of natural communities. For instance, in some cases herbivore damage was exclusively based on chewing insects or on all insect species present on the plants without considering differences between insect feeding guilds. It is important, however, to include multiple interspecific interactions, since escape from one guild of enemies does not necessarily imply escape from other guilds (Agrawal et al. 2005). In addition, predicting herbivore pressure based on only foliar damage (Carpenter and Cappuccino 2005; Genton et al. 2005; Hill and Kotanen 2009; Vasquez and Meyer 2011) or insect faunal richness (Memmott et al. 2000; Ernst and Cappuccino 2005; Liu et al. 2006; Engelkes et al. 2012), may not always accurately predict the impact of herbivory that plants actually experience (Vasquez and Meyer 2011). The distinction of other feeding guilds, such as carnivores (predators, parasitoids) is also important, because the invasion success of exotic plants may also be a consequence of herbivore release enhanced by the pressure from the natural enemies on herbivores. However, studies on invasive plants have, thus far, focused almost exclusively on plant-herbivore interactions, while the impact of carnivores has been largely ignored (Harvey et al. 2010b; Engelkes et al. 2012; Harvey and Fortuna 2012).

The functional structure of arthropod communities can also vary in space and time (Root and Cappuccino 1992), hence it is important to sample multiple plant populations at different times during the growing season to account for seasonal variability in the invertebrate community. Furthermore, many of the studies testing the ERH focus only on species that are already recognized as invasive. The study of mechanisms underlying invasion success should as well include exotic species that are on the threshold of becoming invasive in their new range, for example in their "lag-phase", after which time they can become successful invaders (Verhoeven et al. 2009; Richardson and Pysek 2012). This lag-phase has been reported for most exotic species as the time from initial establishment to subsequent spread, a process which can take from few generations up to century or more (Hobbs and Humphries 1995).

Successful invasion is not only dependent on the characteristics of the invading species but also on the characteristics, dynamics, and history of the site being invaded. The diversity and structure of plant community can affect positively or negatively the abundance and diversity of arthropods associated to that community (Agrawal et al. 2006; Scherber et al. 2010; Schuldt et al. 2010; Kostenko et al. 2012), which is also locally maintained by, and in turn maintains a diversity of parasites and predators (Siemann et al. 1998; Bezemer et al. 2010a). Some studies have shown that contextual aspects of the native plant community can affect the degree of damage inflicted by native herbivores on the exotic plant (Prieur-Richard et al. 2002). For instance, exotics with close native relatives in the invaded area can rapidly accumulate enemies, since a shift of enemies is more likely among closely related hosts (Strong et al. 1984; Louda and Rand 2003; Hill and Kotanen 2009). Therefore, if plant enemies can readily spread from natives to related exotics, then the exotics with close relatives in the native flora might experience reduced enemy release, which may limit their ability to spread.

Thus far, studies reporting the community-wide effects of exotic plants on insect assemblages are scarce, but most of the existing studies with inter-continental invaders (Ernst and Cappuccino 2005; de Groot et al. 2007; Gerber et al. 2008) and intra-continental range expanders (Engelkes et al. 2008; Engelkes et al. 2012) have shown negative impacts of the exotic plants on insect abundance and richness. We tested the hypothesis that a range-expanding plant species harbours fewer invertebrates, such as herbivores and carnivores, and suffers less damage, than related native plant species. In order to test this hypothesis we performed a community study to compare vegetation composition and invertebrate assemblages on the intra-continental range expander Bunias orientalis L. and its native confamilials, Brassica rapa L., Sinapis arvensis L. and Brassica nigra L. Koch (all Brassicales: Brassicaceae). All species are ruderal and have similar habitat preferences in the Netherlands (Tamis et al. 2005). Additionally, to assess the sensitivity of exotic-native plant comparisons to natural variation in plant and invertebrate abundance, we examined these communities over the course of an entire growing season.

Bunias orientalis, originated from western Asia and extreme south-eastern Europe, has been established in parts of central and northern Europe since the 18th century (Steinlein et al. 1996). It remained rare and local until the 1980s, since when it has become increasingly abundant in countries throughout Europe, including Germany, Czech Republic, Poland and Norway (Steinlein et al. 1996). However, in the Netherlands it is still only naturalized in a lag-phase (Tamis et al. 2005; Harvey et al. 2010a). This exotic species is perennial and has an extended growing season. However, no large native Brassicaceae in the Netherlands are perennials; all are annuals or biennials with short seasonal growing phenologies (1-2 months). Here, we selected three native species which are approximately the size of Bu. orientalis and which grow at different times of the year (May through September) in the following sequence: Br. rapa, S. arvensis and Br. nigra. Nonetheless, because of differences in life history traits, the exotic and the native species can have different resource allocation strategies, including investment in defence against herbivores, which may vary temporally during the growing season. However, since the selected natives grow sequentially in the field and exploit similar habitats as Bu. orientalis in the Netherlands, our approach enables us to compare and contrast invertebrate communities on these plants and to determine if and to what extent they may differ.

Material and Methods

Plant species and populations

The selected brassicaceous species, exotic and natives, co-occur in the same eutrophic and disturbed habitats, along roadsides, fallow fields and primary succession grasslands in the Netherlands (Tamis et al. 2005). Bunias orientalis is a long-lived perennial that has expanded its range northwards and westwards within Europe in recent years, and in some regions has become a serious invasive weed (Steinlein et al. 1996; Harvey et al. 2010a). The species can occur in dense patches with individual plants harbouring up to hundred leaves and flowering stems 1.5 m height (Dietz et al. 1999; Harvey et al. 2010a), and has an extended growing season from early spring to fall (Harvey and Fortuna 2012, personal observations). All three selected native species are annuals with short life-cycles, although Br. rapa is occasionally biennial. In much of Europe, the three native species exhibit little overlap in their life cycles. In the Netherlands, Brassica rapa starts flowering in late April, S. arvensis usually flowers in late May and June, and Br. nigra generally starts flowering in July (Van der Meijden 2005).

These species were selected because they are abundant and ubiquitous, and serve as major food plants for spring and summer generations of generalist and specialist herbivores (Gols et al. 2008a,b; Harvey et al. 2010a). We studied each exotic-native pair in natural populations situated in the province of Gelderland which is located in the east and central region of the Netherlands. Plants were sampled during the growing season of 2010-2011. We sampled three populations per focal plant species, which included eight roadside populations and four populations in reserve areas, two along the Rhine River, and the other two along the Waal River (Figure 1, Table 1). Populations of Bu. orientalis had a smaller size than those of the three native crucifers, since this exotic species is not as common as the others (Table 1).

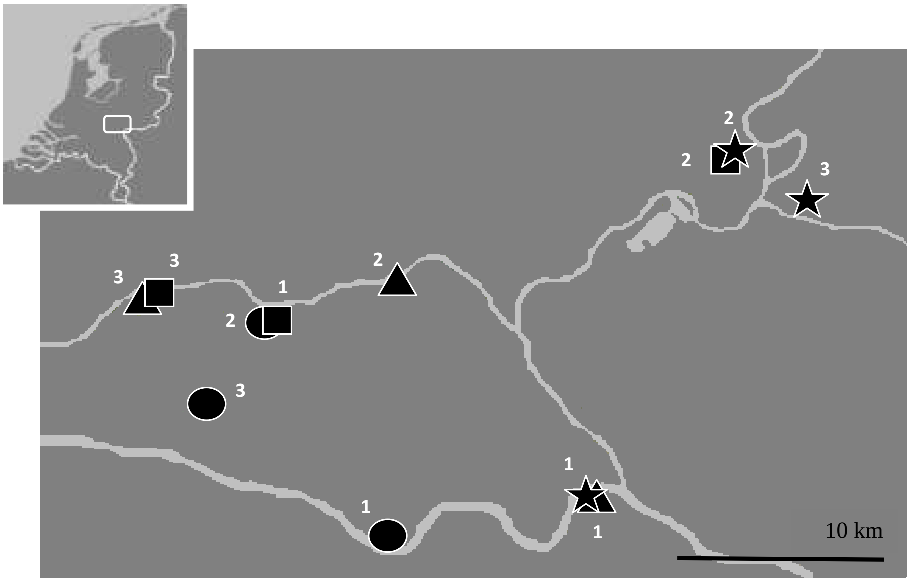

Figure 1 Distribution map of the sampled populations of the exotic plant, Bunias orientalis (stars), and the related native plants Brassica rapa (circles), Sinapis arvensis (squares) and Brassica nigra (triangles) in the province of Gelderland, the Netherlands. Numbers correspond to the different populations of each plant species (see Table A1).

Table 1 Location (GPS coordinates) and size of the sampled populations of the exotic plant, Bunias orientalis, and the three native relatives Brassica rapa, Sinapis arvensis and Brassica nigra. All populations were roadsides, except population 1 of Bu. orientalis and population 3 of Br. nigra, which were in Millingerwaard natural reserve along the Waal River, and populations 1 and 2 of Br. nigra, which were in a natural area along the Rhine River.

	Population 1		**Population 2**		**Population 3**	
Plant species	**Location**	**Size**	**Location**	**Size**	**Location**	**Size**
Bunias orientalis	51°52' N, 6°00' E	40	52°01' N, 6°06' E	80	52°00' N, 6°09' E	> 1,000
Brassica rapa	51°51' N, 5°51' E	100	51°57' N, 5°45' E	200	51°54' N, 5°43' E	> 5,000
Sinapis arvensis	51°57' N, 5°48' E	80	52°01' N, 6°06' E	500	51°57' N, 5°41' E	> 1,000
Brassica nigra	51°52' N, 6°00' E	50	51°58' N, 5°51' E	> 10,000	51°57' N, 5°40' E	> 5,000

Invertebrate sampling and identification

Each native plant population was sampled three times during the growing season, except population 2 of Br. rapa, which was accidentally mowed before the third sampling time. Sampling of native populations was consecutive, with some overlapping, because of the different plant phenologies. Therefore, populations of Br. rapa were sampled first, then S. arvensis populations and finally Br. nigra populations (Figure 2). Populations of Bu. orientalis were sampled six times during the growing season in order to ensure they overlapped with the three native species (Figure 2). Within each population 10 randomly chosen plants were sampled with a minimal distance of 5m between each individual. All invertebrates on each plant were collected using first a leaf vaccum (Pro Force Tanaka THB 2510N coupled with fine mesh bag of 80x80 cm) for 1 minute and a mouth-aspirator for the remaining time, making a total of 5 minutes. Sampled plants were labelled to prevent repeated sampling. All invertebrates, except eggs and Lepidoptera larvae, were stored in 70% ethanol in labelled containers until further identification. Eggs and Lepidoptera larvae, healthy and parasitized, were reared in acclimatized chamber (21±2ºC, 60% RH, 16:8h L:D photoperiod) and identified after emergence.

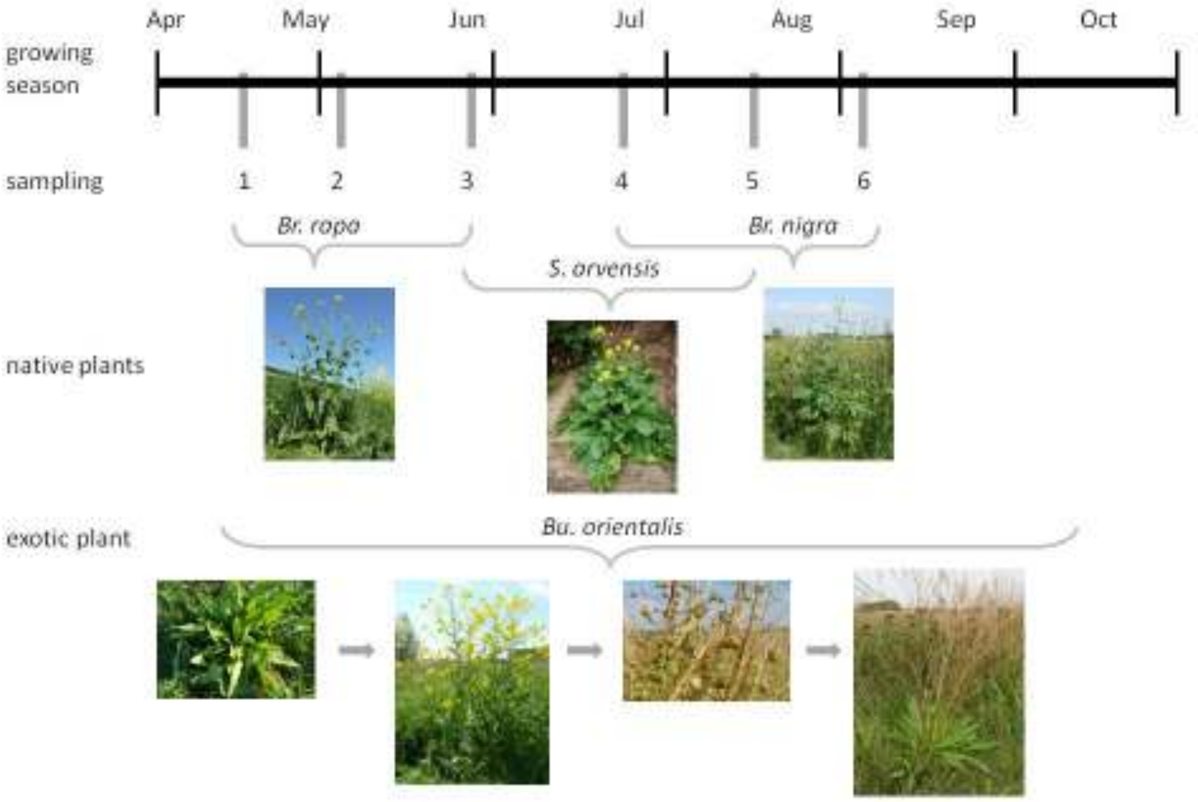

Figure 2 Sampling events (1-6) of the invertebrate community on the exotic plant species, Bunias orientalis, and the three native plant species, Brassica rapa, Sinapis arvensis, Brassica nigra along the growing season of 2010 in the Netherlands.

All collected invertebrates were identified to order, family or species level (Betrem 1966; Van Achterberg 1982; Finnamore and Michener 1993; Unwin 2001; Chinery 2007; Harde and Severa 2009; and specialist consultation) and then divided into trophic guilds and further subdivided into feeding guilds (Table 2 and see Table S1 for details on the collected taxonomic groups). The group of transients includes all collected invertebrates that are not herbivorous or predacious and that do not directly feed on living plant material (e.g. leaf litter, fungi and detritus feeders), or herbivores that are known to use non-brassicaceous species as host or food plants. Parasitized larvae of Lepidoptera were included in the dataset as one larva and either one or two parasitoids, depending on the trophic level (primary and/or secondary parasitoids). Batches of eggs were also included in the dataset as one adult individual of the respective order/ family. The number of leaves and flowers was measured in each sampled plant to correct for plant size. The relative presence of each feeding guild in the invertebrate community was determined as the number of individuals of each guild over the total number of invertebrates collected per plant. Herbivore load was then expressed per leaf as the sum of all herbivore guild loads (leaf chewers, sap feeders, seed eaters) collected in each individual plant. Additionally, herbivore leaf damage was investigated after invertebrate sampling for all ten plants sampled per plant population. For each plant, leaves were scored as either damaged (visual damage by herbivores in at least 5% of all leaf area) or undamaged. The ratio of damaged leaves to total number of leaves per plant was determined as the percentage of herbivore damage. Carnivore pressure was determined per plant as the ratio of carnivores (predators, parasitoids) to herbivores. This is a measure of only potential carnivore pressure on the herbivore community, as intra-guild predation may occur, or else some natural enemies may be more specialized (Finke and Denno 2003). Actual carnivore-prey relationships were not verified.

Table 2 Classes of feeding guilds and associated orders (families, species) for trophic guilds and transient invertebrates. Trophic guilds were classified into of herbivores, pollinators, carnivores and transients. Invertebrates were identified to order or family level for classification into feeding guilds. The group 'Transients' were defined as non-herbivorous or non-carnivorous invertebrates that do not have a direct link with the plant or herbivorous invertebrates that are known to have a non-Brassicaceae host plant.

Trophic guild	Feeding guild	Order	Family (species)
Herbivores	Leaf chewers	Coleoptera	Apionidae, Cerambycidae, Chrysomelidae, Curculionidae, Elateridae, Kateretidae, Lagriidae, Mordellidae, Nitidulidae, Oedemeridae, Scarabaeidae
		Hymenoptera	Tenthredinidae (larvae)
		Lepidoptera	All larvae
		Orthoptera	
		Thysanoptera	
		Gastropoda	
	Sap feeders	Hemiptera	Aphidoidea, Cercopidae, Cicadeliidae, Delphacidae, Lygaeidae, Miridae, Pentatomidae, Psyllidae, Tingidae, Triozidae
	Seed eaters	Coleoptera	Carabidae (Amara sp, Demetrias sp, Dromius atricapilus)
Pollinators	Pollen/ nectar feeders	Hymenoptera	Apidae
		Lepidoptera	Adults

Table 2 (continued)

Trophic guild	**Feeding guild**	**Order**	**Family (species)**
Carnivores	Predators	Coleoptera	Cantharidae, Coccinellidae, all larvae
		Hemiptera	Anthocoridae, Nabidae, Reduviidae
		Neuroptera	
		Odonata	
		Araneae	
		Opiliones	
		Pseudoscorpionida	
	Parasitoids	Hymenoptera	
Transients	Others	Acarina	
		Coleoptera	Anthicidae, Corylophidae, Cryptophagidae, Hydrophilidae, Latridiidae, Staphylinidae
		Hemiptera	Coreidae (Coreus marginatus), Aradidae, Miridae (Stenodema calcarata, Capsus ater, Leptopterna ferrugata), Aphididae (Atheroides serrulates), Diaspidae
		Hymenoptera	Formicidae, Tenthridinidae (adults)
		Dermaptera	
		Diptera	
		Ephemeroptera	
		Mecoptera	
		Psocoptera	
		Chilopoda	
		Diplopoda	
		Isopoda	
		Collembola	

Leaf toughness and fresh weight

For each exotic-native species pair, leaves were collected once in the phenological peak of the species growth season for measurements of fresh weight and leaf toughness. Ten individual plants were sampled randomly from one population of each plant species. Three leaves were collected per plant at different canopy height: bottom, middle and at the top. Leaves were kept in a box with ice elements to prevent dehydration (Garnier et al. 2001) until further measurements. Within 2 hours of sampling, leaf fresh weight was recorded and their toughness was measured using a penetrometer. This device measures the maximum load (kg/cm^2) required by a steel punch rod (blunt-ended, 3.18 mm of diameter) to penetrate a leaf. To standardize the location of leaf tissue sampled (Aranwela et al. 1999), the measurements were performed on the underside of the leaves and excluding the midribs. Mean leaf toughness and fresh weight were determined by averaging these parameters for all three leaves sampled from each individual plant.

Vegetation survey

The vegetation survey was conducted within a 1 m diameter circle (equivalent area of 0.8 m^2) around a focal plant of the exotic species and each of the three native species (Figure S1). The vegetation census was carried out for each exotic-native species pairs: 1) Bu. orientalis-Br. rapa, 2) Bu. orientalis-S. arvensis, and 3) Bu. orientalis-Br. nigra. Thus, the census was conducted once for each of the native species, around the phenological peak of their life cycle, whereas the exotic species was sampled three times along its growing season to compare with each of the native species. Five individual plants were randomly chosen per plant population to perform the vegetation survey. Only fully grown plants, flowering or early seed setting, were used as focal plants. In each survey, all vascular plants inside the circle were identified based on Streeter et al. (2011), and the number of different species and plant cover was recorded. Plant cover was estimated using the Braun-Blanquet cover-abundance scale (Table 3), which is used as a measure of species importance within plant communities (Wikum and Shanholtzer 1978). Therefore, species cover was estimated from the vertical plant shoot-area projection, which corresponds to a percentage in the sampled circular area. Species richness was determined for each exotic-native plant pair as the number of plant species associated with the focal species. For each species, plant occurrence was determined based on the percentage of total sampling circles (15) in which the species was found, and mean plant cover was estimated by averaging the plant cover from the circles where the species was found.

Table 3 Braun-Blanquet cover-abundance scale and scale conversion to midpoint of the cover range.

Braun-Blanquet scale	Range of cover (%)	Midpoint of cover range (%)
5	75-100	87.5
4	50-75	62.5
3	25-50	37.5
2	5-25	15.0
1	<5 (numerous individuals)	2.5
+	<5 (few individuals)	0.1

Statistical Analysis

All univariate analyses were performed using GenStat 15th edition (VSN International, UK), and multivariate analyses using CANOCO version 4.55 (Ter Braak and Šmilauer 2002). The exotic-native plant pairs were analyzed separately in all analyses.

Invertebrate sampling and identification: Differences in relative presence of invertebrate feeding guilds between exotic-native pairs were analyzed for each sampling period using unbalanced design ANOVA (except for the first sampling period of Bu. orientalis-Br. rapa, where regular ANOVA could be applied) with plant species and feeding guild as main factors and population nested within plant species. The two-way interaction between plant species and feeding guild was included in the model. Feeding guild percentage data was arcsin √ transformed in all three plant pairs to meet the assumptions of normality. To test whether plant origin affected the herbivore load (herbivores per plant leaf) on the exotic and the native species, we used non-parametric Kruskal-Wallis test for the three plant comparisons. Plant species effect was then tested for each sampling period using a χ^2 probability test. A non-parametric test was chosen since requirements of normality and homoscedasticity could not be fulfilled for the two comparisons, Bu. orientalis-Br. rapa and Bu. orientalis-S. arvensis. Percentage of leaf damage due to herbivory and potential carnivore pressure (carnivores per individual herbivore) were analyzed for each exotic-native pair using unbalanced design ANOVA with plant species and period (3 periods of sampling) as main factors and population nested within plant species. The two-way interaction between the main factors and the three-way interaction between all factors were also included in the model. To fulfil the requirements of normality and homogeneity of variances, percentage data of leaf damage was √ transformed, and carnivore pressure data was 1/ √(x+1) transformed in all three plant pairs.

Leaf toughness and fresh weight: Leaf toughness of plants from each exotic-native plant pair was compared using one-way ANOVA for Bu. orientalis-S. arvensis pair with plant species as main factor. Prior to analyses, leaf toughness was √ transformed to improve normality of the data. Leaf toughness of the other two pairs, Bu. orientalis-Br. rapa and Bu. orientalis-Br nigra, was compared based on non-parametric Mann-Whitney U-test. Leaf fresh weight for each plant pair was compared using one-way ANOVA with plant species as main factor. Prior to analyses, leaf weight of Bu. orientalis-Br. rapa pair was √ transformed to fulfil assumptions of normality and homoscedasticity, and leaf weight of Bu. orientalis-Br nigra pair was log (x+1) transformed to improve normality of data.

Vegetation survey: To compare species richness between the exotic-native plant pairs, Bu. orientalis and Br. rapa or Br. nigra, a general linear model ANOVA was used with focal plant species as main factor and population nested within plant species. Number of plant species was √ transformed for the Bu. orientalis and Br. nigra pair to meet assumptions of normality and equal variances. Species richness in Bu. orientalis and S. arvensis pair was analysed by using non-parametric Mann-Whitney U test. Differences in the plant community (species, plant cover) between exotic and native focal species were analysed using linear multivariate analysis, canonical analysis (CA) and canonical correspondence analysis (CCA) as the longest gradient resulted from detrended correspondence analysis (DCA) was >3 (Lepš and Šmilauer 2003; see Table S2). In the CCA analysis, focal species and plant population, representing the environmental variables, were included in the model as main factors, as well as their interaction. To separate partially the direct effects of focal species and population, a model was fitted using the factor of interest as an explanatory (environmental) variable and the other as a covariable. All eigenvalues values were positive. Significances in multivariate analysis were inferred by Monte Carlo permutation tests (unrestricted 999 permutations), which test the null hypothesis that there is no effect of environmental variables on species composition of the plant community. Coefficients of the canonical analysis were used to display the variation in plant community among all focal species (Figure 3, Table S3.1) and each exotic-native plant pair separately (Figure S3, Table S3.2-4). Differences in the plant cover of the focal species for each exotic-native pair within the sampling area were compared using Student's t-test.

Results

Invertebrate sampling

In total, more invertebrate individuals were collected on the exotic plant Bunias orientalis (11 213) compared to the native Brassica rapa (6 081), whereas less invertebrates were collected on the exotic species (7 056 and 10 652) compared to the native species Sinapis arvensis (8 109) and Brassica nigra (10 796) along the growing season (Table S1). The relative presence of the invertebrate community in the three exotic-native pair comparisons was affected by both plant species and feeding guild, resulting in a significant two-way interaction between these two factors (Table 4). When looking at the relative presence of each feeding guild, the invertebrate community on the natives showed a dominance of leaf chewers and sap feeders compared to that on the exotic species (Figure 4, Table S3). In addition, in the exotic species comparison with Br. rapa and S. arvensis there is a trend for an increase in carnivore presence along the growing season (Figure 4a, 4b, Table S3). Transient invertebrates were dominant in relation to the other groups in both the exotic and the native species (Figure 4, Table S3). Transients were also more abundant in the exotic species compared to the natives, particularly in the comparison with S. arvensis and Br. nigra (Figure 4, Table S3).

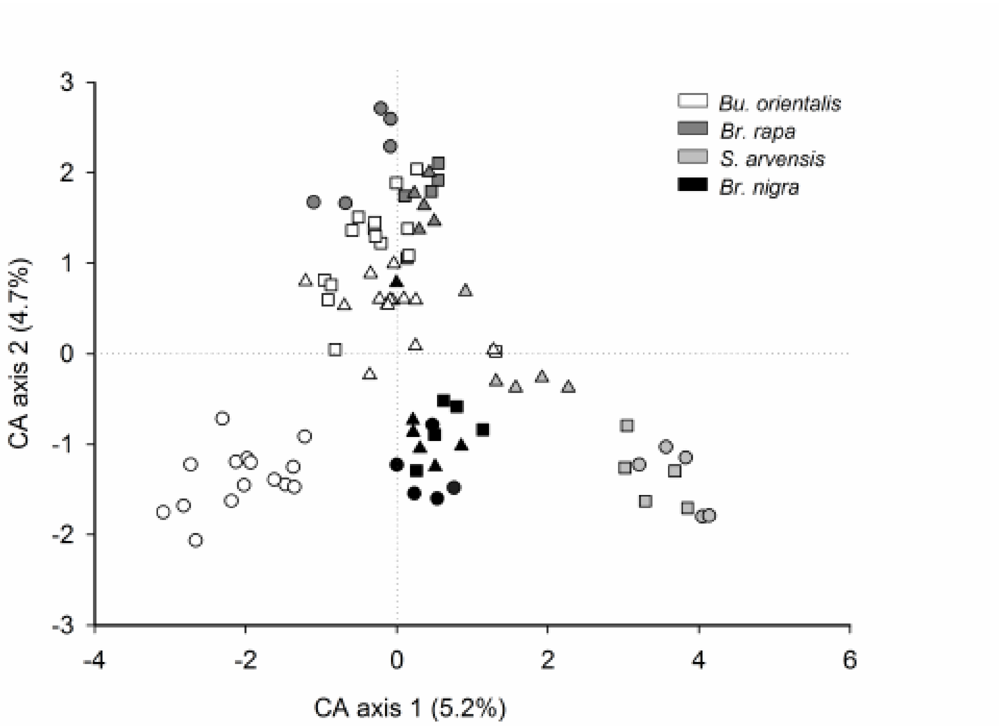

Figure 3 Two dimensional canonical analysis (CA) plot of the plant community (species, plant cover) associated with the exotic plant species, Bunias orientalis, and the three native species, Brassica rapa, Sinapis arvensis, and Brassica nigra. Different point shapes represent the sampled populations: population 1 represented by circles; population 2 represented by squares; population 3 represented by triangles. Each point represents the plant community surrounding each individual plant of the focal species (exotic or native). Percentages of total explained variation by CA axes are given in parentheses.

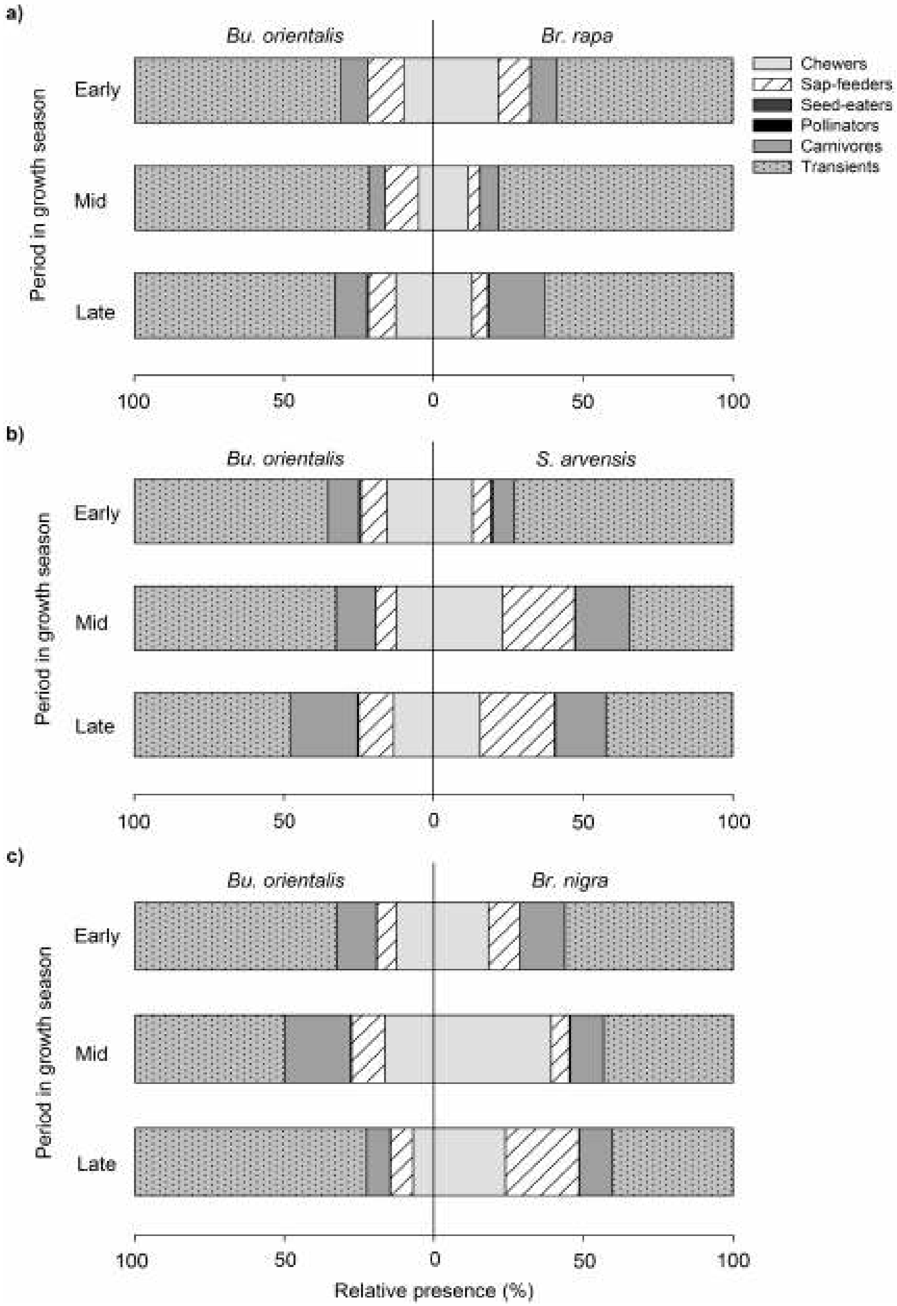

Figure 4 Relative presence (%) of each feeding guild in the invertebrate community on the exotic plant Bunias orientalis and on the three native species: a) Brassica rapa; b) Sinapis arvensis and; c) Brassica nigra along the growing season (early, mid and late period).

Table 4 F-test for the fixed effects and variance component estimates from unbalanced ANOVA of the effect of plant species (Ps) and feeding guild (Fg) class on the relative presence of the invertebrate community on each exotic-native plant pair: 1) Bunias orientalis (Bo) and Brassica rapa (Br); 2) Bunias orientalis and Sinapis arvensis (Sa); 3) Bunias orientalis and Brassica nigra (Bn).

		Bo - Br			**Bo - Sa**			**Bo - Bn**		
Time	**Factors**	df	F	P	df	F	P	df	F	P
Early	Ps	1	1.50	0.22	1	0.42	0.52	1	2.80	0.096
	Ps * Pop	4	1.08	0.37	4	2.45	0.046	4	2.12	0.078
	Fg	5	273.37	< 0.001	5	299.40	< 0.001	5	278.59	< 0.001
	Ps * Fg	5	4.27	< 0.001	5	2.70	0.021	5	3.46	0.005
Mid	Ps	1	11.30	< 0.001	1	5.17	0.024	1	1.69	0.20
	Ps * Pop	4	8.42	< 0.001	4	2.04	0.090	4	1.06	0.38
	Fg	5	822.81	< 0.001	5	176.51	< 0.001	5	139.16	< 0.001
	Ps * Fg	5	10.12	< 0.001	5	17.31	< 0.001	5	12.22	< 0.001
Late	Ps	1	0.86	0.35	1	0.07	0.80	1	4.13	0.043
	Ps * Pop	4	1.45	0.23	4	1.31	0.27	4	0.60	0.66
	Fg	5	258.55	< 0.001	5	66.16	< 0.001	5	130.24	< 0.001
	Ps * Fg	5	2.52	0.031	5	1.86	0.10	5	19.09	< 0.001

On average, the exotic Bu. orientalis had a much higher maximum number of leaves (166 ± 36 leaves) than any of the other native plants species (49 ± 8 leaves in Br. Rapa; 41 ± 4 leaves in S. arvensis; and 51 ± 10 leaves in Br. nigra). In addition, the exotic plant had a far higher total number of flower buds (146 ± 29 flowers) than the native species (30 ± 5 flowers in Br. Rapa; 34 ± 5 flowers in S. arvensis; 146 ± 29 flowers in Br. nigra) in the peak of the flowering period. The herbivore load per leaf was, therefore, significantly lower on Bu. orientalis than on all the three native species (Table 5; Figure 5a, 5c, 5e). For the Bu. orientalis-Br. rapa comparison the effect of plant species differed along the growing season (Table 5). The herbivore load on the exotic species was significantly lower than on the native Br. rapa in the early period, but it did not differ in the mid and late period of the growing season (Figure 5a). Herbivore leaf damage in all three exotic-native plant comparisons was differently affected by plant species, population and sampling period, resulting in a significant three-way interaction for this variable (Table 6). There was a strong effect of plant species on leaf damage, with the exotic species suffering less herbivore attack than the native species (Table 6, Figure 5b, 5d, 5f). This effect was, however, dependent on the plant population and sampling period (Table 6). The period in the growing season had a strong effect on leaf damage of Bu. orientalis-S. arvensis and Bu. orientalis-Br. nigra pairs, with the native species receiving significantly more damage than the exotic species in mid and late period of the natives growing season (Table 6; Figure 5d, 5e).

Table 5 Chi-square probability test from the non-parametric Kruskal-Wallis to test the effect of plant species on the herbivore load per leaf of the exotic and the three native species along the growing season. Exotic-native plant pairs: 1) Bunias orientalis (Bo) and Brassica rapa (Br); 2) Bunias orientalis and Sinapis arvensis (Sa); 3) Bunias orientalis and Brassica nigra (Bn).

	Bo - Br				**Bo - Sa**				**Bo - Bn**			
Time	n_{Bo}	n_{Br}	H	P	n_{Bo}	n_{Sa}	H	P	n_{Bo}	n_{Bn}	H	P
Early	30	30	15.47	< 0.001	30	30	11.97	< 0.001	30	30	28.49	< 0.001
Mid	16	21	0.90	0.34	30	30	25.42	< 0.001	30	29	13.08	< 0.001
Late	30	18	1.55	0.21	28	30	8.47	0.003	28	30	30.34	< 0.001

Table 6 F-test for the fixed effects and variance component estimates from unbalanced ANOVA of the effect of plant species (Ps) and sampling period (Ti) on the percentage of leaf damage by herbivory in each exotic-native plant pair: 1) Bunias orientalis (Bo) and Brassica rapa (Br); 2) Bunias orientalis and Sinapis arvensis (Sa); 3) Bunias orientalis and Brassica nigra (Bn).

	Bo - Br			**Bo - Sa**			**Bo - Bn**		
Factors	df	F	P	df	F	P	df	F	P
Ps	1	12.76	< 0.001	1	271.29	< 0.001	1	87.54	< 0.001
Ps * Pop	4	9.34	< 0.001	4	17.29	< 0.001	4	6.73	< 0.001
Ti	2	1.39	0.25	2	51.36	< 0.001	2	4.14	0.018
Ps * Ti	2	4.85	0.009	2	15.31	< 0.001	2	8.98	< 0.001
Ps * Pop * Ti	7	3.34	0.002	8	4.25	< 0.001	8	2.55	0.012

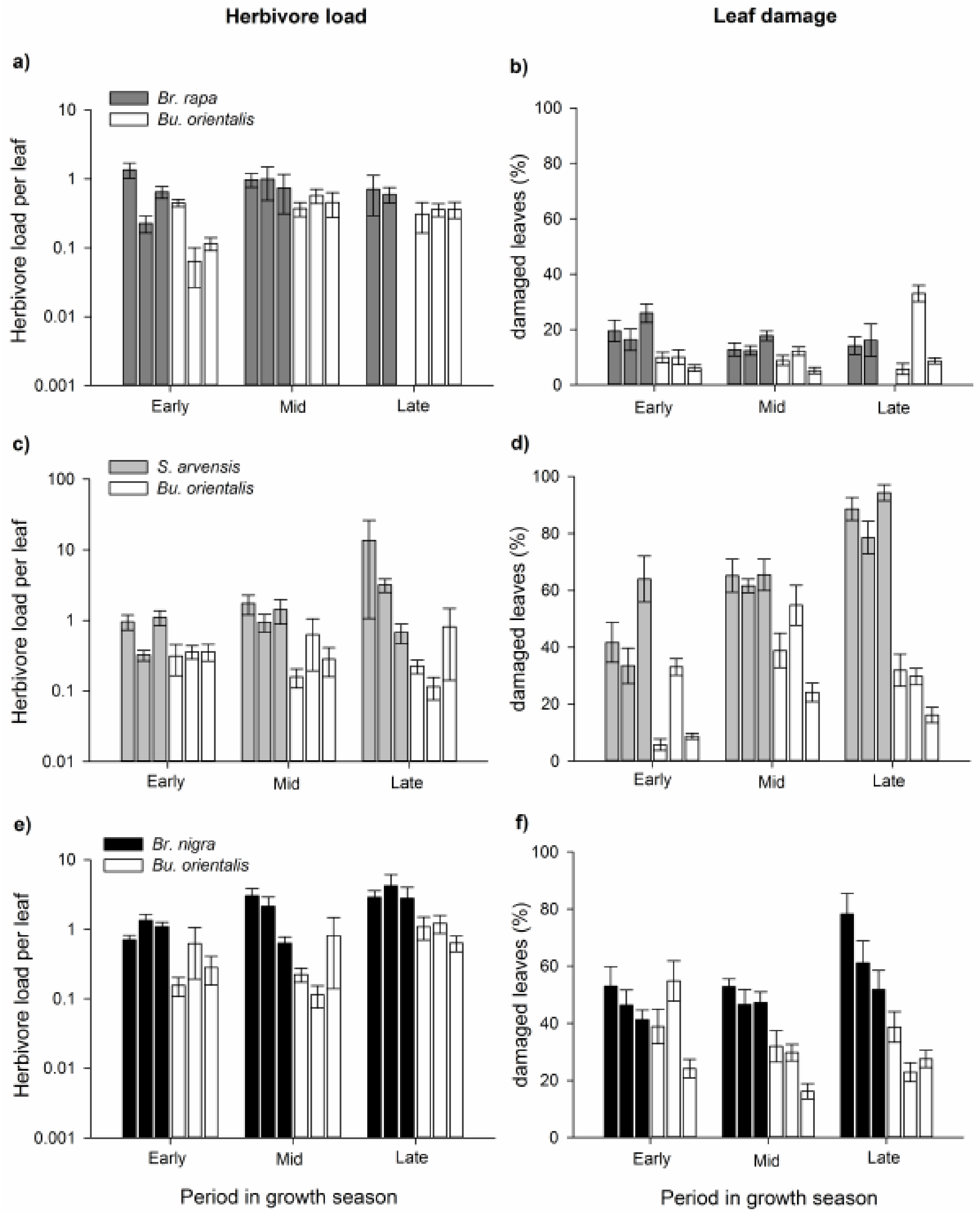

Figure 5 Herbivore load per leaf (a, c, e) and leaf damage due to herbivory (b, d, f) for each exotic-native plant species pair: Bunias orientalis and Brassica rapa (a, b); Bunias orientalis and Sinapis arvensis (c, d); Bunias orientalis and Brassica nigra (e, f). Three populations per plant species were sampled per period of the native species growth season (early, mid, late), except in the late period of Brassica rapa (two populations). Given are means ± SE. Each bar represents values for one plant population.

The carnivore pressure (carnivores per herbivore individual) on Bu. orientalis did not differ from that on the two native species, Br. rapa and S. arvensis. Nevertheless, it was significantly greater on Bu. orientalis than on the native Br. nigra (Table 7, Figure 6). The effect of plant species on the carnivore pressure in this exotic-native pair was, however, dependent on the sampling period (Table 7), being stronger in mid and late period of the species growth season (Figure 6).

Table 7 F-test for the fixed effects and variance component estimates from unbalanced ANOVA of the effect of plant species (Ps) and sampling period (Ti) on the potential carnivore pressure in each exotic-native plant pair: 1) Bunias orientalis (Bo) and Brassica rapa (Br); 2) Bunias orientalis and Sinapis arvensis (Sa); 3) Bunias orientalis and Brassica nigra (Bn).

	Bo - Br			**Bo - Sa**			**Bo - Bn**		
Factors	df	F	P	df	F	P	df	F	P
Ps	1	2.47	0.12	1	2.00	0.16	1	34.84	< 0.001
Ps * Pop	4	3.39	0.012	4	1.85	0.13	4	2.31	0.066
Ti	2	5.11	0.008	2	1.69	0.19	2	2.46	0.09
Ps * Ti	2	1.57	0.21	2	1.79	0.17	2	4.00	0.022
Ps * Pop * Ti	7	1.68	0.12	8	1.13	0.36	8	1.42	0.20

Leaf toughness and fresh weight

In all exotic-native plant pairs, Bu. orientalis plants were found to have significantly tougher leaves than those of the native species (Bu. orientalis-Br. rapa: $U = 0.0$, $P < 0.001$; Bu. orientalis –S. arvensis: $F_{1,19} = 21.42$, $P < 0.001$; Bu. orientalis-Br. nigra: $U = 9.0$, $P = 0.001$; Figure S2b). Leaf weight of Bu. orientalis plants was more varied along the three sampling periods. Leaves of the exotic species were significantly heavier than those of Br. rapa ($F_{1,19}=57.5$, $P<0.001$), while the native S. arvensis had significantly heavier leaves than the exotic species ($F_{1,19}=6.67$, $P=0.019$). No difference was found between the leaves of the exotic species and the native Br. nigra ($F_{1,19}=0.36$, $P=0.556$), both species showed a high leaf weight (Figure S2a).

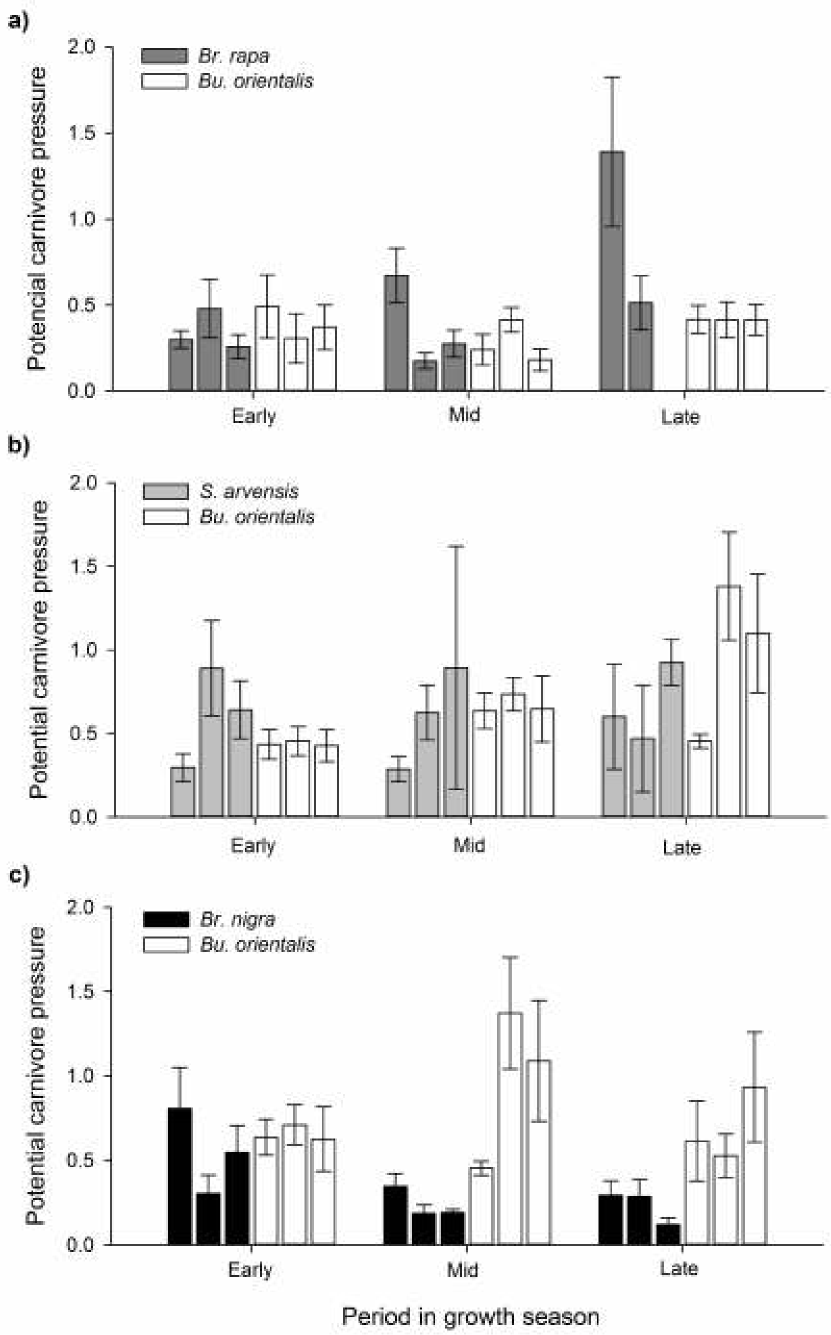

Figure 6 Potential carnivore pressure for each exotic-native plant species pair: a) Bunias orientalis and Brassica rapa; b) Bunias orientalis and Sinapis arvensis; c) Bunias orientalis and Brassica nigra. Three populations per plant species were sampled per period of the native species growth season (early, mid, late), except in the late period of Brassica rapa (two populations). Given are means ± SE. Each bar represents values for one plant population.

Vegetation census

The total number of plant species recorded in Bunias orientalis populations ranged between 58, 42 and 44 along the three sampling times, and between 43, 53 and 39 plant species in the populations of the native species, Br. rapa, S. arvensis and Br. nigra respectively (Table S5). The mean species richness was not significantly different between any of the three exotic-native plant comparisons (Bu. orientalis-Br. rapa: $F_{1,29} = 3.81$, $P = 0.063$; Bu. orientalis–S. arvensis: $U = 70.0$, $P = 0.076$; Bu. orientalis-Br. nigra: $F_{1,29} = 0.40$, $P = 0.531$). There was a tendency, however, for the populations of Bu. orientalis to harbour richer plant communities compared to those around Br. rapa populations (Figure S3). The identified plant communities were mainly composed by native species, and only three of the species found were exotic, such as Heracleum mantegazzianum and Persicaria amphibia, which were both associated with Bu. orientalis plants, and Conyza canadensis associated with Br. nigra plants (Table S5). Canonical analysis revealed a clear separation between the exotic and the related native plant species in all three plant-pairs based on the plant community composition (Figure 3, Figure S4, Table S5). However, these differences in plant community composition were dependent on the plant population (CCA: $P_{\text{interaction}} < 0.001$, Table 8). Roadside populations tend to have different floral community composition compared to those in the reserve areas (Figure 3, Figure S4). The most common species (present in at least 50% of the circles) associated with Bu. orientalis was Carex recta, Lamium album (Table S5.1) and Elymus caninus (Table S5.3), while Anthriscus sylvestris, Bromus sterilis, Elymus caninus and Urtica dioica were the most common species associated with Br. rapa populations (Table S5.1). In S. arvensis populations the most common species was Daucus carota, whereas Glechoma hederacea, Poa annua and Urtica dioica were the most common species present in Br. nigra populations. In addition, for all exotic-native pairs it was common to find other individuals of the same focal species in the sampled area around the focal plants. However, when other individuals were present, Bu. orientalis was as abundant as the other native species (Bu. orientalis-Br. rapa: $t = -0.03$, $df = 4$, $P = 0.98$; Bu. orientalis–S. arvensis: $t = 0.69$, $df = 4$, $P = 0.53$), except compared to Br. nigra, which covered a significantly greater area than the exotic species (Bu. orientalis-Br. nigra: $t = 3.56$, $df = 4$, $P = 0.023$).

Table 8 F-ratio (Canoco test statistic) and P-values from Monte Carlo permutation test for testing the significance of relationships with environmental variables, focal species (Fs), population (Pop) and their interaction, in the analysis of the plant community composition associated to each exotic-native plant pair: 1) Bunias orientalis (Bo) and Brassica rapa (Br); 2) Bunias orientalis and Sinapis arvensis (Sa); 3) Bunias orientalis and Brassica nigra (Bn).

	Bo - Br		**Bo - Sa**		**Bo - Bn**	
Factors	F	P	F	P	F	P
Fs	3.26	0.001	2.71	0.001	2.31	0.001
Pop	3.02	0.001	2.62	0.001	2.12	0.001
Fs * Pop	3.47	0.001	2.64	0.001	2.62	0.001

Discussion

According to the ERH, invasions are more likely to be successful if introduced plants suffer lower fitness-related costs as a result of damage from enemies (e.g. herbivores and pathogens) compared to their native range, as well as, to native species in the new range. The results found in this study partially support the ERH, as we found smaller herbivore load on the exotic Bu. orientalis than on its native confamilials, Br. rapa, S. arvensis and Br. nigra. Similar results with insects have been shown by other field studies on invasive and native plants (Memmott et al. 2000; Ernst and Cappuccino 2005; Engelkes et al. 2012). The results for Bu.orientalis and the three native relatives show that the mechanisms posted in the ERH for inter-continental invasions can also play an important role in intra-continental range-expanding species, which was also recently shown by Engelkes et al. (2012). Additionally, the exotic species also experienced reduced foliar herbivory compared to the natives species, and this varied with the site and along the season.

Measuring visible leaf damage can provide an underestimation of total herbivore damage, because some feeding guilds can imply smaller damage than the others, and damage to other important tissues, such as roots or seeds, may be missed (Vasquez and Meyer 2011). However, given the temporal, spatial and species-wide breadth of this study, we can assume that it provides a good baseline for estimating plant damage, and that it reveals less aboveground enemy regulation experienced by the Bu. orientalis in the Netherlands compared with three related plants. It is thus far unknown how susceptible is Bu. orientalis to enemies in its native range. Nonetheless, if this exotic species has escaped from some of its important co-evolved enemies, this in turn could in time contribute to rapid range expansion

(MacKay and Kotanen 2008), as it is happening in many parts of central and eastern Europe (Harvey et al. 2010a). Our results also emphasize the importance of studying plant-enemy interactions on species that, although not in the rapid expansion phase, can become problematic weeds in the novel range. A better understanding of the determinants of species naturalization (e.g. forming self-sustaining populations that do not spread substantially) is important because besides including a critical stage of the invasion process, it also permits pro-active management by targeting potentially invasive species before they start to exert ecological impacts in ecosystems and economic costs to human society (Hobbs and Humphries 1995; Richardson and Pysek 2012).

We considered herbivore load and foliar damage as proxies for enemy pressure on plants, while predator pressure was interpreted as a proxy for potential top-down control on the herbivores. Plants can be attacked by a suite of enemies, which can vary in their feeding modes and in time. In our study, the phytophagous community on both exotic and native species was dominated by chewers and sap feeders, whereas other feeding guilds such as seed eaters, leaf miners and gallers were rarely found. Vacant herbivore niches are predicted to occur on exotic plants in the new range as the colonisation of insects more intimately associated with the plant (e.g. leaf miners and gallers) is likely to take longer than the acquisition of external feeding herbivores (e.g. leaf chewers and sap feeders) (Strong et al. 1984). However, differences in relative proportion of various feeding guilds may be also related to the sampling technique used in this study. Concealed invertebrates, such leaf miners, seed eaters and gallers are difficult to collect with a leaf vacuum or mouth aspirator, although damage from leaf miners was found in some cases when we inspected plants for foliar damage. Neverthless, we encountered less leaf chewers and sap feeders on the exotic plant than on the natives, and this decrease in herbivore load was more pronounced in the middle of Bu. orientalis growing season, particularly when compared with S. arvensis and Br. nigra native plants.

Herbivore communities in natural ecosystems are regulated by a combination of top-down and bottom-up forces, such as predator pressure, plant related-traits (structure and quality), and the composition of plant communities (Strong et al. 1984; Schmitz et al. 2000; Halaj and Wise 2001). In our study, potential top-down carnivore pressure on herbivore communities was higher on the exotic than on the native species in the middle period of Bu. orientalis growing season (comparison with Br. nigra) at the peak of invertebrate abundance. Similarly, a study by Engelkes et al. (2012) showed higher predator pressure on several species of exotic range expanders than related native plants. Higher carnivore pressure on Bu. orientalis later in the growing season may contribute to a reduction in herbivores compared with Br. nigra, which is the last of the large native Brassicaceae to be common in the Netherlands. Other studies have found a similar pattern on invasive species, where higher predator biomass was related to an increase in herbivore biomass in the species native range (Memmott et al. 2000), and high predator pressure was concomitant with low percentages of invertebrate herbivores (Holmquist et al. 2011). However, predator-prey cycles are also dependent on other factors, such as plant's nutrional quality (Ode 2006), which changes over the season, apparent competition (Holt 1977) or direct competition. For instance, invertebrates included in the transient group, which do not feed on the focal plant, can still interact with herbivores and predators, by providing alternative prey, or by competing with other insects for food and shelter. Invertebrates belonging to the transient group had a tendency to be more abundant on the Bu. orientalis, which could dilute the top-down effect of the carnivores on this plant species. As suggested by Engelkes et al. (2012), future studies on exotic and native species should include experimental predator removal to help determine whether predator pressure is driving or following the herbivore loads on these plant species.

Invertebrate populations are also influenced by bottom-up forces of plant-related traits. Our results showed that Bu. orientalis had much tougher leaves than leaves of the native plants. In a recent review, Onoda et al. (2011) reported leaf toughness as a key factor in leaf mechanical resistance and that strongly influences plant-herbivore interactions. However, other plant traits of Bu. orientalis can be involved in resistance against herbivores besides leaf toughness, such as trichome densities and chemical defence. Higher trichome densities in Bu. orientalis leaves compared to other brassicaceous species, including Br. rapa, have been suggested to play an important role in the low performance of neonates from specialist herbivores, such as Athalia rosae L. or Phaedon cochleariae F., on this exotic plant (Travers-Martin and Müller 2008; Kühnle and Müller 2009). The negative impact of leaf toughness and hairiness on feeding behaviour and survival of neonates is known for several herbivorous species (reviewed in Zalucki et al. 2002). Moreover, some studies with exotic plants have linked high levels of plant secondary compounds on the exotics with smaller herbivore loads compared to native congeners (Engelkes et al. 2008; Cano et al. 2009). Glucosinolates (GS), plant secondary metabolites characteristic of brassicaceous species, have been also shown to play an important role in the invasive success of some Brassicaceae (Müller 2009). Although Bu. orientalis produces higher levels of GS than the natives S.

arvensis or Br. nigra (Harvey et al. 2010a), these seem to not play a crucial role for generalist and specialist foliar herbivores (Buschmann et al. 2006; Harvey et al. 2010a), even though some specialist such as P. cochleariae and some Pierids develop very poorly on this exotic species (Kühnle and Müller 2009; Harvey et al. 2010a). Thus, it has been suggested that some other as yet unidentified secondary metabolite(s) may be present in leaf tissues of Bu. orientalis and is at least partially responsible for negatively affecting native insect herbivores in the new range (Kühnle and Müller 2009; Harvey et al. 2010a). Some studies have showed, however that a specific flavonoid and hydroxycinnamic acid derivades contained in Bu orientalis leaves may be good candidates for the feeding deterrent effect of this exotic plant (Dietz and Winterhalter 1996; Kühnle and Müller 2009).

Besides acting in plant defence, GS breakdown products can also function as allelopathic agents and benefit brassicaceous species in competition with other plants (Roberts and Anderson 2001; Renwick 2002; Siemens et al. 2002). Recently, it was reported that Bu. orientalis exhibits high constitutive levels of GS in the roots, which may be related with an allelopathic function (van Geem et al. 2013). Studies of Dietz et al. (1996) have shown that both root exudates and leaf litter leachates of Bu. orientalis do not have a strong impact on the seedling growth of other species cooccuring with the exotic species. Nevertheless, the impact of Bu. orientalis on the belowground soil communities, as well as the role of these communities in the establishment of the exotic species has not yet been studied. For instance, some invasive brassicaceous species such as Alliaria petiolata (M. Beib. Cavara and Grande) in North America are known to reduce the abundance of arbuscular mycorrhiza and alter native communities by creating a plant–soil biota feedback that facilitates invasion (Roberts and Anderson 2001; Reinhart and Callaway 2006). Successful range expanders have also been found to be better defended against both root and shoot enemies compared to their native congeners (Engelkes et al. 2008). Future studies with exotic plants should therefore include interactions with organisms from physically separated compartments (e.g. plant roots and shoots) to better understand the factors underlying the success (or failure) of these species in their new ranges (Agrawal et al. 2005; Harvey et al. 2010b). If aboveground and belowground enemies both negatively affect introduced plants less than natives, the results may be synergistic and may reinforce invasive ability; however if these effects vary in opposite directions they may cancel out.

Plant community context can affect the degree of damage experienced by exotic species. It has been observed that invaded communities with high plant species richness can have increased damage by herbivores (Prieur-Richard et al. 2002). However, in natural grassland systems arthropod abundance may not be a simple function of plant species richness, and it has been emphasized that plant community composition (specie identity, abundance) have also an important role in the abundance patterns of the arthropod assemblages (Dennis et al. 1998; Perner et al. 2005). We found no differences, however, between plant species richness of the communities associated with the exotic plant and the natives, although the CCA analysis showed a clear separation of the plant communities. Differences among plant species according to plant populations showed that plant communities and the associated herbivore fauna are likely to differ across sites, which can affect comparisons between exotics and natives (Vasquez and Meyer 2011). This difference in plant communities was clear particularly between the roadside populations and those in a nature reserve area close to the river Waal. Therefore, it is not clear from our data to which extent invertebrate communities on Bu. orientalis and the native species can be explained solely by differences in plant community or by the influence of the exotic species on those communities. Other community studies have measured herbivore pressure on exotic and native plant species in different sites (Carpenter and Cappuccino 2005; Hill and Kotanen 2009). However, thus far, none of them had compared plant community composition with the associated invertebrate community on these species as it was done in the present study. Additionally, community-level studies should also incorporate knowledge on the invertebrate communities on exotic and native species within a single site independently of species relatedness. This is important to test the ERH, because plants in the field compete with both related and unrelated species, and generalist herbivores on neighboring plants may move over to the target species under investigation. This alternative approach to the community-level studies complements both phylogenetically-based experiments and broader-scale studies that examine a large number of species across multiple sites (Vasquez and Meyer 2011).

In their review, Keane and Crawley (2002) further predict that the release from enemy pressure facilitates increased plant performance of exotic species in the introduced range. This prediction has also been conceptualized as the evolution of increased competitive abilities (EICA) of exotic invasive species (Blossey and Nötzold 1995). Although plant performance was not measured directly in this study, the exotic species produced a much higher number of leaves and flowers than the native species. This measurement per se may not be very informative without the study of interspecific interactions between Bu. orientalis and plants in its native range. However, we can expeculate that by allocating greater resources to growth and reproduction the exotic plant might also gain a competitive advantage in relation to close related natives, which are shown to have a greater herbivore pressure than the exotic species. This is not always a linear relationship, however, because greater herbivore pressure might not always translate in high impact of enemies on plant fitness (Maron and Vila 2001; MacKay and Kotanen 2008). Native plants may also be better defended against local generalist herbivores than the exotic species, or have increased levels of tolerance to herbivory. Furthermore, assuming that tolerance incurs fitness costs (Strauss and Agrawal 1999), an increased tolerance can also be translated in decreased competitive ability against new plant intruders in native communities. Therefore, more experimental work is needed to understand how altered patterns of selection in both native and exotic species might influence the evolution of plant tolerance during the invasion process.

Importantly, because of differences in life history traits, the exotic and the native plants can exhibit different strategies for resource allocation, including investment in defence and tolerance against herbivores, during the growing season. In addition, the extended growing period of Bu. orientalis compared to the native species may facilitate plant shifts by invertebrate communities to the exotic plant, especially in the end of the season when few brassicaceous species are growing and specialist herbivores may have few alternate host plants. Finally, since the exotic species has been expanding its range to regions that contain closely related native species, specialist enemies of native species might readily switch to attack the exotic species. Over time, native organisms can adapt to even the most uniquely defended introduced plants (Graves and Shapiro 2003; Keeler and Chew 2008). A recent study has shown that approximately 100 native herbivores insect species in the Netherlands are using exotic plants as host plants (Meijer et al. 2012). Ultimately, an enemy exclusion experiment on natural communities of coexisting exotic and native plants must be conducted over the entire growing season, including measurements of plant performance (plant growth, plant abundance and population dynamics) in order to empirically test the ERH. In addition, as proposed by Agrawal et al. (2005), these experiments should be conducted for more than a year, since the magnitude and direction of escape from enemies can vary from year to year.

In conclusion, this study reports that an exotic range-expanding plant species have smaller herbivore loads, experience less foliar herbivory, have higher potential top-down carnivore pressure and increased bottom-up control compared with three native confamilial plant species. This is a clear indication that the ERH can be an important ecological mechanism underlying the range expansion of Bu. orientalis. However, ERH is only one of the many hypotheses that can explain the abundance and/or impact of a potential invader. For example, climatic variables selection for invasive genotypes, human disturbance, resource availability, competition and mutualism are other important factors that were not investigated here. Therefore, future research should seek to identify the relative importance of multiple factors that may be responsible for the success of some exotic invasive species.

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Supplementary material

Table S1 Total number of invertebrate individuals collected on individual Bunias orientalis plants and on plants of the three native species: 1) Brassica rapa; 2) Sinapis arvensis; 3) Brassica nigra, growing in natural populations in Gelderland, The Netherlands.

Trophic guild	Feeding guild	Order	Taxonomic group	Bunias orientalis	Brassica rapa
Herbivores	Leaf chewers	Coleoptera	Apionidae	3	5
			Cerambycidae	0	1
			Chrysomelidae	46	56
			Curculionidae	117	23
			Elateridae	4	2
			Kateretidae	1	2
			Mordellidae	6	1
			Nitidulidae	25	80
			Oedemeridae	27	2
			Scarabaeidae	16	0
		Total		245	172
		Hymenoptera	Tenthredinidae (larvae)	11	10
		Lepidoptera (larvae)	Macrolepidoptera		
			Pieridae	1	0
			Geometridae	7	7
			Nymphalidae	0	1
			Noctuidoidea		
			Noctuidae	3	3
			Arctiidae	1	0
			Microlepidoptera	2	1
		Total		14	12
		Orthoptera		1	2
		Thysanoptera		693	848
		Gastropoda		178	54
	Sap feeders	Hemiptera	Cercopidae	28	1
			Cicadeliidae	558	155
			Delphacidae	66	24
			Lygaeidae	25	8
			Miridae	62	2
			Pentatomidae	2	3
			Psyllidae	10	0
			Tingidae	1	3
			Triozidae	0	2
			Aphidoidea		
			Aphididae	110	41
			Callaphididae	1	1
			Pemphigidae	1	0
		Total		905	325

Table S1 (continued)

Trophic guild	Feeding guild	Order	Taxonomic group	Bunias orientalis	Brassica rapa
Herbivores	Seed eaters	Coleoptera	Carabidae (Amara sp, Demetrias sp, Dromius atricapilus)	5	2
Pollinators	Pollen/ nectar feeders	Hymenoptera	Apidae	9	5
		Lepidoptera (adults)	Noctuidoidea		
			Noctuidae	1	0
			Microlepidoptera	3	4
			Pterophoridae	0	1
		Total		4	4
Carnivores	Predators	Coleoptera (adults)	Cantharidae	4	1
			Coccinellidae	12	3
		Coleoptera (larvae)		7	42
		Total		23	46
		Hemiptera	Anthocoridae	1	0
			Nabidae	2	1
		Total		3	1
		Neuroptera		1	2
		Odonata		0	1
		Araneae		205	178
		Opiliones		49	8
		Pseudoscorpionida		0	4
	Parasitoids	Hymenoptera	Aphelinidae	3	2
			Braconidae	47	57
			Ceraphronidae	7	10
			Cynipoidea	8	2
			Diapriidae	13	3
			Dryinidade (larvae)	3	1
			Encyrtidae	12	0
			Eulophidae	29	15
			Eurytomidae	1	2
			Ichneumonidae	48	23
			Megaspilidae	6	1
			Mymaridae	33	20
			Platygastridae	96	12
			Proctotrupidae	1	0
			Pteromalidae	25	11
			Scelionidae	18	6
			Torymidae	2	1
		Total		352	166
Transients	others	Acarina		654	335
		Coleoptera	Anthicidae	6	0
			Corylophidae	3	0
			Cryptophagidae	12	6
			Hydrophilidae	1	0
			Latridiidae	16	5
			Staphylinidae	20	9
		Total		58	20

Table S1 (continued)

Trophic guild	Feeding guild	Order	Taxonomic group	Bunias orientalis	Brassica rapa
Transients	others	Hemiptera	Miridae (Stenodema calcarata, Capsus ater, Leptopterna ferrugata)	49	13
			Aphididae (Atheroides serrulates)	2	0
			Diaspidae	2	0
		Total		53	13
		Hymenoptera	Formicidae	46	33
			Tenthridinidae (adults)	1	5
		Total		47	38
		Dermaptera		0	2
		Diptera		688	417
		Mecoptera		1	0
		Psocoptera		2	2
		Chilopoda		1	1
		Diplopoda		10	0
		Isopoda		75	329
		Collembola		6926	3084
Total				11213	6081

Table S1 (continued)

Trophic guild	Feeding guild	Order	Taxonomic group	Bunias orientalis	Sinapis arvensis
Herbivores	Leaf chewers	Coleoptera	Apionidae	6	7
			Cerambycidae	2	0
			Chrysomelidae	42	107
			Curculionidae	78	50
			Elateridae	7	4
			Kateretidae	6	5
			Lagriidae	1	0
			Mordellidae	10	7
			Nitidulidae	22	293
			Oedemeridae	48	1
			Scarabaeidae	20	0
		Total		242	474
		Hymenoptera	Tenthredinidae (larvae)	20	2
		Lepidoptera (larvae)	Macrolepidoptera	2	14
			Pieridae	1	11
			Geometridae	0	1
			Psychidae	1	1
			Noctuidoidea	6	10
			Noctuidae	4	8
			Arctiidae	1	2
			Microlepidoptera	3	6
		Total		11	30
		Orthoptera		2	5
		Thysanoptera		802	838
		Gastropoda		107	40
	Sap feeders	Hemiptera	Cercopidae	57	14
			Cicadeliidae	315	123
			Delphacidae	9	3
			Lygaeidae	7	12
			Miridae	56	59
			Pentatomidae	16	20
			Psyllidae	3	2
			Tingidae	2	1
			Triozidae	7	30
			Aphidoidea		
			Aphididae	337	2590
			Callaphididae	9	297
			Chaitophoridae	5	3
			Pemphigidae	1	0
		Total		824	3154
	Seed eaters	Coleoptera	Carabidae (Amara sp, Demetrias sp, Dromius atricapilus)	9	1

Table S1 (continued)

Trophic guild	Feeding guild	Order	Taxonomic group	Bunias orientalis	Sinapis arvensis
Pollinators	Pollen/ nectar feeders	Hymenoptera	Apidae	9	3
		Lepidoptera (adults)	Macrolepidoptera		
			Hesperiidae	1	0
			Microlepidoptera	7	15
		Total		8	15
Carnivores	Predators	Coleoptera (adults)	Cantharidae	13	11
			Coccinellidae	3	7
		Coleoptera (larvae)		40	105
		Total		56	123
		Hemiptera	Anthocoridae	0	2
			Nabidae	11	7
		Total		11	9
		Neuroptera		2	0
		Araneae		183	113
		Opiliones		22	3
	Parasitoids	Hymenoptera	Aphelinidae	4	0
			Braconidae	53	75
			Ceraphronidae	17	19
			Cynipoidea	11	21
			Diapriidae	9	14
			Dryinidade (larvae)	3	3
			Encyrtidae	14	3
			Eulophidae	77	40
			Eurytomidae	6	3
			Ichneumonidae	14	29
			Megaspilidae	6	4
			Mymaridae	63	45
			Platygastridae	59	46
			Proctotrupidae	2	0
			Pteromalidae	42	32
			Scelionidae	20	5
			Torymidae	1	1
			Trichogrammatidae	2	0
		Total		403	340
Transients	others	Acarina		540	206
		Coleoptera	Anthicidae	4	0
			Corylophidae	1	0
			Cryptophagidae	4	2
			Latridiidae	37	7
			Staphylinidae	5	6
		Total		51	15

Table S1 (continued)

Trophic guild	Feeding guild	Order	Taxonomic group	Bunias orientalis	Sinapis arvensis
Transients	others	Hemiptera	Miridae (Stenodema calcarata, Capsus ater, Leptopterna ferrugata)	30	1
			Aphididae (Atheroides serrulates)	2	0
		Total		32	1
		Hymenoptera	Formicidae	26	60
			Tenthridinidae (adults)	0	1
		Total		26	61
		Dermaptera		9	8
		Diptera		760	504
		Mecoptera		2	1
		Psocoptera		1	1
		Chilopoda		1	0
		Diplopoda		17	0
		Isopoda		34	34
		Collembola		2872	2128
Total				7056	8109

Table S1 (continued)

Trophic guild	Feeding guild	Order	Taxonomic group	Bunias orientalis	Brassica nigra
Herbivores	Leaf chewers	Coleoptera	Apionidae	8	6
			Cerambycidae	2	0
			Chrysomelidae	54	139
			Curculionidae	29	16
			Elateridae	3	1
			Kateretidae	5	11
			Lagriidae	1	0
			Mordellidae	5	2
			Nitidulidae	7	25
			Oedemeridae	21	4
			Scarabaeidae	4	0
		Total		139	204
		Hymenoptera	Tenthredinidae (larvae)	10	7
		Lepidoptera (larvae)	Macrolepidoptera		
			Pieridae	4	0
			Psychidae	1	0
			Noctuidoidea	10	10
			Noctuidae	6	9
			Arctiidae	1	1
			Microlepidoptera	3	14
		Total		18	24
		Orthoptera		2	0
		Thysanoptera		994	2484
		Gastropoda		121	345
	Sap feeders	Hemiptera	Cercopidae	39	13
			Cicadeliidae	465	489
			Delphacidae	37	49
			Lygaeidae	12	18
			Miridae	32	73
			Pentatomidae	23	29
			Psyllidae	1	15
			Tingidae	4	2
			Triozidae	10	21
			Aphidoidea		
			Aphididae	290	2283
			Callaphididae	9	229
			Chaitophoridae	11	9
		Total		933	3230
	Seed eaters	Coleoptera	Carabidae (Amara sp, Demetrias sp, Dromius atricapilus)	6	3

Table S1 (continued)

Trophic guild	Feeding guild	Order	Taxonomic group	Bunias orientalis	Brassica nigra
Pollinators	Pollen/ nectar feeders	Hymenoptera	Apidae	0	5
		Lepidoptera (adults)	Macrolepidoptera		
			Hesperiidae	1	0
			Noctuidoidea		
			Noctuidae	0	1
			Microlepidoptera	7	4
		Total		8	5
Carnivores	Predators	Coleoptera (adults)	Cantharidae	9	11
			Coccinellidae	15	1
		Coleoptera (larvae)		146	22
		Total		170	34
		Hemiptera	Anthocoridae	0	5
			Nabidae	12	4
			Reduviidae	0	1
		Total		12	10
		Neuroptera		5	3
		Araneae		249	208
		Opiliones		15	15
	Parasitoids	Hymenoptera	Aphelinidae	7	7
			Braconidae	55	51
			Ceraphronidae	31	32
			Cynipoidea	6	7
			Diapriidae	12	10
			Dryinidade (larvae)	3	6
			Encyrtidae	10	6
			Eulophidae	102	86
			Eurytomidae	6	5
			Ichneumonidae	6	6
			Megaspilidae	1	1
			Mymaridae	91	78
			Platygastridae	53	58
			Proctotrupidae	2	0
			Pteromalidae	31	40
			Scelionidae	37	59
			Sphecidae	0	2
			Trichogrammatidae	4	1
		Total		457	455
Transients	others	Acarina		2655	300
		Coleoptera	Anthicidae	10	0
			Cryptophagidae	7	1
			Latridiidae	55	3
			Staphylinidae	7	1
		Total		78	5

Table S1 (continued)

Trophic guild	Feeding guild	Order	Taxonomic group	Bunias orientalis	Brassica nigra
Transients	others	Hemiptera	Coreidae (Coreus marginatus)	1	9
			Aradidae	1	0
			Miridae (Stenodema calcarata, Capsus ater, Leptopterna ferrugata)	8	2
			Aphididae (Atheroides serrulates)	0	1
		Total		10	12
		Hymenoptera	Formicidae	19	13
		Dermaptera		9	4
		Diptera		707	996
		Ephemeroptera		0	1
		Mecoptera		1	2
		Psocoptera		1	0
		Diplopoda		9	0
		Isopoda		284	52
		Collembola		3739	2379
Total				10652	10796

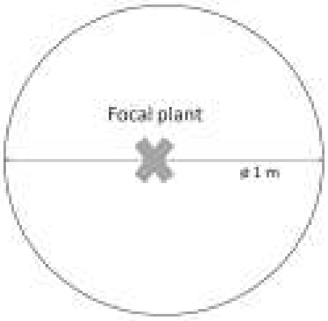

Figure S1 Sampling area used for assessment of the plant communities surrounding the focal species in each exotic-native pair.

Table S2 Longest gradient in detrended correspondence analysis (DCA), and percentage of variance of plant community data (species variables) explained by the first four axis in the canonical analysis (CA), or by the focal species, population and their interaction (environmental variables) in the canonical correspondence analysis (CCA).

		Br - Bo	**Sa - Bo**	**Bn - Bo**
DCA	Longest gradient	6.63	8.21	4.71
CA (% variance)	Axis 1	12.40	10.10	12.30
	Axis 2	11.20	8.50	9.90
	Axis 3	9.40	7.40	7.10
	Axis 4	6.50	7.30	6.30
CCA (% variance)	Focal sp (1)	10.80	9.10	7.90
	Population (2)	10.10	8.80	7.30
	Interaction (1.2)	28.60	23.40	23.20

Table S3 Canonical coefficients for the first two axes of the CA plot for plant communities associated with 1) all focal plant species the exotic Bunias orientalis (Bo) and the three natives Brassica rapa (Br), Sinapis arvensis (Sa) and Brassica nigra (Bn); or individually with each exotic-native plant pairs: 2) Br versus Bo 3) Sa versus Bo 4) Bn versus Bo.

Focal species	Population	Individual plant	Scores 1	Scores 2
Br. rapa (1)	1	1	-0.081	2.594
		2	-1.101	1.675
		3	-0.084	2.291
		4	-0.681	1.662
		5	-0.212	2.711
	2	1	0.546	1.919
		2	0.104	1.744
		3	0.455	1.795
		4	0.133	1.058
		5	0.551	2.110
	3	1	0.230	1.774
		2	0.294	1.371
		3	0.358	1.637
		4	0.428	2.004
		5	0.494	1.462
Bu. orientalis (1)	1	1	-1.477	-1.445
		2	-2.664	-2.065
		3	-1.623	-1.392
		4	-2.190	-1.626
		5	-1.220	-0.915
	2	1	-0.964	0.817
		2	-0.913	0.602
		3	-0.591	1.363
		4	-0.869	0.763
		5	-0.819	0.050
	3	1	-0.362	-0.233
		2	-0.057	0.590
		3	-0.095	0.596
		4	0.246	0.086
		5	-1.208	0.802
S. arvensis (2)	1	1	3.826	-1.148
		2	3.568	-1.030
		3	3.212	-1.226
		4	4.048	-1.801
		5	4.135	-1.790
	2	1	3.680	-1.295
		2	3.023	-1.260
		3	3.051	-0.792
		4	3.289	-1.628
		5	3.849	-1.704

Table S3 (continued)

Focal species	**Population**	**Individual plant**	**Scores 1**	**Scores 2**
S. arvensis (2)	3	1	2.271	-0.375
		2	1.923	-0.264
		3	1.315	-0.306
		4	1.575	-0.374
		5	0.911	0.688
Bu. orientalis (2)	1	1	-2.309	-0.718
		2	-2.729	-1.224
		3	-3.093	-1.751
		4	-2.824	-1.680
		5	-2.131	-1.192
	2	1	0.153	1.088
		2	-0.303	1.380
		3	-0.511	1.509
		4	0.265	2.040
		5	1.309	0.025
	3	1	0.092	0.607
		2	-0.132	0.544
		3	-0.007	0.786
		4	1.281	0.042
		5	-0.350	0.888
Br. nigra (3)	1	1	0.470	-0.785
		2	0.233	-1.544
		3	0.533	-1.599
		4	0.758	-1.479
		5	-0.002	-1.227
	2	1	0.261	-1.290
		2	0.498	-0.892
		3	1.139	-0.841
		4	0.619	-0.516
		5	0.795	-0.585
	3	1	0.854	-1.021
		2	0.507	-1.248
		3	0.216	-0.870
		4	0.212	-0.735
		5	0.305	-1.046
Bu. orientalis (3)	1	1	-1.371	-1.252
		2	-2.025	-1.451
		3	-1.984	-1.155
		4	-1.936	-1.200
		5	-1.358	-1.472
	2	1	0.145	1.382
		2	-0.212	1.223
		3	-0.009	1.888
		4	-0.295	1.454
		5	-0.285	1.301
	3	1	-0.231	0.606
		2	-0.040	0.998
		3	0.249	0.595
		4	-0.120	0.539
		5	-0.696	0.533

Table S3 (continued)

Focal species	Population	Individual plant	Scores 1	Scores 2
Br. rapa (1)	1	1	2.513	0.3183
		2	4.0651	2.7933
		3	2.9205	1.4401
		4	3.0409	2.123
		5	3.2262	1.6943
	2	1	0.8404	-2.303
		2	0.762	-0.9805
		3	1.2489	-1.1289
		4	0.1607	-1.0514
		5	1.1034	-1.96
	3	1	0.5585	-2.524
		2	0.6067	-1.8076
		3	0.5168	-2.1843
		4	1.2039	-2.313
		5	0.4972	-2.0991
Bu. orientalis (1)	1	1	-2.3912	1.0076
		2	-2.9695	1.7076
		3	-2.469	1.0344
		4	-2.5222	1.0947
		5	-1.9959	0.6206
	2	1	-1.0523	0.4308
		2	-1.069	0.3809
		3	0.2468	0.2222
		4	-1.1251	0.4841
		5	-1.3572	0.0755
	3	1	-1.4973	-0.1153
		2	-0.8021	-0.413
		3	-0.6622	-0.4883
		4	-0.8351	-0.7357
		5	-0.4369	0.698

Table S3 (continued)

Focal species	Population	Individual plant	Scores 1	Scores 2
S. arvensis (2)	1	1	-3.0458	5.1818
		2	-2.1096	1.8805
		3	-2.173	1.8935
		4	-2.6408	3.3166
		5	-2.7174	3.7228
	2	1	-1.9807	-0.1451
		2	-1.9068	0.3998
		3	-1.8554	-0.1406
		4	-2.1672	0.1715
		5	-2.1871	0.1989
	3	1	-1.2582	-0.1133
		2	-1.6996	-0.1316
		3	-1.6139	-0.9077
		4	-1.3008	-0.8855
		5	-1.0788	-1.0344
Bu. orientalis (2)	1	1	4.8923	0.6243
		2	5.7651	0.9324
		3	6.0636	1.0248
		4	5.4772	0.7685
		5	4.3291	0.3629
	2	1	-0.3171	-0.6127
		2	-0.5385	-1.6715
		3	0.1795	-1.4224
		4	-0.8061	-1.947
		5	-1.262	-0.3861
	3	1	-0.7393	-2.2528
		2	-0.1771	-1.7013
		3	-0.7312	-1.9791
		4	-1.0977	-1.4205
		5	-0.2972	-2.1469

Table S3 (continued)

Focal species	Population	Individual plant	Scores 1	Scores 2
Br. nigra (3)	1	1	-1.1714	-0.8907
		2	-1.4907	-0.0902
		3	-1.7449	0.0219
		4	-1.742	-0.0908
		5	-1.371	-0.0256
	2	1	-1.2932	-0.116
		2	-1.1444	-0.7903
		3	-1.8924	-1.6058
		4	-1.6624	-1.1601
		5	-1.5924	-1.2476
	3	1	-0.8539	-0.2768
		2	-0.8914	0.0811
		3	-0.8429	-0.303
		4	-0.9862	-0.4207
		5	-1.1941	-0.2553
Bu. orientalis (3)	1	1	-0.0629	1.8201
		2	0.2771	2.6351
		3	0.1814	2.5634
		4	0.6008	2.7233
		5	-0.5079	1.7775
	2	1	2.7098	-0.9976
		2	2.0644	-0.3189
		3	3.1562	-1.0366
		4	2.5334	-0.7187
		5	2.7792	-0.2649
	3	1	1.9578	-0.8311
		2	1.8035	-0.5236
		3	0.8522	-0.7922
		4	1.3118	-0.4186
		5	1.136	0.1658

Table S4.1 Total number of invertebrate individuals per feeding guild collected along the growing season (early, mid, late period) on the plants of the native-exotic plant pair: Brassica rapa (BR) and Bunias orientalis (BO).

	Early time		Mid time		Late time	
Feeding guild	**BR**	**BO**	**BR**	**BO**	**BR**	**BO**
Leaf chewers	361	181	592	419	145	542
Sap feeders	115	227	135	329	75	349
Seed eaters	1	0	0	0	1	5
Pollinators	1	1	6	0	2	12
Carnivores	125	164	173	261	108	208
Transients	1131	1230	2696	4973	414	2312
Total	1734	1803	3602	5982	745	3428

Table S4.2 Total number of invertebrate individuals per feeding guild collected along the growing season (early, mid, late period) on the plants of the native-exotic plant pair: Sinapis arvensis (SA) and Bunias orientalis (BO).

	Early time		Mid time		Late time	
Feeding guild	**SA**	**BO**	**SA**	**BO**	**SA**	**BO**
Leaf chewers	407	542	739	332	243	310
Sap feeders	175	348	1108	188	1871	288
Seed eaters	0	5	1	2	0	2
Pollinators	10	12	5	1	3	4
Carnivores	137	208	281	214	170	255
Transients	1741	2312	815	1503	403	530
Total	2470	3427	2949	2240	2690	1389

Table S4.3 Total number of invertebrate individuals per feeding guild collected along the growing season (early, mid, late period) on the plants of the native-exotic plant pair: Brassica nigra (BN) and Bunias orientalis.

	Early time		Mid time		Late time	
Feeding guild	**BN**	**BO**	**BN**	**BO**	**BN**	**BO**
Leaf chewers	658	332	1441	310	965	642
Sap feeders	384	187	166	288	2680	458
Seed eaters	1	2	1	2	1	2
Pollinators	0	1	6	4	4	3
Carnivores	256	214	212	255	257	439
Transients	1306	1503	1052	530	1406	5480
Total	2605	2239	2878	1389	5313	7024

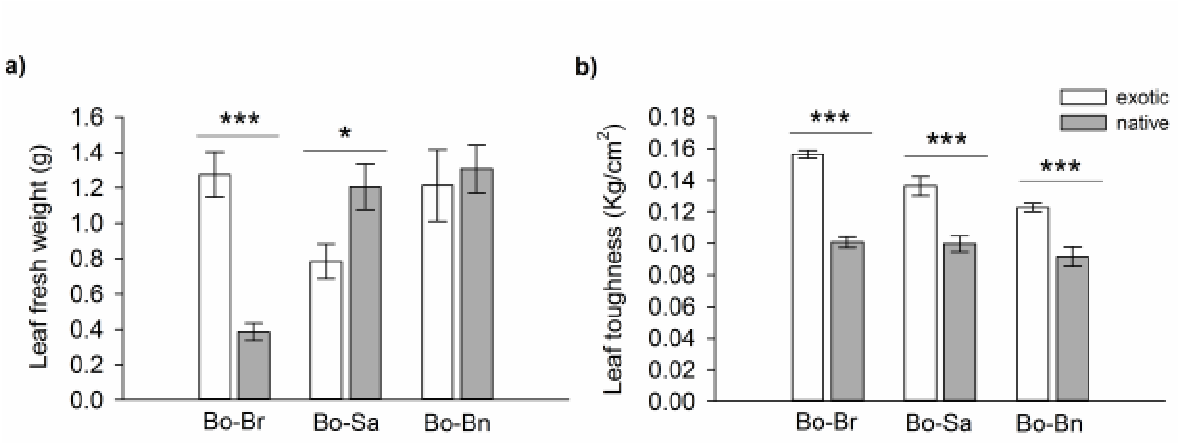

Figure S2 Leaf fresh weight (a) and leaf toughness (b) of each exotic-native plant pair: Bunias orientalis (Bo) versus Brassica rapa (Br), Sinapis arvensis (Sa), or Brassica nigra (Bn). Given are means ± SE. Significant differences ($P < 0.05$; $P < 0.001$) between exotic and native plant species are indicated by asterisks (*; ***) based on one-way ANOVA or Mann-Whitney U-test. N=10 per plant species.

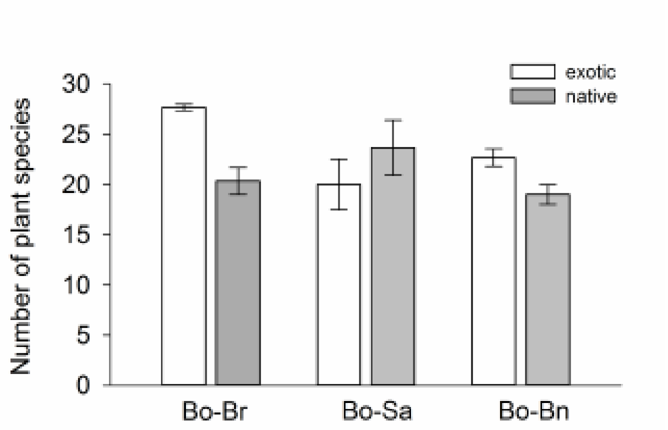

Figure S3 Plant richness (number of species) recorded in 0.8 m^2 around the focal plant of each exotic-native plant pair: Bunias orientalis and Brassica rapa (Bo-Br); Bunias orientalis and Sinapis arvensis (Bo-Sa); Bunias orientalis and Brassica nigra (Bo-Bn). Given are means ± SE.

Table S5.1 Plant occurrence and cover (%) of the species recorded in 1 m^2 around each focal plant of the native-exotic pair Brassica rapa and Bunias orientalis. Plant occurrence is based on the percentage of total circular areas (15) in which the species was found. Plant cover is based on the percentage of Braun-Blanquet cover-abundance scale. Given are means (SE). Non-native plant species are represented with an asterisk (*).

	Br. rapa		**Bu. orientalis**	
Family/ Species	**Occurrence (%)**	**Mean cover (%)**	**Occurrence (%)**	**Mean cover (%)**
Apiaceae				
Aegopodium podagraria			13	2.5 (0)
Anthriscus sylvestris	60	5.6 (2.4)		
Daucus carota	7	0.1 (N/A)	20	18.3 (10.2)
Foeniculum vulgare	7	0.1 (N/A)	7	0.1 (N/A)
Heracleum mantegazzianum*			7	2.5 (N/A)
Heracleum sphondylium			20	18.3 (10.2)
Pimpinella major			7	0.1 (N/A)
Asteraceae				
Achillea millefolium	7	0.1 (N/A)	13	1.3 (1.2)
Artemisia vulgaris			20	15.0 (0)
Bellis perennis	7	0.1 (N/A)		
Centaurea jacea	7	15.0 (N/A)		
Cirsium arvense			27	1.3 (0.7)
Cirsium vulgare	13	15.0 (0)	13	0.1 (0)
Crepis biennis	7	0.1 (N/A)		
Crepis foetida	7	0.1 (N/A)		
Crepis mollis			7	0.1 (N/A)
Jacobaea vulgaris	7	15.0 (N/A)	13	0.1 (0)
Leontodon hispidus	7	0.1 (N/A)		
Senecio vulgaris	13	7.6 (7.5)		
Tanacetum vulgare			20	17.5 (10.9)
Taraxacum officinale	20	1.7 (0.8)		
Blechnaceae				
Unknown sp1			7	0.1 (N/A)
Boraginaceae				
Myosotis discolor	7	2.5 (N/A)		
Symphytum officinale			7	0.1 (N/A)
Symphytum sp.			7	0.1 (N/A)
Brassicaceae				
Arabidopsis thaliana			13	1.3 (1.2)
Brassica rapa	60	26.4 (6.2)		
Bunias orientalis*			87	19.3 (4.5)
Caryophyllaceae				
Cerastium arvense	7	2.5 (N/A)		
Saponaria officinalis			33	26.0 (7.3)
Convolvulaceae				
Calystegia sepium	13	0.1 (0)		
Convolvulus arvensis	13	18.8 (18.7)		

Table S5.1 (continued)

	Br. rapa		**Bu. orientalis**	
Family/ Species	**Occurrence (%)**	**Mean cover (%)**	**Occurrence (%)**	**Mean cover (%)**
Cyperaceae				
Carex hirta			7	2.5 (N/A)
Carex praecox	13	50.0 (12.5)	7	0.1 (N/A)
Carex recta			53	40.9 (5.4)
Carex sp.			7	0.1 (N/A)
Equisetaceae				
Equisetum arvense	33	29.0 (9.4)	27	16.3 (15.4
Euphorbiaceae				
Euphorbia esula			20	5.9 (4.6)
Fabaceae				
Trifolium repens	7	2.5 (N/A)		
Trifolium subterraneum			7	0.1 (N/A)
Vicia hirsuta	7	0.1 (N/A)	33	0.1 (0)
Vicia sepium			13	38.8 (23.8)
Fumariaceae				
Fumaria officinalis			7	0.1 (N/A)
Fumaria sp.			7	0.1 (N/A)
Geraniaceae				
Erodium cicutarium	7	0.1 (N/A)		
Geranium columbinum	20	0.1 (9.8E-18)		
Geranium molle	27	1.9 (0.6)		
Geranium pyrenaicum	13	0.1 (0)		
Geranium sp.			7	0.1 (N/A)
Lamiaceae				
Clinopodium vulgare			20	5.9 (4.6)
Glechoma hederacea	27	1.9 (0.6)	33	1.5 (0.6)
Lamium album			60	4.2 (2.1)
Lamium sp.			7	0.1 (N/A)
Unknown sp2			7	0.1 (N/A)
Plantaginaceae				
Plantago lanceolata	7	0.1 (N/A)	13	0.1 (0)
Plantago major			13	1.3 (1.2)
Unknown sp4	7	2.5 (N/A)		
Veronica officinalis			27	1.9 (0.6)
Poaceae				
Agrostis sp1			20	6.7 (4.2)
Agrostis sp2			7	2.5 (N/A)
Bromus sterilis	67	25.0 (4.3)		
Dactylis glomerata	27	7.6 (4.3)	47	48.6 (9.0)
Elymus caninus	60	31.9 (8.2)	13	15 (0)
Phleum phleoides	47	3.3 (2.0)	27	2.5 (0)
Poa annua	7	37.5 (N/A)	33	33.5 (8.8)
Poa pratensis	7	0.1 (N/A)		

Table S5.1 (continued)

Species	Br. rapa		Bu. orientalis	
	Occurrence (%)	**Mean cover (%)**	**Occurrence (%)**	**Mean cover (%)**
Polygonaceae				
Persicaria amphibia*			27	0.7 (0.6)
Rumex conglomeratus			13	0.1 (0)
Rumex crispus	20	5.1 (5.0)		
Ranunculaceae				
Ranunculus bulbosus	20	10.0 (5.0)		
Ranunculus sp	7	0.1 (N/A)		
Thalictrum sp.			7	0.1 (N/A)
Rosaceae				
Argentina anserina			7	62.5 (N/A)
Crataegus monogyna			7	2.5 (N/A)
Fragaria sp.			7	0.1 (N/A)
Potentilla reptans	13	1.3 (1.2)	7	2.5 (N/A)
Potentilla sp.			7	0.1 (N/A)
Rosa sp.			7	0.1 (N/A)
Rubus caesius	20	30.8 (15.8)		
Unknown sp5			7	0.1 (N/A)
Rubiaceae				
Galium aparine	40	1.7 (0.5)	40	1.3 (0.5)
Galium odoratum	33	14.5 (6.4)		
Verbascum blattaria			13	1.3 (1.2)
Urticaceae				
Urtica dioica	53	30.3 (6.8)	33	23.0 (8.9)

Table S5.2 Plant occurrence and cover (%) of the species recorded in 1 m^2 around each focal plant of the native-exotic pair Sinapis arvensis and Bunias orientalis. Plant occurrence is based on the percentage of total circular areas (15) in which the species was found. Plant cover is based on the percentage of Braun-Blanquet cover-abundance scale. Given are means (SE). Non-native plant species are represented with an asterisk (*).

	S. arvensis		**Bu. orientalis**	
Family/ Species	**Occurrence (%)**	**Mean cover (%)**	**Occurrence (%)**	**Mean cover (%)**
Apiaceae				
Aegopodium podagraria			7	2.5 (N/A)
Anthriscus sylvestris	7	2.5 (N/A)		
Apium graveolens	7	0.1 (N/A)		
Daucus carota	60	1.4 (0.4)	33	1.1 (0.6)
Heracleum sphondylium			27	32.5 (11.3)
Pastinaca sativa			20	0.9 (0.8)
Achillea millefolium			20	1.7 (0.8)
Artemisia vulgaris	13	15.0 (0)	13	7.6 (7.5)
Centaurea jacea			13	15.0 (0)
Asteraceae				
Cichorium intybus	7	15.0 (N/A)		
Cirsium arvense	13	7.6 (7.5)	13	0.1 (0)
Cirsium vulgare	7	0.1 (N/A)		
Crepis foetida	7	2.5 (N/A)		
Hieracium prenanthoides	7	0.1 (N/A)		
Jacobaea vulgaris			7	0.1 (N/A)
Lactuca serriola	40	3.0 (2.4)		
Lactuca virosa	27	3.8 (3.7)		
Lapsana communis	20	0.9 (0.8)		
Matricaria recutita	40	9.2 (6.1)		
Senecio sylvaticus	13	0.1 (0)		
Senecio vulgaris	7	0.1 (N/A)		
Sonchus oleraceus	7	0.1 (N/A)		
Tanacetum vulgare	20	0.9 (0.8)	27	19.4 (10.5)
Taraxacum officinale	7	0.1 (N/A)		
Taraxacum sp.			7	0.1 (N/A)
Boraginaceae				
Anchusa arvensis			13	18.8 (18.7)
Brassicaceae				
Alliaria petiolata	20	5.9 (4.6)		
Brassica nigra	7	15.0 (N/A)		
Bunias orientalis*			53	8.8 (4.7)
Capsella bursa-pastoris	7	2.5 (N/A)		
Erysimum cheiranthoides	7	0.1 (N/A)		
Sinapis arvensis	53	6.00 (2.7)		
Sisymbrium officinale	20	0.9 (0.8)		
Caryophyllaceae				
Saponaria officinalis			27	38.1 (9.7)
Stellaria media	13	7.6 (7.5)		

Table S5.2 (continued)

	S. arvensis		**Bu. orientalis**	
Family/ Species	**Occurrence (%)**	**Mean cover (%)**	**Occurrence (%)**	**Mean cover (%)**
Convolvulaceae				
Convolvulus arvensis			20	6.7 (4.2)
Cyperaceae				
Carex praecox			33	43.0 (9.0)
Carex recta			33	24.0 (5.5)
Carex viridula viridula			7	0.1 (N/A)
Equisetaceae				
Equisetum arvense			33	38.5 (16.7)
Euphorbiaceae				
Euphorbia cyparissias			13	2.5 (0)
Fabaceae				
Vicia sepium			33	13.5 (6.9)
Fumariaceae				
Fumaria sp.	7	15.0 (N/A)		
Geraniaceae				
Geranium dissectum	7	0.1 (N/A)		0.1 (N/A)
Geranium molle	7	0.1 (N/A)	7	
Geranium pusilum	7	0.1 (N/A)		
Lamiaceae				
Glechoma hederacea	7	2.5 (N/A)	27	5.0 (3.4)
Lamium album	13	7.6 (7.5)	13	0.1 (0)
Lamium purpureum			7	0.1 (N/A)
Unknown sp3	33	13.5 (6.9)		
Onagraceae				
Chamerion augustifolium			13	7.6 (7.5)
Plantaginaceae				
Plantago lanceolata	7	2.5 (N/A)	7	0.1 (N/A)
Poaceae				
Arrhenatherum elatius	47	45.0 (12.5)	13	8.8 (6.3)
Dactylis glomerata	13	1.3 (1.2)	20	22.5 (7.5)
Elymus caninus	13	37.5 (0)	20	30.0 (7.5)
Elymus repens	13	1.3 (1.2)		
Holcus lanatus	7	0.1 (N/A)	20	38.3 (13.7)
Lolium perenne	13	1.3 (1.2)	7	62.5 (N/A)
Phalaris arundinacea	7	0.1 (N/A)	7	15.0 (N/A)
Poa pratensis			20	1.7 (0.8)
Vulpia myuros	33	18.5 (8.2)		
Zea mays	7	0.1 (N/A)		
Polygonaceae				
Persicaria amphibia*			20	14.2 (11.7)
Polygonum aviculare	33	9.0 (3.6)		
Polygonum rurivagum	27	0.7 (0.6)		
Rumex conglomeratus	7	0.1 (N/A)		
Rumex crispus	13	0.1 (0)	7	0.1 (N/A)
Rumex obtusifolius	7	0.1 (N/A)	7	2.5 (N/A)

Table S5.2 (continued)

Family/ Species	S. arvensis Occurrence (%)	S. arvensis Mean cover (%)	Bu. orientalis Occurrence (%)	Bu. orientalis Mean cover (%)
Primulaceae				
Anagallis arvensis	20	0.9 (0.8)		
Rosaceae				
Potentilla argentea			7	2.5 (N/A)
Rubus caesius	20	5.1 (5.0)		
Rubus fructicosus	20	10.0 (5.0)		
Rubiaceae				
Galium aparine	20	5.1 (5.0)	20	5.1 (5.0)
Scrophulariaceae				
Linaria vulgaris			7	15.0 (N/A)
Verbascum nigrum			13	7.6 (7.45)
Urticaceae				
Urtica dioica	33	19.0 (7.9)	40	13.0 (7.8)
Violaceae				
Viola biflora	20	1.7 (0.8)		

Table S5.3 Plant occurrence and cover (%) of the species recorded in 1 m^2 around each focal plant of the native-exotic pair Brassica nigra and Bunias orientalis. Plant occurrence is based on the percentage of total circular areas (15) in which the species was found. Plant cover is based on the percentage of Braun-Blanquet cover-abundance scale. Given are means (SE). Non-native plant species are represented with an asterisk (*).

	Br. nigra		**Bu. orientalis**	
Family/ Species	**Occurrence (%)**	**Mean cover (%)**	**Occurrence (%)**	**Mean cover (%)**
Apiaceae				
Aegopodium podagraria			20	10.0 (5.0)
Anthriscus sylvestris			7	2.5 (N/A)
Daucus carota	13	1.3 (1.2)	33	2.0 (0.5)
Heracleum sphondylium			27	4.4 (3.6)
Asteraceae				
Achillea millefolium			27	4.4 (3.6)
Achillea ptarmica	13	1.3 (1.2)		
Arctium lappa	13	0.1 (0)		
Arctium minus	13	26.3 (11.3)		
Artemisia vulgaris			20	10.8 (4.2)
Centaurea jacea			27	8.2 (4.0)
Cirsium arvense	20	15 (0)	7	0.1 (N/A)
Cirsium palustre	33	4.0 (2.8)	13	0.1 (0)
Conyza canadensis*	13	1.3 (1.2)		
Crepis foetida	7	0.1 (N/A)		
Hieracium premanthoides	7	2.5 (N/A)		
Jacobaea vulgaris	13	0.1 (0)	7	0.1 (N/A)
Lactuca virosa	27	5.0 (3.4)		
Lapsana communis	7	15.0 (N/A)		
Matricaria recutita	13	7.6 (7.5)		
Tanacetum vulgare	20	10.8 (4.2)	27	20.0 (14.5)
Taraxacum officinale	13	0.1 (0)		
Boraginaceae				
Anchusa arvensis			13	7.6 (7.5)
Brassicaceae				
Brassica nigra	100	60.5 (8.1)		
Bunias orientalis*			87	26.4 (5.8)
Caryophyllaceae				
Saponaria officinalis			27	17.5 (7.3)
Silene latifolia			7	2.5 (N/A)
Convolvulaceae				
Calystegia sepium	13	18.8 (18.7)	7	2.5 (N/A)
Convolvulus arvensis	7	2.5 (N/A)	7	0.1 (N/A)
Cuscuta europaea	13	32.5 (30.0)		
Cyperaceae				
Carex pendula	7	2.5 (N/A)	7	15.0 (N/A)
Carex praecox			40	34.6 (11.5)
Carex sp.	13	20 (17.5)		

Table S5.3 (continued)

	Br. nigra		**Bu. orientalis**	
Family/ Species	**Occurrence (%)**	**Mean cover (%)**	**Occurrence (%)**	**Mean cover (%)**
Equisetaceae				
Equisetum arvense			33	40.5 (11.0)
Euphorbiaceae				
Euphorbia cyparissias			13	0.1 (0)
Fabaceae				
Securigera varia			7	62.5 (N/A)
Trifolium pratense	7	2.5 (N/A)		
Trifolium subterraneum			7	2.5 (N/A)
Vicia hirsuta			13	0.1 (0)
Vicia sepium			7	2.5 (N/A)
Geraniaceae				
Geranium dissectum	7	0.1 (N/A)		
Geranium pusilum	7	0.1 (N/A)		
Lamiaceae				
Glechoma hederacea	73	24.8 (9.7)	27	14.4 (8.4)
Lamium album			20	10.8 (4.2)
Scutellaria columnae			7	15.0 (N/A)
Scutellaria minor	7	2.5 (N/A)		
Stachys palustris	7	0.1 (N/A)		
Plantaginaceae				
Plantago lanceolata	20	10.8 (4.2)	20	5.9 (4.6)
Plantago major			7	0.1 (N/A)
Poaceae				
Calamagrostis sp.	7	2.5 (N/A)		
Dactylis glomerata	7	15 (N/A)	20	17.5 (10.9)
Elymus caninus	27	8.8 (3.6)	67	43.3 (7.9)
Holcus lanatus			27	1.9 (0.6)
Phalaris arundinacea			13	8.8 (6.3)
Poa annua	67	52.5 (5.5)	33	57.5 (5)
Poa pratensis	13	15.0 (0)	7	2.5 (N/A)
Vulpia myuros			7	0.1 (N/A)
Polygonaceae				
Persicaria amphibia*			20	0.9 (0.8)
Polygonum aviculare	13	15.0 (0)		
Polygonum bistorta			13	7.6 (7.5)
Rumex crispus			7	0.1 (N/A)
Rumex obtusifolius	13	1.3 (1.2)		
Rosaceae				
Rubus caesius	13	1.3 (1.2)		
Rubus fructicosus	13	8.8 (6.3)		
Rubiaceae				
Galium aparine	40	20.0 (10.2)	7	0.1 (N/A)
Scrophulariaceae				
Linaria vulgaris			7	15.0 (N/A)
Verbascum nigrum			20	1.7 (0.8)
Urticaceae				
Urtica dioica	80	22.1 (6.6)	33	14.5 (6.4)

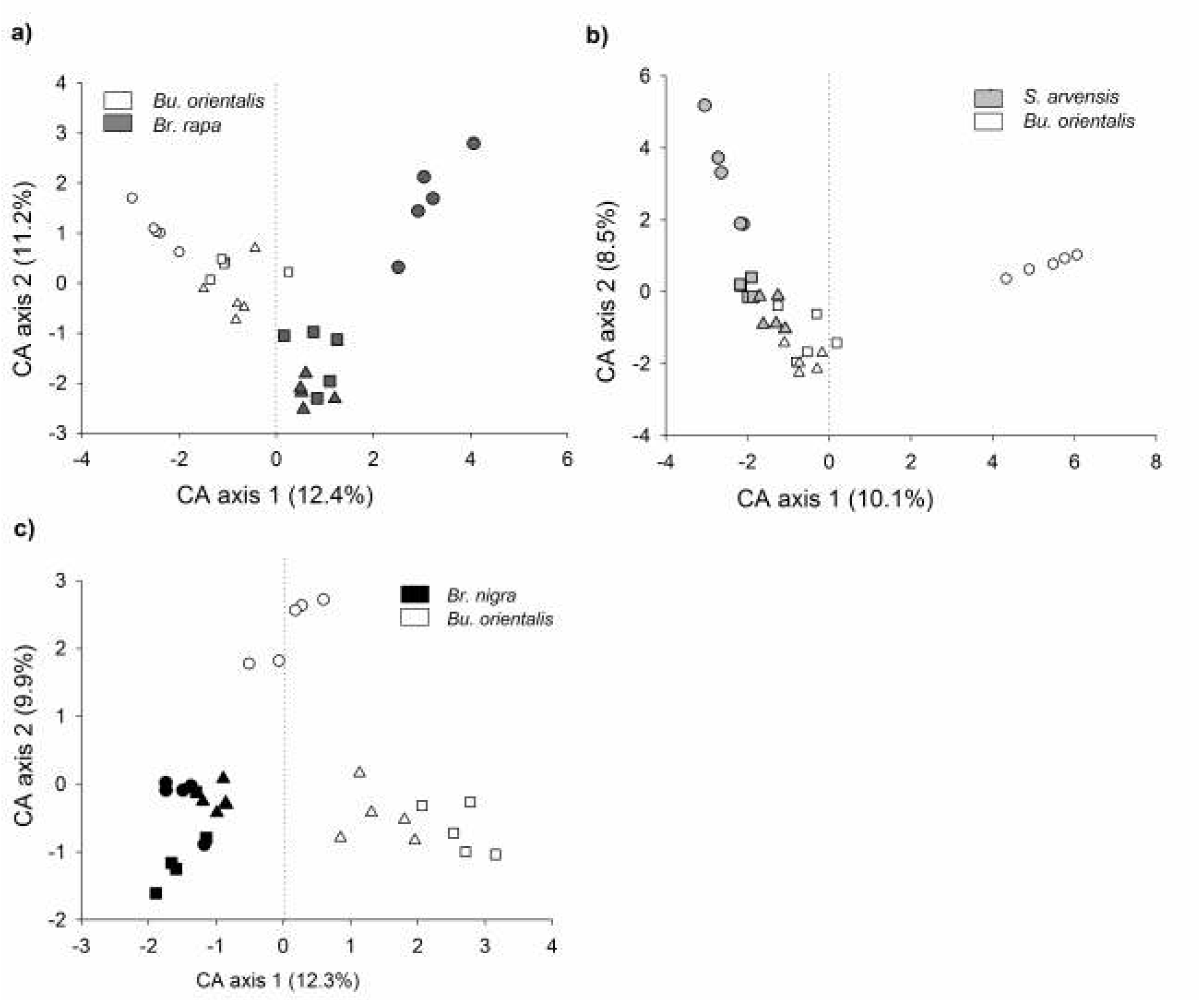

Figure S4 Two dimensional canonical analysis (CA) plots of the plant community associated with each exotic-native plant pair: a) Bunias orientalis and Brassica rapa; b) Bunias orientalis and Sinapis arvensis; c) Bunias orientalis and Brassica nigra. Different point shapes represent the sampled populations: population 1 represented by circles; population 2 represented by squares; population 3 represented by triangles. Each point represents the plant community surrounding each individual plant of the focal species (exotic or native). Percentages of total explained variation by CA axes are given in parentheses.

CHAPTER 3

Chemical and structural effects of invasive plants on herbivore–parasitoid⁄predator interactions in native communities

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Abstract

The introduction and/or spread of exotic organisms into new habitats is considered a major threat to biodiversity. Invasive plants have been shown to negatively affect native communities, competing with and excluding other plants and disrupting a wide range of trophic interactions associated with them. In spite of this, thus far few studies have explicitly studied the mechanisms underlying the displacement and potential local extinction of native herbivores and their natural enemies up to the third trophic levels and even higher. Here, we formulate hypotheses on how structural and chemical characteristics of invasive plants may affect the plant finding abilities of herbivores and the host or prey-finding behaviour of parasitoids and predators. The sudden incursion of an invasive plant into a native plant community may fragment native habitats and thus create physical and structural barriers that impede dispersal and plant finding ability for herbivores and prey or host finding ability for predators and parasitoids. At the same time, invasive plants may produce odours that are attractive to native insects and thus interfere with interactions on native plants. If invasive plants are both attractive and toxic to native insects, they may constitute 'traps' that are possibly beneficial against insect pests in agro-ecosystems but have conservation implications for native herbivores and their natural enemies. However, we also suggest that some herbivores, and by association, their parasitoids, may benefit from the establishment and spread of exotic plants because they increase the amount of available resources for them to exploit.

Introduction

Over the past century, an increasing number of anthropogenic processes have combined to threaten ecological communities, ecosystems and biomes across the biosphere. These processes include habitat loss and fragmentation, various forms of pollution, climate change and biological homogenization through the introduction of exotic species into non-native ecosystems (Kappelle and van Vuuren 1999; Novacek and Cleland 2001; van der Putten et al. 2004; Thuiller 2007; McGeoch et al. 2010). The scale of these threats has increased in recent years, especially with concomitant increases in population and per capita impacts on nature due to increases in technology. For example, the integration of the global economy, combined with habitat destruction, has accelerated the introduction and/or spread of plants and animals into new habitats. Once they occupy new habitats, a small number of invasive organisms flourish and are capable of displacing native biota, driving local extinctions, and ultimately threatening the functioning of ecosystems and interfering with a range of important services that emerge from them (Vitousek et al. 1996; Pejchar and Mooney 2009; Ehrenfeld 2010).

Predicting the impacts of invasive species on food webs and communities is one of the biggest contemporary challenges facing ecologists. Because they occur at the basal end of the food chain, invasive plants have the capacity to seriously disrupt native communities from the bottom up, competing with and excluding other plants and interfering with a wide range of trophic interactions associated with them (Cronin and Haynes 2004; Harvey and Gols 2011; Paine et al. 2011). Some invasive plants are able to colonize a broad range of native ecosystems and are able to displace native vegetation, quickly transforming these ecosystems into ones that are profoundly different in structure and composition from the original native ecosystems.

Several hypotheses have been proposed to describe that factors that help to promote the success of invasive plants. Two of these deal exclusively with the effects of the second trophic level (e.g. herbivores and plant pathogens) on the plants and vice-versa. For example, the ‘enemy-release hypothesis’ (ERH) posits that invasive plants have escaped from a wide range of co-evolved specialist natural enemies in their native range and thus experience less damage from native generalist enemies in their new range (Keane and Crawley 2002; Agrawal et al. 2005; Jogesh et al. 2008). Several studies, for example, have indeed reported that invasive plants have lower herbivore loads than related native plants (Memmott et al. 2000; Ernst and Cappuccino 2005). Furthermore, it has also been shown that some of the most successful invaders posses certain characteristics, such as novel secondary compounds, that are not found amongst native plants in the new range (Cappuccino and Arnason 2006). The ‘novel-weapons hypothesis’ (NWH) describes plants which produce unique morphological or chemical traits that confer protection for the invader against potentially new enemies in the native range that are not evolutionarily adapted to the new traits (Callaway and Maron 2006; Callaway et al. 2008). The garlic mustard, Alliaria petiolata (Brassicaceae) is native to Eurasia where it is locally common but rarely dominant. By contrast, this species is highly invasive in North America, where it aggressively out-competes native vegetation, including other forbs and even trees (Stinson et al. 2006). Alliaria petiolata produces a cocktail of toxins in its leaf tissues, including the glucopyraniside alliarinoside, as well as cyanide, which are absent or rare in other North American plant taxa (Cappuccino and Arnason 2006; Cipollini and Gruner 2007). The plant is highly toxic to the caterpillars of native white butterflies, including Pieris oleracea Harris and P. virginiensis Edwards (both Lepidoptera: Pieridae), whose adult females readily oviposit onto the plant. This has been cited as a major factor in the regional decline of these insects over the past 20 years (Keeler et al. 2006; Keeler and Chew 2008).

Going up: from two to three trophic levels and higher

One of the biggest shortcomings of current studies with invasive plants is that they have primarily focused on interactions involving only two directly interacting trophic levels i.e. the first and second. Moreover, the vast majority of these studies have been based on unraveling the various factors that enable a small minority of exotic plants to become invasive pests in their new range (e.g. Keane and Crawley 2002; Thomas and Reid 2007). However, it has long been known that a more complete appreciation of the factors that regulate the structure and functioning of terrestrial communities must include natural enemies of herbivores, such as pathogens, predators and parasitoids, as well as antagonistic interactions even further up the food chain (Hairston et al. 1960; Price et al. 1980; Hunter and Price 1992; Hunter 2003).

Recent meta-analyses have shown that top-down as well as bottom-up forces are involved in regulating terrestrial biomass (Schmitz et al. 2000; Halaj and Wise 2001; Romero and Koricheva 2011). If this is true, studies exploring the various factors that determine the success or failure of a plant to become an invasive pest in a new habitat should include natural enemies of herbivores, although this has rarely been done (but see Cronin and Haynes 2004). Thus far, current knowledge of invasive plants on native food webs (including natural enemies) is largely descriptive, for instance based on quantitative food web analyses

(Memmott and Waser 2002; Heleno et al. 2009). By contrast, few studies with invasive plants have thus far explored a range of eco-physiological mechanisms that determine: (a) the effects of primary and secondary plant compounds (nutrients and toxins) on the development and survival of herbivores and their natural enemies, and (b) structural and chemical aspects of invasive plants that affect foraging and host location behaviour in herbivorous insects and thus might lead to differential consequences on various members of an interacting food chain. The first area has recently been discussed by Harvey et al. (2010a,b). This article will address the second aspect. Our main hypothesis is that changes in the structural and chemical environment at small and medium spatial scales will affect interactions between native herbivores and their antagonists. These effects may lead to the local loss in insect abundance and/or diversity, and have consequences for the structuring of food webs. Whilst many of these effects will be negative through either the disruption of foraging behaviour of herbivores, parasitoids and/or predators, or through variable responses exhibited by the organisms across different trophic levels, we argue that alien plants may also benefit some native insects.

Structural and chemical characteristics of plants as they affect the foraging and dispersal behaviour of herbivores, parasitoids and hyperparasitoids

Vegetation complexity is characterized by two main trait-mediated aspects: structural and chemical complexity. These traits are often species-specific and diversity within and between habitat patches will strongly affect the expression of these traits. Chemical and structural complexity of plants will affect flight, host-finding, orientation, and oviposition behaviour in insects and both may act synergistically in affecting these parameters. It should also be stressed that habitats in which invasive plants establish themselves are never in stasis, but exhibit dynamic patterns of growth, biomass and species diversity that may vary considerably even over the course of a single growing season (Figure 1). This may mean that the growing phenology of seasonal invaders and the life-cycles of arthropods in their habitats may overlap to varying degrees. Furthermore, many insect herbivores and their natural enemies that are found in habitat patches may have more than a single generation per year, and thus different generations of insects may be exposed to a structural and chemical milieu that differs profoundly according to the composition of the plant community (Gols et al. 2011). For example, there is a high seasonal fluctuation in the toxicity of the invasive crucifer, A. petiolata, in North America (Haribal and Renwick 2001). The theoretical and empirical literature is replete with studies that have examined the effects of structural and chemical complexity of the plant community on the biology and ecology of herbivorous insects and their natural enemies (Root 1973; Kareiva 1985; Andow 1991; Grez and Gonzalez 1995; Renwick 2002; Meiners and Obermaier 2004; Tsharntke and Brandl 2004; Gols et al. 2005; Hambäck et al. 2006; Randlkofer et al. 2010). However, thus far this has rarely been done in exploring the potential community-related effects of invasive plants. Below we separately examine each of these areas in more detail.

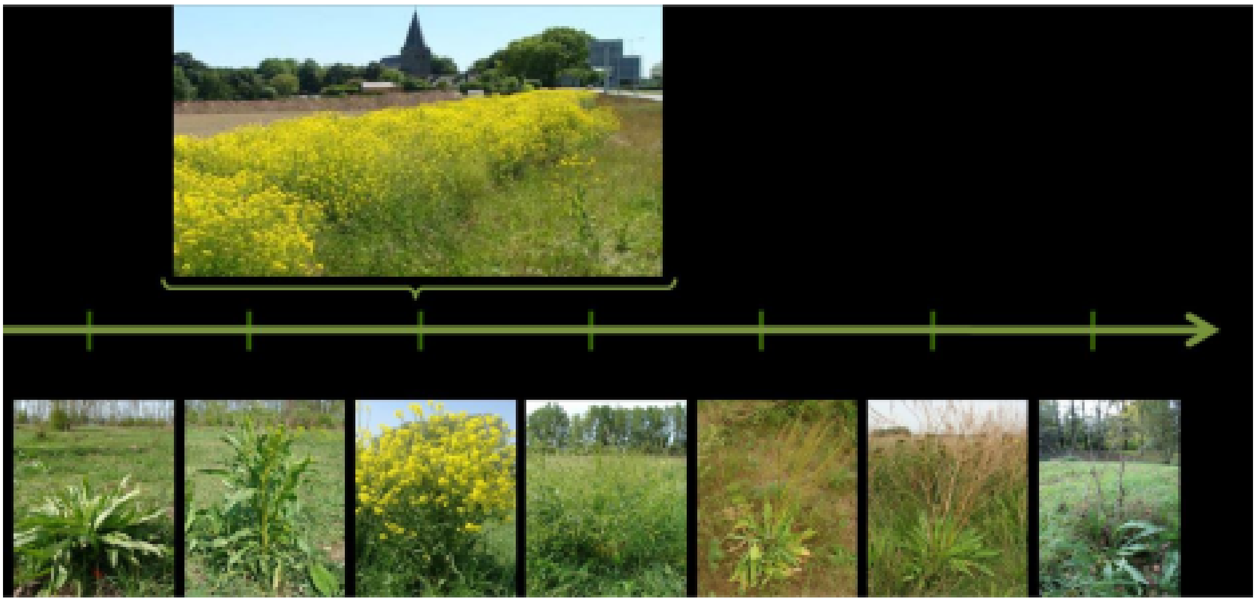

Figure 2 Phenology of the exotic plant Bunias orientalis across a single growing season in a natural population in the Netherlands. This species, which is native to central and western Asia, has recently become a highly invasive pest weed over much of central and northern Europe. The plant is a perennial that can live up to 15 years, although every winter it dies back to the root-soil interface. In early spring, rosette leaves begin to grow and flowering shoots appear in May when the plant begins to flower. Following the production of seeds (which are ripe by late July), the first rosette temporarily dies back; however, a second set of rosette leaves begins to grow soon thereafter, and remains apparent until the first frost at the end of October, when the first frosts kill the plant above the soil surface. In the peak of the summer Bu. orientalis plants grow several flowering stems and can dominate extensive areas along disturbed habitats, such roadsides as shown on the picture above taken in Drempt, the Netherlands, June 2010.

How invasive plants affect structural heterogeneity in native habitats

After they have become well established in a native community, invasive plants may displace native plants and end up occupying much of the latter's habitat, thereafter much smaller patches of native vegetation. This in turn may significantly affect insect herbivores and their natural enemies which are associated with the native plants. A large body of evidence has shown that the physical characteristics of habitats can affect intra- and inter- patch dispersal movements of insects that are associated with plants within the patches (Roland et al. 2000; Goodwin and Fahrig 2002; Cronin 2004; Cronin and Haynes 2004; Bukovinszky et al. 2005; Gols et al. 2005; Bezemer et al. 2010b; reviewed by Andow 1991; and Randlkofer et al. 2010). In theory at least, the disruption of dispersal and foraging behaviour in herbivores and their natural enemies could deleteriously affect the population dynamics of predator-prey or host-parasitoid associations and, if serious enough, eventually lead to the local extinctions of species or species interactions.

The effects of invasive plants on native consumer-based interactions are also likely to vary at the spatial and temporal scales in which they occur. Early in the season, when annual plants have only begun to grow, the structure of the plant community is likely to be much simpler than later in the season after which there has been a dramatic increase in biomass. However, even in simple landscapes and at smaller scales, the architecture of the plant stem, young shoots and leaves can either facilitate or impede insect movement amongst vegetation. If the invasive plant is taller than its native neighbors, or else produces much more densely packed foliage, then this may prove to be a major impediment in the efficiency of insects to cross habitat patches, to locate suitable food plants, or to disperse to adjacent habitat patches (Figure 2B). In one of the few studies to explicitly test the effects of an invasive plant on a native tri-trophic interaction, Cronin and Haynes (2004) examined landscape-level interactions involving prairie cordgrass, Spatina pectinata (Poaceae) an obligate specialist herbivore, the plant-hopper Prokelisia crocea (Homoptera: Delphacidae) and its specialist egg parasitoid, Anagrus columbi (Hyemoptera: Mymaridae) in mudflat plots with and without an invasive grass, smooth brome, Bromus inervis (Poaceae). The authors found that the presence of brome facilitated dispersal of the insects much more rapidly through habitat matrices and negatively affected the abundance and persistence of both species. They also found that the presence of brome resulted in a 4-5 fold increase in local extinction rates of the insects, with the parasitoid being more adversely affected than its herbivore host. It is not known if the invasive plant impeded trophic interactions through structural or chemical (or a combination of both) traits.

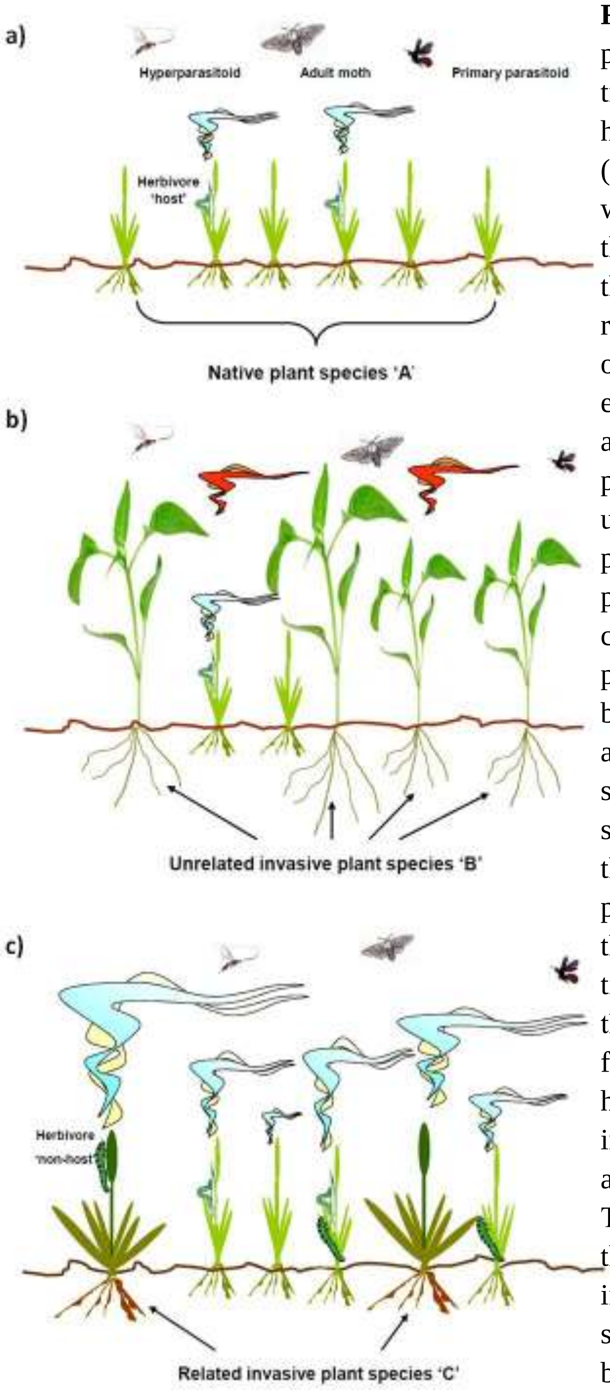

Figure 2 Conceptual diagrams showing the potential effects of invasive plants on a four-trophic level interaction involving plants, herbivores, a parasitoid and hyperparasitoid. In (a), a native plant species grows in patches where it is dominant. A specialist herbivore (in this case a moth) oviposits on the plant which, through feeding damage from the caterpillar, releases volatiles (blue plumes) that attracts one of its specialist natural enemies, a primary endoparastioid. In turn, plant related cues attract a primary hyperparasitoid of the primary endoparasitoid. In (b), a larger unrelated invasive plant species fragments patches in which the native plant grows. The plant provides structural (and perhaps chemical) barriers, such as repellents (red plumes) that interfere with the foraging behaviour of the moth, its primary parasitoid and hyperparasitoid. In (c), the invasive plant species is a close relative of the native plant species, and invades the same patches where the native plant normally occurs. The invasive plant produces volatiles that are attractive to the herbivore and its parasitoid, but the plant tissues are toxic to both of them and the plant thus represents a potential evolutionary 'trap' for these consumers. Another generalist herbivore readily feeds on both plants, increasing the level of volatiles emitted that are attractive to the herbivore and its parasitoid. This leads to 'associational susceptibility' of the specialist herbivore and its parasitoid to the invasive plant. Ultimately, the effects of structural and chemical impediments imposed by invasive plants on native insects will critically depend on how different species and trophic levels respond to them.

A number of studies with either natural or artificial plant assemblages have begun to disentangle the effects of structural barriers on the foraging behaviour of insects. Gols et al. (2005) found that the parasitoid, Diadegma semiclausum (Hyemoptera: Ichneumonidae) took longer to find larvae of its host, Plutella xylostella (Lepidoptera: Plutellidae) on the host food plant, cabbage, when the wasps were released into cages containing barley and cabbage as opposed to cabbage alone. Barley plants were taller than cabbage plants and the wasps were physically impeded during foraging flights by the presence of barley. However, some insects may forage more efficiently in complex habitats. For instance, White and Andow (2006) found that parasitism of the European corn borer by its specialist parasitoid, Macrocentrus grandii (Hymenoptera: Braconidae) was reduced by almost 100% when roots of host plants were infested with larvae of the corn rootworm. The authors revealed that root-infested plants were much smaller and grew in patches that were less dense than plants growing in the absence of rootworms, and that the parasitoids preferred to forage in structurally complex, dense habitats whilst avoiding more open habitats. These results reveal that the effects of changes in the composition structure of the plant community, potentially mediated by the presence of invasive plants, are likely to be association-specific depending on biological and life-history characteristics of species in the trophic chain under investigation.

At smaller scales, characteristics of plants, such as the shape and size of the leaves, the density in which leaf tissues grow, and the surface structure of leaf tissues can affect interactions between herbivores and their natural enemies (Grevstad and Klepetka 1992; Mulatu et al. 2006; Olson and Andow 2006). Sticky glands or trichomes, for instance, that are present on the leaf surfaces or undersides of a novel invader may either trap native insects that are not adapted to them or else impede the movement of insects on the leaf surface (Romeis et al. 1998; Lovinger et al. 2000). Additionally, the structure of the host plant may shape the odour plumes and the way that the chemical cues are perceived by insects when searching for host plants or prey (Chapman 1988). Plant structural traits (e.g. vegetation height, density, plant connectivity) can also affect microclimatic conditions, such as air flow and turbulence, which affect the emission and spread of plant volatiles within the host habitat (Randlkofer et al. 2010). These parameters will come into play if native insects must navigate through stands of invasive plants to find a native plant that is embedded in the same habitat. However, thus far studies of these plant characteristics have been restricted to native-plant insect interactions or research involving cultivars. There is clearly an urgent need for studies exploring the importance of similar mechanisms in invasive plants and how these may affect the local demographics of native insect assemblages.

How invasive plants may affect chemical heterogeneity in native habitats

Invasive plants may play an important role by interfering with the chemical heterogeneity of the introduced community. Plant volatiles, as part of this chemical complexity, are known to influence arthropod movements and plant orientation in the native host habitat (Schoonhoven et al. 2005; Figure 2a). Therefore, the foraging behaviour and fitness success of herbivores and carnivores might be influenced by novel odour blends emitted by invading plants, and this in turn may have repercussions on community composition and functioning. Although plant volatiles belong mainly to three chemical groups (e.g. terpenoids, phenylpropanoids, fatty acid derivates) and some compounds are common to most of the plants (e.g. green leaf volatiles), there is still enormous inter-specific variation in the types of plant volatiles released in nature. The specificity is expressed in the qualitative and quantitative composition of the different odour blends and on the arthropod response to the volatile bouquet (Bruce et al. 2005; Baldwin 2010). For instance, specific volatile compounds such as isothiocyanates, the breakdown products of glucosinolates that are characteristic of plants in the order Brassicales (e.g. Brassicaceae), may vary profoundly amongst different species in this order and may therefore influence the plant preference behaviour of different crucifer specialists in a wide range of ways (Hopkins et al. 2009; Müller et al. 2009). In addition, plant volatiles can vary amongst cultivated strains of wild plants (Poelman et al. 2008; Gols et al. 2011).

The chemical complexity of specific habitats is closely related to the vegetation structure and diversity within the habitat. Odour plumes in adjacent habitats may differ significantly because of differences in the species richness, abundance and structure of these habitats. Therefore, when an invasive plant colonizes a native habitat, the effect on the arthropod community will depend on several factors, including the response of native plants in the habitat and the higher trophic levels associated with them. In this context, if the invasive plant is introduced into a native plant community where there are few or no related plant species present, the odour blend emitted by the invasive plant may overwhelm volatile blends from the native plants. In this way it might dilute or mask the detection of volatiles released by the native plants by native arthropods. Odour masking has been shown to affect the foraging behaviour of parasitoids, and has even been suggested as a factor in reducing population levels of herbivorous insects in mixed cropping systems (Bukovinszky et al. 2005; Schoonhoven et al. 2005). In an even more extreme scenario, novel volatile cues released by the non-host plant may act as repellents for herbivore and carnivores within their host plant-host patch, decreasing their fitness success (Soler et al. 2007; Figure 2b). On the other hand, if the invasive plant colonizes habitats containing related native plants and shares a similar secondary chemistry with them it might attract herbivores and carnivores associated with the natives because of their similar volatile cues (Renwick 2002; Randlkofer et al. 2010 and references therein; Figure 2c). Furthermore, plant volatiles can also indirectly influence arthropod populations through the attraction of mutualists such as ants and many species of pollinators (Heil and Bueno 2007). Some volatile compounds can also act as repellents to both plant enemies and mutualists, including herbivores, parasitoids and pollinators (Soler et al. 2007; Kessler et al. 2008; Figure 2b). In addition, volatile metabolites (e.g phytohormones) can also be emitted in sufficient quantities to adversely affect the growth of neighboring plants through their effects on microbes associated with their rhizo- and phyllo-spheres. These compounds can indirectly mediate interactions between native and invasive plants, altering the plant community through plant-microbe associations (Baldwin 2010). At the other end of the spectrum, novel volatiles that are not recognized by herbivores or their natural enemies may actually benefit these consumers by making it easier to recognize cues that are attractive. Clearly there is a wide array of potential effects that may be only disentangled at the level of individual associations.

Chemical heterogeneity in native habitats is strongly influenced by plant community diversity (Randlkofer et al. 2010). Different scenarios may occur according to the diversity of the plant community where the exotic plant is introduced and how arthropods respond to the volatiles emitted by the invader (Figure 3). In a species-rich habitat, the odours of an exotic plant may attract more arthropods to the patch than would be the case when it is absent (Gohole et al. 2005). The exotic plant may be considered an evolutionary ‘trap’, however, if its odours are attractive to herbivores for oviposition, but which are not suitable for the development of their offspring (Keeler et al. 2006; Keeler and Chew 2008; Harvey et al. 2010a; Fortuna et al. 2012). On the other hand, if plant volatiles emitted by the invader have a repellent effect on arthropod foraging behaviour, this might negatively affect the ability of herbivores and natural enemies to locate suitable plants within their habitat and changes in the strength of food web interactions might be expected (Cronin and Haynes 2004; Cronin and Reeve 2005; Figure 3). In disturbed habitats, fast growing invaders (e.g. early successional species) may find optimal conditions in which to spread rapidly, creating large stands where the invading species eventually becomes dominant (Anderson et al. 1996; Woitke and Dietz 2002). In this scenario, patch colonization by arthropods may be enhanced if the volatile blend emitted by the novel plant is attractive. Consequently, native herbivores may rapidly switch to the invasive plant. Louda et al. (2001) found that native herbivores of the bull thistle, Cirsium altissimum (Asteraceae) readily attacked an invasive thistle, C. vulgare (Asteracea) suppressing establishment and spread of the latter species. This rapid switch may have been because the volatile blends emitted from both the native and exotic thistles were attractive to the native herbivores, although no mechanism was explored. On the other hand, if chemical cues emitted by an invasive plant are repellent to many native herbivores, even when the plant may be nutritionally suitable, then this may allow the plant to escape from its antagonists in support of the ERH.

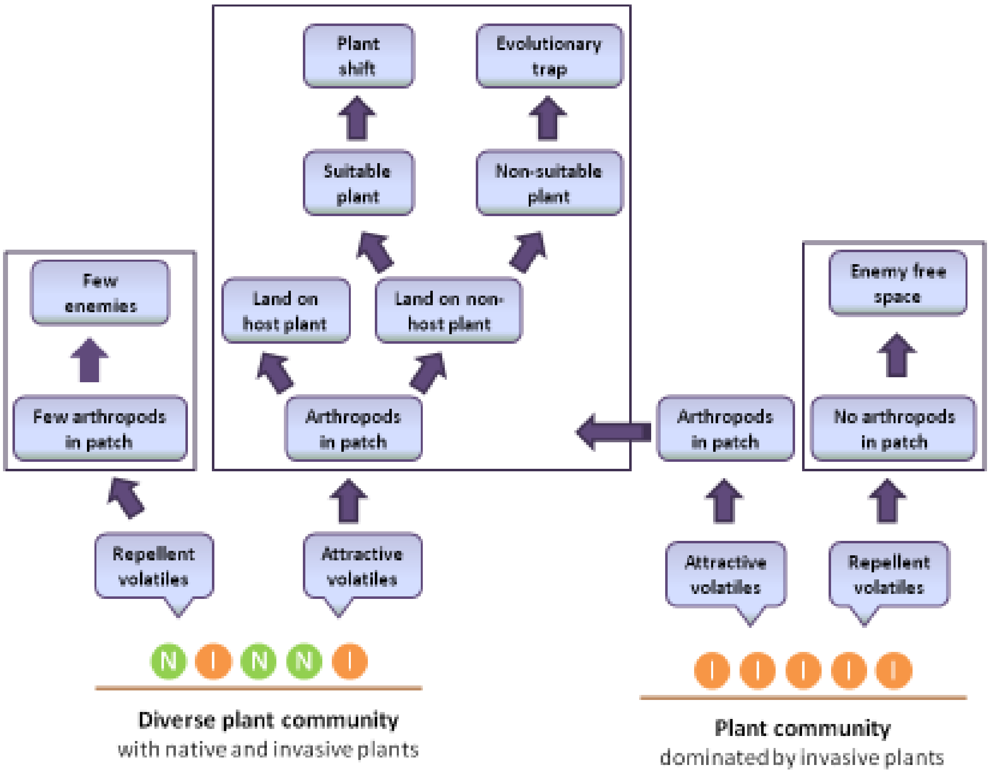

Figure 3 Flow diagram showing the different scenarios describing the potential effects of plant volatiles on arthropod communities based on the diversity of the native (N) plant communities where the invasive (I) plant is introduced. Attractive volatiles emitted by the invaded plant community may lead to a plant shift by local fauna if the invasive plant is suitable or to an evolutionary trap if the invasive plant is not suitable. Alternatively, if invasive plant volatiles trigger a repellent response on local arthropods, the patch will hold few or no plant enemies and the invasive plant may find a time window that enables it to spread and eventually become dominant, supporting the ERH hypothesis.

The majority of plants in the field are attacked by a wide array of herbivores, including species that exploit the plant in quite different ways. Hence, the chemical heterogeneity of a given habitat will change according to the herbivore-induced plant volatiles emitted by the native plant community where the exotic is introduced. In addition, the amount of volatile compounds released by plants under herbivore attack varies with the herbivore species and such differences might also be detected by natural enemies (Schoonhoven et al. 2005). Herbivore-damaged plants may increase their production of secondary metabolites that are involved in direct and indirect defence, which will contribute therefore for an increase of volatile emission in the habitat as a whole. These qualitative and quantitative changes of the odour plumes might alter the foraging behaviour of herbivores and their natural enemies. For instance, Soler et al. (2007) showed that the foraging behaviour of Cotesia glomerata (Hymenoptera: Braconidae) a parasitoid of an aboveground herbivore can be influenced by belowground herbivory through changes in plant volatile blend. Additionally, plants do not only react to herbivore feeding, but they can adjust their metabolism upon egg deposition by insects (Hilker et al. 2002; Hilker and Meiners 2006). Studies have shown that herbivore oviposition can suppress constitutive and herbivore-induced volatiles (Bruce et al. 2010; Peñaflor et al. 2010), which may influence a plant's response to herbivory and affect the interactions with associated organisms, such egg parasitoids (Fatouros et al. 2005). Assuming that an exotic plant introduced in similar systems might be initially less susceptible to herbivore attack than its native neighbors, we might predict that host preference behaviour of herbivores and parasitoids might change based on qualitative and quantitative differences in the chemical induction of the invader compared with the more heavily-damaged natives. Moreover, natural enemies will often have to choose between plants containing host and non-host herbivores and plants infested with only non-host species (Figure 2c). It has been reported that some parasitoid species are attracted to infochemicals emitted from the leaves of plants containing non-host herbivores (Vos et al. 2001; Snoeren et al. 2010). In extreme cases, parasitoids are as equally attracted to certain species of 'clean' (e.g. host-un-infested) plants as they are to closely related plants infested with hosts (Bukovinszky et al. 2005). Chemical signals from exotic plants and how the insects perceive them may therefore affect local diversity by differentially affecting the behaviour of various species in food chains.

The perception of volatiles in the invaded habitat is restricted to the sensorial capacity of arthropods to distinguish the different compounds in the odour plumes. For host plant recognition the insect requires a highly sophisticated detection mechanism enabling it to identify the correct volatile blend against a background of different compounds that are constantly being emitted by non-host plants. A host plant is recognized if the herbivore is attracted by the correct combination of sensory inputs. In contrast, a plant is considered as a non-host plant when wrong chemical cues are detected or there is a repellent response by the insect. Several studies have been shown that the majority of chemical receptors found on the antennae of herbivores and parasitoids do not only respond to the volatiles of a single host plant. Instead, they rely on the recognition of particular blends of volatiles distributed generally among a range of plant species found in their habitat (Rojas 1999; Bruce et al. 2005; Gouinguené et al. 2005; Jönsson and Anderson 2008; Ngumbi et al. 2009). Behavioural studies suggest that the blend composition of plant volatiles is crucial because specific mixtures are more attractive than individual compounds (Fraser et al. 2003; Natale et al. 2003). For instance, blends of ubiquitous compounds have been found to attract the specialist wheat midge, Sitodiplosis mosellana (Diptera: Cecidomyiidae). The midge is highly sensitive to subtle changes in the ratios of host-plant volatiles and exhibits no response when the incorrect ratio of ubiquitous compounds is presented (Birkett et al. 2004). The presence of an invasive plant may similarly alter the blend ratio of volatile compounds in native plant communities which will have repercussions on the host plant-host recognition behaviour of herbivores and their natural enemies.

The effects of invasive plants on native insect communities via associational resistance and susceptibility

Characteristics of plants and habitat patches have long been known to strongly influence the demographics of insect herbivores and their natural enemies through differences in structural and chemical properties amongst the plants and their neighbors. Several hypotheses have been proposed to explain the relationship between vegetation complexity in a habitat and the diversity of herbivorous and carnivorous arthropods. The ‘resource concentration hypothesis’ (Root 1973) posits that plants growing in more diverse habitats harbor less herbivores than plants growing in less diverse habitats. This is because herbivores are able to locate and colonize larger patches of suitable food plants more easily. The ‘natural enemy hypothesis’ (Root 1973) predicts that natural enemies will be more abundant (and thus reduce herbivore populations more effectively) in structurally complex habitats, because these will contain more micro-niches where the insects can hide as well as larger numbers of potential hosts or prey. One of the shortcomings of these hypotheses is that they were originally postulated on the basis of crop plants (e.g. cultivated cabbage) growing in agricultural landscapes. However, agricultural fields are generally simple environments, where interstitial vegetation has been removed. This makes them often much more susceptible to herbivores, or else population ‘sinks’ where insect populations become saturated.

The species diversity and structural and chemical complexity of natural and semi-natural plant communities is often many times greater than that found in agro-ecosystems. In these communities, interactions between different plant species may act in synergy in effecting the behaviour of herbivores and their natural enemies. For instance, specific plant associations may enhance (known as 'associational susceptibility') or decrease (known as 'associational resistance') the attractiveness of focal plants to herbivores, predators or parasitoids (Barbosa et al. 2009). Thus far, this area of research has focused almost exclusively on interactions involving native plants and their insect communities. Associational resistance or susceptibility may be mediated by chemical or structural traits in plants that influence the behaviour of insects within habitat patches (Andow 1991; Stiling et al. 2003; Hambäck et al. 2006; White and Andow 2006; Adati et al. 2011; Jactel et al. 2011). Recent studies have suggested that increased plant floral diversity around the perimeter of cropping systems may even enhance the abundance of hyperparasitoids, thus negating the benefits of attracting primary parasitoids through associational susceptibility (Araj et al. 2009). It is almost inevitable that novel, invasive plants will also facilitate the resistance and/or susceptibility of native plants to interactions with higher trophic levels, although thus far this area has received little attention.

Invasive plants may also act as chemical lures and traps for native insects, with potential benefits as well as costs in terms of biological control and conservation (Renwick 2002). In the vicinity of agricultural systems for example, invasive plants that become established may attract native herbivores that may not be able to detoxify or excrete their toxic allelochemicals. For instance, Harvey et al. (2010b) found that larvae of the small and large cabbage white butterflies (Pieris rapae and P. brassicae), which are major pests in collard crops, performed very poorly when were fed on leaves of the invasive crucifer, Bunias orientalis (Brassicaceae). In spite of this, adult female butterflies of both species, and especially P. rapae, oviposit onto leaves of this plant.

Over time invasive plants may serve as food plants for a diverse range of native insect herbivores (Louda et al. 2001). If an invasive plant is of high nutritional quality for a certain herbivore species, then over time this might facilitate a plant shift, whereby that herbivore becomes locally (or more widely) adapted to the novel plant (Grosman et al. 2009). This may reduce pressure from this herbivore on other native food plants with which it has long been associated. At the same time, strongly co-evolved natural enemies of the herbivore may 'follow' their host/prey and also develop a new and intimate relationship with it and its new food plant over time. In reducing herbivore pressure because of the presence of the invader, native plants may develop associational resistance as a result of herbivore switching (Andow 1991; Barbosa et al. 2009).

The potential benefits of invasive plants on native insects

The effects of invasive plant species on native insects, at least in terms of their demographics and populations dynamics is poorly understood, but the effects are not necessarily always negative. In particular, if the invasive plant possesses traits, such as allelochemistry, that are also found in potentially less common native plant species, then the presence of a more common invasive plant may benefit native insect communities. Native plants may decline due to a range of anthropogenic stresses including habitat loss and climate change, meaning that successful exotics may ultimately substitute as food plants. Indeed, several studies have reported host-plant switches by native generalist and specialist herbivores to invasives. For instance, larvae of the southern white butterfly, Pontia protodice (Lepidoptera: Pieridae) which is native to the southeastern United States, feed on a range of well established exotic cruciferous plants (Brassicaceae) that originate from Eurasia (Kingsolver 1985). Similarly, Chew (1981) found that P. oleracea (Lepidoptera: Pieridae), which occurs over much of the United States and southern Canada, oviposits and feeds on several exotic crucifers including Brassica rapa, Br. nigra, Sisymbrium altissimum and Raphanus raphanistrum. In California, 34% of the states native specialist butterfly species were found to feed on exotic plants (Graves and Shapiro 2003). Urban butterflies in California were even found to be dependent on alien plants for their survival (Shapiro 2002). The specialist herbivore, the Baltimore checkerspot butterfly, Euphydryas phaeton (Lepidoptera: Nymphalidae) has recently expanded its dietary breath from feeding exclusively on turtlehead, Chelone glabra (Scrophulariaceae) to also feeding on the introduced weed, ribwort plantain Plantago lanceolata (Plantaginaceae) (Bowers et al. 1992). However, herbivores performed less well on the alien plant, in terms of reduced pupal mass and relative growth rate on plantain, suggesting that there may be a trade-off between plant quality and accessibility. Generalist herbivores, such as woolly bear caterpillars (Lepidoptera: Arctidae) also feed on a wide variety of abundant alien plants in North America, including plantain, Plantago lanceolata (Plantaginaceae) and dandelion, Taraxacum officinale (Asteraceae; Dethier 1980).

By association, natural enemies may follow their herbivore prey or hosts to the novel plant, although this is not always a given (Grosman et al. 2009). The ability of predators and parasitoids to adapt to novel plants and to enjoy realized fitness is dependent on the completion of several hierarchal steps involving the location of suitable habitat, plant location, prey/host acceptance and palatability (Vinson 1976). Although these processes have been well studied with insect herbivores and their natural enemies in native communities, little is known about shifts involving several trophic levels from native to alien plants. Several studies have shown that the buckeye butterfly, Junonia coenia, and several of its predators are now intimately associated with the alien plant, P. lanceolata in western North America (Stamp and Bowers 1992; 1993; 1996). However, the time scales over which shifts occur, and the degree of success up to the terminal end of the food chain has been little studied.

Conclusions and future directions

It is clearly evident that there are many gaps in our understanding of invasive plants on the population ecology of multitrophic level interactions involving insect herbivores, their natural enemies such as parasitoids and predators, and even higher up in the food chain (e.g. hyperparasitoids). To fill in these gaps we need many more studies combining various interdisciplinary approaches and that are carried out over a wide range of scales to better elucidate the local and broader effects of invasive plants on native communities. Cronin and Reeve (2005) argued that most predator-prey models are inadequate in predicting the dynamics of trophic interactions because they generally ignore landscape-level processes. For instance, predator-prey models are spatially unrealistic, ignoring the size and structure of habitat patches and their edges and how these parameters affect dispersal, foraging and mate-finding behaviour in insects. At the same time, the models usually focus on only two-trophic levels (e.g. host and parasitoid) whilst ignoring the role plant related traits such as stem and shoot architecture on host location and nutritional quality on herbivore and parasitoid development. Additionally, given the potential importance of carnivores in regulating prey numbers and how plant volatile cues are crucial in the foraging behaviour of carnivores (Vet and Dicke 1992; Steidle and van Loon 2003, Bukovinszky et al. 2005), studies with invasive plants should include a larger number of interactions that include higher trophic levels, such as parasitoids and hyperparasitoids (Harvey et al. 2010b).

Importantly, most studies with invasive plants have focused their effects on native communities long past the establishment phase when they are already dominant. Patterns of weed spread show that many exotic species have long ‘sleeper’ or ‘lag’ phases after their introduction before they begin to dominate local ecosystems. Hobbs and Humphries (1995) argued that early detection and treatment of invasions before the rapid spread occurs may enable the development of more effective management strategies. Thus far, little is still known about the impact of invasive species on native communities during the establishment and lag phases. Many intra-continental plant invasions are now occurring as result of anthropogenic processes such as climate warming whereby plants are colonizing more poleward biomes from their natural ranges in lower latitudes (Engelkes et al. 2008). A challenging way to understand how species responses change from the lag phase to later stages of invasion is to study the ecology of these species along environmental and/or geographical gradients. Studies comparing changes in population patterns and effects of exotic species on local communities across spatial gradients can help to reconstruct how the invasion occurred and which factors favored or limited its spread at different phases of invasion (Dietz and Edwards 2006).

Thus far, the effects of differences in habitat structure on multitrophic interactions have been primarily based on population-related studies, whilst often ignoring traits and characters of the species involved. For instance, the effects of invasive plants on native arthropod communities may be contingent on certain life-history and morphological traits exhibited by higher trophic levels (Fortuna et al. 2012). These may include differences in feeding strategies, degree of dietary specialization, morphological traits like the presence or absence of wings, reproductive biology, and in parasitoids the stage of host attacked. To be honest, the importance of these parameters has even rarely been investigated in native communities, where differences in habitat characteristics have instead considered herbivores and their natural enemies on their trophic status whilst ignoring more intimate aspects of their relationships with each other. However, a better understanding of results such as those generated by Cronin and Haynes (2004) may be obtained by incorporating an evolutionary perspective of the interactions between different trophic levels.

In addition, although several studies have focused on insect orientation based on host-plant volatile emissions, most of these are based on behavioural essays in Y-tubes or flight chambers where the insect response is measured toward a single plant or a small group of plants. Future research should include a more realistic picture of invaded communities by measuring their structural and chemical complexity under field conditions. There is a clear need to develop a better understanding of the species composition of communities with invasive plants and to compare them with less disturbed native communities. Furthermore, experiments measuring insect preference and performance in both the laboratory and in the field can tell us much about the impact of invasive plants in native communities (Fortuna et al. 2013). These studies should be combined with the measurement of allelochemicals in plant tissues and headspace analyses to measure volatile emissions. Additionally, conceptual models of odour plume diffusion under different conditions, including air turbulence, light intensity, and species composition, may help to understand better the effect of novel community assemblages on insect foraging behaviour.

Although the field of invasion ecology has grown immensely over the past 20 years, there are still large gaps in the empirical literature, particularly with respect to the effects of invasive plants on native communities. We suggest that this gap can be filled by addressing some of the following questions:

1. How do alien, invasive plants affect the foraging and dispersal behaviour and development of native insects?
2. Are these effects mediated by primary and secondary chemistry or by structural and architectural characteristics of the plant or are the factors inter-related?
3. Do these effects differ for different members of an interacting trophic chain?
4. If the effects of invasive plants are disproportionate amongst i.e. herbivores and their natural enemies, can invasive plants create stronger trophic cascades or, alternatively, reduce the strength of top-down control?
5. How do the spatial and temporal dynamics of the spread of invading populations affect native multitrophic interactions?
6. What are the longer term implications of invasive plants on native food webs and their persistence?

Addressing these questions will require a multidiscliplinary approach integrating population and evolutionary ecology as well as ecophysiology. However, by doing so we will be able to better appreciate both large- and small scale effects of invaders on multitrophic interactions in native plant-arthropod communities.

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CHAPTER 4

Effects of an invasive plant on the performance of two parasitoids with different host exploitation strategies

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Abstract

In their new range, exotic plants create the possibility for novel interactions to occur with native consumers. Whereas there is evidence that these novel interactions can be negative for native insects, alien plants that are closely related to native species may in fact act as important food sources for native insects during the growing season. Thus far, studies with invasive plants have mostly focused on plant-herbivore interactions. However, to better understand how top-down and bottom-up processes may affect the success of potential invaders we also need to consider the effects of invasive plants on higher trophic levels. We examine multitrophic interactions on an exotic invasive crucifer, Bunias orientalis, and a native crucifer, Brassica nigra. The performance of a specialist herbivore, Pieris brassicae, and two of its gregarious endoparasitoids, the koinobiont Cotesia glomerata and the idiobiont Pteromalus puparum, was investigated. Emphasis was laid on parasitoid host-resource use strategies and how these may be differently affected by the quality of the exotic food plant. Pieris brassicae larvae performed poorly on the exotic plant, with lower survival, longer development time and a lower pupal mass, than on the native plant. The exotic plant affected the performance of the two parasitoid species in different ways. Cotesia glomerata survival was strongly co-ordinated with the survival of its larval host, showing also high mortality. Adult wasps that survived on Bu. orientalis had an extended development time and small body size. By contrast, Pt. puparum survival was similar on pupal hosts reared on both plant species. Our results show that constraints imposed by differing plant quality of native and exotic plants on trophic interactions can depend on resource use strategies of the species involved, suggesting that effects of exotic species should be elucidated on a case-by-case basis.

Introduction

The introduction of exotic organisms into non-native ecosystems is considered to be a major threat to biodiversity. Invasive organisms have the potential to disrupt trophic interactions, which may ultimately lead to the unravelling of food webs and the weakening of ecosystem services with huge economic costs (Pimentel et al. 2000). However, recent evidence suggests alien species can also become integral components of the ecosystems that they invade. A recent study by Pattemore and Wilcove (2012) argues that invasive organisms often take on important biological roles, such as pollinators or pest controllers, once held by the species they either displaced or that have become rare due to other anthropogenic causes (e.g. climate change, habitat destruction). This suggests that our approach to the effects of alien species should be elucidated on a case-by-case basis (Davis et al. 2011).

Plants, amongst the most important of invasive organisms, can exhibit disproportionate effects on food webs because they occur at the bottom of the food chain. To understand the effects of exotic plants on natural and managed ecosystems, it is important to understand the potential mechanisms that allow a very small percentage of exotic species to become invasive. Several hypotheses have been proposed to address the relationship between phenotype and invasion success. For example, exotic plants may possess pre-adapted traits, such as chemistry or structure that are novel in the new range, enabling them to repel or resist attack from native herbivores and pathogens (Keane and Crawley 2002; Mitchell and Power 2003; Cappuccino and Arnason 2006). According to 'ecological fitting', described by Janzen (1985), organisms are able to persist in novel environments if a suite of evolved traits they bring from their native habitat enables them to enjoy realized fitness in the new habitat. Therefore, in the short term, phenotypic characteristics of many associations that occur in nature are not necessarily the result of long co-evolution, but of recent novel interactions (Agosta and Klemens 2008).

However, although some exotic plants might be released from the natural enemies from their native range, interactions between these plants with competitors and natural enemies in the new range are still very likely to occur (Gerber et al. 2008; Verhoeven et al. 2009). Consequently, rather than escaping from their consumers, exotic plants might simply interact with new insect assemblages in their new range (Müller-Schärer and Steinger 2004; Liu and Stiling 2006). Invasive plants can have negative effects on native insects, with implications for their conservation. For example, the garlic mustard, Alliaria petiolata, which is native to Eurasia, has become a dominant weed in much of eastern North America. It has displaced native food plants of several native white butterflies, Pieris oleracea and P. virginiensis, that in turn readily lay their eggs on A. petiolata but whose larvae die early during their development (Keeler and Chew 2008). In contrast, in some cases exotic plants can be beneficial to native consumers (Graves and Shapiro 2003; Pattemore and Wilcove 2012; Harvey and Fortuna 2012). For instance, in North America exotic plants (e.g. Plantago lanceolata, Taraxacum officinale, as well as several Brassicas) now constitute important food sources for the larval stages of many native butterflies species, including P. oleracea (Graves and Shapiro 2003). This shows that exotic plants have the potential to become an integral part of the native flora and to fit in naturally with many native fauna (Davis et al. 2011; Pattemore and Wilcove 2012).

Furthermore, although many studies have demonstrated either positive or negative effects of novel interactions on the behaviour and development of naïve herbivores (Wolfe et al. 2004; Keeler and Chew 2008; Harvey et al. 2010a; Oduor et al. 2011), multitrophic interactions, i.e. between exotic plants, herbivores and their natural enemies, have been little studied (Cronin and Haynes 2004; Stenberg 2012). Because of this, important information on ecophysiological aspects of these interactions is lacking (Harvey et al. 2010b; Harvey and Fortuna 2012). Carnivorous insects have long been recognised to play a significant role in affecting the structure and function of terrestrial communities (Price et al. 1980). Parasitoids generally rely on a limited amount of resources that are obtained from a single host. Because of this, their ontogeny is strongly correlated with host quality, defined as variation in the host size or nutritional value, which consequently affects parasitoid fitness (Price 1973; Godfray 1994; Harvey 2005). Many studies have shown that differences in plant quality can affect parasitoid development via its effect on their host (Campbell and Duffey 1979; Harvey 2005; Bukovinszky et al. 2008). However, parasitoid performance is not always affected by lower quality host resources (Gols et al. 2008b), showing that the effects of plants on trophic interactions is generally highly association-specific (Harvey 2005). Amongst parasitoids species, host resources are exploited in two different ways. Idiobiont parasitoids are species that develop in non-growing hosts, such as eggs, paralyzed larvae or pupae. In contrast, koinobiont parasitoids attack hosts that continue to grow after parasitism (Askew and Shaw 1986). Mackauer and Sequeira (1993) proposed three models, one for idiobionts, and two for koinobionts, to predict the effects of host quality on parasitoid development. The idiobiont model predicts that offspring development is dependent on the quality and amount of resources available at the time of oviposition, because idiobionts exploit host resources that are effectively static (Mackauer and Sequeira 1993). Consequently, host size can generally be taken as an index of host quality and large hosts are usually assumed to be of higher quality because they contain more resources for parasitoid development (Godfray 1994). Alternatively, the koinobiont models predict that host quality is dependent on the rate of host growth after parasitism and on the final size of the host, because host resources exploited by koinobionts are dynamic. Although the models have been empirically tested at the level of two (host-parasitoid) trophic level interactions (e.g. see review by Harvey 2005) they have rarely been tested when incorporating the food plant as an additional constraint on host (and in turn parasitoid) development.

The Turkish rocket Bunias orientalis L. (Brassicales: Brassicaceae) is a perennial wild crucifer native to SE-Europe and SW-Asia (Tutin et al. 1993). Over the past 30 years it has become a highly invasive species in many parts of northern and central Europe establishing on roadsides, edges of arable land and more recently, in meadows, orchards and vineyards (Steinlein et al. 1996). At the same time, the plant remains less common (although increasing) in other parts of Western Europe, including The Netherlands (Harvey et al. 2010a). Bunias orientalis is a good example of a ruderal invader, which behaves opportunistically and is well adapted to anthropogenic disturbance regimes such as mowing and soil perturbation (Steinlein et al. 1996).

In the current study we examine the development and survival of the specialist herbivore, P. brassicae, and two of its gregarious primary parasitoids, the koinobiont larval parasitoid Cotesia glomerata L. (Hymenoptera: Braconidae) and the idiobiont pupal parasitoid Pteromalus puparum L. (Hymenoptera: Pteromalidae), on the exotic invasive crucifer, Bu. orientalis, and on the native crucifer, Brassica nigra L. (Capparales: Brassicaceae). Brassica nigra is an annual crucifer abundant in The Netherlands and serves as a main food plant for the larvae of pierid butterflies, including P. brassicae (Feltwell 1982; Gols et al. 2008b; Harvey et al. 2010a). This study aimed to reveal (a) how local insect assemblages respond both developmentally and behaviourally to the novel plant, and (b) how natural enemies of important herbivores may prove to be an impediment in the control of the invasive plant. We tested two hypotheses: (1) the native plant is of higher quality for the herbivore development than the invasive plant; (2) the koinobiont parasitoid will suffer larger costs in fitness than the idiobiont, because host quality is less predictable for the former parasitoid at the time of oviposition.

Material and Methods

Plants

The Turkish rocket, Bunias orientalis, is a perennial long-lived plant with an extensive growing season, from April to October. The seeds germinate in spring producing a dense rosette and in May reproductive individuals develop flowers. The hard fruits ripen by late July and a second rosette is then produced, which survives until October (Steinlein et al. 1996). The black mustard, Brassica nigra, is an annual species with a short life cycle that grows naturally in The Netherlands. It is native to Europe and other parts of North Africa (Oduor et al. 2011). Seeds germinate in spring, but plants do not flower until mid-July, setting seeds in early September. Similar to Bu. orientalis, Br. nigra grows in ruderal sites, producing a significant plant biomass, which serves as a major food plant for the summer generations of specialist herbivores, such as P. brassicae (Gols et al. 2008b). Brassicaceous species are known to have important defences against enemies, which include qualitative defences as the production of glucosinolates, secondary metabolites typical for this family (Renwick 2002), and quantitative defences based on digestibility reducers, as cellulose, tannins, proteinase inhibitors, and trichomes (Travers-Martin and Müller 2008). Both Bu. orientalis and Br. nigra biosynthesise glucosinolates, in which sinalbin is the main compound in Bu. orientalis, and sinigrin in Br. nigra (Harvey et al. 2010a). Seeds of Bu. orientalis were collected from an established wild population growing in a field adjacent to the Max Planck Institute for Chemical Ecology in Jena, Germany (N 50°52'42" E 11°35'77") in September 2006. Brassica nigra seeds were obtained from a wild, local population growing along the riverbank of the Nederrijn in Wageningen, The Netherlands (N 51°57' 37" E 5°40'45") in August 2005. The seeds of Bu. orientalis were extracted by cracking the seed coat as mentioned by Harvey et al. (2010a). Seeds of both plant species were then germinated in plastic boxes (18x13x6 cm). After one week, the seedlings were transferred to 1.2 L pots filled with potting soil (30% sand, 5% clay, and 65% peat). Plants were grown in a greenhouse at 22 ± 1ºC (day) and 16 ± 1ºC (night), with 40-70% RH and a photoperiod of L16:D8 hours, at the Netherlands Institute of Ecology, Wageningen. Supplementary illumination was supplied by metal halide bulbs (minimum 225 µmol photons m^{-2} sec^{-1} PAR) when the natural illumination dropped below 500 µmol photons m^{-2} sec^{-1} during the light period. Plants were watered daily and after 4 weeks, 0.5 of regular Hogland nutrient solution was supplied twice a week. Brassica nigra plants used in the experiments were 5 weeks old and Bu. orientalis plants were 6 weeks old.

Insects

The large white cabbage butterfly, Pieris brassicae (Lepidoptera: Pieridae), is widely distributed, naturally occurring over much of Eurasia, where it may have more than a single generation per year. Larvae of P. brassicae are specialist chewer herbivores of plants that produce glucosinolates. Mated female butterflies lay batches of 30 to 100 eggs on the host food plant. After emerging, first larval instars (L1-L3) feed gregariously on leaves and flowers until they disperse over the food plant in the third instar. After completing 5 instars, the larvae pupate either on or off the food plant (Feltwell 1982). Cotesia glomerata (Hymenoptera: Braconidae) is a gregarious koinobiont endoparasitoid that parasitizes L1-L3 instar larvae of P. brassicae and related species in the Pieridae. Considered a successful larval parasitoid of P. brassicae, C. glomerata can regionally inflict 100 % losses of the butterfly progeny (Feltwell 1982). The female parasitoid lays 20 to 30 eggs per larval host. After egg hatching, C. glomerata larvae feed primarily on host hemolymph and fat body until it reaches the final instar (L5), when emerges from the side of its larval host. After egression the parasitoid larvae spin yellow cocoons on the host plant and pupate (Geervliet et al. 2000; Harvey 2000). Pteromalus puparum (Hymenoptera: Pteromalidae) is a gregarious idiobiont endoparasitoid that attacks pupae of several butterfly families, including the Pieridae (Harvey et al. 2011). The female wasps host feed and uses the host hemolymph as protein source for the production of eggs (Takagi 1986; Jervis et al. 2008). At oviposition, Pt. puparum females inject permanently paralyzing venom to delay host development. The female parasitoid lays approximately 150 eggs per gram of host (Takagi 1986), which in average corresponds to approximately 35 eggs per P. brassicae pupa (Harvey et al. 2011). After egg hatching, the parasitoid larvae feed on the internal host tissue and pupate between the internal membrane and the cuticle of the host pupa. Adult wasps emerge after a few days (Harvey et al. 2011).

All insects used in the experiments were obtained from laboratory cultures that originated from cabbage fields in the surroundings of Wageningen, The Netherlands. The Pieris brassicae culture was maintained on Brussels sprout plants (B. oleracea var. gemmifera cv Cyrus) at the Department of Entomology, Wageningen University. Cotesia glomerata and Pt. puparum were obtained from parasitized larvae and pupae of P. brassicae maintained on B. oleracea plants at the Department of Terrestrial Ecology of Netherlands

Institute of Ecology, Wageningen. The herbivore and parasitoids were reared in acclimatized room at 22 ± 2ºC, 60% RH and a photoperiod of L16:D8 hours. Newly emerged adult wasps were placed in separate cages and fed with water and honey ad libitum. To ensure the female wasps were mated, both females and males were placed together in a Petri dish with drops of honey one day before the experiment. For parasitism, we used females that were 5 to 10 days old, when there is maximal egg production (Jervis et al. 2008).

Experimental set up

Herbivore and parasitoid development on Bunias orientalis and Brassica nigra

To test the effects of plant quality on the herbivore performance, larvae of P. brassicae were reared on either Bu. orientalis or Br. nigra plants. Furthermore, parasitoid fitness measures were evaluated by allowing C. glomerata and Pt. puparum wasps to parasitize and develop in P. brassicae hosts (larvae and pupae respectively) that had been reared on each plant species.

Pieris brassicae performance

Four plants of the same species were introduced in a cage (65x48x40 cm). First instar larvae (L1) of P. brassicae, less than one-day old, were placed in groups of twenty on middle-aged leaves of a single plant, and were allowed to feed freely on the other plants of the cage. We considered two treatments for each plant species: non-parasitized larvae (control) (N = 92) or parasitized larvae by C. glomerata [N = 70 (L2 hosts) or N = 84 (L3 hosts)] on Bu. orientalis, and non-parasitized larvae (control) (N = 52) or parasitized larvae by C. glomerata (N = 30) on Br. nigra. Seven replicates (cages) were set up for each treatment on Bu. orientalis, except for L3 parasitized larvae (4 replicates). Five replicates were set up for each treatment on Br. nigra. The cages were then kept in climate-controlled room at 22 ± 0.5 ºC, 50% RH and photoperiod of L16:D8 hours. Larval development was checked daily and fresh food plants were supplied regularly. The number of P. brassicae larvae in each cage was reared on no less than 20 plants of each species. The day of pupation was recorded when new pupae were formed and less than 24h later, when the cuticle had solidified, the pupae were removed from the cages and weighed on an analytical balance (Mettler-Toledo AG104, accuracy ±1 mg). The egg-to-pupa development time of P. brassicae was determined as the number of days between egg hatching and pupation. The proportion of survival was determined as the number of P. brassicae larvae that reached the pupal stage divided by the number of larvae that was placed initially on each plant species.

Parasitoid performance

Females of Cotesia glomerata that had been maintained in rearing cages were presented with second (L2) or third (L3) instar larvae of P. brassicae that had been feeding on either plant species. Parasitism was performed inside a small plastic vial, where P. brassicae larvae were presented individually on the tip of a brush to a single parasitoid female. Parasitism event was considered successful when the ovipositor was inserted into the host for 5 to 10 s according to the protocol of Harvey (2000). Each female wasp parasitized a maximum of 10 individual hosts. Parasitized larvae were placed on each plant on which they had been reared previously, in groups of up to approximately 30 individuals, and were allowed to feed until parasitoid larvae egressed from the host's body. Cocoons from individual broods were placed in Petri dishes (10 cm diameter) and checked daily until adult wasps emerged.

One-day old pupae of P. brassicae from the control treatment were placed individually in a Petri dish (10 cm diameter) for parasitism by individual Pt. puparum females over the course of 24 h. The handling time of Pt. puparum is longer than C. glomerata; it takes up to several hours for a wasp to lay a full brood (Harvey et al. 2011). After 24 h, the female wasp was removed, and the parasitized pupae were monitored daily until the emergence of adult parasitoids. Newly emerged C. glomerata and Pt. puparum wasps were killed by freezing, sexed and weighed on an analytical microbalance (Mettler-Toledo MT5, accuracy ± 0.001 mg). Adult fresh mass was averaged per clutch and calculated using a maximum of 15 randomly chosen individual wasps of each sex. Egg-to-adult development time of the parasitoids was recorded in days. Parasitoid clutch size was defined as secondary clutch size, which included both adult wasps that emerged successfully from the host, as well as parasitoid larvae that died after egression (in case of C. glomerata), or pupated successfully but died prior to adult emergence (both C. glomerata and Pt. puparum). Parasitoid sex ratio was measured as the proportion of males to females in the total number of adult wasps emerging from each clutch. Survival was measured as the proportion of parasitoid clutches that emerged from the P. brassicae larvae or pupae parasitized initially.

The above experiments were undertaken from June 2008 to April 2009 in 3 repeated trials. The performance of herbivore and parasitoids on both plant species was measured in trial 1 and 3. In trial 2 only the exotic plant species was used as host plant. In trials 2 and 3,

C. glomerata performance was tested on both L2 and L3 instars of P. brassicae growing on Bu. orientalis. For each host plant species herbivore and parasitoid performance data from the different cages were pooled.

Statistical Analysis

Herbivore and parasitoid survival was analysed using a Chi-squared test that was performed on Excel pop-tools. Performance data of P. brassicae (pupal mass and egg-to-pupa development time) was analysed by a non-parametric Mann-Whitney U-test, because the data did not meet the assumptions of normality and homoscedasticity, even after data transformation. Similarly, the performance data of the parasitoids (adult fresh mass, egg-to-adult development time, clutch size and sex ratio) was analysed by a Mann-Whitney U-test. To meet the assumption of independent samples, the data of individual parasitoids (adult fresh mass and egg-to-adult development time data) was averaged per host according to wasp sex. We only included in the analysis mixed parasitoid broods since females producing only male offspring may not have been successfully mated. Analyses were carried out using the PASW® Statistics 18 software package of SPSS Inc. The analysis of P. brassicae survival and performance included data from the two trials where the effect of the two host plant species was compared (trial 1, 3). Additionally, data from trial 2 was included in the herbivore analysis for the comparison between the different host instars of P. brassicae on Bu. orientalis. Analysis of C. glomerata survival included data from all three trials, but analysis of performance of this parasitoid only included data from trial 2 and 3, due to the small sample size related with high mortality of C. glomerata on the exotic plant. Finally, the analysis of survival and performance of Pt. puparum was based on data from trials 1 and 3.

Results

Pieris brassicae performance

In total, 182 P. brassicae larvae completed their development on Br. nigra, while, despite a much larger initial sample size, only 144 larvae survived on Bu. orientalis. Survival of P. brassicae larvae to pupal stage was, therefore, strongly dependent of the plant species (χ^2_1= 74.64, $P < 0.001$). The survival was approximately 71% on the native crucifer, Br. nigra, while only 25% of the P. brassicae larvae were able to complete the development on the exotic plant, Bu. orientalis (Figure 1). Similarly, plant species had a significant effect on P.

brassicae pupal fresh mass (Z = -12.61, P < 0.001) and egg-to-pupa development time (Z = -14.73, P < 0.001). Herbivore pupae were heavier on Br. nigra compared to Bu. orientalis (Figure 2a) and development time was longer on the exotic plant, compared to the native plant (Figure 2b).

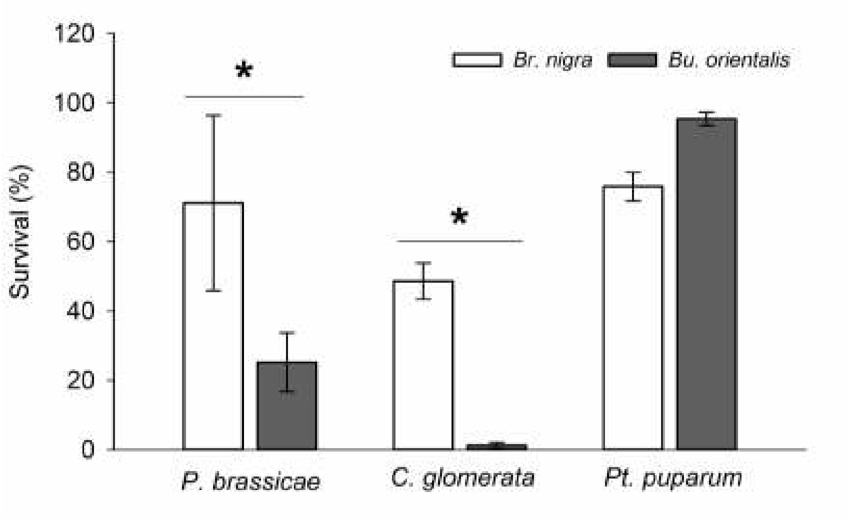

Figure 1 Survival of Pieris brassicae, Cotesia glomerata and Pteromalus puparum reared in the greenhouse on Brassica nigra and Bunias orientalis plants. Given are means ± SE; * indicates significant differences between plant species (Chi-squared test: P < 0.001). Pieris brassicae sample size on Br. nigra, N = 258, and on Bu. orientalis, N = 645. Cotesia glomerata sample size on Br. nigra, N = 143, and on Bu. orientalis, N = 493. Pt. Puparum sample size on Br. nigra, N = 103, and on Bu. orientalis, N = 46.

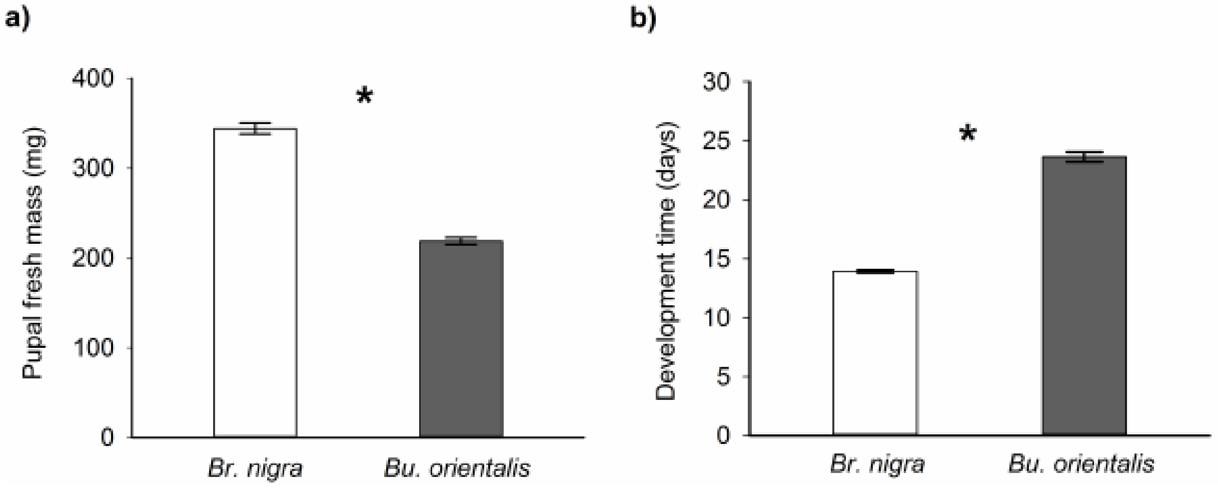

Figure 2 Pupal fresh mass (a) and egg-to-pupa development time (b) of Pieris brassicae reared in acclimatized chamber on Brassica nigra (white bars) and Bunias orientalis (grey bars) plants. Given are means ± SE; * indicates significant differences between plant species (Mann-Whitney U-test: p < 0.001). P. brassicae sample size on Br. nigra, N = 182, and on Bu. orientalis, N = 144.

Parasitoid performance: Cotesia glomerata

In the same way, the survival of C. glomerata differed according to the plant species where its larval host had fed on (χ^2_1= 79.40, P < 0.001). The wasps had a significantly higher survival (49%) on hosts developing on the native plant than on the exotic plant, where only approximately 1% of the wasps survived (Figure 1). However, the survival of C. glomerata on Bu. orientalis was significantly higher (14%) when the wasps parasitized an older stage of the host, L3 instead of L2 larvae (χ^2_1= 10.93, P = 0.001). In total 1,593 individual C. glomerata wasps were collected from 57 P. brassicae hosts on Br. nigra, and on Bu. orientalis 119 or 872 wasps were collected from 7 L2 hosts or 36 L3 hosts, respectively. Clutch size varied significantly between the two plant species (Z = -2.52, P = 0.012). Brood sizes were smaller when developing in hosts growing on Bu. orientalis than on Br. nigra (Figure 3a). However, offspring sex ratio was not significantly different in hosts growing on the two plant species (Z = -1.75, P = 0.080). Furthermore, plant species had a significant effect on adult C. glomerata body mass in both females (Z = -2.48, P = 0.013) and males (Z = -2.97, P = 0.003). In addition, adult fresh mass varied significantly with the parasitoid sex on Br. nigra (Z = -5.43, P < 0.001), but not on Bu. orientalis (Z = -1.57, P = 0.116). Overall, adult wasps were smaller on the exotic plant compared to the native plant. However, female wasps were significantly heavier than males on the native plant species, but not on the exotic species (Figure 3b). Moreover, female (Z = -2.83, P = 0.005) and male (Z = -3.478, P = 0.001) wasps developed significantly faster on Br. nigra compared to Bu. orientalis, but no differences were found in the development time between sexes within each host plant species. Both sexes had a similar development time on the native plant (Z = -1.19, P = 0.235) and on the exotic plant (Z = -0.072, P = 0.942) (Figure 3c).

Parasitoid performance: Pteromalus puparum

Pteromalus puparum survival did not vary significantly with plant species (χ^2_1 = 1.57, p = 0.211). The wasps had a relatively high survival on the exotic plant, 95 %, compared to the native plant, 76% (Figure 1). A total of 2, 117 Pt. puparum individuals were collected from 43 P. brassicae hosts on Br. nigra, and 257 wasps from 10 hosts on Bu. orientalis. Plant species did have a significant effect on the parasitoid clutch size (Z = -3.49, P < 0.001). Larger broods emerged from pupae reared on Br. nigra plants compared to Bu. orientalis (Figure 3d).

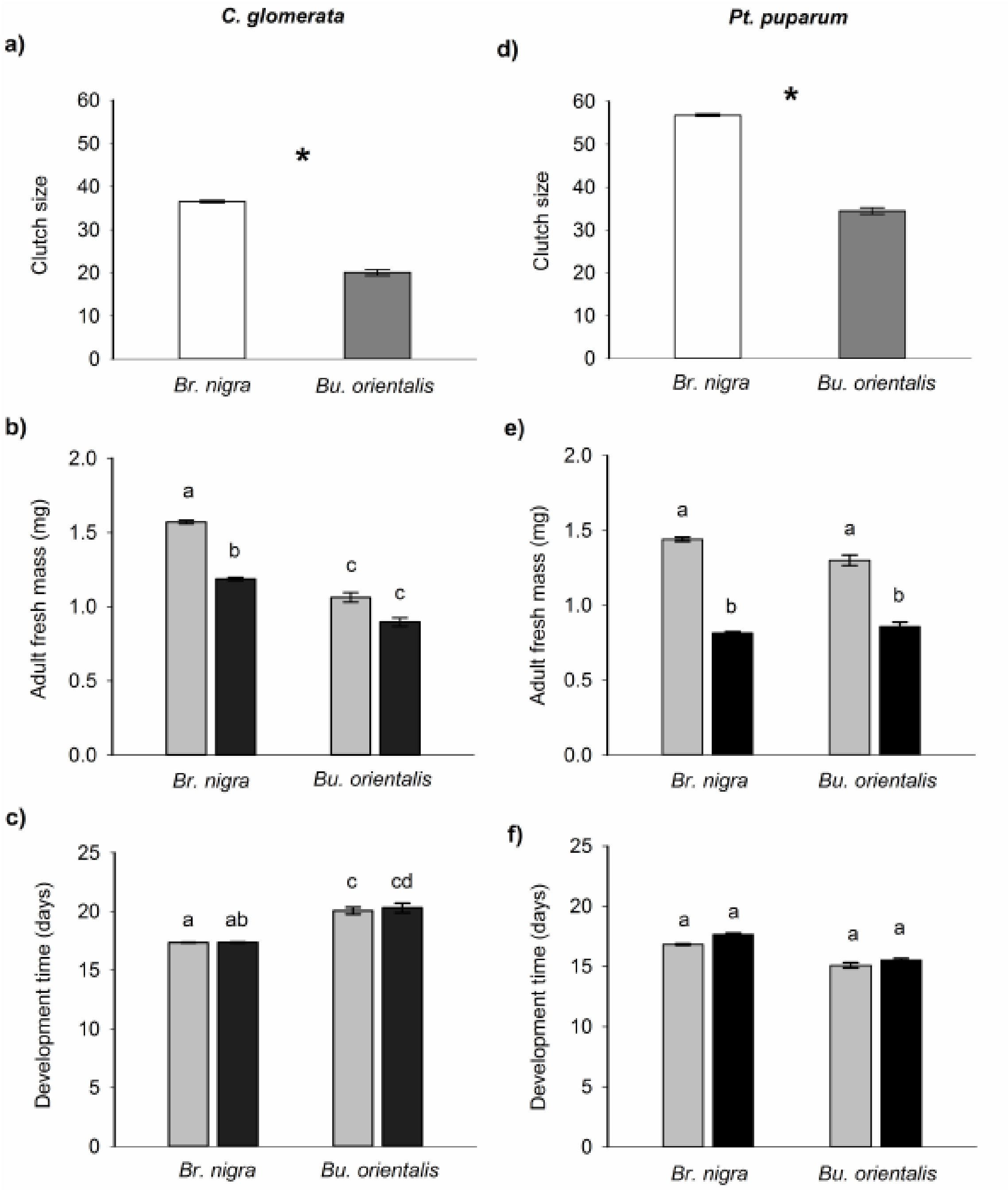

Figure 3 Clutch size, adult fresh mass and egg-to-adult development time of Cotesia glomerata (a,b,c) and Pteromalus puparum (c,d,e) reared in acclimatized chamber in Pieris brassicae hosts on Brassica nigra and Bunias orientalis plants. Given are means ± SE for females (light grey bars) and males (black bars); * indicates significant differences between plant species (Mann-Whitney U-test: $P < 0.001$). For adult fresh mass, C. glomerata sample size on Br. nigra, N = 1223, and on Bu. orientalis, N = 101; Pt. puparum sample size on Br. nigra, N = 945, and on Bu. orientalis, N = 166. For egg-to-adult development time, clutch size and sex ratio, C. glomerata sample size on Br. nigra, N = 1593, and on Bu. orientalis, N = 119; Pt. puparum sample size on Br. nigra, N = 2117, and on Bu. orientalis, N = 257.

However, no differences were found between the offspring sex ratio on the two plant species (Z = -0.53, P = 0.593). Neither was there an effect on Pt. puparum female (Z = -1.93, P = 0.054) and male (Z = -0.62, P = 0.535) fresh mass. Nevertheless, females were significantly heavier than males both when reared on Br. nigra (Z = -7.25, P < 0.001) and Bu. orientalis (Z = -2.45, P = 0.014) (Figure 3e). No significant effect of plant species was found neither on female (Z = -1.31, P = 0.191) or male (Z = -1.07, P = 0.286) egg-to-adult development time between and within plant species. Both females and males developed at the same rate on either the native (Z = -0.71, P = 0.478) or the exotic plant (Z = -0.08, P = 0.934) (Figure 3f).

Discussion

Our study shows that Bu. orientalis is a lower quality food plant for the specialist herbivore, P. brassicae when compared to the native plant Br. nigra. The survival of the herbivore was extremely low on the exotic plant. Additionally, those larvae that were able to survive on Bu. orientalis were smaller, and developed much more slowly than the ones reared on Br. nigra. Similar results have been observed in the development of other specialist-feeding pierids on exotic cruciferous plants. For example, survival of Pieris oleracea larvae is very low on the highly invasive weed Alliaria petiolata in North America, even though female butterflies often choose this plant for oviposition (Keeler and Chew 2008). The unsuitability of Bu. orientalis shown in this study was also reported in previous work, where Bu. orientalis plants were found to be highly toxic to several herbivores, including other crucifer specialists (Travers-Martin and Müller 2008; Kühnle and Müller 2009; Harvey et al. 2010a). For example, the turnip sawfly Athalia rosae L. (Hymenoptera: Tenthredinidae) had a high mortality rate on Bu. orientalis and the insects that survived had a longer developmental time and lower adult mass compared to sawflies reared on native crucifers (Travers-Martin and Müller 2008). Thus far, only few herbivores are known to commonly feed on invasive populations of Bu. orientalis in the field, including slugs (Buschmann et al. 2005), flea beetles (pers. obs. C. Müller), and the cabbage moth Mamestra brassicae L. (Lepidoptera: Noctuidae) (Harvey et al. 2010a). Pieris brassicae is known to oviposit and develop on many wild brassicaceous species. For instance, it has been documented that this herbivore develops on several novel crucifer species whose distributions do not naturally overlap with local populations of P. brassicae (Feltwell 1982). However, it appears that Bu. orientalis contain some, as of yet unidentified, compound that is toxic to specialist herbivores (Harvey et al. 2010a). The chemical profile of the plant is dominated by the aromatic glucosinolate sinalbin, which is found in several other native crucifers that are highly palatable plants for P. brassicae and other specialist herbivores (Harvey et al. 2010a). Conversely, phenylpropanoids, as coumarins and flavonoids, and hydroxycinnamic acids derivates, also known for their bioactivity, have also been found in tissues of Bu. orientalis plants (Dietz and Winterhalter 1996; Kühnle and Müller 2009). Furthermore, the low suitability of Bu. orientalis for the development of P. brassicae larvae might also be due to mechanical defences through morphological plant traits (Awmack and Leather 2002). Chemical defences are believed to be less severe on insect performance compared to mechanical defences in well-adapted crucifer specialists (Traw and Dawson 2002b; Travers-Martin and Müller 2008). Indeed, a previous study has reported a high trichome density on Bu. orientalis leaves compared to other Brassicaceae species (Travers-Martin and Müller 2008). Additionally, leaves of this exotic species are thicker, and thus potentially more difficult for the jaws of young larvae to penetrate, than the leaves of other native crucifers (pers. obs. T. Fortuna). The negative impact of trichomes and leaf toughness on feeding behaviour and survival of larvae is known for several herbivores (Traw and Dawson 2002a,b; Zalucki et al. 2002) and it might play a role on the unsuitability of Bu. orientalis for this specialist herbivore as well.

The different effects of the two food plants on parasitoid performance of C. glomerata and Pt. puparum support the predictions of idiobiont and koinobiont models of Mackauer and Sequeira (1993). The development of C. glomerata was strongly co-ordinated with that of its host, P. brassicae, with high immature mortality, as well as extended development time and small adult size for the surviving wasps on Bu. orientalis. By contrast, the development of Pt. puparum was similar on pupal hosts reared on both plant species. As predicted by Mackauer and Sequeira (1993) host quality is difficult, and perhaps impossible, to predict for the koinobiont, C. glomerata, at the time of oviposition. This is because this parasitoid attacks small, early instar hosts that must pass through several instars and attain almost the size of healthy caterpillars to support the nutritional requirements of the parasitoid progeny (Vet et al. 1994; Harvey 2000). Thus, the fitness of the parasitoid brood is dependent on the quality of the host after it is parasitized (Harvey 2005). Although many koinobionts endoparasitoids can detect the presence of hetero- or conspecific eggs or larvae in the body of their host during oviposition (Godfray 1994), it is still unknown if these parasitoids can detect plant-related differences in host quality with their ovipositor. Our results suggest that specific components of the larval host diet have an important negative effect on the parasitoid development. Given that P. brassicae does not sequester glucosinolates during its development (Müller et al. 2003) it is unlikely that glucosinolates affect C. glomerata performance. Brassica nigra and Bu. orientalis may also differ in levels of other defence-related compounds (e.g. digestibility reducers), or levels of primary metabolites (e.g. amino acids, proteins) that are also important factors determining food plant quality for insect herbivores (Slansky 1986). Hence, future research will be necessary to unravel what makes Bu. orientalis unsuitable for specialist herbivores and their larval parasitoid.

In contrast with the negative effects of plant quality on P. brassicae and C. glomerata, the performance of Pt. puparum was little affected. Although few P. brassicae survived to the pupal stage on the exotic plant, those that did were suitable for the development of Pt. puparum. Parasitoid survival was high on both plant species, and there was no difference in development time and adult wasp size. The major difference was that Pt. puparum produced smaller broods on P. brassicae pupae that had been reared on Bu. orientalis, compared with pupae reared on Br. nigra. Therefore, as Mackauer and Sequeira (1993) proposed, host quality for idiobiont parasitoids, is often dependent on the nutritional history of the host prior to parasitism. Pteromalus puparum, like other gregarious idiobiont parasitoids, is able to assess quantitative differences in the host and to adjust their clutch size accordingly (Schmidt and Smith 1985; Harvey et al. 2011). We observed Pt. puparum females drumming the host pupae with their antennae and walking along the pupal surface for several minutes before deciding to oviposit. Pteromalus puparum has also been shown to discriminate host size through the detection of chemical and/or physical changes of the host hemolymph, and to regulate the number of eggs it lays accordingly (Takagi 1986).

Although our results show that the performance of the idiobiont parasitoid is much less affected than that of the koinobiont by the identity of the plant species, one should also interpret these results within a broader context of the population demographics. For instance, even though the pupal parasitoid performed well on hosts reared on both plant species, it is important to point out that very few P. brassicae caterpillars actually survived to the pupal stage on the exotic plant. From this it can be assumed that a much larger pool of P. brassicae eggs would be required on Bu. orientalis than on Br. nigra to sustain a viable population of Pt. puparum in the field. Consequently, from an ecological perspective it can be concluded that Bu. orientalis is a poor plant for Pt. puparum, whereas this can be broadened to an ecophysiological perspective for the host and C. glomerata.

Thus far, few studies have examined the effects of alien plants on native food webs (Gerber et al. 2008), and on the dynamics of tightly linked trophic interactions between native insect herbivores and their natural enemies, such as parasitoids (Cronin and Haynes 2004; Stenberg, 2012). Here, we have shown that the effect of the exotic plant, Bu. orientalis on the development of a native herbivore and two of its parasitoids may depend on their resource exploitation strategies. A broader species comparison is needed to draw definite conclusions on this. Future studies should explore and compare the dynamics and persistence of multitrophic interactions in alien plants and related natives, as well as elucidate the mechanisms underlying these processes. In this way we can gain further insights into the effects that alien plants may have on native insect communities, and be able to understand how this might vary with the biology and ecology of the species involved (Harvey et al. 2010b). Additionally, although alien plants have usually been considered as being negative for native biota (Cronin and Haynes 2004; Keeler and Chew 2008; Harvey et al. 2010a), some alien species may actually benefit native herbivores (and by association their natural enemies) if their native food plants decline. For instance, it is known that some butterflies in the United States are now locally dependent on alien plants for their survival (Graves and Shapiro 2003). If their natural enemies are not following this host-plant shift, exotic plants may provide an enemy-free space for herbivores. In this way, herbivores may avoid their enemies by selecting novel, alien plants in their habitat (Vet and Dicke 1992; Harvey and Fortuna 2012). Hence, alien plants offer an exciting opportunity to explore novel interactions with native consumers with which they have no recent evolutionary history. By investigating the mechanisms underlying the success (or failure) of these novel interactions, we may better understand why only a small percentage of alien plants become highly invasive pests.

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CHAPTER 5

A tritrophic approach to the preference-performance hypothesis involving an exotic and a native plant

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Abstract

Exotic plants often generate physical and chemical changes in native plant communities where they become established. A major challenge is to understand how novel plants may affect trophic interactions in their new habitats, and how native herbivores and their natural enemies might respond to them. We compared the oviposition preference and offspring performance of the crucifer specialist, Pieris brassicae, on an exotic plant, Bunias orientalis, and on a related native plant, Sinapis arvensis. Additionally, we studied the response of the parasitoid, Cotesia glomerata to herbivore-induced plant volatiles (HIPV) and determined the volatile blend composition to elucidate which compound(s) might be involved in parasitoid attraction. On both host plants we also compared the parasitism rate of P. brassicae by C. glomerata. Female butterflies preferred to oviposit on the native plant and their offspring survival and performance was higher on the native plant compared to the exotic. Although, headspace analysis revealed qualitative and quantitative differences in the volatile blends of both plant species, C. glomerata did not discriminate between the HIPV blends in flight-tent bioassays. Nevertheless, parasitism rate of P. brassicae larvae was higher on the native plant under semi-field conditions. Overall, P. brassicae oviposition preference may be more influenced by bottom-up effects of the host plant on larval performance than by top-down pressure exerted by its parasitoid. The potential for dietary breadth expansion of P. brassicae to include the exotic Bu. orientalis and the role of top-down processes played by parasitoids in shaping herbivore host shifts are further discussed.

Introduction

It has been long established that plants differ in their suitability for insect herbivores and in many species immature stages are often relatively immobile (Ehrlich and Raven 1964). Therefore, natural selection should favor female insects with an ability to discriminate between hosts of different suitability for the development of their progeny (Jaenike 1978; Mayhew 1997; Thompson 1988). The ‘preference-performance’ or ‘the mother-knows-best’ hypothesis suggests that females will maximize their fitness by laying eggs on plant species where their offspring will develop more successfully and thus have higher fitness returns (Jaenike 1978). Although some studies have shown a positive correlation between female preference and offspring performance (reviewed by Gripenberg et al. 2010; Singer et al. 1988), others report that this relationship is weak or is even absent altogether (Chew 1977; Larsson and Ekbom 1995; Ohsaki and Sato 1994; Rausher 1979; Valladares and Lawton 1991). Evaluating the factors influencing oviposition decisions in insects may be critically dependent on several parameters, such as the availability of high quality food plants in the habitat or dynamic changes in the physiological state of the foraging female (i.e. egg load, age, hunger level). In combination, these factors may make optimal foraging decisions in insects strongly context- and trait-dependent (Jaenike 1990; Papaj 2000).

Several ecological factors have been proposed to explain apparent mismatches between choice and performance in insects (Mayhew 1997; Thompson 1988; Thompson and Pellmyr 1991). One of these factors is the recent addition of a novel plant species to the habitat. It has been documented that females may sometimes choose plant species that are of low quality and in some instances even fatal for the development of their offspring (Chew 1977; Keeler and Chew 2008; Kühnle and Müller 2009; Larsson and Ekbom 1995). The inability of females to discriminate among plant species may occur if novel plants lack the required oviposition cues to reject them (Harvey and Fortuna 2012); or conversely, if these plants may possess oviposition stimulants, even when they are unsuitable as food plants (Renwick 2002). In this case, there may not have been sufficient time for females to evolve recognition cues that enable them to reject toxic novel plants or, when they do oviposit on these plants, for their progeny to adapt to the novel phytotoxins (Thompson 1988). These mechanisms are predicted by important hypotheses in invasion ecology, such as the enemy release (Keane and Crawley 2002) and the novel weapons hypotheses (Callaway and Ridenour 2004), which have been proposed to explain the success of exotic invasive plant species in the new range. However, these mechanisms may be short lived and oviposition "mistakes" may in time also function as the 'raw material' for host plant shifts. Selection might favor females from populations that have broadened the number of host plant species in their diet, thereby saving time in searching for suitable host plants (Chew 1977; Graves and Shapiro 2003; Thompson and Pellmyr 1991).

Another important factor that can explain the lack of correlation between female preference and larval performance is differential mortality risk imposed by pressures from natural enemies, such as predators or parasitoids (Price et al. 1980). Ohsaki and Sato (1994) showed that the differences in plant preference of three Pieris butterflies resulted from a trade-off between the avoidance of natural enemies (e.g. parasitoid wasps) and the intrinsic quality of several cruciferous food plants. Thus, although a plant species may be sub-optimal nutritionally, it might also be unattractive to natural enemies of the herbivores and provide them with 'enemy-free space' (Price et al. 1980; Stamp 2001; Thompson 1988). Despite the many studies focused on the mechanisms underpinning female oviposition behaviour and offspring performance (reviewed by Gripenberg et al. 2010), few have included the role of natural enemies in molding preference-performance relationships (Ohsaki and Sato 1994; Shiojiri et al. 2002), and even fewer have considered a tritrophic approach to plant preference-performance on exotic plant species (Harvey et al. 2010b).

Resource exploitation by herbivores and their natural enemies occurs in habitats that are structurally and chemically heterogeneous. Individuals that use the information associated with the food sources (e.g. plant, prey or host) most efficiently will generally enjoy higher fitness benefits (Vet and Dicke 1992). Plant volatiles are often induced upon herbivore damage, and it is well established that natural enemies, such as parasitoids, use these volatile cues to locate host patches and hosts themselves (Vet and Dicke 1992). Although the functional and ecological importance of herbivore-induced plant volatiles (HIPVs) in parasitoid attraction has been discussed for many years (Gols et al. 2011; Takabayashi and Dicke 1996; Turlings et al. 1990), little is still known about the specificity of these plant compounds or mixtures of compounds in determining parasitoid host-finding behaviour. The establishment and rapid spread of an exotic plant into a native community may fragment habitats and create barriers (e.g. chemical, physical) to the dispersal and host-finding abilities of herbivores and their natural enemies (Cronin and Haynes 2004; Harvey and Fortuna 2012). Furthermore, recent studies have shown that herbivore pressure was lower on exotic plant species than on native congeners, while potential top-down predator pressure was higher on exotic plants than on native ones (Engelkes et al. 2012). These findings suggest that lower herbivore loads on exotic species can also be related to high predation or parasitism rates on these species in their new range.

In this study we compared plant preference-performance behaviour of a specialist crucifer herbivore, Pieris brassicae L. (Lepidoptera: Pieridae), on two related wild species, the exotic Turkish rocket, Bunias orientalis L. (Capparales: Brassicaceae), and the native charlock mustard, Sinapis arvensis L. (Brassicales: Brassicaceae). Additionally, in flight chamber bioassays we compared the host-finding behaviour of the P. brassicae endoparasitoid, Cotesia glomerata L. (Hymenoptera: Braconidae) on the two plant species, and measured parasitism rate under semi-field conditions outdoors. We addressed the following 6 questions: (1) Is oviposition choice of P. brassicae female butterflies different between the exotic and the native plant species? (2) Does the performance of their offspring differ between these food plants? (3) Do females prefer the host plant on which their offspring perform better? (4) Do the chemical profiles of HIPV emitted by the native and the exotic plant species differ? (5) Are C. glomerata wasps differentially attracted to the HIPV emitted by the native versus exotic plants? (6) Does this attraction result in different parasitism rates of P. brassicae? Based on our findings, we discuss the potential effects that exotic invasive plants may have on native trophic interactions.

Material and Methods

Plants

Bunias orientalis is a perennial species native to South-eastern Europe and West Asia that had been established, albeit rare, in parts of Western Europe since the 19th century. In the 1980's, it started to spread rapidly in many areas of its introduced range and it has become a highly invasive weed in several countries (Dietz et al. 1999; Harvey et al. 2010a). Previous studies have shown that this plant is unsuitable for the larval development of some crucifer specialists (Fortuna et al. 2012; Harvey et al. 2010a; Kühnle and Müller 2009). However, females of the mustard leaf beetle, Phaedon cochleariae L., accepted Bu. orientalis for oviposition (Kühnle and Müller 2009), and eggs and larvae of P. brassicae have been found on Bu. orientalis in the field in The Netherlands and Germany (T. Fortuna and J. Harvey, pers. observ.). Sinapis arvensis is an annual species and is a major food plant for crucifer specialists during part of the growing season in Europe (Feltwell 1982; Harvey et al. 2010a). Both plant species grow in similar ruderal habitats in dense stands. The native species was selected because its growing phenology, size and secondary chemistry (e.g. glucosinolates) are similar to Bu. orientalis (Harvey et al. 2010a).

Insects

The large white cabbage butterfly, Pieris brassicae, is a specialist herbivore, whose larvae feed exclusively on plants producing glucosinolates, including many species in the Brassicaceae family (Feltwell 1982). Wild broods of P. brassicae in early-mid larval stages were collected from several brassicaceous species growing along roadsides and cultivated fields in Gelderland in June 2009 and 2010. Caterpillars were then reared on Brussels sprouts plants, Brassica oleracea gemmifera cv. Cyrus, until they developed to pupal stage. After emergence, female and male butterflies were placed in an outdoor tent (2.5 x 2.0 x 2.0 m). Wild flowers of non-brassicaceous species were supplied as nectar sources. Naïve mated female butterflies used in the oviposition choice experiment were 3 to 8 days old. Cotesia glomerata is a gregarious koinobiont endoparasitoid that attacks young larvae of several species of pierid butterflies; P. brassicae is its preferred host in Europe (Feltwell 1982). To locate their hosts, C. glomerata females rely on infochemicals (e.g. volatile cues) both from the plant and its host (Geervliet et al. 1997). Parasitoid wasps were obtained from parasitized P. brassicae caterpillars feeding on Brussels sprouts plants. Newly emerged adult wasps were placed in a rearing cage (30 x 30 x 30 cm) containing water and honey ad libitum. Females and males were placed together in a Petri dish with honey 1 day before the experiments, to ensure that female wasps were mated. For flight-tent and semi-field experiments we used naïve-mated female wasps that were 5 to 10 days old when egg loads are fully matured (Jervis et al. 2008). All insects were reared in a controlled climate room (22 ± 2ºC, 60% RH, 16L:8D).

Experimental design

Seeds of Bu. orientalis were collected from roadside populations growing in Gelderland (N 52°00' E 6°09'/ N 51°58' E 5°42'/ N 51°58' E 5°40'/ N 51°52' E 6°00'), and seeds of S. arvensis were collected from a large wild population in Vlieland (N 53°17' E 5°04'), The Netherlands, in the summer 2008. Bu. orientalis seeds were extracted by cracking the seed coat as described by (Harvey et al. 2010a). Seeds were germinated in plastic containers (18 x 13 x 6 cm) with potting soil (30% sand, 5% clay and 65% peat) and after one week, seedlings were transferred to 1.2 L pots and grown in a greenhouse (22/16 ºC day/night, 60% relative humidity (RH), 16L:8D photoperiod). Natural daylight was supplemented by 400 W metal halide lamps (225 µmol m^{-2} s^{-1} PAR). Plants were watered four times per week, and after 4 weeks, supplied with nutrient-enhanced Hoagland solution twice a week, to compensate for the nutrient depletion in the soil. Since the two plant species have slightly different growth rates, S. arvensis plants used in the experiments were 4-5 weeks old and Bu. orientalis plants were 6-8 weeks old, and not flowering.

Plant-preference of Pieris brassicae in a semi-field experiment

To determine differences in oviposition preference of wild P. brassicae female butterflies we conducted a dual-choice test. One female and two males were placed into an outdoor tent (2.5 x 2.0 x 2.0 m), containing six plants of each species (see experimental design in Figure S1, Supplementary material). Bu. orientalis plants were placed on 20 cm stands to be at an equivalent height of S. arvensis plants. Wild flowers of non-brassicaceous species were provided in each tent as a nectar source for the butterflies. Female butterflies were allowed to oviposit for 24h, and after the number of plants of each species with egg clutches was counted. Tests were conducted during 2 summers, in June 2009 (25 replicates) and June 2010 (8 replicates). Fewer replicates were conducted in 2010 because of extremely hot weather conditions that killed many of the butterflies. Furthermore, we measured the leaf area of both plant species as an alternative factor influencing female oviposition choice. Leaf area of each plant species was determined using the software WinFOLIA® after scanning the surface area of all leaves of twelve plants chosen randomly from different tents.

Statistical Analysis: All univariate analyses were run in GenStat ® 14th edition (VSN International, Hemel Hempstead, UK). Butterflies oviposition choice data was analyzed using generalized linear mixed model (GLMM procedure Payne et al. 2011), with plant species effect as fixed factor and replicate (e.g. one female butterfly) as random effect. The binary trait ‘selected/ unselected plants’ for oviposition followed a binomial distribution with a logit link function. Number of egg clutches in each chosen plant was modeled with generalized linear model (GLM) following a poisson distribution with a logarithm link function. Number of eggs per clutch and total leaf area of each plant species was tested using a two-sample t-test (Payne et al. 2011).

Performance of Pieris brassicae in the greenhouse

Plants with eggs from the oviposition choice test conducted in 2010 were maintained in a greenhouse compartment. To ensure that differences in the offspring performance were due to differences in plant quality and not in genetic variation, we selected egg clutches from replicates where female butterflies oviposited on both plant species. Therefore, only two replicates out of eight were used in this experiment. Ten neonate larvae from hatched eggs were carefully transferred to a new plant and introduced with two extra plants in a cage (35 x 35 x 60 cm) according to the treatment. Four treatments were performed according to where the eggs were laid (oviposition plant) and where the larvae developed (larval development plant): 1) larvae hatched and reared on Bu. orientalis (BO-BO); 2) larvae hatched on Bu. orientalis and reared on S. arvensis (BO-SA); 3) larvae hatched and reared on S. arvensis (SA-SA); 4) larvae hatched on S. arvensis and reared on Bu. orientalis (SA-BO). Larval development was checked daily and new plants were supplied as required. Larval performance was measured based on survival rate, pupal weight and development time. Survival was determined as the fraction of larvae that survived until adult stage from the total number that was placed on the plants. Day of pupation was recorded when pupae were formed and within 24h, when the cuticle had hardened, pupae were removed from the cages and weighed on an analytical balance (Mettler-Toledo AG104, accuracy ±1 mg). After weighing, pupae were individually placed in plastic vials with a perforated lid until adults emerged. Development time was then recorded as the number of days from egg hatching until adult emergence. BO-BO and BO-SA treatments were replicated 10 times and SA-SA and SA-BO treatments 8 times.

Statistical Analysis: Performance data of butterflies' offspring was analyzed using linear mixed model (restricted maximum likelihood method, Payne et al. 2011) with host plant, oviposition and development plant, and their interaction as fixed factor and maternal effect as random factor. In all parameters of performance, the interaction term was never significant, so that was removed from the model. Prior to analyses, survival data was arcsine square-root transformed to meet the assumption of normality. Pupal weight and egg-to-adult development time data was averaged within replicate.

Plant-preference of Cotesia glomerata in a flight-tent experiment

The flight response of C. glomerata females to both plants was tested in a white nylon sheeting tent (2.5 x 2.0 x 2.0 m) that was placed in the greenhouse (22 ± 0.5 °C, 50% RH). Two plants were placed, according to the test (see below), on a table in the middle of the tent, 5 min before the bioassay. One day before testing, naïve wasps were collected from the mass-rearing and placed individually in 5-mL vials with honey and water. Wasps were then released from the vials at the other end of the table with the experimental plants (see Figure S2, Supplementary material), and because wasps do not leave the vials immediately, 10 wasps were released simultaneously. Wasps were allowed to fly freely inside the tent and as soon a wasp alighted on a plant, it was collected and the plant species on which it had landed was recorded. Additional wasps were released when the previous wasps exhibited no flight response within 15 min. A total of 10 wasp responses were recorded per each set of plants (replicate). Plant position was changed between replicates. Individual wasps were used only once.

Test 1 Undamaged (control) vs. Damaged Bu. orientalis (DB20): Herbivore damage was inflicted by 20 L2 P. brassicae larvae that were carefully placed on a clean leaf and allowed to feed for 48 h. To enhance the HIPV emission, damaged plants were presented to the wasps containing host larvae, which habitually fed gregariously on the underside of a leaf. Eight replicates were conducted.

Test 2 Damaged Bu. orientalis (DB20) vs. Damaged S. arvensis (DS20): Plants of the two species were infested by 20 L2 P. brassicae larvae for 48 h. Bu. orientalis plants were placed on 10 cm stands to be at an equivalent height with S. arvensis plants. Sixteen replicates were conducted.

Test 3 Higher host-density on Damaged Bu. orientalis (DB40) vs. Damaged S. arvensis (DS20): Bu. orientalis plants were infested by 40 L2 P. brassicae larvae while S. arvensis plants were infested by 20 L2 P. brassicae larvae for 48 h. Thirteen replicates were conducted.

Moreover, the feeding damage of P. brassicae larvae on both plant species was determined a posteriori with the software WinFOLIA® after scanning the damaged area of the tested plants.

Statistical Analysis: Parasitoid plant preference data was analyzed using GLMM with plant species effect as a fixed factor and replicate (e.g. 10 wasp responses) as a random factor. These data followed a binomial distribution with a logit link function.

Headspace analysis of plant volatiles

Volatile organic compounds (VOC) were collected from Bu. orientalis (N= 9) and S. arvensis (N = 10) plants that had been undamaged or damaged by L2 P. brassicae larvae for 48h. Potted plants were transferred individually to a 17-L glass collection container (41 x 24.5 cm) and placed in a controlled climate cabinet (21 ± 0.5 °C, 70% RH). At the top, the containers were supplied constantly with 200 mL pressurized air and were cleaned over a Zero Air generator to remove hydrocarbons (Parker Hannifin Corp, Tewksbury, USA). Volatiles were trapped on 150 mg Tenax TA and 150 mg Carbopack B steel trap (Markes, Llantrisant, UK) for 60 min at a flow of 100 mL min^{-1}. Volatiles were desorbed from the Tenax traps using an automated thermodesorption cold trap unit (model Unity, Markes) at 200°C for 12 min (He from 30ml/min). The released compounds were cryofocused in an electrically cooled (-10°C) sorbent trap to allow for narrow starting bands. Volatiles were injected into GC in sliptless mode by ballistic heating of the cold trap for 3 min to 270°C. After separation by capillary gas chromatography (column: 30 m x 0.32 mm internal diameter RTX-5 Silms, film thickness 0.33 µm; 40°C to 95°C at 3°C min^{-1}, then to 165°C at 2°C min^{-1}, and after to 250°C at 15°C min^{-1}), volatiles were directly introduced in mass spectrometer (MS) (model Trace, ThermoFinnigan, Austin, USA) operating at 70 eV in EI ionization mode. Mass spectra were recorded with full scan mode (33-300 AMU, 3 scans s^{-1}). Compounds were identified by comparing their mass spectra with those in reference manual (Adams 2007), National Institute of Standards and Technology (NIST 2008, USA; http://www.nist.gov), Wiley 7th edition spectral libraries, and by checking the retention indices. For identification and quantification we used AMDIS 2.1 (Automated Mass spectral Deconvolution and Identification Software). Individual compounds were quantified by measuring peak areas of the total ion fractions of the integrated signal. Peak areas were divided by the total volume (mL) that was sampled over each trap to correct for small differences in sampling time and flow rates over individual traps. To avoid rare compounds of having disproportionate effects on volatile profiles, only compounds with peak area larger than the background and found in more than 80% of the plants in at least one treatment group were analyzed. Finally, peaks of impurities were removed, which resulted in a dataset of 63 volatiles in 38 plants.

Statistical Analysis: Differences in VOC profiles among treatments were analyzed using a supervised multivariate analysis method, orthogonal partial least squares discriminant analysis (OPLS-DA). This method allows us to describe the differences between plant groups in each treatment by comparing with other unrelated chemical variation (Bylesjo et al. 2006). As treatment pairs, we used the three comparisons of the parasitoid choice tests: 1) undamaged and damaged Bu. orientalis plants (control vs. DB20); 2) damaged Bu. orientalis and S. arvensis plants with the same host density (DS20 vs. DB20); 3) or with different host density (DS20 vs. DB40). These comparisons were analyzed using three separate OPLS-DA models and the optimal number of latent variables for each model was evaluated by cross-validation following the procedure described in (Pierre et al. 2011). The VOC discriminating most between two contrasted plant groups in each comparison were collected in a weight vector, explaining the relative importance of each volatile within the total blend. The weight vectors of all three comparisons contained the ranks of the same VOCs, which were represented in a two-dimensional bubble plot to help visualization (Figure4). Based on the position and size of the bubble of each compound together with an arbitrary threshold (0.05) for the weight vectors (Figure4), we defined potential bioactive compounds as candidates responsible for the parasitoid behaviour in the bioassays (Figure3). Multivariate analysis were performed using MATLAB version 7.12 (R2011a; MathWorks, Natick, Ma, USA).

Host-acceptance of Cotesia glomerata in a semi-field experiment

Parasitoid host location and acceptance behaviour was studied in a dual-choice test performed in an outdoor tent (2.5 x 2.0 x 2.0 m), containing six plants of each species (see experimental design in Figure S1, Supplementary material). Prior to the test, ten L2 P. brassicae larvae were placed on the plants and allowed to feed for 24h. Additionally, naïve female wasps of C. glomerata from the mass-rearing were separated in groups of five and placed in 5-mL vial with honey and water. On the test day, one vial was released per tent and wasps were allowed to search for their hosts for 4 h. In each tent, 120 P. brassicae hosts were presented to C. glomerata wasps. The test was replicated nine times and 45 wasps were released in total. After testing, wasps were collected and plants with P. brassicae caterpillars were brought to the greenhouse, where they fed for 4 days to enable the parasitoid development from eggs to larvae. Caterpillars from each plant were placed in 2-mL Eppendorf tubes and kept in the freezer at -20°C until dissection under a stereomicroscope (Leica M205 C). The primary clutch size (number of eggs and larvae inside the host) of C. glomerata was recorded and parasitism rate was determined as the proportion of parasitized caterpillars over the total number of caterpillars collected from each plant after the choice test.

Statistical Analysis: Percentage data on herbivore parasitism rate was analyzed using Chi-squared test to compare the proportion of parasitized caterpillars on each plant species. Total herbivore leaf damage and parasitoid primary clutch size was tested using a two-sample t-test (Payne et al. 2011). Prior to analysis, wasp clutch size was summed within individual plant and averaged per plant species within replicate. Chi-squared test was performed in PopTools (Microsoft Excel add-in; Hood 2010).

Results

Plant-preference of Pieris brassicae in a semi-field experiment

Female butterflies significantly preferred to oviposit on plant of S. arvensis over Bu. orientalis in both years that the test was performed (2009: W = 12.92; P < 0.001; 2010: W = 5.09; P = 0.024; Figure 1a). Overall, 85% of the butterflies that exhibited preference chose the native over the exotic plant. In 2010, despite extremely hot conditions that occurred during the choice test, the butterflies still showed a similar oviposition response as in the previous year. Nevertheless, some P. brassicae butterflies (39%) laid their eggs on both plant species. Host plant species had no significant effect on the number of egg clutches laid by the butterflies in both years (2009: Z = - 0.05; P = 0.956; 2010: Z = - 0.71; P = 0.477). Generally, butterflies laid only a single clutch per plant, independent of the plant species. However, the number of eggs laid per clutch was significantly higher on the exotic plant compared to the native plant (t = - 2.16; P = 0.039) in 2009 (Figure 1b). The average clutch size on Bu. orientalis was approximately 90 ± 14 eggs, whereas on S. arvensis was 60 ± 7 eggs. In 2010, although the average clutch size was larger on the exotic plant (35 ± 17 eggs) compared to the native plant (15 ± 3 eggs) no significant difference was detected (t = - 1.19; P = 0.277; Figure 1b). Total leaf area varied significantly according to the plant species (t = 6.11; P < 0.001). The exotic plant had a leaf area (1424 ± 107 cm^2) that was almost twice as large as that of the native plant (741 ± 35 cm^2).

Performance of Pieris brassicae in the greenhouse

Survival of P. brassicae offspring was significantly affected by the plant on which butterflies oviposited their eggs (W = 11.49; P < 0.001), but not on the plant on which their larvae grew (W = 0.08; P = 0.777). Independent of the plant where the herbivore developed, survival was higher in clutches laid on S. arvensis, 96%, (SA-SA; SA-BO) than on Bu. orientalis, 78%, (BO-BO; BO-SA) (Figure 2a). Pupal weight was significantly affected by the oviposition plant (W = 4.99; P = 0.033) and larval development plant (W = 178.9; P < 0.001). Pupae developing on S. arvensis plants were bigger when they were from S. arvensis-preferring females (SA-SA) than those from Bu. orientalis-preferring females (BO-SA). The same pattern was found on Bu. orientalis plants, where pupae from S. arvensis-preferring females (SA-BO) were larger than those from Bu. orientalis-preferring females (BO-BO) (Figure 2b). Egg-to-adult development time was also significantly affected by the oviposition plant (W = 4.78; P = 0.036) and the larval development plant (W = 43.40; P < 0.001). Caterpillars developed faster on the native plant (SA-SA; BO-SA) than on the exotic plant (BO-BO; SA-BO; Figure 2c).

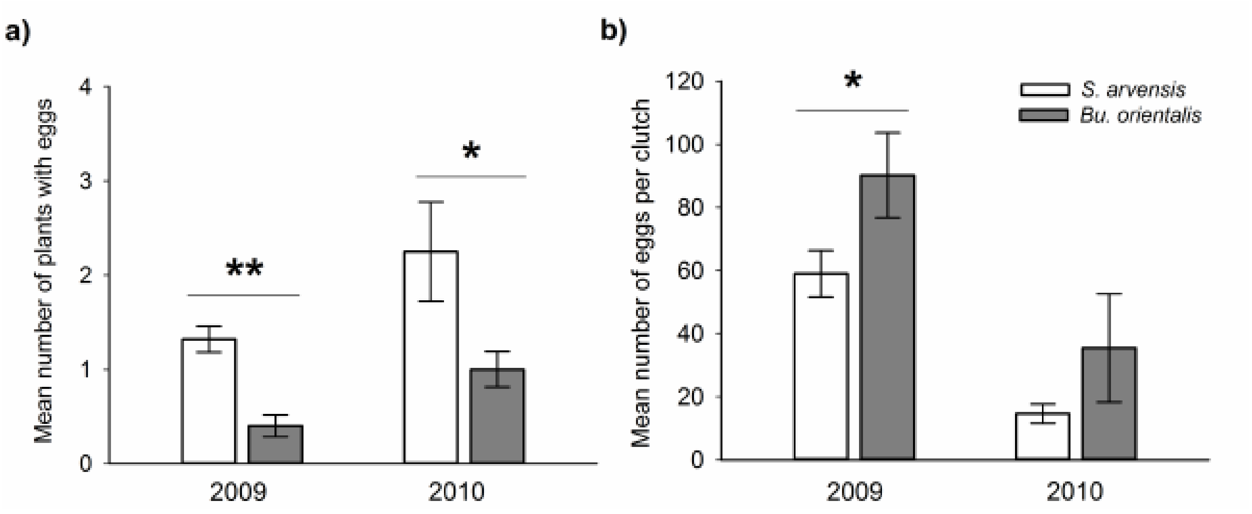

Figure 1 Oviposition behaviour of wild Pieris brassicae butterflies. Females were allowed to lay eggs on Sinapis arvensis and Bunias orientalis plants in a two-choice test for 24h. Butterflies were tested individually in the summer of 2009 and 2010. Given is a) Mean ± SE number of plants preferred for oviposition; b) Mean ± SE number of eggs laid per clutch. Asterisks indicate significant difference in the number of plants with eggs (Wald test: P < 0.001**; P < 0.05*) and in the number of eggs per clutch (two-sample t-test: P < 0.05*). n = 25 (2009); n = 8 (2010).

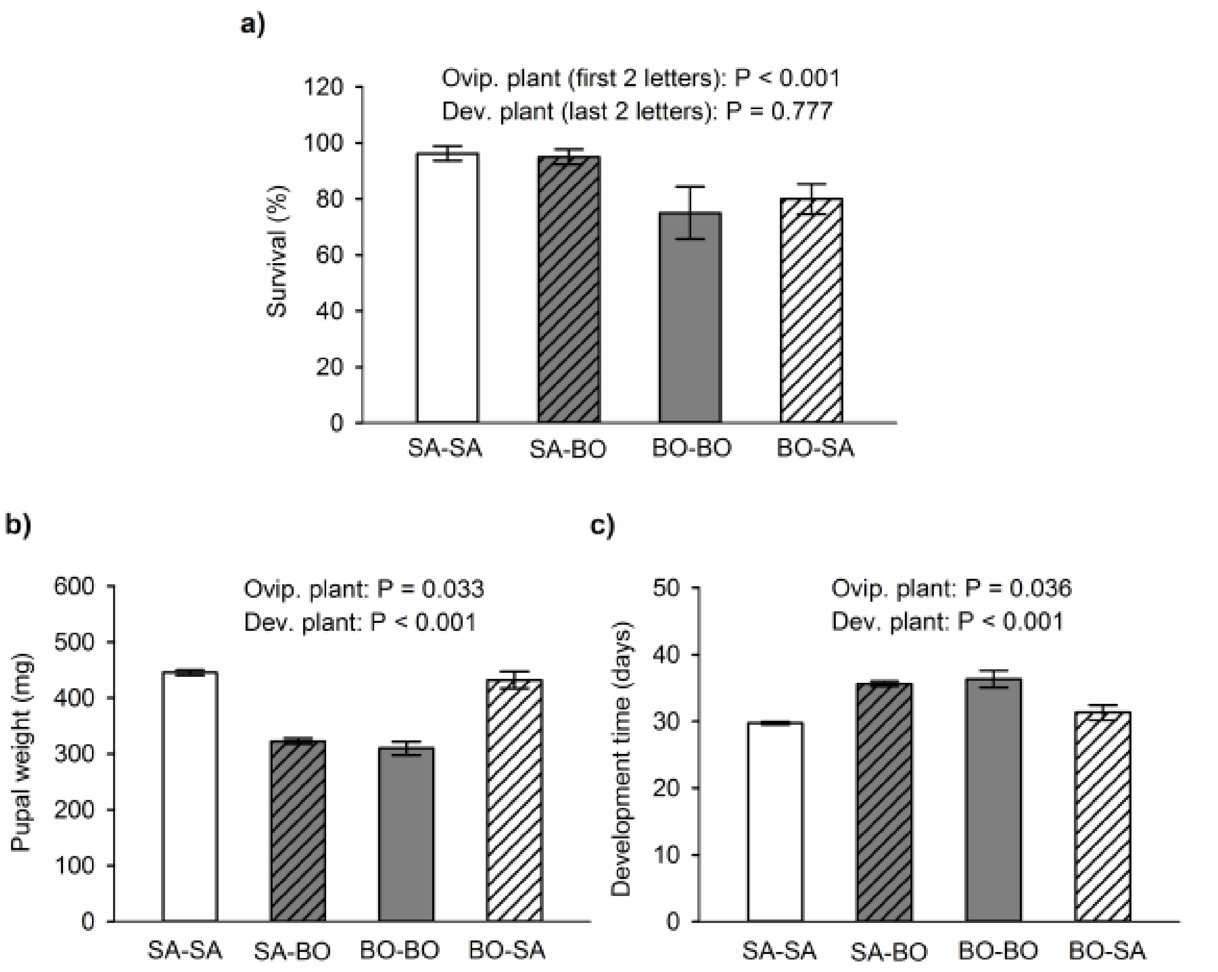

Figure 2 Survival (a), pupal weight (b) and egg-to-adult development time (c) of Pieris brassicae offspring grown on Sinapis arvensis (SA; white bars) or Bunias orientalis (BO; grey bars) plants. Four plant treatments are considered according to the oviposition plant (first two letters SA- or BO-) and larval development plant (last two letters -SA or -BO). Given is mean ± SE for each plant treatment. Significant differences are shown for oviposition plant and larval development plant (Wald test: $P < 0.001$; $P< 0.05$). n = 8 (SA-SA, SA-BO); n = 10 (BO-BO, BO-SA).

Parasitoid plant-preference in a flight-tent experiment

Cotesia glomerata showed a clear preference for Bu. orientalis plants damaged by the larval host, when compared with undamaged plants ($F = 68.15$; $P < 0.001$). Almost 90% of the wasps landed on plants that were infested with P. brassicae larvae, confirming that the parasitoid females are able to locate their hosts on the exotic plant (Figure 3a). However, the parasitoid did not discriminate between host-infested plants of S. arvensis and Bu. orientalis when both plant species were infested with the same host density ($F = 0.80$; $P = 0.379$; Figure 3b). Female wasps only showed a preference for the exotic plant when it was infested with higher host density ($F = 28.58$; $P < 0.001$). Significantly more wasps landed on Bu. orientalis plants that were infested with 40 caterpillars compared to S. arvensis plants infested with 20 caterpillars (Figure 3c). The total leaf area damaged by 20 P. brassicae caterpillars was significantly greater in S. arvensis plants than in Bu. orientalis plants with the same host density (t = -2.26; P = 0.038), but smaller than in Bu. orientalis plants with 40 caterpillars (t = 4.85; P < 0.001).

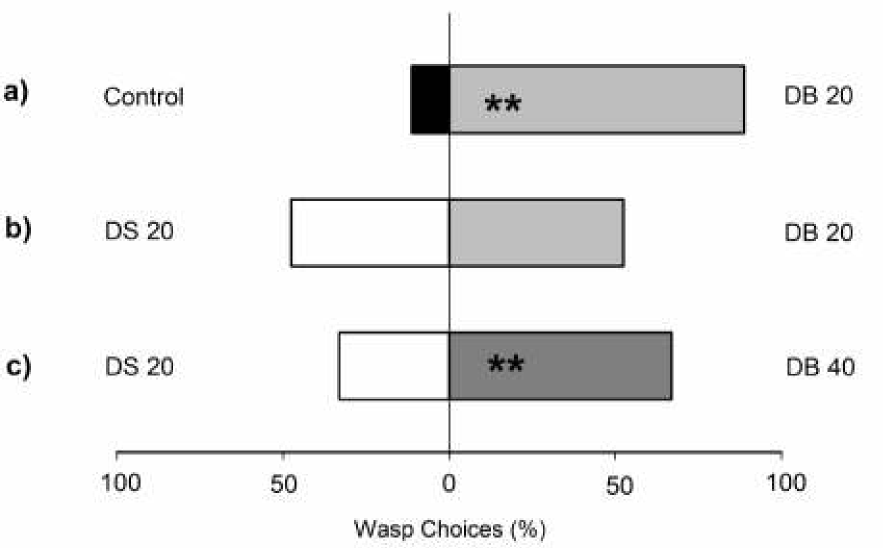

Figure 3 Percentage of choices of Cotesia glomerata females, in a two-choice experiment between: a) undamaged (control) or damaged Bunias orientalis plants by 20 Pieris brassicae caterpillars (DB20); b) Sinapis arvensis (DS20) or Bu. orientalis plants (DB20) both damaged by 20 P. brassicae caterpillars; c) S. arvensis plants damaged by 20 P. brassicae caterpillars (DS20) or Bu. orientalis plants damaged by 40 P. brassicae caterpillars (DB40). ** indicate significant difference in the parasitoid preference tests (F test: P < 0.001). a) n = 8; b) n = 16; c) n = 13.

Headspace analysis of plant volatiles

A total of 63 volatile compounds were detected in the headspace analysis of Bu. orientalis and S. arvensis plants (Table S1, Supplementary material). In both species, control and herbivore-infested plants differed quantitatively in their VOC emissions. However, while in Bu. orientalis there was 3-fold increase of VOC in damaged plants compared to control, in S. arvensis there was a 2-fold decrease of VOC in damaged plants. Despite the total HIPV decrease in S. arvensis damaged plants, the concentration of some compounds increased upon herbivory (see Table S1 in Supplementary material). HIPV profile in Bu. orientalis plants also varied quantitatively among treatments. Volatile emissions were higher in plants damaged by large herbivore load. Between plant species, HIPV profiles were both qualitatively and quantitatively different; damaged plants of Bu. orientalis produced higher compound variety and at a three time higher rate than damaged plants of S. arvensis.

The computation of OPLS-DA based on VOC emission showed that the effect of all three comparisons from the parasitoid behaviour bioassay can be described by low-dimensional multivariate models. The models consisted either in two latent variables, for the first comparison (control vs. DB20), or in one latent variable, for the second and third comparisons (DS20 vs. DB40; DS20 vs. DB20). All models had a significant permutation-based probability ($P = 0.02$; $P < 0.01$; $P < 0.01$, respectively), supporting that each tested comparison is real. Differences and similarities among plant treatments were compared by plotting the discriminate functions of the three OPLS-DA models against each other on a bubble plot (Figure 4). This representation of OPLS-DA in two-dimensions allows, therefore, visual identification of interesting compounds that are important for differentiation between the volatile profiles for each of the plant treatments. Particularly, decanal (9), decyl-cyclohexane (19), ethyl hexanoate (32), 2-hydroxy ethanone (39), 2-butanone (44), menthone isomer (53), unknown terpene (61), polyalcene (62) were in higher concentration in damaged plants of Bu. orientalis compared to control (X-axis) or to damaged plants of S. arvensis (Y-axis; Figure 4). By contrast, octanol (1), 3-methyl-1-butanol (3), benzyl-methanol (5), menthol isomer (7), 4-nonenal (15), anthracene (57), were in the reverse situation (Figure 4). Combining this information with the position and size of the compound bubbles we looked for potential bioactive compounds responsible for the parasitoid behaviour in the bioassays (Figure 3). The volatile candidates have: (1) to increase or decrease similarly in DB20 compared to control, and in DB40 compared to DS20 (upper right or lower left quarter); (2) to show no differences (in- or decrease) between DB20 and DS20 (large bubble size) (Figure 4). A relevant pattern for potential bioactive compounds was followed by p-menthane (24). Additionally, a similar behaviour is followed by the compound 2-hydroxy-1,2-diphenyl ethanone (39) and benzyl-methanol (5), although considering a slightly higher threshold level (0.1) (Figure 4; Table S1 Supplementary material).

Host-acceptance of Cotesia glomerata in a semi-field experiment

Parasitism rate by C. glomerata differed significantly on the two plant species ($\chi^2 = 9.25$; $P = 0.002$). Female wasps parasitized a higher number of P. brassicae larvae on the native plant than on the exotic plant (Figure 5). However, parasitoid primary clutch size was not significantly different between hosts developing on the two plant species ($t = 0.11$; $P =$

0.916). Female wasps typically laid primary broods of 22 to 25 eggs in P. brassicae caterpillars on the two species.

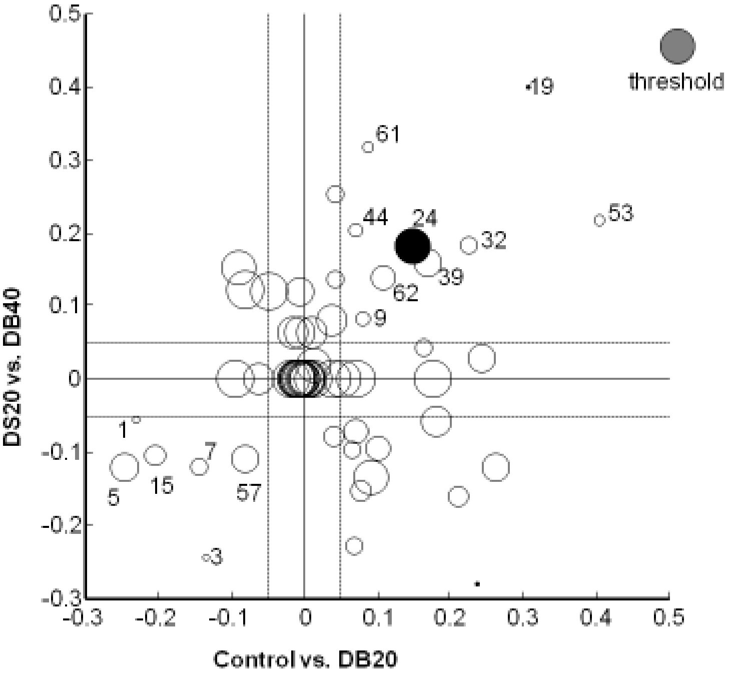

Figure 4 Two-dimensional bubble plot representation of volatiles emitted after different herbivore infestation treatment in Bunias orientalis and Sinapis arvensis plants. Herbivory was induced using 20 or 40 P. brassicae larvae. Plant treatments were compared by plotting the discriminate functions of the three OPLS-DA models against each other. (i) First comparison (X-axis) represents the VOC profile emitted by damaged plants of Bu. orientalis in contrast to control plants (control vs. DB20). (ii) Second comparison (Y-axis) corresponds to HIPV emitted by damaged plants of S. arvensis and Bu. orientalis with different host densities (DS20 vs. DB40). (iii) Third comparison (bubble size) corresponds to HIPV emitted by damaged plants of S. arvensis and Bu. orientalis with the same host density (DS20 vs. DB20). Each bubble represents a volatile compound and its position is determined according to its relative importance (weight vector values) for each of the three comparisons. Increased or decreased levels of a compound are indicated by a positive or negative value in X- and Y-axis (i and ii), or by the size of the circles (iii). The size of the bubble is negatively correlated with the relative importance of the compound in explaining differences in the comparison (iii) i.e. large circle size indicates a compound with relatively low importance in explaining volatile differences between DS20 and DB20 treatments. Numbers correspond to the compounds (referenced in Table S1) with relative importance (-0.05 < weight vector > 0.05) in the first and second comparison. An arbitrary threshold of significance was defined by an absolute weight vector value smaller than 0.05 (grey bubble). The black bubble (24) corresponds to the biomarker p-Menthane. Bu. orientalis: n = 9; S. arvensis n = 10.

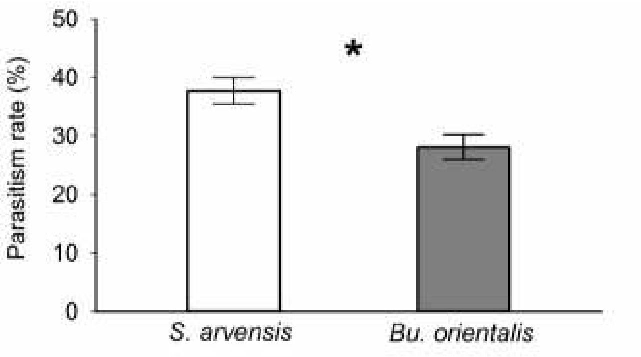

Figure 5 Parasitism rate (%) of Cotesia glomerata females in a two-choice experiment between Sinapis arvensis (white bar) and Bunias orientalis (dark grey bar) plants infested with Pieris brassicae larval hosts. Plants were infested with 10 P. brassicae caterpillars 24 h prior the choice test.* indicates significant difference (χ^2 test: $P < 0.05$). n = 9.

Discussion

Host-plant preference-performance of Pieris brassicae

Our results show a clear preference-performance link in the specialist herbivore, P. brassicae. The butterflies showed an innate oviposition preference for the native host plant, S. arvensis, over the exotic species, Bu. orientalis, in both years the test was conducted. Furthermore, the plant species on which they oviposited affected the survival and development of their offspring. Overall, caterpillars survived better when females oviposited on the native plant, and pupae that had developed on this plant species were larger and completed their development faster, than those which had developed on the exotic plant. These results emphasize the importance of oviposition decisions made by the female butterflies and provide further support for the ‘mother knows best’ hypothesis (Jaenike 1978).

Furthermore, our study supports the results of Gripenberg et al. (2010) who found stronger preference-performance relationships in oligophagous insect herbivores than in either monophagous or polyphagous insect herbivores. Indeed, a positive correlation between female plant choice and offspring performance has been demonstrated in several studies with other oligophagous insects (Kühnle and Müller 2009; Scheirs et al. 2003; Travers-Martin and Müller 2008). For instance, plant preference in females of the sawfly Athalia rosae, a specialist herbivore of brassicaceous species, was influenced by larval performance, and reproductive success was found to be highly dependent on host plant choice over the course of only two generations (Travers-Martin and Müller 2008). In an earlier study, Harvey et al. (2010a) also showed that P. brassicae preferred to oviposit on the native crucifers, Brassica nigra and S. arvensis, over Bu. orientalis. However, the survival of this herbivore, which had been reared for many generations on cultivated cabbages in the laboratory, was much lower on Bu. orientalis compared to the results obtained in the present study (Fortuna et al. 2012; Harvey et al. 2010a). These partly contrasting results suggest that long-term inbreeding in lab-reared insects may reduce their vigor and make them more susceptible to novel plant species. Laboratory cultures may lose much of the genetic variability related to behavioural and physiological plasticity of insects in natural ecosystems (Harvey et al. 2010a). Thus, greater efforts should be made in using wild individuals in these bioassays to account for the natural variation among the insect populations and also because they may possess a greater ability to deal with chemical defences of natural plants (Harvey et al. 2010a).

Host preference of Cotesia glomerata

In the flight-tent bioassays, C. glomerata females detected volatile blends of Bu. orientalis plants damaged by P. brassicae hosts, both when they were tested against undamaged plants and against S. arvensis damaged by lower host density. However, female wasps did not discriminate between Bu. orientalis and S. arvensis with the same host density. This behaviour is common to parasitoid species whose hosts are specialists but that feed on different plant species within the same family (Vet and Dicke 1992). As a consequence, different parasitoid generations can also be associated with different host-associated plants within a growing season (Gols et al. 2011). In these cases, natural selection may favor a flexible response of naïve parasitoids to plant infochemicals due to the variation among blends emitted by different plant species. Moreover, to effectively expand their host-foraging arena, parasitoids may actively choose not to discriminate between subtle differences until they become more sensitized to these differences through experience (Vet et al. 1998). However, despite similar attraction to the host plant cues, in our semi-field experiment the wasps parasitized slightly more P. brassicae larvae on S. arvensis than on Bu. orientalis plants. This may be due to differences in the spatial scale of the two experiments, which result in a different localized perception of the parasitoids to plant- and host-related cues. During host plant selection behaviour C. glomerata rely more on general plant cues (e.g. HIPV) common to the Brassicaceae family, whilst during the host selection phase the parasitoid also uses contact cues based partly on host quality-related parameters, such as host size and nutritional status (Vinson 1976). Additionally, other plant-related traits such as height and visibility may have also influenced differently the parasitoid host searching behaviour in the two experimental bioassays.

Many parasitoids, including C. glomerata, are known to have evolved highly efficient mechanisms to perceive chemical cues that are associated with high quality hosts (Geervliet et al. 1998). Moreover, our study highlights the importance of validating the results obtained in flight-chamber bioassays with field or semi-field experiments. Plant choice experiments in parasitoids have mostly been conducted in y-tubes (Bukovinszky et al. 2005) and flight assays (Geervliet et al. 1996; Geervliet et al. 1998; Gols et al. 2008b), which only tell us where the wasps alight, but not whether they actually locate and accept hosts on the tested plants. Field experiments can indicate whether and to what extent behavioural and developmental parameters measured in the laboratory relate to conditions that occur in more complex situations in natural habitats (Geervliet et al. 2000). In addition, it has been suggested that flight-chambers enclosed environment may lead to less contrasting odour-sources or volatile interference, which could also affect plant preference by parasitoids (Vos et al. 2001).

Role of the volatiles in Cotesia glomerata attraction

The volatile bouquet emitted by P. brassicae-damaged plants of Bu. orientalis was more diverse and concentrated than that emitted by S. arvensis plants. These results agree with previous studies, where volatile emissions varied considerably in quality and quantity, both within and among Brassicaceous species (Bukovinszky et al. 2005; Gols et al. 2011; Gols et al. 2008b). However, despite the qualitative and quantitative differences in HIPV emitted by the two plant species, C. glomerata did not discriminate between these plants during its host-searching behaviour. Similar results were found in the studies of Geervliet et al. (1997; 1996) where C. glomerata females did not discriminate between different plant cultivars infested with hosts, even when the volatile profiles of those cultivars were found to be qualitatively different.

Nevertheless, results from OPLS-DA models showed that the cyclic alkane p-menthane might be a good candidate to explain the parasitoid behavioural response in the flight-tent bioassays. This alkane is known to be involved in terpenoid biosynthesis as a parent structure of monoterpenes (Bernards 2010). For example, it has been demonstrated in the menthol biosynthesis pathway of Mentha species that p-menthane is the precursor of the monoterpene limonene, which is in turn the precursor of menthone (Croteau et al. 2005).

Also, in our system a menthone isomer is emitted by both plant species, which can be related to a similar biosynthesis pathway, where p-menthane is used as a terpenoid precursor. Monoterpenes, due to their volatile character, are known to have important biological activities as aroma components, and they can mediate tritrophic interactions acting as parasitoid wasp attractants (Mumm and Dicke 2010). It has already been shown that *C. glomerata* females have the ability to detect terpenes with their antennal chemical receptors (Smid et al. 2002; Vet et al. 1995). In addition, this parasitoid species seems to rely on more general plant cues associated with its host. Therefore, it is likely that female wasps have evolved to detect terpenoid precursors, because they are more common and less variable than the compounds at the end of the terpene biosynthetic pathway. Moreover, these precursor compounds are released during an earlier stage of host infestation, which might indicate more suitable host stages for the parasitoid.

In spite of numerous factors affecting parasitoid host location, in particular the role played by HIPVs, it is still unknown how parasitoids perceive and integrate shifts in the composition of complex VOC mixtures, and how they learn to respond to these changes within complex odour plumes (Dicke and Vet 1999; Harvey and Fortuna 2012). For instance, herbivorous spider mite *Tetranychus urticae* and its specialist enemy mite, *Phytoseiulus persimilis* were shown to perceive volatile mixtures as a whole rather than as a collection of individual components (van Wijk et al. 2011). Further investigations based on gas chromatography coupled to electroantennography (GC-EAG) are required to reveal if compounds, such as the ones identified in the present study, elicit a response in the chemoreceptors of the parasitoid (Dicke and Baldwin 2010). Using this technique, Smid et al. (2002) identified the volatiles emitted by *P. brassicae*-damaged cabbage plants to which the parasitoid evoked EAG reaction in its antennae. Among those volatile compounds, four were found in the present study from either *Bu. orientalis* or *S. arvensis* plants (e.g. 2-hexenal, octanal, limonene, decanal) and although they seem to not explain the parasitoid choice based on OPLS-DA, they are likely building stones of the whole odour used by the parasitoids.

Ecological significance

In their new range exotic plant species can disrupt trophic interactions and food webs through physical and chemical interference as well as by displacing native plants (Cronin and Haynes 2004; Harvey and Fortuna 2012). However, in time they may ultimately generate new communities to which local native consumers may adapt. This is particularly the case if the exotic plants are closely related to the natives and thus produce chemicals to which the native herbivores are already pre-adapted (Harvey and Fortuna 2012). As we showed, some P. brassicae butterflies oviposited on Bu. orientalis plants in our semi-field experiment and the survival of their progeny was relatively high on this plant. On the other hand, as predicted by the enemy release hypothesis other native plant species, such as S. arvensis, are still likely to be highly preferred, because of long co-evolutionary history between the butterflies and these ubiquitous native plants. This may end up reducing the rate at which the novel plant will be used as an oviposition site. However, P. brassicae and other crucifer specialist herbivores may benefit by incorporating Bu. orientalis into their diet as an alternative host plant, particularly in late summer when other crucifers are scarce. Acceptance of a new plant species for oviposition by a female is an important first step towards dietary expansion. It has been argued that in general behavioural plasticity (such as oviposition preference) should undergo more rapid evolutionary changes than morphological or physiological plasticity (such larval growth on different plants), and that for this reason behavioural plasticity will be more likely to be an initiator of new direction in evolution (West-Eberhard 1989). Hence, the ovipositing females can take the leading role in the evolution of host plant utilization. For example, in the garlic mustard-Pieris oleracea association in United States, the butterflies readily accept this invasive plant for oviposition, although their larvae develop very poorly on it (Keeler and Chew 2008). However, more recently, in habitats where the garlic mustard has been well established for few decades and is the dominant crucifer, larval survivorship was shown to be positively correlated with the mother’s preference, suggesting that P. oleracea is adapting to this plant (Keeler and Chew 2008).

Similarly, P. brassicae may also gain the advantage of incorporating Bu. orientalis into its diet if this plant becomes more abundant, which is occurring in parts of central and northern Europe. In this scenario, native specialist herbivores would be positively selected to adapt to the novel food plant (Chew 1977). In the Netherlands, P. brassicae can have two (or three) generations within the growing season. Given that the native annual crucifers also have very short life cycles (i.e. typically 1-2 months) and varying seasonal phenologies, different generations of this butterfly probably develop on different plant species (Gols et al. 2011). Bunias orientalis grows from April until October, thus different generations of the butterfly may have the ability for developing on the same species over an entire growing season. The progeny of a female can remain in the natal patch, without having to spend potentially costly time searching for new plants in new habitats. In this instance, a preference-performance correlation may be produced within a single generation, because individuals that survive on a particular host species will tend to pass on preference-performance genes to their progeny (Singer et al. 1988). Additionally, individual Bu. orientalis plants often produce enough leaf biomass to support a large brood of P. brassicae, whereas annual crucifers, like S. arvensis, rarely do (J Harvey, pers. obs.).

In addition, the evolution of habitat preference may also be driven by top-down pressure mediated by natural enemies. For instance, enemy-pressure may be greater when herbivores develop on some plant species, and thus selection of certain plant types enables the herbivores to escape their natural enemies, i.e. enemy-free space (Camara 1997; Stamp 2001; Thompson 1988). Bunias orientalis may create an enemy-free space for P. brassicae if their larvae suffer lower parasitism rates from C. glomerata than larvae developing on native species. In some herbivore species it has been shown that the evolution of plant preference can be driven by both intrinsic quality of food plants and interactions with predator and/or parasite loads. In other pierids, plant preference may be based on trade-offs, whereby butterflies choose nutritionally sub-optimal plants but where their progeny are less susceptible to attack by parasitoids (Ohsaki and Sato 1994).

By affecting the foraging efficiency of natural enemies, such as predators and parasitoids, plants can affect the impact that those enemies have on herbivore populations and thus ultimately interfere with predator-prey or parasitoid-host dynamics (Dicke and Vet 1999). Parasitoids can increase their efficiency in host finding by learning plant-related cues (e.g. HIPV) and temporarily specialize on available and profitable plants (Geervliet et al. 2000). Cotesia glomerata, for example, learns to prefer plant species that are most profitable in terms of host encounter rate (Geervliet et al. 1998). By delaying herbivore development, Bu. orientalis increases the chance that the parasitoid encounters the more suitable younger host stages (the slow-growth-high-mortality hypothesis, Benrey and Denno 1997). Additionally, since the butterflies tend to lay larger broods on the exotic plant, this may also increase HIPV emission, leading to greater parasitoid attraction (Vet et al. 1998), as observed in our bioassay. These factors may allow C. glomerata to easily locate its P. brassicae host on the novel Bu. orientalis with which it may have no co-evolved relationship. Therefore, the few herbivores that may realize host shifts to the novel plant species can be subjected to higher parasitism pressure, and ultimately release the exotic plants from local herbivores in the invaded environment. Importantly, exotic plants offer an exciting opportunity to investigate the evolutionary forces involved in potential host shifts of herbivores and their natural enemies, ultimately providing insight into some of the many factors that influence the success of novel plants in their new range.

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Supplementary material

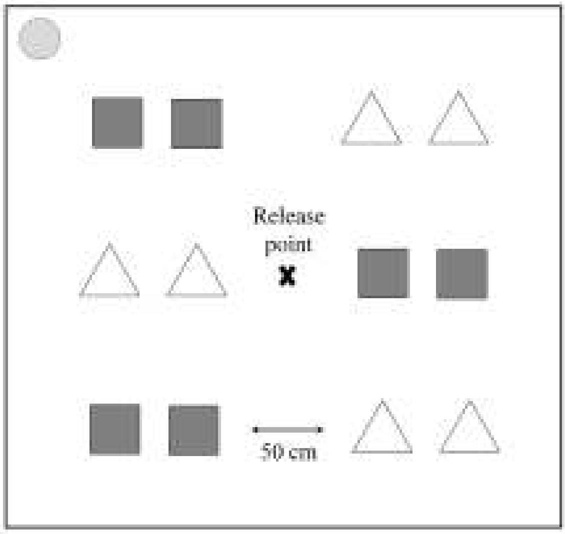

Figure S1 Scheme of the experimental design of Pieris brassicae oviposition choice test and Cotesia glomerata host-acceptance test under semi-field conditions. Distribution of the individual plants that served as target-plants for herbivore oviposition or parasitoid host location inside the experimental tent. In each tent six plants of Sinapis arvensis (white triangle) and six plants of Bunias orientalis (grey square) were distributed along three rows. Herbivore or parasitoid were released in the center of the tent and were allowed to oviposit for 24h or to parasitize for 4h, respectively. Wild flowers of non-brassicaceous species (light grey circle) were provided in the tents as nectar sources for P. brassicae butterflies.

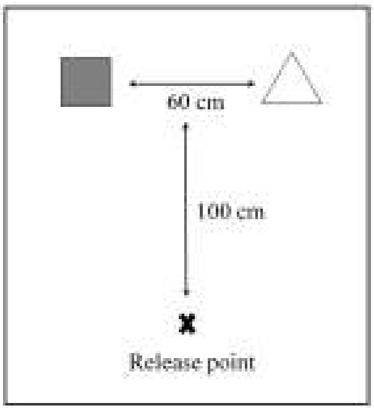

Figure S2 Scheme of the experimental design of Cotesia glomerata flight response test in the greenhouse. Plants of the different treatment (triangle and square) were placed on one side of a table opposite to the wasp release point. Sinapis arvensis and Bunias orientalis infested plants were presented to the wasps with the caterpillars feeding on the abaxial side of the leaf. A total of 10 wasp responses were recorded per replicate.

Table S1 Peak areas transformed as $\log_{10}$ (X) of volatile organic compounds (VOCs) of Bunias orientalis and Sinapis arvensis plants exposed to herbivory (DB, DS), or no herbivory (B or S control). Herbivory was caused by 20 (DB20, DS20) or 40 (DB40) P. brassicae larvae. N.d. = not detected. n = 9 (B control, DB20, DB40). n = 10 (S control, DS20).

#	Volatiles [a]	Compound class	RI [b]	Peak area/ mL sample [c]					Weight vector [d] (Bcon-DB20)	Weight vector (DS20-DB40)	Weight vector (DS20-DB20)
				B control	DB20	DB 40	S control	DS20			
1	1-octanol	Alcohol	1072	3.78±0.72	2.98±0.77	3.13±0.79	3.79±0.83	3.96±0.66	-0.2321	-0.0556	-0.3038
2	1-decanol	Alcohol	1273	0.45±0.45	2.99±0.58	3.86±0.17	n.d.	0.22±0.22	0.0099	0.0631	0.0543
3	3-methyl-1-butanol	Alcohol	731	1.40±0.57	0.98±0.49	1.01±0.51	3.65±0.06	3.12±0.35	-0.1345	-0.2446	-0.2600
4	2-nonen-1-ol	Alcohol	1180	0.52±0.52	4.32±0.20	4.50±0.20	1.31±0.54	n.d.	-0.0062	0.0000	0.0000
5	benzyl-methanol	Aromatic	1110	3.16±0.40	0.95±0.48	0.93±0.46	0.92±0.47	3.18±0.13	-0.2480	-0.1206	-0.1027
6	pentadecanol	Alcohol	1782	0.38±0.38	3.24±0.11	3.04±0.39	n.d.	n.d.	-0.0106	0.0000	0.0000
7	menthol isomer	Cyclic alcohol	1173	1.39±0.28	0.92±0.30	0.57±0.29	0.95±0.39	0.96±0.33	-0.1437	-0.1203	-0.1685
8	1-octadecanol	Alcohol	2085	1.62±0.45	1.77±0.45	1.39±0.46	2.58±0.32	2.62±0.12	0.0118	0.0184	0.0235
9	decanal	Aldehyde	1204	0.17±0.17	1.21±0.39	0.59±0.32	2.21±0.11	0.84±0.29	0.0799	0.0822	0.1959
10	octanal	Aldehyde	1002	0.39±0.39	3.23±0.18	3.10±0.40	n.d.	n.d.	-0.0032	0.0000	0.0000
11	(E)-citral	Aldehyde	1269	1.31±0.26	1.08±0.28	0.86±0.34	n.d.	0.33±0.22	-0.0801	0.1215	0.0034
12	2-hexenal	Aldehyde	847	0.46±0.46	3.75±0.18	3.95±0.15	n.d.	0.25±0.25	-0.0155	0.0632	0.0496
13	hexanal	Aldehyde	800	0.10±0.10	1.75±0.45	1.39±0.36	2.15±0.38	0.95±0.39	0.0786	-0.1549	0.1402
14	(Z)-3-hexenal	Aldehyde	798	0.32±0.32	2.14±0.42	2.58±0.33	n.d.	n.d.	0.0049	0.0000	0.0000
15	4-nonenal	Aldehyde	1093	1.45±0.57	1.57±0.51	1.33±0.53	2.75±0.47	1.90±0.56	-0.2061	-0.1049	-0.1375
16	methyl-indane	Aromatic	1144	0.96±0.40	0.97±0.49	1.20±0.47	2.37±0.40	2.28±0.27	0.1654	0.0423	0.1715
17	hexyl-cyclohexane	Cyclic alkane	1235	0.48±0.32	0.29±0.29	n.d.	1.70±0.46	2.58±0.29	0.0000	0.0000	-0.0633
18	heptyl-cyclohexane	Cyclic alkane	1339	1.34±0.51	2.99±0.38	2.93±0.37	2.97±0.36	2.71±0.36	0.2428	0.0292	0.0987
19	decyl-cyclohexane	Cyclic alkane	1653	1.98±0.11	2.27±0.10	2.09±0.27	2.36±0.05	1.81±0.21	0.3068	0.4003	0.3184
20	1-pentyl-cyclohexane	Cyclic alkane	1171	n.d.	2.13±0.42	1.54±0.49	n.d.	n.d.	0.0000	0.0000	0.0000
21	hexyl-cyclopentane	Cyclic alkane	1138	0.31±0.31	2.53±0.26	2.60±0.37	n.d.	n.d.	0.0129	0.0000	0.0000
22	hexadecane	Alkane	1600	0.95±0.39	1.51±0.48	1.01±0.31	2.19±0.37	2.05±0.24	0.2372	-0.2796	0.3165
23	n-pentadecane	Alkane	1500	0.28±0.28	2.13±0.69	2.08±0.57	3.64±0.15	2.07±0.48	0.0436	0.1352	0.1740
24	**p-menthane**	**Cyclic alkane**	**980**	**2.88±0.55**	**3.31±0.45**	**3.16±0.60**	**2.99±0.53**	**3.42±0.40**	**0.1488**	**0.1835**	**0.0226**
25	2,6 dimethyl-nonane	Alkane	1024	n.d.	0.60±0.40	n.d.	2.50±0.29	1.00±0.41	0.0000	0.0000	0.0704
26	Phenylacetylene	Aromatic	871	2.19±0.56	2.81±0.38	2.55±0.50	2.18±0.48	3.11±0.16	-0.0909	0.1528	0.0328
27	p,alpha-dimethyl styrene	Aromatic	1088	1.88±0.27	1.66±0.45	2.27±0.33	1.94±0.33	2.40±0.32	0.2625	-0.1207	-0.0934
28	coumarin	Aromatic	1427	0.34±0.34	2.81±0.41	2.49±0.48	2.27±0.52	0.24±0.24	0.0380	0.0805	0.0457
29	2-ethyl-1-hexanoic acid	Carboxylic acid	1125	0.41±0.41	3.39±0.16	3.58±0.13	n.d.	n.d.	-0.0179	0.0000	0.0000

Table S1 (continued)

#	Volatiles [a]	Compound class	RI [b]	Peak area/ mL sample [c]					Weight vector [d] (Bcon-DB20)	Weight vector (DS20-DB40)	Weight vector (DS20-DB20)
				B control	**DB20**	**DB 40**	**S control**	**DS20**			
30	acetic acid	Carboxylic acid	576	1.24±0.50	2.26±0.45	1.92±0.48	3.31±0.13	2.38±0.40	0.0922	-0.1343	0.0394
31	benzoic acid	Aromatic	1181	0.39±0.39	3.22±0.17	3.38±0.15	n.d.	0.41±0.28	-0.0061	0.1199	0.0920
32	ethyl hexanoate	Ester	1001	1.10±0.30	1.76±0.35	1.29±0.38	2.06±0.26	1.16±0.32	0.2242	0.1832	0.1775
33	pentyl ester salicylic acid	Ester	1570	0.36±0.36	1.87±0.61	2.87±0.40	n.d.	n.d.	0.0392	0.0000	0.0000
34	isopropyl ester octanoic acid	Ester	1235	0.36±0.36	2.80±0.20	2.78±0.38	n.d.	n.d.	-0.0082	0.0000	0.0000
35	hexyl salicylate	Ester	1673	2.94±0.10	2.03±0.51	1.26±0.50	0.56±0.38	0.88±0.45	0.0670	-0.0974	0.1673
36	ethyl palmitate	Ester	1995	0.40±0.40	2.97±0.40	3.48±0.13	n.d.	n.d.	-0.0085	0.0000	0.0000
37	2-acetyl furan	Furan	909	0.45±0.30	1.53±0.32	1.00±0.40	1.58±0.31	1.40±0.38	0.0392	-0.0786	-0.1646
38	2-methyl furan	Furan	585	0.49±0.33	1.41±0.36	0.73±0.38	1.97±0.19	1.64±0.29	-0.0474	0.1187	0.0013
39	2-hydroxy-1,2-diphenyl ethanone	Ketone	1805	1.46±0.51	2.58±0.52	3.14±0.41	2.38±0.40	3.03±0.19	0.1687	0.1603	0.1023
40	acetonyl acetone	Ketone	928	0.50±0.50	4.18±0.17	4.35±0.15	n.d.	0.33±0.33	-0.0074	0.0632	0.0475
41	2-heptanone	Ketone	890	0.35±0.35	2.52±0.36	2.96±0.16	n.d.	n.d.	0.0036	0.0000	0.0000
42	2-tetradecanone	Ketone	1597	0.92±0.46	1.54±0.50	1.20±0.40	2.65±0.20	2.12±0.35	0.0681	-0.2279	0.1716
43	acetoin	Ketone	709	1.65±0.53	2.57±0.49	3.14±0.07	1.95±0.53	2.43±0.43	0.1802	-0.0574	0.0730
44	2-butanone	Ketone	576	0.79±0.52	2.76±0.56	2.88±0.44	2.98±0.35	0.82±0.37	0.0714	0.2041	0.2218
45	6-methyl-2-heptanone	Ketone	951	3.33±0.14	3.42±0.24	3.43±0.12	n.d.	n.d.	0.1771	0.0000	0.0000
46	2-octanone	Ketone	990	2.23±0.42	1.60±0.51	1.95±0.49	2.21±0.48	3.01±0.07	0.0715	-0.0722	-0.1244
47	delta-nonalactone	Lactone	1386	0.69±0.47	2.45±0.63	3.20±0.43	0.37±0.37	n.d.	0.0698	0.0000	0.0000
48	gamma-hexalactone	Lactone	1052	1.54±0.61	2.24±0.56	3.26±0.09	n.d.	n.d.	-0.0955	0.0000	0.0000
49	beta-myrcene	Monoterpene	990	0.41±0.41	3.38±0.15	3.16±0.42	n.d.	n.d.	-0.0088	0.0000	0.0000
50	camphene	Monoterpene	942	1.48±0.48	1.87±0.47	2.03±0.39	2.98±0.10	2.43±0.28	0.1008	-0.0948	0.1286
51	alpha-pinene	Monoterpene	928	0.93±0.46	0.29±0.29	n.d.	2.77±0.21	1.15±0.47	-0.0626	0.0000	-0.0473
52	limonene	Monoterpene	1026	0.32±0.32	2.66±0.13	2.55±0.33	n.d.	n.d.	-0.0100	0.0000	0.0000
53	menthone isomer	Cyclic ketone	1152	2.67±0.51	4.16±0.10	2.80±0.71	4.01±0.04	1.39±0.58	0.4054	0.2181	0.2293
54	alpha-terpineol	Monoterpene alcohol	1189	0.28±0.28	2.62±0.36	1.94±0.52	0.17±0.17	n.d.	0.0520	0.0000	0.0000
55	2-cyclohexyl-phenol	Aromatic	1583	0.36±0.36	2.92±0.17	3.09±0.16	n.d.	n.d.	-0.0096	0.0000	0.0000
56	2,4-bis (1,1-dimethyl ethyl)-phenol	Aromatic	1512	1.10±0.37	1.73±0.45	1.76±0.45	1.81±0.41	0.62±0.41	0.2115	-0.1600	-0.1648

Table S1 (continued)

#	Volatiles [a]	Compound class	RI [b]	Peak area/ mL sample [c]					Weight vector [d] (Bcon-DB20)	Weight vector (DS20-DB40)	Weight vector (DS20-DB20)
				B control	**DB20**	**DB 40**	**S control**	**DS20**			
57	anthracene	Aromatic	1768	1.04±0.53	0.57±0.38	0.86±0.43	0.24±0.24	2.38±0.41	-0.0803	-0.1084	-0.0810
58	dimethyl trisulfide	Sulfide	960	0.47±0.47	3.93±0.18	3.75±0.48	n.d.	n.d.	-0.0035	0.0000	0.0000
59	diphenyl sulfide	Sulfide	1576	0.68±0.45	3.04±0.20	2.85±0.42	1.07±0.36	1.80±0.41	0.0420	0.2525	0.1824
60	carbon sulfide	Sulfide	541	0.51±0.51	4.28±0.14	4.43±0.13	n.d.	n.d.	-0.0111	0.0000	0.0000
61	unknown terpene	Terpene	1424	1.06±0.54	3.60±0.22	3.38±0.47	1.46±0.41	2.45±0.33	0.0878	0.3186	0.2493
62	polyalcene	Alkene	1579	2.13±0.55	2.14±0.57	1.54±0.62	3.44±0.24	2.89±0.36	0.1084	0.1384	0.1291
63	linalool isomer	Terpene alcohol	1099	0.43±0.43	3.64±0.18	3.80±0.18	n.d.	n.d.	0.0038	0.0000	0.0000

a. The table contains only those VOCs that were found in 80 % of the plants for each treatment group. The numbers in the first column refer to the points in the bubble plot, Fig. 4. Compounds were identified based on mass spectra (MS) reference manuals (Adams 2007), NIST 2008, Wiley 7th ed. MS library, and our own MS and linear retention indices (LRI) literature library. All herbivore damaged plants had P. brassicae larvae feeding on their leaves at the time of VOC collection. **b.** RI=retention index on the GC-MS; column RTX5-MS.One terpene could not be sufficiently identified based on their RI value and mass spectrum using our criteria, and was designated as unknown terpene. **c.** Peak area of VOC is mean (± SE) per mL of sample. **d.** OPLS-DA weight vector is the relative importance of each volatile in the total blend per plant of the two groups compared in each comparison.

CHAPTER 6

Variation in plant defences among populations of a range-expanding plant: consequences for native trophic interactions

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Abstract

- While plant-herbivore-enemy interactions have been studied extensively in inter-continental plant invasions, such knowledge remains scarce for intra-continental range expansions, despite their rapid spread due to global changes. In their new range, range-expanding plants may encounter the same insect species that co-occur with them in their native range.
- Using an ecological and metabolomics approach, we compared the performance of a specialist and a generalist herbivore and a parasitoid, as well as, plant defence traits between native and exotic (invasive and non-invasive) populations of the Turkish rocket, Bunias orientalis, a range-expanding plant species across parts of Eurasia.
- The generalist herbivore Mamestra brassicae and its parasitoid Microplitis mediator performed better on the exotic plant populations than on the native populations. Conversely, survival of the specialist herbivore Pieris brassicae varied between plant populations, but was generally lower on native plants. Plant populations differed significantly in metabolic fingerprints and trichome densities, as well as in the expression of secondary metabolites (e.g. glucosinolates). Exotic populations exhibit higher concentration of sinalbin glucosinolate, but much lower concentrations of glucoputranjivin, and lower trichome densities than their native conspecifics.
- In generally, exotic populations of Bu. orientalis are less defended than native conspecifics, potentially due to a higher herbivore pressure in the native range. Enemy release and potential reallocation of resources into less costly defence traits and/or other fitness components may explain the success of this range-expander in new habitats.

Introduction

The introduction of plants and animals from their native ranges into non-native ecosystems and their concomitant establishment and spread is now considered one of the most important threats to biodiversity worldwide. A number of exotic species become dominant pests in their new ranges, out-competing and displacing native species, disrupting trophic chains and food webs and consequently undermining key ecosystem processes. Plant invasion research has, thus far, focused on inter-continental invasions, e.g. from Eurasia to North America (and vice-versa), although it is now known that intra-continental range-expanding plant species can also exhibit invasive properties (Engelkes et al. 2008). However, little is known about the consequences of range-expansion of plants on ecological parameters such as the ones shaping plant-herbivore-carnivore interactions in their new range (van der Putten 2012).

The origin of exotic plants may determine the success of various plant-related defence traits against herbivores in the new range. The standard interpretation of an exotic plant is a species that is of inter-continental origin and has usually been accidentally or intentionally introduced by man into a non-native habitat (Richardson et al. 2000a). However, this definition is incomplete, because some continents are separated by large bodies of water (which greatly impede dispersal), whereas others are not. Currently, many plant species successfully expand their ranges within continents due to indirect anthropogenic processes such as land use changes and climate warming (Engelkes et al. 2008). The identity and genetic composition of the potential enemies, such as herbivores and pathogens, as well as higher trophic levels, such as parasitoids and predators that are encountered by range-expanding plants in the new habitat might not be as different as the ones in the native range as in classic invasion scenarios. Additionally, the changing environment may also provide the natural enemies with equal opportunities of spread and establishment. For instance, Asian plants that have been introduced into North America might encounter far more potentially novel herbivores than the same plants that are introduced into Europe, because Europe and Asia represent a contiguous land mass.

When establishing into new habitats range-expanding plants might encounter the same species of herbivores that habitually feed on them in the native range, but which are not adapted to them in the new range. This is especially true if the exotic plant populations contain novel defences that are absent in related plants in the new habitat (Callaway and Aschehoug 2000; Cappuccino and Arnason 2006; Mitchell et al. 2006). Current hypotheses explaining the factors that enable invasive plants to thrive in the new range have generally been formulated on the basis of inter-continental invasions. For example, the enemy-release (ERH; Keane and Crawley 2002) and the evolution of increased competitive ability (EICA) hypotheses (Blossey and Nötzold 1995) predict that exotic invasive plants incur less damage from novel native herbivores. This can facilitate rapid evolutionary shifts of resource investment to other functions, such as growth and reproduction, rather than defence, enabling invasive plants to spread and out-compete native vegetation in the invaded communities. Alternatively, exotic plants may still be attacked by generalist herbivores in the new range (Verhoeven et al. 2009). Hence, these plants may increase their investment in relatively cheap qualitative chemical defences (e.g. phytotoxins) against generalist herbivores, whilst reducing more costly quantitative defences (e.g. cellulose, trichomes) aimed at both specialist and generalist herbivores (shifting defence hypothesis, SDH) (Müller-Schärer et al. 2004; Joshi and Vrieling 2005). Therefore, studies of potential shifts in resource allocation of successful intra-continental range-expanding plants must also take into account the identity and dietary breath of potential plant enemies in the new range.

Plant-herbivore interactions are mainly driven by the specific plant quality. Certain plant-related defence traits appear to play a prominent role in enabling the plants to resist attack from native herbivores. These include the production of toxic secondary metabolites and morphological structures, such as sticky glands and trichomes (Schoonhoven et al. 2005). The effectiveness of different plant defences will depend on various factors, including the novelty of these defence traits to local herbivore assemblages, and thus whether the herbivores are adapted to counter the plant traits. The effectiveness of chemical defences, for instance, may vary depending on the dietary breath of the herbivores and whether the novel plant contains toxins that are found in native plants that they normally attack (Ehrlich and Raven 1964; van der Meijden 1996). Plant secondary metabolites may deter feeding by both generalist and specialist herbivores, but the same compounds may stimulate feeding and oviposition by other specialists (Blau et al. 1978; Lankau, 2007; Gols et al. 2008a). Furthermore, herbivore attack is often highly variable in space and time, hence plant populations respond by exhibiting variations in defensive traits that are ecologically important in a given habitat (Mithen et al. 1995; Macel et al. 2004; Gols et al. 2008a; Wolf et al. 2011).

The Turkish rocket, Bunias orientalis L. (Brassicales: Brassicaceae) is a range-expanding plant from extreme south-eastern Europe and western Asia that has become invasive in recent years in parts of northern and central Europe, including Germany and

Czech Republic (Steinlein et al. 1996; Dietz et al. 1999; Harvey et al. 2010a). At the same time, Bu. orientalis is less common in parts of western Europe, such as France and the Netherlands (Harvey et al. 2010a). Previous studies have shown that Bu. orientalis is of poor quality for the development of brassicaceous specialist herbivores from German and Dutch populations (Travers-Martin and Müller 2008; Kühnle and Müller 2009; Harvey et al. 2010a; Fortuna et al. 2012). By contrast, a Dutch population of one generalist herbivore species, the cabbage moth Mamestra brassicae L. (Lepidoptera: Noctuidae), readily feeds on the exotic plant populations and achieves high fitness on it (Harvey et al. 2010a; Harvey and Gols, 2011). Like all Brassicaceae, Bu. orientalis produces glucosinolates (GS), secondary metabolites that play a role in defence against herbivores (reviewed in Renwick 2002), and thus may also influence the success of plant invasions (Cipollini et al. 2005; Müller 2009). In addition to GS chemistry, high trichome densities and elevated concentrations of enzymes such as proteinase inhibitors in Bu. orientalis may contribute to reduced survival of specialist herbivores, such as Athalia rosae L. (Hymenoptera:Tenthredinidae) (Travers-Martin and Müller 2008). Furthermore, plant defence traits do not only impact the herbivores, but also the performance of their natural enemies. The performance of insect natural enemies, such as parasitoids is affected by differences in the quality of their host's diet, which is often mediated by plant secondary metabolites (Harvey 2005; Ode 2006; Gols et al. 2008a;b). Recent studies have reported that the development of two larval parasitoids was negatively affected when reared on exotic populations of Bu. orientalis (Harvey and Gols 2011; Fortuna et al. 2012). However, it is unknown how generalist herbivores and their natural enemies perform on Bu. orientalis populations originating from both its native and exotic ranges.

Metabolomic fingerprinting in combination with targeted analysis of plant defences is particularly powerful for studying the mechanisms underlying ecology and evolutionary processes, such as plant resistance to herbivores (Macel et al. 2010; Sutter and Müller 2011). Here, using a combined ecological and metabolomics approach, we examined differences in metabolic pattern and morphological traits (trichomes, specific leaf area) between Bu. orientalis populations originating from the native and exotic, invasive or non-invasive, range. For simplification exotic invasive plant populations are named thereafter as invasive, while exotic non-invasive are named as only exotic. We conducted development bioassays to compare the performance of the generalist herbivore Mamestra brassicae and its endoparasitoid, Microplitis mediator Haliday (Hymenoptera: Braconidae) on Bu. orientalis plants from the different populations. We also tested the performance of the specialist herbivore, Pieris brassicae L. (Lepidoptera: Pieridae) on single populations of Bu. orientalis from each origin. The herbivores and the parasitoid were collected from Dutch populations, and therefore are sympatric with exotic populations of Bu. orientalis in the Netherlands, but not with the invasive and native plant populations. However, both herbivore species and Mi. mediator are also native and abundant in parts of Eurasia where Bu. orientalis is native (Güçlü et al. 2006; Secmen et al. 2010). The range-expanding populations of this plant are, therefore, potentially encountering new populations of the same herbivores (and their natural enemies) which may interact with the plant in its native range.

We tested the following hypotheses: (1) the performance of the specialist and generalist herbivores will be differentially affected by plant quality characteristics at the population level; (2) the insects will perform better on local (Dutch) populations of Bu. orientalis with which they may be locally adapted; (3) the performance of the parasitoid Mi. mediator will closely reflect that of its host M. brassicae. A previous study reported very low survival of Cotesia glomerata L. (Hymenoptera: Braconidae), an important parasitoid of P. brassicae, when reared on one of the invasive Bu. orientalis populations (Fortuna et al. 2012), thus it was not included here; (4) the chemical and mechanical plant defence traits exhibit intraspecific variation in native and exotic, invasive and non-invasive, populations of Bu. orientalis; (5) this variation reflects differences in the performance of specialist and generalist insect herbivores, as well as in the performance of the parasitoid in the plant new range.

This is, as far as we know, the first study employing untargeted metabolic fingerprinting combined with targeted analysis (GS analysis) to study defence patterns of both native and non-native plant populations in a multitrophic framework. We also take into consideration the geographic origin of the insects, something that is often overlooked in plant-insect interaction studies. We argue that invasion ecology needs to consider more intimate processes that incorporate the ecophysiology of interactions involving the plants and their insect herbivores and in turn, their natural enemies. In this way we will be able to isolate mechanisms involved in insect responses to potentially novel defence traits in invasive plants at both microscopic and macroscopic scales.

Materials and methods

Plant and insects

Turkish rocket seeds were collected from several plants from nine populations growing in disturbed sites in the invasive (Czech Republic, Germany), exotic (The Netherlands) and native (Turkey) geographical range. Seeds from Czech Republic (CZ) were collected and pooled from three sites (50º32’ N, 14º6’ E/ 50º31’ N, 13º58’ E/ 50º30’ N, 13º57’ E). In Germany, seeds were obtained from a population in Jena (JE: 50º52’ N, 11º35’ E) and one in Würzburg (WU: 49º50’ N, 9º51’ E). In the Netherlands, the three populations were located in Drempt (DE: 52º0’ N, 6º9’ E), Driel (DI: 51º58’ N, 5º51’ E) and Wageningen (WA: 51º58’ N, 5º40’ E). Dutch populations are considered exotic non-invasive because Bu. orientalis offspring does not form dominant stands far from the parental plants (Richardson et al. 2000a). In Turkey, the three populations were located near Rize, on the Basköy plateau, 2000 m altitude (T1: 40º44’ N, 040º45’ E/ T3: 40º43’ N, 040º47’ E/ T4: 40º44’ N, 040º44’ E).

The generalist herbivore M. brassicae is native to the Palearctic, and is frequently considered a pest in cabbage and other crops (Theunissen et al. 1985). Microplitis mediator is a solitary larval endoparasitoid native to Eurasia that attacks first (L1) to third (L3) instar larvae of M. brassicae and a few closely related hosts in the Noctuidae (Gols et al. 2008a). Pieris brassicae is also native to most of Eurasia and is a specialist leaf chewer of Brassicaceae known to feed on several species within this family (Feltwell 1982). All insects were obtained from an insect culture reared on Brussels sprouts plants, Brassica oleracea gemmifera cv. Cyrus, maintained at the Laboratory of Entomology of Wageningen University, The Netherlands.

Insect Performance

Bunias orientalis seeds were germinated as described by Harvey et al. (2010). Seedlings were transferred to 2.1-L pots with soil (‘Lentse potgrond’ no. 4, Lent, The Netherlands). Plants were grown in a greenhouse compartment (18-25ºC, 50-70% r.h., 16L:8D photoperiod). If the light intensity dropped below 500 µmol photons m^{-2} sec^{-1} during the light period, supplementary illumination was supplied by high pressure mercury lamps (225 µmol photons m^{-2} sec^{-1}). Plants were watered every other day, and after 4 weeks, fertilized once a week with Kristallon Blauw (16N: 6P: 20K: 3Mg, concentration 2.5 mg/L). Watering and fertilization continued during the experiments.

Four weeks after transplanting seedlings, the pots were randomly allocated to one of the following three treatments: non-parasitized and parasitized herbivores and undamaged control plants. All treatments were replicated seven times, except the control treatment (three times). Each replicate consisted of three Bu. orientalis plants that were placed into a finely meshed cage (35 x 35 x 60 cm). Undamaged control plants were also placed in cages but were not exposed to herbivory. As mortality tends to be high during the first larval instar, neonates were placed in Petri dishes with excised leaves and moistened filter paper. For the non-parasitized herbivore treatment, twenty neonates of M. brassicae were randomly allocated to labeled Petri dishes (9 cm diameter) with middle-aged leaves that were excised from plants according to their replicate (cage). After three days of feeding, the caterpillar mortality was recorded and ten of the surviving larvae, randomly chosen, were carefully placed on the plants inside their respective cages. Caterpillars were allowed to move freely within each cage. Because mature larvae normally burrow into the soil to pupate, caterpillars close to pupation were placed in containers (18 x 13 x 6 cm) with a 3-cm layer of vermiculate and some leaf material of the respective plant population. The containers were checked daily and fresh pupae were collected and weighed on an analytical balance. Larval development time was determined as the number of days between egg hatching and pupation. The percentage of larvae that survived until the pupal stage was calculated. For the parasitized herbivore treatment, thirty neonates of M. brassicae were randomly allocated to Petri dishes (14 cm diameter) with excised leaves as described before. After three days, the caterpillars were presented individually to Mi. mediator mated female wasps in small plastic vials which were allowed to sting the host once. Twenty-four hours later, parasitized caterpillars were placed on the Bu. orientalis plants inside each cage. After pupation, parasitoid cocoons were collected from each cage and placed individually in Petri dishes until adult wasp emergence. At emergence, the date of eclosion was recorded and parasitoids were transferred to labeled glass vials, dried in an oven (at 60ºC during 3 days), sexed and weighed on a microbalance. Development time was calculated as the number of days between parasitism (egg laying) and adult emergence. Parasitoid survival was defined as the ratio between the number of hosts from which adult wasps emerged and the total number of hosts parasitized initially.

The performance of P. brassicae was also compared on three Bu. orientalis populations (invasive range, CZ; exotic range, DI; and native range, T1) that were also used for chemical analysis. Seeds were germinated and grown in a greenhouse with the same conditions as described above. Four to five weeks after seedling transplantation, four plants were randomly allocated to fine meshed cages (35 x 35 x 60 cm) in a total of three cages per population. After egg hatching, twenty neonates of P. brassicae were carefully placed on a middle-aged leaf of one of the four plants in each cage. Larvae were allowed to feed and move freely within each cage. When caterpillars developed into pre-pupae, the cages were checked daily. Pupae were collected and their weight and pupation day was recorded. The percentage of larvae that survived until the pupal stage was also determined.

Chemical analyses of leaf tissues

The chemical analyses were performed on the same plants as used in the M. brassicae bioassay. To compare the metabolic fingerprints and glucosinolate profiles in leaf tissues of Bu. orientalis, only a subset of the samples of the plant populations, on which the herbivore showed the most contrasting performance, were initially analysed. This included CZ from the invasive range, DI from the exotic non-invasive range and T1 and T4 from the native range. Additionally, for the targeted analysis of glucosinolates leaf samples from the rest of the plant populations were also analysed. To investigate the effect of herbivory on the plant metabolome, leaf samples were taken from undamaged control plants (-H, n=3) and plants damaged by unparasitized M. brassicae larvae (+H, n=6-7), all plants of the same age. Furthermore, to investigate the effect of age, leaf samples were also taken from undamaged plants at two time points, at the beginning of the insect performance biossays (4-weeks old plants) and at the end when larval development was completed (8-weeks old plants). Five leaf discs (9 mm diameter) were sampled per plant from the upper-middle part, avoiding the main veins, of middle-aged leaves. Leaf discs from all three plants within each replicate were pooled in 2-mL Eppendorf vials. Samples were immediately frozen in liquid nitrogen and stored at -80ºC until they were freeze-dried at -50ºC for 72 h. For untargeted metabolic fingerprinting and targeted analysis of glucosinolates, the lyophilized leaf samples were prepared following the protocol by Kutyniok and Müller (2012). Details on sample preparation for uHPLC-ToF-MS measurements and metabolite quantification are given in the Supplementary material (see Appendix S1).

Leaf morphology

Trichome density was determined on abaxial and adaxial leaf surfaces of Bu. orientalis plants from all nine populations grown in the greenhouse. A leaf square (area 4 cm^2) was cut from the upper-middle part, avoiding the main veins, of middle-aged leaves. Three leaves were sampled per plant of eight plant replicates within each population. Leaf squares were then photographed with a microscope camera (Carl Zeiss AxioCam MRc 5) coupled to a stereo-microscope and images were processed with Image software AxioVision LE, Release 4.8. The number and type of trichomes were counted in a 1 cm^2 area randomly chosen in each picture. Three types of trichomes were identified according to their length and shape: A) long and straight with a wide base; B) short and straight; C) Y-shaped with variable length (Figure 4d). Simple and branched trichome types have been described for brassicaceous species, including the family lineage of Bu. orientalis (Beilstein et al. 2006). The leaf samples were freeze-dried at -50ºC for 72 h and their dry weights were recorded. Specific leaf area (SLA, $cm^2.g^{-1}$) was calculated as the ratio between the leaf area (4 cm^2) and sample dry weight. Plants used to estimate trichomes and SLA were 7 weeks old.

Statistical Analyses

All analyses were performed using GenStat 14th edition (VSN International, UK), except for the chemical data that were analyzed using R software version 2.15.1 (R Development Core Team, 2011) and SAS version 9.3 (SAS Institute Inc., USA).

Insect performance: Differences in M. brassicae and Mi. mediator survival among plant populations were analyzed using a one-way ANOVA with plant population as main factor. Insect performance data were analyzed using a linear mixed model (restricted maximum likelihood method: REML), with plant population as fixed factor and cage as random factor. In the parasitoid performance analysis, sex and its interaction with plant population were also included as fixed factors. Pairwise comparisons were performed a posteriori using Fisher's LSD tests. Prior to analysis, M. brassicae survival proportions were arcsine square-root transformed; M. brassicae pupal fresh mass and Mi. mediator adult dry mass and development time were log transformed to fulfil assumptions of normality and homoscedasticity. The survival data of P. brassicae was analyzed using the non-parametric Kruskal-Wallis test, as assumptions of normality and homoscedacity could not be met.

Chemical analyses: To visualize differences in metabolic fingerprints between plant populations and treatment groups, principal components analyses (PCA) was performed. The resulting scores of the samples (PC2-PC3 as described in Kutyniok and Müller 2012) were tested for significant differences with Kruskal-Wallis tests followed by post hoc Mann-

Whitney test with Bonferroni correction. For metabolomic data pre-treatment see Appendix S2, in Supplemental material. Differences in total GS concentrations and that of the two most abundant GS (p-hydroxybenzyl GS, methylethyl GS) were analysed with respect to plant population and treatment (herbivory or plant age). Herbivory effect was tested by comparing plant chemistry in control and herbivore-treated leaf tissues collected at the end of the experiment. Plant-age effect was tested by comparing samples of undamaged leaves collected just prior to herbivory and collected from control plants at the end of the experiment. GS data were analysed using a two-way ANOVA with plant population, treatment and their interaction as main factors. As Levene's test revealed unequal variances among groups in p-hydroxybenzyl GS and 1-methylethyl GS in response to herbivory, a two-way ANOVA was conducted using actual variances. ANOVA analyses were followed by post hoc Tukey-Kramer tests. Prior to analyses, total GS and p-hydroxybenzyl GS concentrations were log transformed; 1-methylethyl GS concentrations were log (x+1) transformed. Differences in 1-methylethyl GS concentrations among plant populations to examine plant age effect were tested using the non-parametric Mann-Whitney test, as both assumptions of normality and homoscedasticity could not be met.

Leaf morphology: Differences in density and type of trichomes between different plant populations were analysed using a generalized linear mixed model (GLMM) with plant population, trichome type and their interaction as fixed factors and individual plant as random factor. GLMM were fitted with a Poisson distribution and a logarithm link function. Specific leaf area data was analysed using one-way ANOVA with plant population as main factor.

Results

Insect performance: Mamestra brassicae and Microplitis mediator

Overall, larval survival of M. brassicae was significantly affected by plant population ($F_{8,52}$ = 16.6, P < 0.001). Survival of larvae was lower on native Bu. orientalis (less than 40%), than on exotic and invasive plants (higher than 80%; Figure 1a). Plant population also affected early larval survival ($F_{8,54}$ = 3.00, P = 0.007). Survival during the first three days after hatching was high (between 78% and 99%); however, no clear relationship was found between survival and plant origin (Figure S1). Plant population had also a significant effect on pupal biomass ($F_{8,48}$ = 11.4, P < 0.001), as well as egg-to-pupa development time ($F_{8,49}$ = 9.22, P < 0.001). Pupae were heavier and developed faster on plants from the exotic and invasive range than on plants from the native range (Figure 1b, 1c). Parasitoid survival, biomass and development time were all affected by the plant population on which the host was reared (survival: $F_{8,53} = 3.64$, $P = 0.002$; biomass: $F_{8,44} = 3.09$, $P = 0.007$; development time: $F_{8,45} = 3.16$, $P = 0.006$). Compared to the results for the herbivore, the effects of plant population were similar but less pronounced for Mi. mediator with respect to survival and development time, whereas the relationship between plant geographical origin and parasitoid biomass was absent (Figure 1d, 1e, 1f). Development time was also affected by the offspring sex ($F_{1,440} = 25.5$, $P < 0.001$), with males developing faster than females (Figure 1f).

Insect performance: Pieris brassicae

Pieris brassicae development to pupal stage was very variable on Bu. orientalis plants, irrespective of their origin. Overall, it was a poor quality food plant for this herbivore. No significant differences were found in the survival of P. brassicae between plant populations ($H = 2.289$, d.f. = 2, $P > 0.10$). However, there was a non-significant trend for a lower larval survival on Bu. orientalis plants from the native range compared to those on plants from the non-native origin. For instance, the larval survival was 0% in all three replicates of the native plant population (T1); whereas 0%, 0% and 50% on the exotic plants (DI), where few larvae survived; and 0%, 65% and 95% on the invasive plants (CZ), where more larvae survived until pupation (Figure S2). Since no larvae survived on the native populations, data on the herbivore development time and biomass on the other two plant populations are not presented.

Chemical analysis of leaf tissues

Metabolic fingerprints: The methanolic extracts of Bu. orientalis of all four populations (CZ, DI, T1, T4) contained more than 19,000 peaks of polar compounds, including fragments and adducts. Plant volatile compounds were, therefore, not analyzed in this study. The fingerprints of the different plant populations could be separated along the second and third principle component (PC2-PC3) for the herbivore-damaged plants (PC2: $\chi^2_3 = 19.0$, $P < 0.001$; PC3: $\chi^2_3 = 9.87$, $P = 0.02$, Figure 2a), and marginally for the control plants along PC2 (PC2: $\chi^2_3 = 7.52$, $P = 0.06$; PC3: $\chi^2_3 = 0.06$, $P = 0.996$).

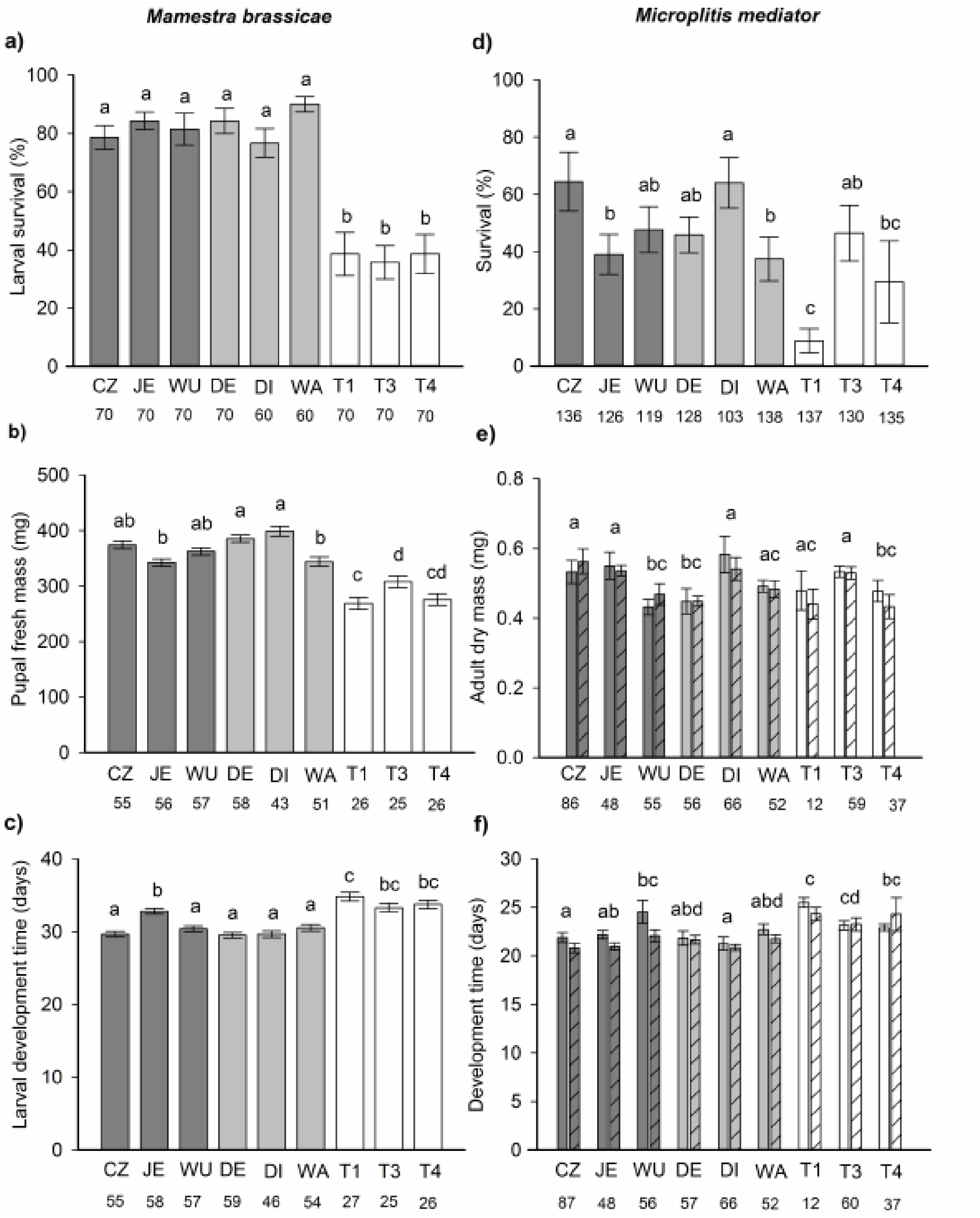

Figure 1 Performance of Mamestra brassicae and Microplitis mediator on plants from different Bunias orientalis populations. Survival, body mass and development time of the herbivore M. brassicae **(a-c)** and its endoparasitoid Mi. mediator **(d-f)** on plants from Bu. orientalis populations from its invasive range (dark grey bars: CZ, JE, WU), exotic range (light grey bars: DE, DI, WA) and native range (white bars: T1, T3, T4). Mi. mediator data is shown for females (solid bars) and males (diagonal-stripped bars). Bars give the mean values (± SE). Sample sizes are given below the x-axis. Different letters indicate significant differences at P< 0.05.

The metabolic fingerprints of the two native populations differed from those of the exotic and invasive population. Moreover, differences between herbivore-damage and control plants were more pronounced in the two native than in the exotic and the invasive population (Figure 2a). In addition, samples taken from undamaged plants differing in age could be separated along PC2 for the 4-weeks old plants (PC2: χ^2_3 = 19.6, P < 0.001; PC3: χ^2_3 = 1.87, P = 0.60, Figure 2b), and marginally for the 8-weeks old control plants (PC2: χ^2_3 = 7.59, P = 0.055; PC3: χ^2_3 = 0.13, P = 0.99). Geographical origin related differences are more pronounced in younger than older plants of all populations. PC1 explained 20.7% of the variance in the herbivory-control analysis and 19.4% in the ageing effect analysis, but did not contribute to class separation, indicating that it primarily showed instrumental variation and not biological variation.

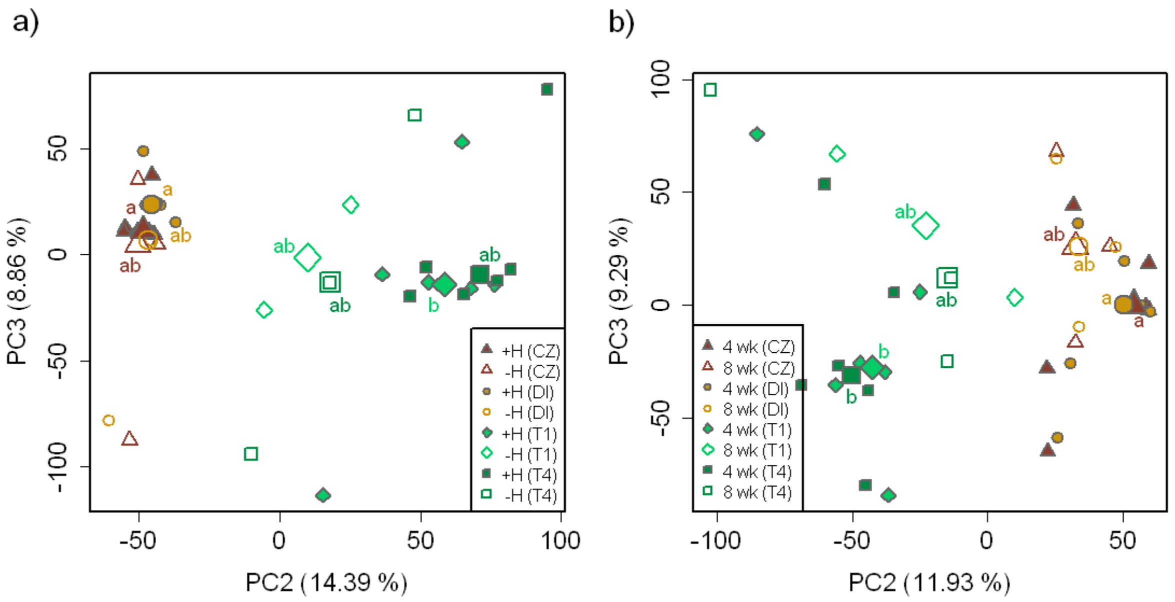

Figure 2 PCA scores plot of the metabolic fingerprints of plants from different Bunias orientalis populations. Representation of the metabolic fingerprints of methanolic extracts from leaf tissue of Bu. orientalis populations from its invasive (CZ, red triangles), exotic (DI, yellow circles) and native range (T1, green diamonds; T4, green squares). Large symbols represent the centroids of the population clustering mean values of PC2-PC3 scores. **(a)** Profile of samples collected from 8-weeks old plants with herbivores (+H, close symbols, n=6-7) or without herbivores (-H, open symbols, n=3). Plants were induced by feeding of 10 Mamestra brassicae larvae for four weeks. **(b)** Fingerprints of samples collected at the beginning (4-wk, close symbols, n=6-7) and at the end (8-wk, open symbols, n=3) of the experiment. In total, 19,602 peaks (metabolites with fragments and adducts) were found by uHPLC-ToF-MS, using positive and negative electronic ionization. Percentage of explained variation by each PC is given in parentheses. Different lowercase letters indicate significant differences at P<0.05 between the scores of the PC2 axis.

Glucosinolates: GS analysis of leaf tissues revealed strong quantitative variation between the four Bu. orientalis populations, CZ, DI, T1 and T4 (Figure 3). The GS profile of the invasive and exotic plants were dominated by the aromatic p-hydroxybenzyl GS (sinalbin) and only traces of the aliphatic GS 1–methylethyl GS (glucoputranjivin) and 4-methylsulfinylbutyl GS (glucoraphanin) were detected (Figure 3a). In contrast, leaves of the native plants contained high concentrations of both p-hydroxybenzyl GS and 1–methylethyl GS (Figure 3b). Levels of 4-methylsulfinylbutyl GS were less than 0.3µmol/g DW in all populations. This was true for all populations used in the herbivore bioassays (data not shown). Overall, the total GS concentration was similar in the four plant populations (herbivory comparison: $F_{3,29} = 0.43$, $P = 0.73$; age comparison: $F_{3,31} = 1.46$, $P = 0.24$) and increased significantly in all populations after M. brassicae herbivory ($F_{1,29} = 54.6$, $P < 0.001$). The effect of plant age on total GS concentration depended marginally on the plant population (population x age interaction $F_{3,31} = 2.74$, $P = 0.06$; Table S1; Figure 3c). With respect to individual GS, the p-hydroxybenzyl GS concentration was lower in the two native populations than in the introduced populations ($F_{3,29} = 37.36$, $P < 0.001$; Table S1) and increased in all populations in response to herbivory ($F_{1,29} = 39.27$, $P < 0.001$). Thereby, the effect of plant age on p-hydroxybenzyl GS depended on plant population (population x age interaction $F_{3,31} = 5.70$, $P = 0.003$; Table S1; Figure 3a). The concentration of 1–methylethyl GS was very low in the invasive and exotic populations and dramatically higher in the native populations ($F_{1,29} = 13.6$, $P = 0.004$; Table S1), and it only increased significantly in response to herbivory in the native T4 plants (population x herbivory interaction $F_{3,29} = 7.57$, $P = 0.016$; Table S1). Concentrations of 1–methylethyl GS were not affected by plant aging in any of the four populations ($P > 0.05$; Table S2; Figure 3b).

Leaf morphology: Trichomes

Trichome density on Bu. orientalis leaves also revealed considerable quantitative and qualitative variation among the nine plant populations (Figure 4, 5). On average, the total density of trichomes was higher on the native plants than on the invasive and exotic plants on both adaxial ($F_{8,63} = 26.55$, $P < 0.001$) and abaxial ($F_{8,63} = 11.00$, $P < 0.001$) leaf surfaces (Figure 5). The densities of the three trichome types also differed on both surfaces (adaxial: $F_{2,619} = 3160.03$, $P < 0.001$; abaxial $F_{2,619} = 3331.20$, $P < 0.001$).

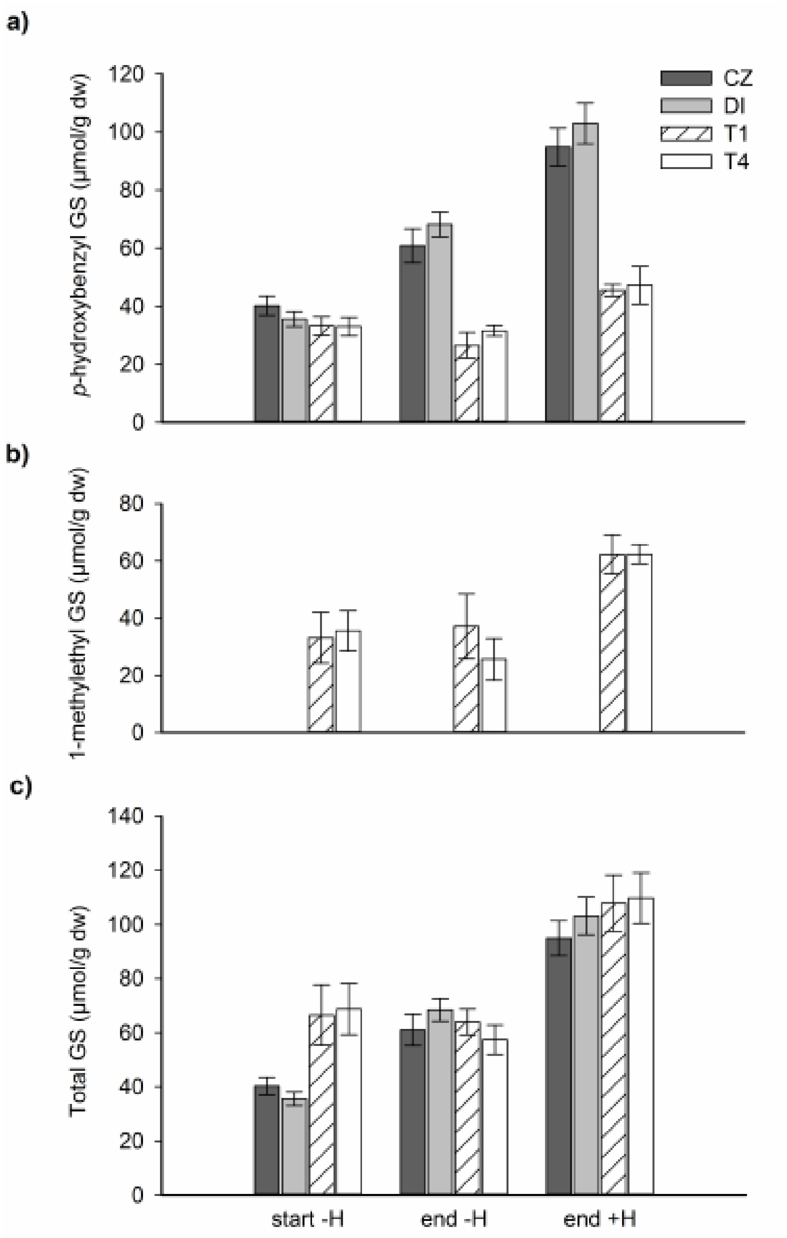

Figure 3 Glucosinolate profile of plants from different Bunias orientalis populations. Mean concentration (± SE, µmol/g dw) of **a)** p-hydroxybenzyl GS (sinalbin); **b)** 1-methylethyl GS (glucoputranjivin) and; **c)** total GS in leaf tissue of plants from Bu. orientalis populations from its invasive range (CZ, dark grey bars), exotic range (DI, light grey bars) and native range (T1, white striped bars; T4, white bars). Samples were taken from 4-weeks old plants without herbivores (start, -H, n=6-7) and 8-weeks old plants (end) without herbivores (-H, n=3) or with herbivores (+H, n=6-7). +H plants were induced by feeding of 10 M. brassicae larvae for four weeks.

However, these differences in density and types of trichome depended on the plant population (population x type interaction adaxial: $F_{16,619} = 362.98$, $P < 0.001$; abaxial $F_{16,619} = 362.98$, $P < 0.001$). Although the three trichome types were present in all plant populations, some types were more abundant in some populations than in others. For instance, the Y-shaped trichome type C was more abundant in all native populations, particularly on the adaxial leaf surface, whereas in most of the invasive and exotic populations the straight-short trichome type B was predominant, particularly in the abaxial leaf surface. The straight-long trichome type A was present in lower densities than the other two types on both leaf surfaces in all plant populations. The density of this trichome type was especially low on the adaxial leaf surface of plants from the native range, where trichome type C is dominant (Figure 4, 5).

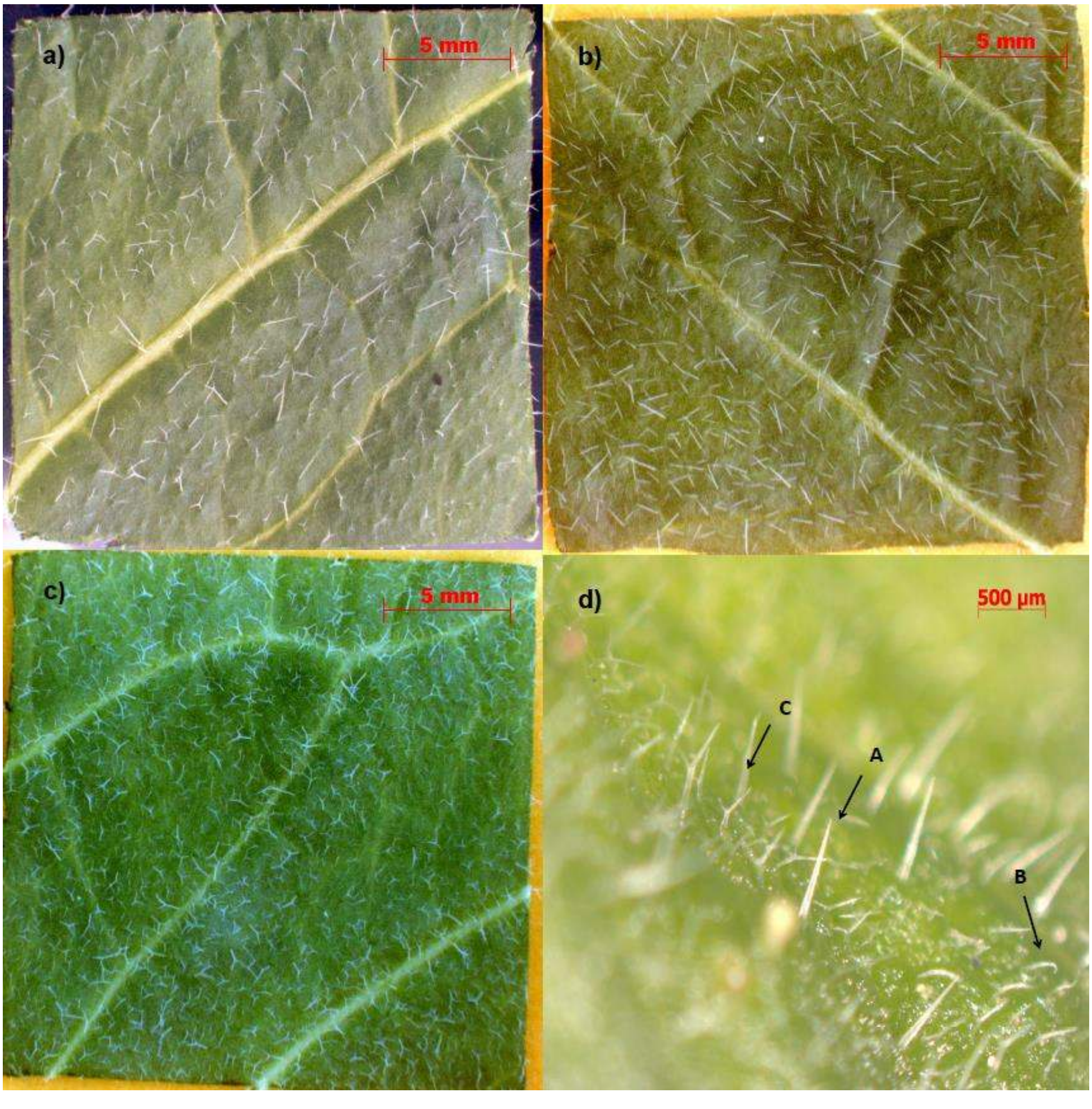

Figure 4 Leaf trichomes on plants from different Bunias orientalis populations. Trichome distribution on the adaxial surface of Bu. orientalis leaves from populations where the species is: a) invasive (CZ), b) exotic (DI) and, c) native (T1). d) The three types of trichomes that were identified according to their shape and length: A. long straight hair with a wide insertion on the leaf surface; B. short straight hair; C. short or long hair with Y-shape. Bars in a) to c) = 5mm; bar in d) = 500 µm.

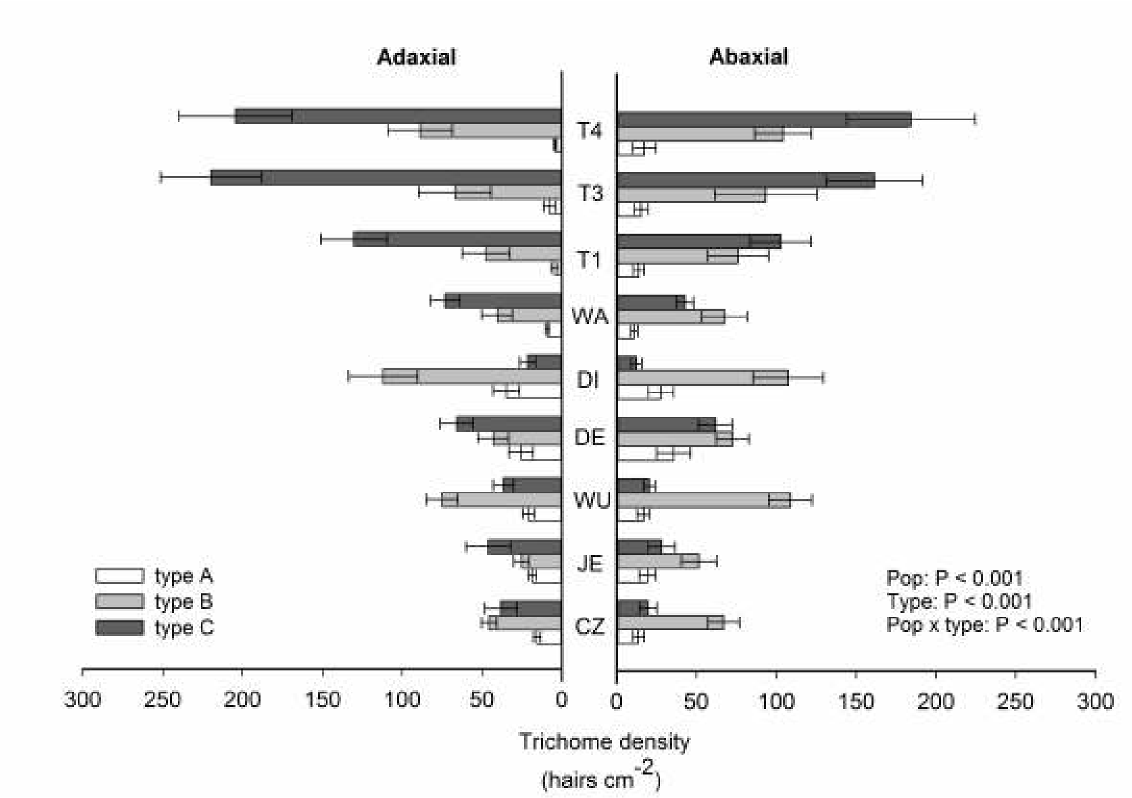

Figure 5 Leaf trichome densities on plants from different Bunias orientalis populations. Mean (± SE, hairs.cm^{-2}) density of trichomes on adaxial and abaxial surface of Bu. orientalis plants (n=8) from populations of different origin: invasive (CZ, JE, WU), exotic (DE, DI, WA) and native range (T1, T3, T4). Three types of trichomes were identified: (A) long and straight with a wide base; (B) short and straight; (C) Y-shaped and variable length. Three middle-aged leaves were sampled per plant of each population. Significance of the main effects, plant population and trichome type, are based on a generalized linear mixed model (GLMM). The interaction between population and trichome type is significant and therefore was included in the model (P<0.001).

Leaf morphology: SLA

Overall, the specific leaf area of Bu. orientalis plants varied on average between 166 and 227 cm^2.g^{-1} (Figure S3), but did not differ significantly among the nine plant populations ($F_{8,63}$ = 1.53, P = 0.165).

Discussion

Our results reveal that the performance of the generalist herbivore M. brassicae and its parasitoid Mi. mediator (originating from the Netherlands) was more negatively affected on the native populations of Bu. orientalis than on the exotic, invasive and non-invasive, conspecific populations. Survival of a Dutch population of the specialist herbivore P.

brassicae was lower than M. brassicae on the three Bu. orientalis populations tested, and this herbivore did not survive at all on plants from the native range.

In introduced areas, exotic plants may face different biotic and abiotic pressures from those experienced in their native range. If the cost of maintaining certain traits, such as defence is high, then plants may reallocate resources to other functions, such as growth and competition, and invest less in defence in the new range when attackers are absent. This has been shown, for example, for inter-continental plant invaders, such as in populations of Silene latifolia Poiret (Caryophyllaceae) (Wolfe et al. 2004) and Pastinaca sativa L. (Apiaceae) (Zangerl and Berenbaum 2005) in North America, in which both species escaped a single species of co-evolved lepidopteran insect herbivores. In contrast, introduced populations of some brassicaceous species in North America do not exhibit the same pattern (Bossdorf et al. 2004; Müller and Martens 2005; Oduor et al. 2011). For instance, introduced populations of Lepidium draba L. (Brassicaeceae) were not less defended than their European native conspecifics, producing higher GS amounts in the new range. Probably the reason is that several European specialist herbivores have also been introduced along with the plants in North America, and more importantly, the plants were also readily attacked by several generalists, against which GS are more effective.

Bunias orientalis differs from the examples above because it has expanded its range within the same contiguous land mass. Therefore, in its native and introduced ranges likely encounter the same native herbivore species. Mamestra brassicae and P. brassicae are both native and widespread across most of Eurasia, and are common in the plant’s native range where the populations were sampled (Güçlü et al. 2006; Secmen et al. 2010). The insects studied here were of Dutch origin and performed poorly on the native populations, and the specialist herbivore performed more poorly than the generalist. This result reveals that Bu. orientalis is a low-quality plant for the development of Dutch P. brassicae development. A previous study reported that several specialist herbivores originating from the Netherlands developed very poorly on Dutch Bu. orientalis plants, whereas generalist tended to performed better (Harvey et al. 2010a). Wide-ranging generalist herbivores may be better able to cope with novel range-expanding plants, as they possess physiological adaptations to a broad array of plant chemotypes (Futuyma and Moreno 1988; Sorensen et al. 2005; Verhoeven et al. 2009). Specialists, on the other hand, possess more refined mechanisms enabling them to excrete, detoxify or sequester specific defensive compounds of a narrower range of closely related plants (Wittstock et al. 2004). Importantly, these finely-tuned adaptations may limit the extent to which dietary specialists are able to exploit novel plant types in their habitat (Berenbaum and Zangerl 1994; Garcia-Robledo and Horvitz 2012).

The ability of native herbivores to incorporate exotic plants into their diet critically depends on a number of behavioural and physiological criteria. Alliaria petiolata (M. Bieb.) Cavara and Grande (Brassicaceae), which is highly invasive in North America, is native in Europe where it is suitable for the development of several pierid species including P. brassicae. In the invasive range, the plant contains ‘novel’ secondary metabolites that deter feeding by Pieris oleracea Harris (Lepidoptera: Pieridae), a native North American pierid species (Renwick 2002; Keeler and Chew 2008). Nevertheless, in areas where A. petiolata has been established for more than a century, P. oleracea is adapting to this novel food plant species (Keeler and Chew, 2008). As this example shows, it is not surprising that Dutch populations of P. brassicae may be physiologically constrained and unable to effectively cope with chemical or physical deterrents produced by range-expanding plants like Bu. orientalis. Given that several other brassicaceous plants in the Netherlands are abundant and suitable for P. brassicae, it can be argued that there has not been strong selection in local populations of this herbivore to adapt to Bu. orientalis. On the other hand, the more general detoxification apparatus of Mamestra brassicae allows this herbivore to cope more easily with the deterrent(s) present in Bu. orientalis. It would be interesting, however, to investigate whether local populations of P. brassicae oviposit and develop on this plant species in its native range.

The majority of the studies comparing native and non-native populations of invasive species have been conducted in a bi-trophic framework (plant-herbivore), whereas only few have included interactions with higher trophic levels, such as parasitoids (Ode et al. 2004; Harvey and Gols 2011; Fortuna et al. 2012). Recent meta-analyses have shown that top-down as well as bottom-up forces are involved in regulating terrestrial biomass (Schmitz et al. 2000; Romero and Koricheva 2011). If this is true, studies exploring the various factors that determine the success or failure of a plant to become invasive in a new habitat should include natural enemies of herbivores, although this has rarely been done (but see Cronin and Haynes 2004). Our results show that the parasitoid is similarly or only slightly less negatively impacted by adverse effects determining quality of Bu. orientalis as a food plant. To determine whether the presence of range-expanding plants has differential consequences on the food chain, future studies should compare the foraging behaviour of both herbivores and their natural enemies in habitats with native and exotic plants versus habitats consisting of native food plant species only (Harvey and Fortuna 2012).

The metabolic fingerprints significantly differed between the native and non-native Bu. orientalis populations. Moreover, herbivore-induced changes in the metabolome were more dramatic in the native populations than in the non-native populations, and origin-related differences in the metabolic profiles were more pronounced in younger than older plants. Targeted chemical analysis of glucosinolates revealed that plants from the non-native range produced mainly p-hydroxybenzyl GS, whereas in addition to this aromatic GS plants from the native range contained high concentrations of the aliphatic 1-methylethyl GS. Furthermore, the concentrations of GS increased with increasing age of the plants and in response to herbivore feeding, a pattern that is characteristic for many Brassicaceae (Cipollini et al. 2005; Müller and Martens 2005; Harvey et al. 2010a). Previous work suggested that p-hydroxybenzyl GS may not play a major role in plant resistance against M. brassicae (Harvey et al. 2010a; Harvey and Gols 2011). By contrast, negative effects of aliphatic GS on the performance of generalist herbivores have been widely reported (Chew 1988; Mithen et al. 1995; Lankau 2007), and particularly in M. brassicae (Gols et al. 2008a). Hence, it is possible that the low survival of this herbivore on the native plant populations is related to the high concentrations of aliphatic GS, which may also act synergistically with the mechanical defences (e.g. trichomes) of Bu. orientalis. It is unlikely that 1-methylethyl GS is responsible for the poor performance of P. brassicae, as this herbivore has evolved an efficient mechanism to detoxify GS (Wittstock et al. 2004; Smallegange et al. 2007). In addition, myrosinase activities, which catalyse the conversion of GS into more toxic hydrolysis products, may also differ among the Bu. orientalis populations (Renwick 2002). For example, in Lepidium draba, myrosinase activities were found to be higher in plants from the invasive range compared to those of the native range (Müller and Martens 2005). Apart from GS, other metabolites differing strongly between the populations may also have influenced the herbivores' performance negatively. Trichome production and structure in Bu. orientalis also differed significantly on the different plant populations, with those on the native plants having the highest densities, particularly the trichomes with barbed, forked tips. The density of leaf trichomes can influence both host-plant selection behaviour and performance (i.e., growth, survival) of both generalist and specialist insect herbivores (Agrawal 1999; Traw and Dawson 2002a,b). Non-glandular trichomes are often considered as relatively soft 'weapons' in plant defence compared with phytotoxins. However, they are less likely to result in counter-adaptations of insects, which can therefore reduce total damage caused by a wide range of herbivores (Schoonhoven et al. 2005).

Our findings of higher investment in qualitative defences (e.g. p-hydroxybenzyl GS) and lower investments in quantitative defences (e.g. leaf trichomes) among the non-native populations of Bu. orientalis support predictions of the SDH. According to this hypothesis, communities dominated by generalists in the invasive range select for plants with low levels of quantitative defences and high concentration of less costly qualitative defences (Doorduin and Vrieling 2011). Therefore, levels of p-hydroxybenzyl GS in non-native populations of Bu. orientalis may be associated with defence against other generalist herbivores and pathogens present in the new range. Some chemical defences in Bu. orientalis may also have alternative benefits (e.g. allelopathy), for instance in the belowground environment, which could contribute to invasiveness and select for its maintenance in the new range (Dietz and Winterhalter 1996; Cipollini et al. 2012). Additionally, breakdown products of GS, such as isothiocynates, have also volatile proprieties, which may provide cues to parasitoids to locate their hosts in the exotic plant (Scascighini et al. 2005; Gols et al. 2011). The reduced concentration of 1-methylethyl GS in non-native populations comparatively to the native ones, however, does not fit with SDH predictions. These results show that GS, p-hydroxybenzyl GS and 1-methylethyl GS, may undergo different selection pressure and that we may actually deal with different chemotypes in the different habitats (Macel and Klinkhamer 2010; Wolf et al. 2011).

In conclusion, our study shows that changes in plant defence traits both chemical and mechanical also occur in populations of an intra-continental range expanding plant species. The performance of the herbivores and the parasitoid was in general lower in native plant populations than in non-native ones. These results suggest that native populations of Bu. orientalis are generally better well-defended than populations in the new range, suggesting a potential higher herbivore pressure in the plant’s native range in relation to the new range. If so, in the new range the range-expanding plant might re-allocate investment in less costly defence traits and/or in other functions rather than defence, such as growth and reproduction, which can also play an important role in its invasive success. In addition, insects performed similarly on the abundant invasive (German and Czech Republic) and more scarce exotic (Netherlands) populations of Bu. orientalis, suggesting that factors that influence insect performance did not differ among these populations. Alternatively, plant characteristics associated with the belowground environment, e.g. resistance against soil herbivores and pathogens that were not expressed in aboveground tissues may differ. Comprehensive surveys, in the future, of local insect communities in the various geographic ranges and their degree of adapation to plant ecotypes may establish the importance of insect herbivores (and their natural enemies) as a selection pressure on plant resistance traits. Finally, although we emphasize herbivory as an important selective pressure in plant invasiveness, other factors like abiotic and soil related characteristics are likely to vary with plant range expansion and to affect plant defence traits. These factors can interact with herbivore pressure to mediate coevolutionary interactions between range-expanding plant species, herbivores and higher trophic levels in ways that are currently poorly understood, but deserve further attention.

Acknowledgements

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Supplementary material

Appendix S1 Samples preparation for uHPLC-ToF-MS measurements.

After grinding in a mill, aliquots of 15 mg DW per replicate were weighed and extracted threefold in 90% methanol (LC-MS grade, Fisher Scientific UK Limited, Loughborough, UK) with 1 µg/ml hydrocortisone (>98%, Sigma-Aldrich CHEMIE GmbH, Steinheim, Germany) as an internal standard. Supernatants were filtered through 0.2 µm PTFE membrane syringe filters (15 mm, Phenomenex, Torrance, USA). Samples were analysed immediately after preparation with an ultra-high performance liquid chromatography (uHPLC) equipped with a Grom Sil 120 ODS-4 HE column (150 x 2 mm, 3 µm particle size, Alltech Grom GmbH, Rottenburg-Hailfingen, Germany) and coupled to a time of flight (ToF) mass spectrometer (MS; 1290 Infinity uHPLC and 6210 ToF-MS, Agilent Technologies, Santa Clara, USA) in positive and negative ionization mode. Sample elution of methanolic plant extracts was accomplished at a 19 min gradient from ultrapure water (A; TKA, Thermo Electron LED GmbH, Niederelbert, Germany) to acetonitrile (B; LC-MS grade, Fisher Scientific UK Limited, Loughborough, UK), both containing 0.1% formic acid (98%, Fluka, Seelze, Germany), with a flow of 0.8 $ml.min^{-1}$ from 2% B (hold for 3 min), up to 98% B (in 16 min), followed by a cleaning cycle. Samples were measured in positive and negative mode from 100 to 1000 m/z with a fragmentor voltage of ± 140 V, skimmer voltage of 60 V, gas temperature of 350 °C, and a drying gas flow of 12 $L.min^{-1}$. Blank samples, with only solvent and no leaf material, were treated in parallel at each extraction day to check for the instrumental reproducibility. Based on these uHPLC - ToF-MS data, three GS could be extracted from negative ionization data. GS were identified by their exact ion masses, by their UV spectra and by comparison to standards. GS quantification was based on the relation of their peak areas to the peak area of the internal standard peak hydrocortisone. Response factors were incorporated, which had been determined by co-injection of hydrocortisone and 2-propenyl glucosinolate, as representative of aliphatic GS, and p-hydroxybenzyl glucosinolate, as representative of aromatic GS (Phytoplan, Heidelberg, Germany), in various concentrations.

Appendix S2 Metabolomic data pre-treatment.

Chromatograms gained by uHPLC-ToF-MS were exported from MassHunter Workstation software (version B.03.01, Agilent Technologies Inc., USA). Isotopes were excluded during the procedure to reduce redundant data originating from one metabolite. Peak selection, grouping of associated peaks, and retention alignment was performed using the xcms software package (version 2.15.1, 1,2 in R; for parameters settings see Kutyniok and Müller 2012). The resulting data matrices of positive and negative ionisation mode measurements were pooled for further analysis. These data were normalized to the internal standard intensity.

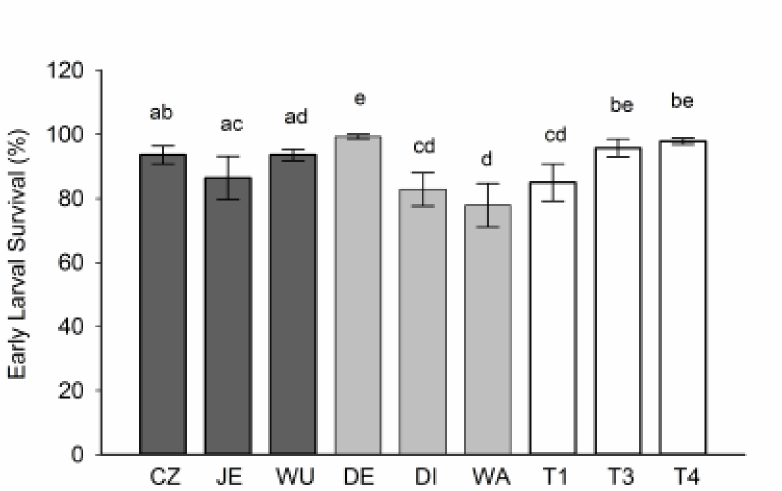

Figure S1 Survival of early larval stage (L1) of Mamestra brassicae on different populations of Bunias orientalis. M. brassicae L1 larvae fed for 3 days on plants of Bu. orientalis populations from its invasive range (dark grey bars: CZ, JE, WU), exotic range (light grey bars: DE, DI, WA) and native range (white bars: T1, T3, T4). Bars give the mean values (± SE). Different letters indicate significant differences at $P< 0.05$.

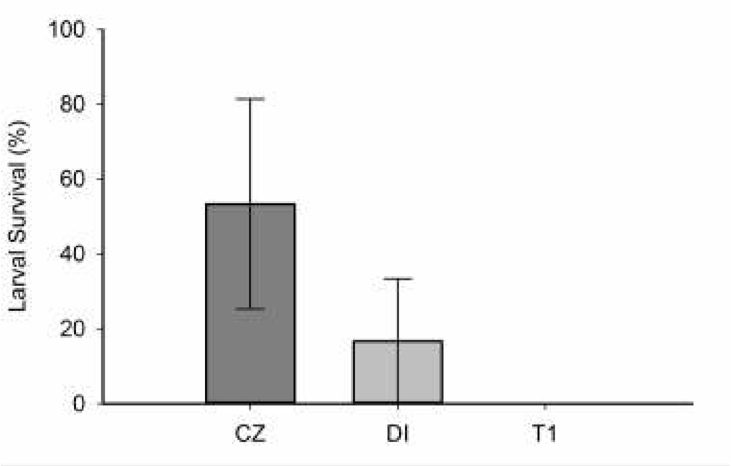

Figure S2 Larval survival of Pieris brassicae on different populations of Bunias orientalis. Pieris brassicae caterpillars fed until pupation on plants of Bu. orientalis populations from the plant invasive range (dark grey bar: CZ), exotic range (light grey bars: DI) and native range (white bars: T1). Bars give the mean values (± SE).

Table S1 Results of Tukey-Kramer post hoc test. P-values for testing differences in the concentration of total GS, p-hydroxybenzyl GS and 1-methylethyl GS between populations (CZ, DI, TI, T4) of Bunias orientalis and treatments (herbivory, plant age). Leaf samples were collected from plants with (+H) or without (-H) M. brassicae herbivory (herbivory effect), and at the beginning (start) or the end of the experiment (age effect). *P< 0.05, **P<0.01, ***P<0.001.

	Effect	Pop	Treat	Pop	Treat	t-values	Adj P-values	
Total GS								
Herbivory	Treatment		end, -H		end, +H	-7.39	< 0.0001	***
Age	Treatment		end, -H		start, -H	2.34	0.026	*
	Pop*Treat	CZ	end, -H	CZ	start, -H	1.99	0.055	
		DI	end, -H	DI	start, -H	3.07	0.004	**
		T1	end, -H	T1	start, -H	0.22	0.826	
		T4	end, -H	T4	start, -H	-0.59	0.561	
p-hydroxybenzyl GS								
Herbivory	Population	CZ		DI		-1.36	0.209	
		CZ		T1		7.29	0.002	**
		CZ		T4		7.16	< 0.0001	***
		DI		T1		8.68	0.002	**
		DI		T4		8.79	< 0.0001	***
		T1		T4		-0.81	0.452	
	Treatment		end, -H		end, +H	-6.27	0.0001	***
Age	Population	CZ		DI		-0.01	0.992	
		CZ		T1		4.32	0.0002	***
		CZ		T4		3.58	0.001	***
		DI		T1		4.33	0.0001	***
		DI		T4		3.59	0.001	**
		T1		T4		-0.78	0.440	
	Treatment		end, -H		start, -H	2.51	0.018	*
	Pop*Treat	CZ	end, -H	CZ	start, -H	2.55	0.016	*
		DI	end, -H	DI	start, -H	3.93	0.0004	***
		T1	end, -H	T1	start, -H	-1.31	0.200	
		T4	end, -H	T4	start, -H	-0.10	0.924	
1-methylethyl GS								
Herbivory	Population	CZ		DI		-0.54	0.619	
		CZ		T1		-25.91	< 0.0001	***
		CZ		T4		-40.59	< 0.0001	***
		DI		T1		-25.96	< 0.0001	***
		DI		T4		-40.98	< 0.0001	***
		T1		T4		0.75	0.470	
	Treatment		end, -H		end, +H	-3.69	0.004	**
	Pop*Treat	CZ	end, -H	CZ	start, -H	1.16	0.364	
		DI	end, -H	DI	start, -H	-1.19	0.280	
		T1	end, -H	T1	start, -H	-1.47	0.191	
		T4	end, -H	T4	start, -H	-4.83	0.005	**

Table S2 Results of Mann-Whitney test. P-values to analyze differences in 1-methylethyl GS concentration among plants with different age (start: 4-wk old; end: 8-wk old) of Bunias orientalis populations (CZ, DI, TI, T4).

Population	n (start)	n (end)	U	P-value
CZ	7	3	4.0	0.183
DI	7	3	2.0	0.067
TI	6	3	6.0	0.548
T4	7	3	7.0	0.517

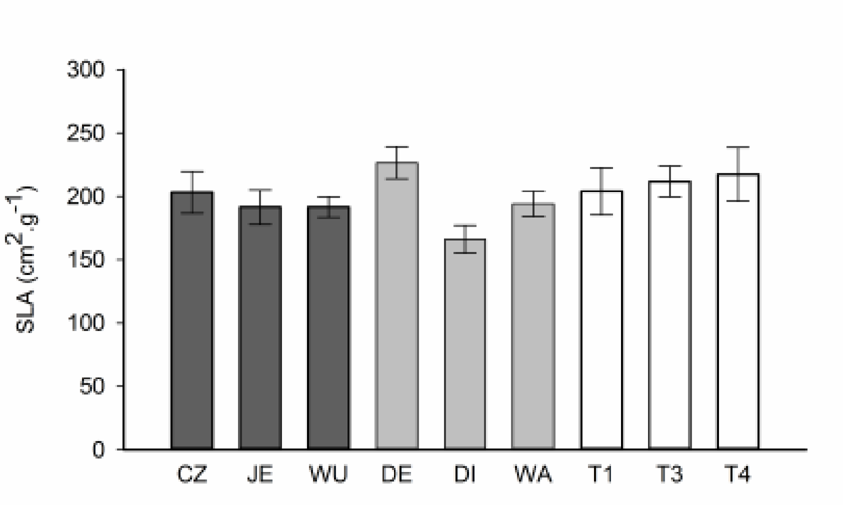

Figure S3 Specific leaf area (SLA) of different populations of Bunias orientalis. Mean SLA values (± SE; $cm^2.g^{-1}$) of Bu. orientalis plants (n=8) from populations in its invasive range (dark grey bars: CZ, JE, WU), exotic range (light grey bars: DE, DI, WA) and native range (white bars: T1, T3, T4). Significance of the main effect, plant population, is tested at P<0.05.

CHAPTER 7

Synthesis and General Discussion

Taiadjana M. Fortuna

Synthesis and Discussion

The main aim of this thesis was to investigate how an exotic range-expanding plant species interacts with aboveground herbivores and their natural enemies in the new range, by developing a better understanding of the mechanisms that mediate interactions between the three trophic levels. Ultimately, by examining how the exotic plant influences the aboveground invertebrate communities and how ‘novel’ interactions can exert different selective pressures in plant resistance traits, I was able to infer some of the factors that may contribute to the successful establishment of the exotic plant in the new range. I tested the hypothesis that the exotic plant Bunias orientalis L. (Brassicaceae) suffers less from aboveground herbivory than related plant species (in the same family) that are native in the invaded habitat (The Netherlands). These results will be discussed in the first section. Then I hypothesized and studied the effects of the exotic plant on the host-finding abilities and performance of native insect herbivores and their natural enemies, particularly of a specialist foliar herbivore and its parasitoids. I focused on plant quality related traits, such as secondary metabolites, and plant volatiles in mediating interactions between the exotic plant, the herbivore and its parasitoids. I tested the hypothesis that when attacked the exotic plant is of lower quality for the performance of the herbivore and its natural enemies than related native plants. The results of these studies will be discussed in the second section. In the last section, I examined variation in plant defence traits of native and non-native (exotic invasive and non-invasive) populations of the range-expanding plant and compared the performance of specialist and generalist herbivores and an endoparasitoid on these plant populations. I tested the hypothesis that intraspecific variation in chemical and mechanical defence traits of the range-expanding plant would be reflected on the performance of the herbivores and the natural enemy of one of them. Finally, I discuss ideas for future research.

Aboveground invertebrate community effects on the exotic range-expanding plant

In their new range exotic plants may enjoy an advantage in interspecific competition with native plants, because while natives are still under control of their enemies, exotic plants may have lost their co-evolved enemies during the invasion process. In the case of intra-continental range-expanding plants, enemy release can occur when plants expand their ranges faster than their enemies and/or when the enemies from the new range do not recognize or feed on the exotic plants (Keane and Crawley 2002; Morrien et al. 2010). On the other hand, the invaded plant communities may provide direct and indirect biotic resistance to the establishment and spread of exotic plants (Maron and Vila 2001). For instance, when the exotic plant establishes in an area with closely related native plants, a shift of enemies is likely to occur and the exotic plant may rapidly accumulate new enemies (Hill and Kotanen 2009). Therefore, both enemy release and novel plant-enemy interactions can affect exotic plants in their new ranges, and whether these plants experience reduced enemy impact depends on the net effects of losing enemies from their native range and gaining new ones in the new range. In the first part of my thesis (Chapter 2), I looked at whether a range-expanding plant species experiences enemy release or biotic resistance from aboveground invertebrate herbivores and carnivores in natural populations of the new range, in the Netherlands.

Although the enemy released hypothesis (ERH) has been widely tested, most of these studies have been based on inter-continental plant invaders, with few examining invasions from species within contiguous land masses. Furthermore, despite this, a considerable number of studies have failed to support the ERH, particularly at the community-level (Colautti et al. 2004; Liu and Stiling 2006). In most of these studies important aspects of natural communities have also been excluded. For instance, leaf damage is often taken as a proxy for herbivore pressure in comparisons between exotic and native species in the invaded range, without looking at the distinction of the invertebrate feeding guilds. This is important, because different feeding guilds, such as leaf-chewing or sap-feeding insect herbivores can incur different magnitudes of damage (Vasquez and Meyer 2011). At the same time, carnivorous insects, such as predators and parasitoids, can exert top-down control of herbivores, releasing the exotic plants from its potentially new enemies (Engelkes et al. 2012). In addition, studies testing the ERH have focused only on plant species that are already invasive, excluding species that although not invasive may be in the threshold of becoming invasive in the new range, such as those species in a well defined 'lag-phase' (Richardson and Pysek 2012). Ultimately, knowledge of the biotic interactions established by exotic plants in the new range can help in applying more pro-active measures in the control and perhaps even in eradicating potentially invasive species before they exert more severe ecological impacts (Hobbs and Humphries 1995).

In Chapter 2, I compared herbivore loads and leaf damage, as well as potential carnivore pressure on herbivores between the exotic range-expanding plant and the three related native species. The three native plant species were selected on the basis of two main criteria: first, they grow in similar habitats as the exotic plant and second, they are common and abundant food plants for specialist herbivores in the Netherlands (Tamis et al. 2005; Harvey et al. 2010a). The invertebrate communities were sampled in roadside and natural plant populations over the course of an entire growing season to account for seasonal variability in plant and invertebrate communities. I tested the hypothesis that the exotic plant would harbour lower herbivore and carnivore loads than native species, as the latter would suffer higher enemy regulation from co-evolved enemies that are locally adapted to these plants. In the same experiment, I determined the diversity of neighbouring plant communities associated with the exotic and the native plant species, which could help to explain the invertebrate assemblages on these plants. Additionally, leaf toughness of each plant species was measured as a proxy for mechanical defence against herbivory.

In support of my hypothesis, the exotic plant harboured smaller herbivore loads and suffered less damaged than its native relatives and this decrease in enemy regulation was maintained over a growing season. Foliar toughness of the exotic plant could partially explain the release from enemies on this species, since this morphological trait can play a key factor in resistance against herbivores (Onoda et al. 2011), particularly against the feeding of early larval instars (Zalucki et al. 2002). In addition, variation in other plant-related traits, such as the content of primary (C/N ratio) and secondary metabolites, or other mechanical defences such as trichomes, which were not measured in this study, could have exerted a synergistic effect in the resistance of the exotic plant against herbivory (Schoonhoven et al. 2005). Other studies have shown that both chemical defences (e.g. flavonoids) and mechanical defences (e.g. trichomes) of Bu. orientalis can negatively impact the performance of specialist herbivores (Travers-Martin and Müller 2008; Kühnle and Müller 2009). Furthermore, potential top-down carnivore pressure was higher on the exotic than on one of the native species that grows later in the season at the peak of arthropod abundance. Natural enemies of herbivores can be extremely important in strengthening or weakening direct interactions between plants and herbivores within food webs (Schmitz et al. 2000). In successful invasive plants, herbivore release can also be enhanced by increased top-down pressure from the herbivore's own natural enemies (Engelkes et al. 2012). Consequently, higher carnivore pressure in the exotic species may also control potential colonization of native herbivores on this species.

Neighbouring plant communities did not differ in plant species richness between the exotic and the related native species, perhaps because these species occupy similar habitats in the Netherlands. However, communities differed in plant species composition and abundance, particularly starting from the middle period of the growth season. Thus, differences in invertebrate assemblages between the exotic and the native plants could also have been the result of differences in their associated plant communities. Variation in species composition can result in plant communities with different chemical and structural complexities, which can affect arthropod diversity and abundance (Strong et al. 1984; Dennis et al. 1998; Cardoso et al. 2007; Harvey and Fortuna 2012). However, it is not clear from my data to which extent invertebrate communities on the exotic and the native species could be solely explained by differences in plant community and/or by the influence of the exotic species on those communities. The integrity of arthropod communities has been suggested to be a linear function of the dominance of alien vegetation (Cardoso et al 2007), and studies have shown that invasive plants can create unstable patches between insect herbivores and their natural enemies (Cronin and Haynes 2004; Harvey and Gols 2011). Most importantly, the results of this study show that the mechanisms stated for the ERH for inter-continental invaders can potentially play an important role in intra-continental range-expanding species. Additionally, enemy release on exotic plants can result from combined bottom-up plant effects on invertebrate communities, as well as top-down pressures of higher trophic levels on plant enemies. Finally, the identity and structure of the plant community in which the exotic species is embedded can affect the diversity of herbivorous and carnivorous insects as well as plant-plant competition for access to light and nutrients.

Effects of the exotic plant on multitrophic interactions in the new range

Plants, amongst the most important of invasive organisms, can exhibit disproportionate effects on food webs because they are at the basis of the food chain. Thus, exotic invasive species have the capacity to disrupt native communities from the bottom-up, out-competing native plants and interfering with a wide range of associated trophic interactions (Ehrenfeld 2010; Vila et al. 2011). Although a robust body of literature has demonstrated the negative impact of invasive plants in the native communities in their new range, few studies have explicitly included interactions with more than two trophic levels, such as interactions between exotic plants, herbivores and their natural enemies (Harvey et al. 2010b; Pearse et al. 2013). In addition, current knowledge of the effects of invasive species on native food webs is largely descriptive, based for example on quantitative food web analyses (Memmott and Waser 2002; Heleno et al. 2009; but see Lopezaraiza-Mikel et al. 2007). Therefore, in

Chapter 3 we formulated hypotheses on how invasive plants can affect chemical and structural complexity of the invaded ecosystems and how this influences the host-finding ability of native herbivores and their natural enemies. Our main hypothesis was that structural and chemical changes in the environment promoted by the establishment of exotic invasive plants at small-medium scales affect interactions between native herbivores and their antagonists. These effects may lead to local losses in insect abundance and diversity and thus have potentially negative repercussions on the structuring of food webs. However, exotic plants may also benefit some native insects by providing them with alternative food plants.

Physical characteristics of habitats can affect patch dispersal movements of insects associated to the plants within the patches (reviewed by Andow 1991; Randlkofer et al. 2010). At smaller scales, plant characteristics, such as shape, size and structure of leaf surface can affect interactions between herbivores and their natural enemies (Grevstad and Klepetka 1992; Olson and Andow 2008). When exotic plants establish in the new range, their structural characteristics, such as plant architecture, can differ from that found in native plants. This may disrupt dispersal and foraging behaviour of herbivores and their natural enemies (Bezemer et al. 2010b) and affect population dynamics of predator-prey or host-parasitoid associations (Cronin and Haynes 2004; Harvey et al. 2014). Sticky glands or trichomes, for instance, that are often present on the leaf surfaces of invasive plants, may impede the movement and feeding of insect herbivores (Travers-Martin and Müller 2008), as well as the movement of their natural enemies (Schoonhoven et al. 2005). Microclimatic conditions, such as airflow and turbulence, can also be affected by plant structural traits (e.g. plant height, density and connectivity) (Randlkofer et al. 2010). Consequently, the emission and spread of plant volatiles in odour plumes can also be affected by the introduction of exotic plants. However, thus far, studies of these plant characteristics have been restricted to native-plant insect interactions or to cultivars, and there is clearly an urgent need for studies exploring similar mechanisms in exotic plants.

Plant volatiles, an integral part of the chemical complexity of ecological communities, are known to influence arthropod movement in native host habitats (Schoonhoven et al. 2005). Therefore, the foraging behaviour and fitness success of herbivores and carnivores might be influenced by novel odour blends emitted by invading plants. If the exotic plant is introduced into a native plant community where there are few or no related plant species present, the novel odour blends might mask the detection of volatiles released by the native plants. Alternatively, novel volatiles that are not recognized by arthropods may actually benefit these consumers by making it easier to recognize cues that are attractive. There is enormous interspecific variation in plant volatiles released in nature, and the specificity of the signal relies on the qualitative and quantitative composition of the odour blends, as well as on the arthropod recognition and response to the volatile bouquet (Bruce et al. 2005). For host plant recognition, insects require a highly sophisticated detection mechanism to identify the correct volatile blend against a heterogeneous background of compounds also emitted by non-host plants. Behavioural studies with insect herbivores and carnivores suggest that the blend composition of plant volatiles is crucial because specific mixtures are more attractive than individual compounds (Fraser et al. 2003; van Wijk et al. 2011). If the exotic plant colonizes habitats containing related native plants it might attract arthropods associated with the natives because of their similar volatile cues (Renwick 2002). If the odour blend of the exotic species is attractive to herbivore females for oviposition, but their offspring cannot develop on the novel food plant, it can be defined as an ‘evolutionary trap’ (Keeler and Chew 2008; Kühnle and Müller 2009).

Some successful invasive plants possess novel secondary compounds that are not found amongst native plants, and this may confer protection against potentially new enemies that are not evolutionary adapted to the new traits (Cappuccino and Arnason 2006; Callaway et al. 2008). On the other hand, the exotic plant can also emit repellent odours, which may enable herbivores and natural enemies to avoid these novel plants leading to potential changes in the strength of food web connectivity (Cronin and Haynes 2004). However, from the perspective of the exotic plant this may allow it to escape from antagonists in the new range in support of the ERH. Finally, volatile blends emitted by exotic plants can be attractive and suitable for the development of native herbivores. In this scenario, herbivores may rapidly switch to the novel plant, and provide local herbivores with alternative food plants (Chew 1981; Graves and Shapiro 2003; Meijer et al. 2012). By association, herbivore natural enemies may follow their prey or hosts to the novel plant (Stenberg 2012). However, if this is not the case, then the novel species can generate enemy-free space for the herbivores (Grosman et al. 2005). Hence, depending on the speed with which natural enemies follow their prey or host to a new host plant, enemy-free space on novel host plants may only exist for a limited period of time. Parasitoids, for instance, can increase their efficiency in host finding by learning plant-related cues, such as herbivore-induced plant volatiles (Vet et al. 1995; Geervliet et al. 2000), and their preference for novel plants may increase if those are the most profitable in terms of host encounter rate.

In Chapters 4 and 5, I tested some of the hypotheses formulated in the previous chapter. In these chapters, I investigate how a local specialist insect herbivore and its parasitoids respond developmentally and behaviourally to the novel plant, and how interactions between the three trophic levels may influence top-down regulation of the exotic plant in the new range.

Invasive plants can have negative effects on native insects with implications for their conservation (Cronin and Haynes 2004; Keeler and Chew 2008). On the other hand, some exotic plants have the potential to become an integral part of the native flora and fit in naturally with native fauna (Shapiro 2002; Davis et al. 2011). Several studies have shown either positive or negative effects of novel interactions on the behaviour and development of native herbivores (Wolfe et al. 2004; Zou et al. 2008; Kühnle and Müller 2009; Harvey et al. 2010a). However, the general importance of top-down limitation of herbivores on exotic plants is unclear, because, few studies have included interactions between invasive plants and higher trophic levels up the food chain (Cronin and Haynes 2004; Stenberg 2012). For instance, parasitoids rely on a limited amount of resources that are obtained from a single host. Hence, their fitness success is strongly correlated with host quality (e.g. size and nutritional status). Many studies have shown that differences in plant quality can also indirectly affect parasitoids as mediated through host diet (Barbosa et al. 1986; Ode 2006; Bukovinszky et al. 2008; Gols and Harvey 2009). Parasitoids can exploit host resources in two ways. Koinobiont parasitoids attack hosts that continue to grow after parasitism (e.g. larvae), whereas idiobionts parasitize non-growing hosts (e.g. eggs or pupae) (Harvey 2005). Although effects of host quality on parasitoid development can differ according to their host exploitation strategy (Mackauer and Sequeira 1993), this has rarely been tested when incorporating an exotic food plant as an additional constraint on host development. In Chapter 4, I tested the hypothesis that a native plant, which is an important food plant for specialist herbivores in the Netherlands, is of higher quality for the herbivore development than the exotic plant. In addition, I tested the hypothesis that koinobiont parasitoids of hosts feeding on the native or the exotic plant suffer larger fitness costs than idiobionts, because host quality is immediately accessed by the latter group upon oviposition, whereas it is not by the former group.

In line with the first hypothesis, I found that the performance of the specialist leaf herbivore Pieris brassicae was significantly lower on the exotic plant than on the native plant. The larvae of this herbivore showed lower survival rate, slower development time and smaller pupal mass than those growing on the native plant. This result was in line with previous studies where the exotic plant Bu. orientalis was found to be toxic to several specialist herbivores (Travers-Martin and Müller 2008; Kühnle and Müller 2009; Harvey et al. 2010a). In contrast, the exotic plant was found to be suitable for the development of some generalist herbivores (Buschmann et al. 2005; Harvey et al. 2010a). Different chemical and mechanical plant-related traits have been suggested to play an important role in Bu. orientalis resistance against specialist herbivores (Dietz and Winterhalter 1996; Travers-Martin and Müller 2008; Kühnle and Müller 2009); however, mechanisms mediating plant unsuitability for these herbivores are not yet clear. Interestingly, in early food choice experiments it was found that P. brassicae larvae would feed on filter paper smeared with Bu. orientalis leaf exudates, and even other kinds of leaves treated with sinalbin (the main glucosinolate in Bu. orientalis) if leaves were not too tough and did not contain other kinds of repellents (Ehrlich and Raven 1964). Therefore, it is likely that mechanical defences, such as cuticle thickness and trichomes, in this plant species may be one of the main impediments for the feeding of specialist herbivores (Travers-Martin and Müller 2008).

The exotic plant differentially affected the performance of the two parasitoid species. The survival of the koinobiont parasitoid was strongly co-ordinated with the survival of its larval host, with high immature mortality and very low potential fitness (e.g. small body size) of survivors when reared on hosts that had developed on the exotic plant. By contrast, the idiobiont parasitoid developed successfully on pupal hosts growing on both plant species. These results corroborate the models predicted by Mackauer and Sequeira (1993), which state that host quality is more difficult to predict for koinobionts at the time of oviposition than for idiobiont parasitoids. This is because, koinobionts attack young host stages, which may still need to grow substantially to support the nutritional requirements of the parasitoid progeny, whereas idiobionts are able to immediately assess quantitative differences in the host, which does not grow more after parasitism (Harvey 2005). This study shows that constraints imposed by differing plant quality of native and exotic plants on trophic interactions can depend on resource use strategies of the species involved, and emphasizes that the effects of exotic plants in invaded communities should be elucidated on a case-by-case. Although a broader species comparison is needed to draw definitive conclusions, one can speculate that koinobiont parasitoids will be under stronger selective pressure to reject hosts growing on the exotic plant than idiobiont parasitoids. In this scenario, native herbivores that might colonize the exotic plant and overcome its defences may suffer stronger top-down control by idiobiont parasitoids, which can release the plant from the attack of enemies. On the other hand, exotic plants may provide enemy-free space for the herbivores if their parasitoids, such as koinobionts, do not follow the host-plant shift, in which case native herbivores will confer biotic resistance to the exotic plant in its new range.

The preference-performance hypothesis (Jaenike 1978) predicts that oviposition preference should correlate with host suitability for offspring development, because females of phytophagous insects are assumed to maximise their fitness by ovipositing on high quality host plants. Host plant choice is influenced by optimal foraging decisions of females, which is determined by bottom-up forces (i.e. food quality, competition) and top-down forces (i.e. natural enemies, diseases) (Stephens and Krebs 1986). Several studies have shown both a positive or poor correlation between female preference and offspring performance (reviewed by Mayhew 1997). Apparent mismatches between choice and performance in insects can be explained by a wide range of different ecophysiological factors that may work independently or in concert. These include the physiological state of the insect (e.g. age, egg load), the ability of the foraging insect to recognize attractive or repellent cues emitted by novel plants (Kühnle and Müller 2009), as well as the differential mortality risks imposed by natural enemies (Price et al. 1980). In Chapter 5, I examined the preference-performance hypothesis of the specialist foliar herbivore, P. brassicae, in choosing between a familiar native plant and the unfamiliar exotic plant. In addition, I studied the host location and host selection behaviour of the most important endoparasitoid of P. brassicae, the gregarious C. glomerata, as well as the factors mediating the parasitoid host plant choice, such as herbivore-induced plant volatiles.

In this chapter, I observed that there was a clear preference-performance link in the specialist herbivore. Female butterflies showed an innate preference to oviposit on the native plant, on which their progeny also developed more successfully than on the exotic plant. These results were in accordance with another study, where P. brassicae oviposit preferentially on native plants (Harvey et al. 2010a), and with results of Chapter 4, where P. brassicae caterpillars also performed better on the native plant. However, in both these studies the survival of P. brassicae caterpillars on the exotic plant was more dramatically reduced than in Chapter 5. These differences in herbivore survival might be explained by two factors. The first is that the insects in the other studies were from a laboratory culture which had been reared for many generations on cultivated cabbage plants, while in this study I used wild individuals that were collected as larvae from a range of native Brassicaceae in the field.

This shows that long-term breeding in laboratory can reduce variation related to behaviour and physiological plasticity of reared insects. Hence, they may become more susceptible to novel plant species, while wild individuals possess greater ability to deal with defences of these plants. The second factor was that in this chapter P. brassicae females oviposited directly on the plants where their progeny developed, while in the other two studies P. brassicae neonates where placed artificially on the plants, a process which may have stressed the insects. I believe that Bu. orientalis is a plant of marginal suitability for P. brassicae, where the threshold between larval survival and precocious death is finely delineated. Thus, even minor stresses during early larval development can be fatal for the insects. As my results show the plant chosen by females for oviposition is highly important for the survival of their progeny, which is in support of the mother knows best hypothesis.

Herbivore-induced plant volatiles released by the exotic species were more diverse and concentrated than those emitted by the native plant. However, despite the qualitative and quantitative differences in the volatile blends of both plant species, the parasitoid females did not discriminate between host-induced plants of the two species, except when host density varied. Nevertheless, parasitism rate of P. brassicae caterpillars was slightly higher on the native plant. This may be due to a different localized perception of female parasitoids to plant- and host-related cues. During host plant location behaviour in the flight-tent bioassay, C. glomerata females rely more on general plant cues (e.g. plant volatiles) that are representative of the Brassicaceae family. This behaviour is common to parasitoid species whose hosts are specialists but that feed on different plant species within the same family so different parasitoid generations may experience different host-associated plants (Vet and Dicke 1992; Gols et al. 2011). In these cases, natural selection will favour a flexible response of naïve parasitoids to plant infochemicals, and only through experience they become more sensitized to subtle differences between food plants (Vet 1999). Under semi-field set up in its host selection behaviour the parasitoid can also use contact cues based on host quality-related parameters (e.g. host size, nutritional status) (Vinson 1976). As shown in Chapter 4 and 5, P. brassicae caterpillars attained smaller size when feeding on the exotic than on the native plants, thus female parasitoids could have rejected these hosts once they access their low quality.

The analysis of plant volatiles emitted by the native and the exotic plant revealed that a precursor in the terpenoid biosynthesis pathway can be a good candidate to explain the parasitoid behavioural response during its host plant location. Terpenoids such as monoterpenes have been shown to act has parasitoid attractants, and C. glomerata females have the ability to detect these compounds with their antennal chemical receptors (Smid et al. 2002). Therefore, detecting terpenoid precursor compounds, which are released in early stage of herbivore damage, might also reflect an adaptive behaviour of the parasitoid to detect more suitable host stages, such as young caterpillars. However, as discussed in Chapter 3, under natural conditions carnivorous insects, such as parasitoids, might have to perceive and integrate shifts in the composition of volatile mixtures emitted by host-associated plants and non-host plants (e.g. exotic plants). Some studies have shown that carnivorous arthropods perceive volatile mixtures as a whole rather than a collection of individual compounds (Smid et al. 2002; Fraser et al. 2003; van Wijk et al. 2011). It is very likely that in the case of C. glomerata other volatile compounds, in addition to the one suggested in this study, are playing an important role as building stones of the whole odour used by parasitoid females in their host-finding behaviour (Gols et al. 2011).

From an ecological perspective, the exotic plant may provide the Pieris-Cotesia system with an exciting opportunity for studying temporal aspects of host-plant shifts. Based on my results, some P. brassicae still accepted the exotic plant species, and although their fitness was reduced on this plant, progeny survival was still fairly high when wild butterflies were allowed to oviposit directly onto them. There may be a strong selection pressure in the future for specialist herbivores to incorporate Bu. orientalis into their ‘menu’ based on two observations. Firstly, this exotic plant species grows in the field for an extensive period of time (6 months) during the growing season, while other brassicaceous species are often short-lived plants (1-2 months). Thus, different generations of this specialist herbivore may have the ability for developing on the same species over an entire growing season. This also means that foraging females could remain in the natal patch and save time in looking for suitable hosts in other patches. Secondly, the exotic plant may also create enemy-free space for P. brassicae caterpillars if they suffer less parasitism than those growing on the native plants (Ohsaki and Sato 1994; Stamp 2001). On the other hand, parasitoids can increase their efficiency in host finding by learning plant-related cues, and C. glomerata is known to increase its preference for plant species they are most profitable in terms of host encounter rate (Geervliet et al. 2000). Host shifts by the specialist herbivore may be then followed by its parasitoid, particularly because hosts take longer to develop on the exotic plant and female butterflies tend to lay bigger broods on this species.

Intraspecific variation in plant defence traits of the range-expanding plant

Plants are adapted to cope with attack from various antagonists, such as herbivores and pathogens. Therefore, they have evolved complex defence strategies, which involve both mechanical defences, such as trichomes, as well as several secondary metabolites, which often act as efficient barriers against generalist herbivores. The specialist-generalist dilemma (van der Meijden 1996) argues that in native areas, intermediate levels of chemical defences (e.g. secondary metabolites) are maintained by opposing selective forces of adapted specialists that use plant defence chemicals as host-plant recognition cues, and of non-adapted generalists that are deterred by the same chemicals. Plants deal with this dilemma by inducing certain metabolites only when attacked by enemies (Karban and Baldwin 1997). This is also based in the fact that biosynthesis of plant resistance traits can be costly, and there are usually tradeoffs in resource allocation to growth or defence (Herms and Mattson 1992). Hence, when released from their specialized enemies, exotic plants may undergo rapid evolutionary shifts of resource investment from defence into other functions, such as growth and reproduction, which can explain their competitive advantage over native plants in the new range (Blossey and Nötzold 1995; Keane and Crawley 2002). Nevertheless, exotic plants may still be attacked by generalist herbivores in the new range. These herbivores are present in all habitats and may colonize new hosts faster than the specialists from the new range. In this case, exotic plants can also shift their resource investment to a less costly form of defence as suggested by the shifting defence hypothesis (SDH) (Müller-Schärer et al. 2004; Joshi and Vrieling 2005).

In Chapter 6, I used a subset of populations from the native range and the non-native range (exotic invasive or non-invasive) of the range-expanding plant to compare the defence patterns between the different plant populations. I investigated the performance of local specialist and generalist herbivores, and a specialist solitary endoparasitoid of the generalist. As plant defence traits, I looked at the metabolic fingerprints combined with target analysis of glucosinolates, important secondary compounds involved in Brassicaceae resistance against herbivores and pathogens, as well at leaf trichome densities. Metabolomic fingerprinting is a technique used for an untargeted screening of the plant metabolome. Its primary scope is not to identify all the metabolites, but to reveal patterns. In this way untargeted analysis of plant metabolites can display general and treatment-specific induction patterns, which may not be found in a targeted study on a selected set of plant compounds (Sutter and Müller 2011). Hence, the combined approach of metabolomic fingerprinting with targeted analysis of plant defences can be a particularly powerful tool for studying the mechanism underlying the process of plant resistance to herbivores (Macel et al. 2010). The hypothesis tested in this study was that chemical and morphological plant defence traits would exhibit intraspecific variation in native and non-native populations of the exotic plant. Furthermore, this variation would reflect differences on the performance of the parasitoid and the generalist and specialist herbivores on the different plant populations.

In line with my first hypothesis I found that metabolic fingerprints significantly differed between native and non-native populations of the exotic plant when grown under the same conditions. Moreover, herbivore-induced changes in the metabolome were more pronounced in native populations than in the non-native ones. The targeted analysis of glucosinolates revealed patterns similar to those found in the whole plant metabolome. Plants from the native range produced much higher quantities of an aliphatic glucosinolate (i.e. glucoputranjivin) and lower levels of an aromatic glucosinolate (i.e. sinalbin) than those from the non-native range. The constitutive levels of total glucosinolates were lower in non-native populations compared to the natives, particularly in young plants, and in all populations there was high inducibility of glucosinolates upon herbivory. The expression of low constitutive levels and high inducibility of defence has been suggested as a cost-saving strategy resulting from reduced selective pressure by herbivores (Koricheva et al. 2004), and it has also been observed in other invasive brassicaceous species (Cipollini et al. 2005). In addition to chemical defences, I also found that the populations of Bu. orientalis differed in trichome production and structure. Plants from the native populations exhibited a higher density of trichomes, particular the forked type, than plants from the non-native populations. Compared to chemical defences, non-glandular trichomes such as the ones found in the exotic plant can reduce total damage by a wide range of herbivores, since they are less likely to result in counter-adaptations of insects (Schoonhoven et al. 2005). The performance of the specialist and generalist herbivores, as well as the parasitoid, was generally lower on native plant populations than on non-native ones. This may be explained by the trichome patterns on these plants, as well as by the expression of aliphatic glucosinolates, which are particularly effective against generalist herbivores (Gols et al. 2008a). Additionally, apart from glucosinolates, other as yet unidentified primary or secondary metabolites that differed among the Bu. orientalis populations could also have affected the performance of the herbivores.

Overall, these results reveal that native populations of Bu. orientalis are better defended than populations in the new range at least against two potentially important herbivores. The variation in plant resistance traits might result from the range-expanding process whereby less defended genotypes of the exotic plants are able to disperse further distances while the defended genotypes are left behind. However, assuming that over time exotic plants in an enemy-free environment may evolve to invest less in defence, the results from Chapter 6 also suggest that plants in the native range might be under stronger herbivore pressure than their exotic conspecifics. If that is the case, exotic populations of this plant species can re-allocate resource investment from defence to other functions such as growth and reproduction. As predicted by the SDH, I found that non-native populations of Bu. orientalis invested more in chemical defences, such as the levels of sinalbin, and less in mechanical defences, such as leaf trichomes. In addition, although I did not directly measure plant performance in this study, I observed that plants from non-native populations produced larger and thinner leaves than plants from native populations when grown under controlled greenhouse conditions. This shift in resource allocation could benefit exotic populations of the range-expanding plant by providing them with an advantage in competition with the native species, which ultimately can explain the successful establishment and spread of Bu. orientalis populations in the new range. Additionally, since I did not find differences in both insect performance and defence traits between exotic invasive and non-invasive populations of Bu. orientalis, it is likely that non-invasive populations, such as those in the Netherlands, may in time overcome the lag-phase and become invasive as their conspecifics in Germany.

Future research directions

A range, or distribution, is the geographical area where a species can be found. The range of plant species, for instance, is determined by numerous biotic and abiotic factors, including climate, soil type, and species interactions. Many studies on exotic plant invasions have focused on the enemy release hypothesis (Keane and Crawley 2002) and on the isolated role of herbivore pressure in the success (or failure) of exotic plants to become invasive. However, other factors like abiotic and soil related characteristics are likely to vary with plant range expansion and affect plant defence traits as well as multitrophic biotic interactions. Here, I have shown that an exotic range-expanding plant can differently affect aboveground interactions between the second and third trophic levels. Little is still known how range-expanding plants interact with soil biota in the new range (Engelkes et al. 2008). Studies with inter-continental invasions have shown that plants may also become released from belowground enemies (Klironomos 2002). The few studies that have looked at these interactions have shown that in new habitats range-expanders can be exposed to less soil-borne pathogens than related natives (van Grunsven et al. 2007; Engelkes et al. 2008), or than native conspecifics (van Grunsven et al. 2010). Plants can change the structure of their soil community, which can affect aboveground plant community structure (Bezemer et al. 2010a). It remains to be investigated how this process influences exotic plant species during the process of range expansion and whether it plays a role in the success or failure of exotic plants to spread in their new ranges.

Another promising research line in plant invasion will be to include interactions with organisms from physically separated compartments (e.g. plant roots and shoots) in the study of interspecific interactions with exotic plants. Above- and belowground herbivores are both capable of influencing plant abundance by selectively consuming plant shoot and root material, thereby changing intra- and interspecific interactions among plants. Additionally, studies examining the interactions between above- and belowground herbivores feeding on the same plant showed that root and shoot feeding herbivores can indeed interact (Masters et al. 2001; Bezemer et al. 2003; Soler et al. 2005). The effects of belowground feeding herbivores on aboveground herbivores cover a wide range of outcomes from positive to negative. For instance, root herbivory can increase the translocation of primary plant metabolites (e.g. soluble nitrogen and carbohydrates) to the leaves and benefit aboveground herbivores by increasing nutrients that are essential for insect growth and reproduction (Masters et al. 2001). On the other hand, root feeding can also lead to the increase of plant secondary metabolites (e.g. glucosinolates) in leaves or to changes in the volatile blends produced by infested plants, which can negatively affect behaviour and development of aboveground herbivores and their natural enemies (Soler et al. 2005; Soler et al. 2007; Soler et al. 2010). Therefore, whether exotic plants are released or not from belowground enemies can change the outcome of aboveground biotic interactions of these species. If aboveground and belowground enemies both negatively affect exotic plants less than natives, the results may be synergistic and may reinforce invasive ability; however if these effects vary in opposite directions they may be cancelled out (Agrawal et al. 2005). Ultimately, we need this understanding to unravel the relative importance of top-down and bottom-up processes in regulating communities in which exotic plants have become established. As far as I know, no studies on inter-continental invaders or intra-continental range-expanders have looked at how belowground-aboveground interactions can influence the invasive success of exotic plants.

The results of this thesis show that exotic plants may produce quite diverse and concentrated volatile blend in comparison with native species, which can play an important role in host finding abilities by both insect herbivores and their natural enemies. This means that exotic plants have the ability to change the chemical complexity of the invaded ecosystems and, by association, the interactions between plants and arthropod communities. Here I demonstrate that the parasitoid shows an innate attraction to both the native and the exotic plant, but it remains unstudied whether the parasitoid would have changed its preference after learning the associated plant cues of the exotic species. Through learning of herbivore-induced plant volatiles parasitoids can improve their host searching efficiency (Vet and Dicke 1992). In parasitoids there is a direct link between host encounter rate and the production of offspring, thus natural selection acts strongly on parasitoid searching efficiency. Therefore, if exotic plants become more abundant and native herbivores exhibit plant shifts to these plants, their parasitoids may learn the new plant cues more efficiently, especially with a perennial such as Bu. orientalis that has foliage all the growing season. As a consequence, parasitism rates may increase. Exotic plants may also offer an interesting opportunity to study the connection between different levels of organization, such as the link between parasitoid behaviour and community processes. For instance, a study by Vos et al. (2001) showed that C. glomerata wasps have difficulty locating hosts when non-hosts are also present on the host plant, due to non-specific and less reliable plant information. As a consequence of increased herbivore diversity on host plants, the strength of host-parasitoid interaction may become weaker and in extreme cases lead to the extinction of parasitoid populations by insufficient parasitism rates. Hence, a differential herbivore diversity in exotic and native plants (Gerber et al. 2008) may potentially drive the persistence or extinctions of herbivore natural enemies, which can have important implications in the strengthening or weakening of food webs in the invaded habitats (Schmitz et al. 2000).

Most of the studies on exotic plant invasions are based on experiments performed in the new range (Liu and Stiling 2006), but in order to know whether plants are released from their enemies, herbivore pressure from the native range and the new range needs to be compared (Hierro et al. 2005). Too little is still known about the abundance, interaction strengths and ecosystem impacts of even the best-studied exotic plants in their native range. For instance, although the results of this thesis suggest that native populations of Bu.

orientalis may be under stronger herbivore pressure than introduced populations, we can only validate the ERH by measuring this parameter in the plant's native range. All too often plant invasions are studied as if they were discrete events, rather than long-term processes placed within a continually changing ecological context (Dietz and Edwards 2006). Thus, the next step is to study the process of range-expansion itself by looking how the biotic interactions occur in plant populations along a physical transect of expansion from the native area to non-native habitats where the plant has become established. In addition, looking at inter- and intraspecific variation (i.e. genetics and ecology) underlying these interactions we can investigate the adaptive potential of exotic plants and their associated arthropod communities. In this way, we can gain further insights into the effects that exotic plants may have on native arthropod communities, and be able to understand how this may vary with the biology, ecology and evolutionary life history traits of the species involved.

From an applied perspective, the study of the potential evolutionary changes in plant resistance traits along the process of range expansion can have important implications for biological control programmes targeted at invasive plants (Müller-Schärer et al. 2004). To investigate the evolutionary process of range expansion we need to examine how the different selection pressures on phenotypes differ between the native and the introduced range, and assess the genetic polymorphism retained throughout the process of range expansion. Knowing that divergence in plant traits may also occur because of founder effects and genetic drift, it is important to compare source populations in the native range with introduced populations of exotic plants. Molecular marker techniques, such as microsatellite markers coupled with more powerful statistical analyses can help disentangle these processes. Lastly, the results in this thesis suggest that some of the current hypotheses proposed to explain the success of inter-continental invaders can also play a role in intra-continental range-expanding plants and their potential to become invasive. Therefore, future studies on plant invasions should also focus on these intra-continental range-expanders, since a suite of anthropogenic processes is increasingly leading to species range shifts.

Conclusions

- The exotic range-expanding plant, Bunias orientalis, exhibited lower herbivore loads and reduced levels of leaf damage compared with related native plants in the new range. Higher potential carnivore pressure on herbivores on the exotic species in the middle period of the growing season was also observed.
- The exotic plant produced tougher leaves than related native species in the new range.
- Compared with the native black mustard Brassica nigra, the exotic Bu. orientalis affected more negatively the survival and performance of the specialist herbivore, Pieris brassicae, and its koinobiont parasitoid, Cotesia glomerata, but not its idiobiont parasitoid, Pteromalus puparum. This may have been due to different resource use strategies in the two parasitoids.
- Female P. brassicae butterflies, collected in the field as larvae, preferentially selected a native plant, the charlock mustard Sinapis arvensis, to oviposit, where their offspring also performed better compared to those feeding on the exotic plant.
- Under flight-tent conditions naïve C. glomerata females did not discriminate between native and exotic host infested plants, although their volatile blends differed both qualitatively and quantitatively. However, under semi-field conditions the parasitoid preferred hosts that were feeding on the native plant.
- Bunias orientalis plants from non-native populations were less well-defended than native conspecifics and differed significantly in their metabolomic fingerprinting, expression of glucosinolates and trichome densities.
- Specialist (P. brassicae) and generalist (Mamestra brassicae) herbivores and a specialist parasitoid of the latter (Microplitis mediator) performed worse when developing on plants from the native than the introduced range.
- The enemy release hypothesis can be an important mechanism underlying the successful range expansion of the exotic plant. Further studies need to examine belowground interactions on this plant, as well as studying above- and belowground multitrophic interactions where Bu. orientalis is native.

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Summary

Due to current global changes many plant species are being intentionally or accidentally moved from their native ranges to novel environments. Some of these species, regarded as exotic, find favourable abiotic conditions to establish new interactions in the invaded ecosystems. If their co-evolved enemies, such as herbivores or pathogens, are absent in the new range, exotic plants can have a competitive advantage over native species, which can promote their invasiveness. Although the ecological impacts of exotic invasive plants are well-studied, most of the studies have focused on inter-continental invasions, while little is still known about how intra-continental range-expanding plants affect multitrophic interactions in the new range. This is particularly important, because recently many exotic plants are expanding their range within the same land masses due to climate change and other global environmental changes. Furthermore, studies of exotic plants and their enemies have usually been conducted within a bi-trophic framework, involving only plants and herbivores. However, a better understanding of the biotic forces regulating invaded communities should include interactions with higher trophic levels, such as natural enemies of herbivores, which have an important role in the structure and functioning of food webs. This thesis explores the mechanisms of interactions between an exotic range-expanding plant and native aboveground herbivores and their natural enemies. In addition, it investigates the role that the new multitrophic interactions can play in the successful establishment of an exotic plant.

To investigate mechanisms underlying the process of range expansion in exotic species, I used as model system a plant in the Brassicaceae, Bunias orientalis L. This plant, originally from extreme south-eastern Europe and Asia, has been expanding its range since 1980's and has become invasive in parts of northern and western Europe. In the Netherlands, however, it is presently considered as naturalized but non-invasive. In Chapter 1, I introduce the process of plant invasion and range expansion, briefly describing how exotic plants can influence interactions with the enemies in the new range. I describe the general aims and the outline of the thesis.

In Chapter 2, using a community approach, I compare the vegetation composition and invertebrate assemblages between the exotic plant and three related native species through the course of an entire growing season in the Netherlands. I tested the enemy-release hypothesis (ERH) to examine whether herbivore loads, foliar damage and carnivore pressure differ between the exotic and the native plants. In support of this hypothesis, the exotic plant species suffered less herbivore damage and harboured smaller invertebrate communities than the native species in the non-native range. On the other hand, potential carnivore pressure on herbivores was higher on the exotic plant than on a native species that grows later in the season at the peak of arthropod abundance. To better understand the reduced levels of herbivory on the exotic species, I measured the leaf toughness of all plant species, and I found that the foliar toughness of the exotic plant was much greater than any of the native species. In addition, I found differences in species composition and abundance between the plant communities associated with the exotic and the native species. Therefore, enemy release on the exotic plant could result from the combined bottom-up effect of plant resistance related traits and top-down pressure of higher trophic levels on plant enemies. The identity and structure of the plant community in which the exotic species is embedded could affect the diversity of herbivorous and carnivorous insects as well. I concluded that the ERH, although mainly applied to inter-continental invaders, also plays a potentially important role in intra-continental range-expanding plants.

A major challenge in studying plant invasions is to understand how novel plants, particularly intra-continental range-expanding species, affect trophic interactions in their new range, and how native herbivores and their natural enemies respond to these species. In Chapter 3, I hypothesize how structural and chemical traits of exotic invasive plant species can affect plant-finding abilities of herbivores and host/prey-finding behaviour of parasitoids and predators in the invaded communities. The invasive plants can create structural barriers that impede dispersal and host plant-finding abilities of arthropods, and/or they can be attractive to native herbivores but be toxic to their larvae, thus acting as an ecological 'trap'. On the other hand, herbivores and their natural enemies may benefit from the establishment of exotic plants because they locally increase the amount of available resources to exploit. I also discuss implications of exotic plants for pest control in agro-ecosystems and for conservation of native arthropod communities.

In Chapters 4 and 5, I test some of the hypotheses formulated in the previous chapter. In Chapter 4, I compare the influence of plant quality of the exotic species and a native species, Brassica nigra, on the performance of a leaf-chewing specialist herbivore and two of its gregarious parasitoids, a larval and a pupal parasitoid. Emphasis was placed on parasitoid host-resource use strategies and how these may be differently affected by the quality of the exotic plant. In Chapter 5, I compare oviposition preference and offspring performance of the specialist herbivore on the exotic plant and on the native plant, Sinapis arvensis. To better understand the mechanisms underlying herbivore host-plant choice, I describe the response of its larval parasitoid to herbivore-induced plant volatiles (HIPV) emitted by the host plants, and determine the volatile blend composition to elucidate which compound(s) can be involved in parasitoid attraction. In addition to host-finding ability, I also examine parasitoid host acceptance behaviour by comparing the parasitism rate of the herbivore on the exotic and the native plant. The results of these chapters show that behaviour and growth of the specialist herbivore is negatively affected by the exotic plant, which in general is of lower quality for herbivore development than the native species. Parasitoids with different host exploitation strategies were, however, differently affected by the exotic plant. The pupal parasitoid (idiobiont) survived better than the larval parasitoid (koinobiont), and the latter parasitized fewer hosts on the exotic than on native plants. I concluded that there is positive selection pressure acting on the specialist herbivore to adapt to the new plant by conferring ‘enemy free space’, which ultimately could support a host plant shift and control the spread of the exotic species in the new range. Conversely, the larval parasitoid did not discriminate between HIPV emitted by both the exotic and the native plant. This means that the herbivores colonizing the exotic plant can be followed by their natural enemies, such as parasitoids, if host encounter rate on the exotic plant increases. Consequently, it could provide the exotic plant with an alternative form of ‘enemy release’ that has been largely unexplored.

When exotic plants expand their range within the same continent, they can encounter the same insect species that co-occur with them in their native range. In Chapter 6, using a biogeographical approach, I examine the effects of intraspecific variation in resistance traits of Bu. orientalis on the performance of two leaf-chewing herbivores, a specialist and a generalist herbivore, and an important larval endoparasitoid of the generalist, originally from the new range. I compare insect performance in plant populations that were collected in the native range, the exotic invasive and the exotic non-invasive range. To elucidate more about plant resistance traits that could explain insect performance, I compared entire plant metabolomic fingerprint and more specifically glucosinolate profiles, as a measure of chemical defence, as well as leaf trichome density and structure, as a measure of mechanical defence, in plants from the different populations. I found considerable intraspecific variation in plant defence traits (trichomes, metabolomic fingerprints, and glucosinolates) in different populations of Bu. orientalis. Plants from the native range were better defended than their conspecifics from the exotic range. This variation matched with the performance of the generalist herbivore and its parasitoid, which developed more poorly in plants from the native range. It is possible that plants in the exotic range, freed of locally adapted specialist herbivores from their native range, have altered investment in defence traits, making them more susceptible to generalist herbivores that are unpredictable and perhaps infrequent. However, other factors such as founder effect and genetic drift may also play an important role. This plant species was initially introduced in the new range in the 18th century by human intervention and only in the 1980's started a rapid expansion in central and northern parts of Europe. To fully understand the evolutionary changes these populations went through during the range expansion, it is crucial to track down the source populations in the plant's native range.

Finally, in Chapter 7, I synthesise and discuss the findings presented in this thesis and suggest future research directions. The next step in future research will be to target source plant populations in its native range and from there study multitrophic interactions between exotic plants and native communities along the range expansion transect. To broaden our view on the complexity inherent in the range expansion process, the study of multitrophic interactions should also include separated physical compartments (roots and shoots), as well as their associated interactions.

Samenvatting

Het klimaat en de omgeving veranderen, op mondiaal niveau. Deze veranderingen hebben ertoe geleid dat veel plantensoorten hun leefgebied kunnen opschuiven naar nieuwe arealen, waar ze al dan niet opzettelijk door de mens worden geïntroduceerd. Enkele van deze uitheemse soorten belanden daardoor in zeer gunstige abiotische omstandigheden en zijn in staat om nieuwe (voedselweb)interacties te vormen in het geïnvadeerde ecosysteem. Op het moment dat hun ge-coëvolueerde vijanden (herbivoren, pathogenen, etc.) afwezig zijn, hebben de uitheemse planten een competitief voordeel ten opzichte van de inheemse planten. Dit competitief voordeel kan vervolgens bijdragen aan het invasie succes. De ecologische impact die uitheemse invasieve plantensoorten hebben is uitgebreid onderzocht. Dit onderzoek betreft meestal intercontinentale invasies (deze planten hebben hun oorsprong op andere continenten). Kennis over hoe intracontinentale uitheemse planten (deze planten verschuiven hun leefgebied op hetzelfde continent als waar ze van oorsprong voorkomen) de multitrofe interacties in hun nieuwe omgeving beïnvloeden is echter gering. Deze kennis is belangrijk omdat klimaatverandering en andere mondiale veranderingen in het milieu ertoe hebben geleid dat veel planten hun areaal kunnen (en bezig zijn te) vergroten op hetzelfde continent. De studie naar invasieve planten en hun vijanden heeft zich tot nu toe voornamelijk geconcentreerd op de bitrofe interactie tussen alleen de plant en diens herbivoren. Om een bredere kennis te krijgen over de biotische factoren die geïnvadeerde gemeenschappen reguleren, moet er echter ook gekeken worden naar de interacties met de hogere trofische niveaus (b.v. natuurlijke vijanden van de herbivoren), omdat deze een belangrijke rol spelen bij het structureren en functioneren van voedselwebben. Dit proefschrift onderzoekt de interactie-mechanismen tussen een uitheemse (intracontinentale) plant en de inheemse bovengrondse herbivoren en hun natuurlijke vijanden. Daarnaast heb ik de rol die de nieuwe multitrofe interacties kunnen spelen in de succesvolle vestiging van de uitheemse plant onderzocht.

Mijn modelsysteem is Bunias orientalis L. (Brassicaceae). Deze plant komt oorspronkelijk uit Zuidoost-Europa en Azië, en is rond 1980 begonnen aan zijn opmars in Europa. In veel delen van Noord- en West-Europa wordt de Bu. orientalis inmiddels als invasief beschouwd. In Nederland wordt de plant gezien als genaturaliseerd maar niet invasief. In het eerste hoofdstuk introduceer ik het proces dat ten grondslag ligt aan planteninvasies (zowel inter- als intracontinentaal), waarbij ik kort inga op hoe de uitheemse planten de interacties met hun natuurlijke vijanden in het nieuwe gebied kunnen beïnvloeden. De doelstellingen en hoofdlijnen van het proefschrift komen in dit hoofdstuk ook aan de orde.

In hoofdstuk 2 vergelijk ik, met behulp van een community approach, de vegetatiesamenstelling en de levensgemeenschap van invertebraten (ongewervelde dieren) op de uitheemse plant en drie nauw verwante inheemse soorten. Hiervoor heb ik ze gedurende hun gehele groeiseizoen in Nederland bemonsterd. Door de herbivore druk, bladschade, en carnivore druk tussen de plantensoorten te vergelijken, kon ik de 'Enemy-Release-Hypothesis' (ERH) testen. In overeenstemming met de voorspellingen van de ERH bleken de uitheemse planten in hun nieuwe leefgebied minder bladschade te hebben en huisvestten ze een kleinere gemeenschap aan invertebraten dan de inheemse plantensoorten. Tijdens de piek in seizoensgebonden abundantie aan geleedpotigen (arthropoden) was de potentiële carnivore druk op de herbivoren hoger op de uitheemse plant dan de op de inheemse soort die op dat moment rijkelijk aanwezig was. Om de verminderde herbivore druk op de uitheemse soort beter te begrijpen, heb ik de hardheid van de bladeren van alle plantensoorten gemeten. Daaruit bleek dat de hardheid van de bladeren van de uitheemse soort hoger was dan die van alle inheemse soorten. Ook vond ik een lagere taxonomische rijkdom en abundantie van invertebraten op de uitheemse dan op de inheemse plantensoorten. Het is daarom zeer goed mogelijk dat er minder herbivoren op de uitheemse plant zitten door het gecombineerde effect van de vraat-resistentie gerelateerde eigenschappen van de plant en grotere druk van de hogere trofische niveaus. De samenstelling en structuur van de plantengemeenschap waarin de uitheemse plant terechtkomt kan ook een effect hebben op de diversiteit aan herbivore en carnivore insecten. Ik kom tot de conclusie dat de ERH (voornamelijk gebaseerd op intercontinentale invasies) ook een potentieel belangrijke rol kan spelen bij het succes van intracontinentale planteninvasies.

Een grote uitdaging in het onderzoek naar planteninvasies is het begrijpen van hoe (intracontinentale) planten in een nieuw gebied de trofische interacties met de inheemse herbivoren en carnivoren beïnvloeden, en hoe deze geleedpotigen reageren op de nieuwe plant. In hoofdstuk 3 kom ik met de hypothese dat de morfologische en chemische eigenschappen van uitheemse invasieve planten een effect hebben op het gedrag van herbivoren, predatoren, en parasitoïden uit de geïnvadeerde levensgemeenschap. De invasieve plant kan eigenschappen bezitten die een barrière vormen voor de verspreiding van geleedpotigen of die hun zoektocht naar geschikte waardplanten minder succesvol maakt. Ook kan de plant aantrekkelijk zijn voor de inheemse volwassen herbivoren maar juist giftig voor hun larven: een ecologische fuik/val. Herbivoren en hun vijanden kunnen daarentegen ook juist profiteren van de vestiging van de uitheemse plant doordat het een lokale vergroting van het voedselaanbod is. In dit hoofdstuk ga ik bovendien in op de gevolgen van de vestiging van uitheemse planten op zowel plaagbestrijding in agro-ecosystemen als natuurlijk behoud van inheemse geleedpotige levensgemeenschappen.

Hoofdstuk 4 en 5 bevatten de resultaten van experimenten die de hypothesen geformuleerd in het vorige hoofdstuk testen. Het experiment in hoofdstuk 4 vergelijkt het effect van het verschil in kwaliteit van de uitheemse en een inheemse plantensoort (Brassica nigra) als voedselplant voor een bladkauwende specialistische herbivoor en twee van diens parasitoïden: een soort die de larve parasiteert en een soort die het popstadium als gastheer heeft. Ik stel hier de vraag hoe parasitoïden hun gastheer het meest effectief kunnen gebruiken en hoe deze precieze strategie beïnvloed kan worden door de voedselkwaliteit van de uitheemse plant. In hoofdstuk 5 vergelijk ik het verschil in ovipositie voorkeur (leggen van eieren) en het verschil in de overleving en groei van het nageslacht van de specialistische herbivoor op de uitheemse plant en de inheemse plant Sinapis arvensis. Om de mechanismen die leiden tot plantenvoorkeur bij geleedpotige herbivoren beter te begrijpen, onderzocht ik de reactie van de larveparasitoïde op zogenaamde ‘herbivore-induced plant volatiles’ (HIPV). De specifieke samenstelling van deze vluchtige plantenstoffen kan de mate van aantrekking van de parasitoïden sterk bepalen. De aantrekking van de parasitoïde door de waardplant heb ik bepaald door parasitisme van de herbivoor op de uitheemse en inheemse plant te vergelijken. De resultaten laten zien dat het gedrag en de ontwikkeling van de specialistische herbivoor negatief wordt beïnvloed door de uitheemse plant. Deze blijkt bovendien over het algemeen minder geschikt te zijn als waardplant voor de herbivoor dan de inheemse soorten. Het effect van de uitheemse plant op de parasitoïden (met elk een ander zoekgedrag) was echter niet uniform. De popparasitoïde (idiobiont) had een hogere overleving op de uitheemse plant dan de larveparasitoïde (koinobiont). De laatstgenoemde parasiteerde bovendien minder gastheren op de uitheemse plant. Ik concludeer daarom dat er een positieve selectiedruk is op de specialistische herbivoren om zich aan te passen aan de nieuwe uitheemse plant door in te spelen op de ‘enemy free space’. Dit kan uiteindelijk leiden tot een acceptatie van de uitheemse plant als waardplant door de herbivoor, wat weer kan resulteren in het inperken van de verspreiding van de uitheemse plant. Anderzijds reageerde de koinobionte parasitoïde niet verschillend op de HIPVs van de uitheemse en inheemse planten. Dit kan betekenen dat de kolonisatie van de herbivoren op de uitheemse plant gevolgd kan worden door de kolonisatie van de natuurlijke vijanden. Dit kan juist resulteren in een nieuw soort ‘enemy release’ voor de uitheemse plant: een alternatieve mogelijkheid die nog niet veel onderzocht is.

Op het moment dat uitheemse planten hun areaal vergroten op hetzelfde continent, kunnen ze in het nieuwe gebied in contact komen met dezelfde insecten als waarmee ze ge-coëvolueerd zijn. In hoofdstuk 6, met behulp van een biogeografische aanpak, onderzoek ik daarom het effect van intraspecifieke variatie in resistentie kenmerken van Bu. orientalis op de prestatie van twee bladkauwende herbivoren (een specialist en een generalist) en een belangrijke koinobionte parasitoïde van de generalist. Deze parasitoïde komt oorspronkelijk uit het nieuwe gebied. Ik heb de prestaties van de insecten vergeleken op planten afkomstig uit populaties uit het oorspronkelijke gebied, het uitheemse invasieve en het uitheemse niet-invasieve gebied. Om meer te weten te komen over de specifieke kenmerken van plantenafweer die een invloed kunnen hebben op de prestaties van de insecten, heb ik naar de gehele metabole vingerafdruk van de plant gekeken. Daarbij heb ik specifiek gelet op de glucosinolaat profielen (een maat voor chemische afweer), en trichomen dichtheid en structuur (als maat van mechanisme afweer). Er bleek een grote intraspecifieke variatie in trichomen, metabole profielen en glucosinolaten te zijn. Planten uit de oorspronkelijke regio waren beter beschermd dan hun soortgenoten uit het nieuwe leefgebied. Deze variatie kwam overeen met de prestatie van de generalistische herbivoor en diens parasitoïde, die minder goed ontwikkelden op planten uit de oorspronkelijke regio. Het is een mogelijkheid dat de planten in het nieuwe leefgebied (waar ze gevrijwaard zijn van hun ge-coëvolueerde specialistische vijanden) de investering in hun verdediging veranderd hebben, waardoor ze juist ten prooi kunnen vallen aan generalistische herbivoren wiens gedrag en aantallen niet te voorspellen zijn. Het behoort echter ook tot de mogelijkheden dat het ‘founder effect’ en genetische drift een belangrijke rol spelen in het verklaren van de intraspecifieke variatie. Bunias orientalis is in de 18^{e} eeuw door de mens in zijn nieuwe leefgebied geïntroduceerd, en is pas vanaf de jaren ’80 begonnen aan zijn opmars in Centraal en Noord-Europa. Om de evolutionaire veranderingen die de planten hebben ondergaan gedurende hun areaalvergroting beter te begrijpen, is het essentieel om de bronpopulaties te vinden.

Tot slot heb ik hoofdstuk 7 gewijd aan de synthese van dit proefschrift, bediscussieer ik de gepresenteerde resultaten en doe ik suggesties voor vervolg onderzoek. Toekomstig onderzoek zal de bronpopulaties moeten vinden van waaruit de plant zijn opmars is begonnen. Vervolgens zal het veel inzicht geven wanneer de multitrofe interacties tussen de uitheemse plant en de inheemse gemeenschappen bestudeerd worden over het transect van oorspronkelijk tot nieuw leefgebied. Om ons begrip omtrent de complexiteit inherent aan intracontinentale plantinvasies te vergroten is het essentieel om in dit onderzoek naar multitrofe interacties rekening te houden met de boven- en ondergrondse anatomie van de plant (wortels en spruiten) en hoe deze gescheiden compartimenten elkaar beïnvloeden.

Sumário

As alterações globais actuais têm levado a que muitas espécies de plantas sejam intencionais ou acidentalmente introduzidas em novos habitats. Algumas destas espécies, denominadas exóticas, encontram condições abióticas favoráveis para estabelecer novas interacções nos ecossistemas invadidos. As plantas exóticas podem possuir uma vantagem adaptativa sobre as plantas nativas, quando por exemplo os seus inimigos, herbívoros ou patogéneos, estão ausentes no novo habitat, conferindo neste caso carácter invasor a muitas destas plantas exóticas. Apesar dos impactos ecológicos das plantas exóticas invasoras serem bem conhecidos, a maioria dos estudos baseia-se em invasões entre continentes, limitando o conhecimento sobre a forma de como as plantas, que expandem a sua distribuição geográfica no mesmo continente, afectam as interacções multi-tróficas no novo habitat. Este conhecimento é particularmente importante, uma vez que devido às actuais alterações climáticas e ambientais, muitas espécies de plantas têm expandido recentemente a sua distribuição geográfica no mesmo continente. Por outro lado, os estudos com plantas exóticas e os seus inimigos têm sido conduzidos maioritariamente emenquadramentos bi-tróficos, envolvendo apenas plantas e herbívoros. No entanto, para um melhor entendimento das forças bióticas que regulam as comunidades invadidas deve também incluir-se interacções com níveis tróficos superiores, como os inimigos naturais dos herbívoros, que têm um papel muito importante quer na estrutura quer no funcionamento das cadeias alimentares. Esta tese explora os mecanismos de interacção entre uma planta exótica de expansão intra-continental e os herbívoros e seus inimigos naturais no novo habitat. Além disso, investiga o papel que as novas interacções multi-tróficas poderão ter no estabelecimento bem-sucedido da planta exótica.

Para investigar os mecanismos inerentes ao processo de expansão geográfica em espécies exóticas, usei como modelo uma planta na família Brassicacea, Bunias orientalis L. Esta planta, originária do extremo sueste da Europa e Àsia, tem expandido a sua distribuição geográfica desde os anos 1980, tendo-se tornado invasora em regiões do norte e oeste europeu. Na Holanda, no entanto, presentemente esta espécies está naturalizada e não é invasora. No capítulo 1, introduzo o conceito do processo de invasão e de expansão geográfica das plantas, descrevendo brevemente como as plantas exóticas influenciam as interacções com os inimigos no novo habitat. Para além disso, descrevem-se também os objectivos gerais e o plano da tese.

No capítulo 2, usando uma abordagem de comunidades, comparou-se a composição vegetativa e a estrutura da comunidade de invertebrados entre a planta exótica e três espécies nativas da mesma família ao longo da época de crescimento na Holanda. Testou-se a "hipótese de libertação de inimigos" (HLI) para investigar se abundância de herbívoros, os estragos foliares e a pressão potencial de carnívoros sobre os herbívoros diferia entre a planta exótica e as espécies nativas. Corroborando esta hipótese, no novo habitat a planta exótica sofreu menos estragos dos inimigos e manteve uma comunidade de invertebrados mais reduzida que as plantas nativas. Por outro lado, a pressão potencial dos carnívoros sobre os herbívoros foi superior na planta exótica em comparação com uma das espécies nativas que apresenta um crescimento mais tardio coincidente com o pico da abundância de artrópodes. Para entender melhor o reduzido nível de herbivoria na planta exótica mediu-se a resistência foliar das plantas, e verificou-se que este parâmetro era muito superior na planta exótica, em comparação com qualquer das espécies nativas. Adicionalmente, a composição e abundância das espécies de plantas diferiu entre as comunidades associadas à espécie exótica e às plantas nativas. Por este motivo, a libertação de inimigos na espécie exótica poderá ter resultado do efeito combinado da resistência da planta e da pressão exercida pelos inimigos naturais sobre os herbívoros. A identidade e estrutura da comunidade de plantas na qual a espécie exótica está inserida poderiam também ter afectado a diversidade de insectos herbívoros e carnívoros encontrados nesta espécie. Conlui que HLI, apesar de ser maioritariamente aplicada a plantas invasoras inter-continentais, poderá ter também um papel importante na expansão geográfica das plantas dentro do mesmo continente.

Um dos maiores desafios no estudo de invasões de plantas passa por compreender como as novas plantas, em particular as de expansão intra-continental, afectam as interacções tróficas no novo habitat, e de como os herbívoros e os seus inimigos naturais respondem a estas espécies. No capítulo 3, colocaram-se diferentes hipóteses de como as características estruturais e químicas das plantas invasoras podem afectar a capacidade dos insectos, herbívoros e carnívoros, para encontrar o hospedeiro, a planta ou o herbívoro, respectivamente no ecossistema invadido. As plantas invasoras podem criar barreiras estructurais que impedem a dispersão e capacidade dos artrópodes para encontrar a planta hospedeira, e/ou serem atractivas para os herbívoros nativos, mas serem tóxicas para o desenvolvimento da sua descendência, actuando neste caso como uma "armadilha ecológica". Por outro lado, os herbívoros e os inimigos naturais podem benefiar do estabelecimento de plantas exóticas por estas aumentarem localmente a quantidade de recursos disponíveis.

Complementarmente, neste capítulo discute-se também as implicações das espécies exóticas no controlo das pragas de culturas em ecossistemas agrários e na conservação das comunidades nativas de artrópodes.

Nos capítulos 4 e 5, testou-se algumas das hipóteses formuladas no capítulo anterior. No capítulo 4, comparou-se a influência da qualidade da planta exótica e de uma espécie nativa, Brassica nigra, no desenvolvimento de um herbívoro especialista e dois dos seus parasitóides gregários, um que ataca estados larvares (koinobionte) e outro as pupas do hospedeiro (idiobionte). Neste capítulo, foi dado ênfase às estratégias de uso do hospedeiro pelos parasitóides, uma vez que poderão ser afectados diferencialmente pela qualidade da planta exótica. No capítulo 5, comparou-se a preferência das fêmeas do herbívoro especialista para colocar os ovos, assim como o desenvolvimento da sua descendência na planta exótica e numa planta nativa, Sinapis arvensis. Para compreender melhor os mecanismos inerentes à escolha da planta hospedeira pelo herbívoro, a resposta do seu parasitóide koinobionte aos voláteis emitidos pela planta hospedeira após o ataque do herbívoro é descrita, assim como a composição da mistura volátil no sentido de elucidar os componentes que possam estar envolvidos na atracção do parasitóide. Os resultados destes capítulos mostram que tanto o comportamento como o desenvolvimento do herbívoro especialista são negativamente afectados pela planta exótica, que no geral é de qualidade inferior para o seu desenvolvimento, em comparação com as plantas nativas. Os parasitóides com diferentes estratégias de uso do hospedeiro foram diferentemente afectados pela qualidade da planta exótica. O parasitóide idiobionte sobreviveu melhor que o koinobionte, e este parasitou menos larvas do hopedeiro na planta exótica do que na planta nativa. Uma vez que a nova planta pode actuar como “espaço livre de inimigo naturais” para o herbívoro, concluí que poderá existir uma pressão de selecção positiva que actua no herbívoro para se adaptar à nova planta, e em último caso resultar na sua deslocação para uma nova planta hospedeira controlando a expansão da planta exótica no novo habitat. Por outro lado, o parasitóide koinobionte não discriminou entre os voláteis emitidos pela espécie exótica e a planta nativa. Este facto pode significar que a colonização da nova planta pelo herbívoro pode ser seguida pelos seus inimigos naturais, como parasitóides, se a taxa de encontro do hospedeiro aumentar na planta exótica. Neste caso, a nova planta poderá estar sujeita a uma outra forma de “libertação de inimigos” que até à data tem sido quase inexplorada.

Quando as plantas exóticas expandem a sua distribuição geográfica dentro do mesmo continente, elas podem encontrar as mesmas espécies de insectos que co-ocorrem no seu habitat nativo. No capítulo 6, usando uma abordagem biogeográfica, examinaram-se os efeitos da variação intra-específica na resistência de Bu. orientalis no desenvolvimento de duas espécies de herbívoros foliares, um especialista e um generalista, e de um importante parasitóide do herbívoro generalista, sendo todas as espécies originárias do novo habitat. Comparou-se o desenvolvimento destes insectos em populações da planta originárias do habitat nativo e do habitat exótico, onde a planta é invasora ou não-invasora. Para elucidar melhor os caracteres de resistência da planta que poderiam explicar o desenvolvimento dos insectos, comparou-se toda a impressão metabólica e mais especificamente o perfil de glucosinolatos, como medida da defesa química, bem como a densidade e estrutura dos tricomas foliares, como medida da defesa mecânica, em plantas das diferentes populações. Mostrou-se que existe uma variação intra-específica considerável nos caracteres involvidos na defesa da planta nas diferentes populações de Bu. orientalis. Plantas da região de origem estavam mais bem defendidas que as suas conspecíficas no novo habitat. Esta variação correspondeu ao desenvolvimento do herbívoro generalista e do seu parasitóide, que se desenvolveram mais dificilmente em plantas da área nativa. É possível que as plantas no novo habitat, livres de herbívoros especialistas da sua área nativa, tenham alterado o seu investimento nos caracteres de defesa, tornando-as mais susceptíveis aos herbívoros generalistas que são mais imprevisíveis e menos frequentes. No entanto, outros factores relacionados com o efeito fundador e deriva genética poderão também ter desempenhado um papel importante. Esta planta foi inicialmente introduzida nos novos habitats no século XVIII por intervenção humana e só nos anos 1980 encetou uma rápida expansão geográfica em zonas do norte e centro da Europa. Por essa razão, para uma melhor compreensão das alterações evolutivas que as diferentes populações desta espécie exótica terão sofrido ao longo da expansão geográfica é fundamental identicar as populações de origem na área nativa.

Finalmente, no capítulo 7, sintetizo e discuto os principais resultados apresentados nesta tese e sugiro ideias para investigação futura. O próximo passo será identificar as populações de origem e a partir destas estudar as interacções multi-tróficas entre as plantas exóticas e as comunidades nativas ao longo do transecto de expansão. Para além disso, de modo alargar o conhecimento sobre a complexidade inerente ao processo de expanção geográfica, o estudo das interacções multitróficas deverá também incluir compartimentos fisicamente separados, tais como a raíz e a parte aérea da planta, bem como a sua interacção.

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About the author

Taiadjana Filipa Marques Fortuna was born on the 10th of January 1983 in Maputo, Mozambique. She was one-year old when she moved to Portugal where she grew up. After finishing high school in 2001, she started the study of Biology at the Science Faculty of Lisbon University (FCUL), which she finished in 2005 with specialization in environment and terrestrial biology (grade 16/20). During her undergraduate studies, she conducted ecology-oriented research projects in the laboratory and in the field using different model systems including both Mediterranean flora and fauna such as lizards, amphibians and arthropods. From May till September 2006, she worked as a field assistant in the department of biology at Dalhousie University and Saint Mary's University (Nova Scotia, Canada) under the supervision of Prof. dr. Marty Leonard and Dr. Hugh Broders. The research focused on the study of the social behaviour of the northern long-eared bat, as well as on the development of a bat echolocation identification protocol using automated ultrasonic detectors. In November 2006 and back to Lisbon, she ingressed in an ecology and environmental management master program at FCUL. Under the supervision of Prof. dr. José Carlos Franco and Prof. dr. Mª Teresa Rebelo, she conducted her research in the Agronomy Institute (ISA) of Lisbon Technical University, where she studied the kairomonal response of the parasitoid Anagyrus sp. near pseudococci to the sex pheromone of its mealybug host, an important pest of vine. The ultimate goal of the project was to contribute to the development of new biological crop protection tactics and reduce use of pesticides. The master thesis was successfully defended in February 2008 with classification of excellent (grade 19/20).

Together with her PhD supervisors, Taiadjana wrote a proposal to FCT in 2007 to pursue a PhD on the multitrophic interactions of the range-expanding plant species, Bunias orientalis. In April 2008, Taiadjana started her PhD-project under supervision of Prof. dr. Louise Vet and Prof. dr. Jeffrey Harvey at the Netherlands Institute of Ecology (NIOO), Wageningen. Since then, she conducted laboratory, field and semi-field experiments and presented her research at many seminars and national and international conferences. The results of her PhD-project are presented in this thesis.

Publication list

Fortuna TM, Woelke JB, Hordijk CA, Jansen JJ, van Dam NM, Vet LEM and Harvey JA (2013) A tritrophic approach to the preference-performance hypothesis involving an exotic and a native plant. Biological Invasions. DOI: 10.1007/s10530-013-0459-2 (in press)

Fortuna TM, Harvey JA and Vet LEM (2012) Effects of an invasive plant on the performance of two parasitoids with different host exploitation strategies. Biological Control 62(3): 213-220.

Harvey JA and **Fortuna TM** (2012) Chemical and structural effects of invasive plants on herbivore–parasitoid/predator interactions in native communities. Entomologia Experimentalis et Applicata 144(1): 14-26.

Franco JC, **Fortuna T**, Silva EB, Suma P, Russo A, Zada A and Mendel Z (2011) Vine mealybug sex pheromone increases citrus mealybug parasitism by Anagyrus sp. near pseudococci (Girault). Biological Control 58(3): 230-238.

Harvey JA, Biere A, **Fortuna T**, Vet LEM, Engelkes T, Morriën E, Gols R, Verhoeven Koen, Vogel H, Macel M, Heidel-Fischer HM, Schramm K and Putten WH (2010) Ecological fits, mis-fits and lotteries involving insect herbivores on the invasive plant, Bunias orientalis. Biological Invasions 12: 3045–3059.

Bezemer TM, Harvey JA, Kamp AFD, Wagenaar R, Gols R, Kostenko O, **Fortuna T**, Engelkes T, Vet LEM, Putten WH and 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.

Silva EB, **Fortuna T**, Franco JC, Campos L, Branco M, Zada A and Mendel Z (2009) Kairomonal response of a parasitic wasp to the sex pheromone of the vineyard mealybug. IOBC wrps Bull 41: 79-82.

Franco JC, **Fortuna T**, Silva EB, Suma P, Russo A, Campos L, Branco M, Zada A and Mendel Z (2008) May vine mealybug sex pheromones improve the biological control of the citrus mealybug? In: Integrated Control in Citrus Fruit Crops (eds. Garcia-Marí F). IOBC wprs Bull 38: 94-98.

Submitted:

Fortuna TM, Eckert S, Harvey JA, Vet LEM, Müller C and Gols R (2013) Variation in plant defences among populations of a range-expanding plant: consequences for native trophic interactions. New phytologist

Van Geem M, Gols R, van Dam NM, van der Putten WH, **Fortuna TM** and Harvey JA (2013) The importance of aboveground-belowground interactions in the evolution and maintenance of variation in plant defence traits. Frontiers in Plant-Microbe Interaction

In preparation

Fortuna TM, Ens H, Woelke JB, Mariette N, Putten WH, Vet LEM and Harvey JA. Comparing vegetation diversity and invertebrate communities on an exotic range-expanding plant and related natives.

PE&RC PhD Training 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)

- The role of above and belowground biotic interactions in plant invasion (2008)

Writing of project proposal (4.5 ECTS)

- Do above and belowground multitrophic interactions differ between native and invasive plants? (2009)

Post-graduate courses (7.5 ECTS)

- Biodiversity and ecosystem services in a sustainable world; PE&RC (2008)
- Life history theory; RSEE (2009)
- WIAS advanced statistics course; design of experiments; WIAS (2009)
- Linear models; PE&RC (2011)
- Generalized linear models; PE&RC (2011)
- Mixed linear models; PE&RC (2011)
- Multivariate analysis; PE&RC (2011)

Laboratory training and working visits (1.2 ECTS)

- Exotic plant resistance to native herbivores in the introduced range; Max-Planck Institute for Chemical Ecology, Jena, Germany (2009)
- Study of plant populations in the native range; University of Rize, Turkey (2010)
- Plant chemical resistance traits; University of Bielefeld, Germany

Deficiency, refresh, brush-up courses (0.8 ECTS)

- Academic writing II (2009)

Competence strengthening / skills courses (3 ECTS)

- Techniques for writing and presenting a scientific paper; WGS (2009)
- PhD Competence assessment; WGS (2010)
- Scientific writing; WGS (2011)

PE&RC Annual meetings, seminars and the PE&RC weekend (1.2 ECTS)

- PE&RC Weekend (2008)
- Plant-Insect interactions workshop (2010)

Discussion groups / local seminars / other scientific meetings (7.5 ECTS)

- Workshop Plant-Insect interactions and chemical defence; Leiden University (2008)
- Department weekly discussion group (2008-2012)
- PhD Monthly meeting group (2008-2012)
- NIOO Days (2008, 2009)
- Netherlands Annual Ecology Meeting (NAEM) (2009-2011)

International symposia, workshops and conferences (9 ECTS)

- NEOBIOTA: 5th European Conference on Biological Invasions; poster presentation (2008)
- 1st International Entomophagous Insect Conference; oral presentation (2009)
- BIOLIEF: 1st World Conference on Biological Invasions and ecosystem functioning; oral presentation (2009)
- NEOBIOTA: 6th European Conference on Biological Invasions; poster presentation (2010)
- SIP: 14th Symposium on Insect-Plant Interactions; poster presentation (2011)
- 3rd International Symposium on Environmental Weeds and Invasive Plants; oral presentation (2011)
- NEOBIOTA: 7th European Conference on Biological Invasions; poster presentation (2012)

Supervision of 3 MSc students

- Effects of root herbivory on an invasive plant via changes in plant quality
- Comparison of invertebrate communities associated with exotic and native related plants and plant resistance traits
- Plant and invertebrate community diversity associated to exotic and native plants

The research presented in this thesis was conducted at the Department of Terrestrial Ecology at the Netherlands Institute of Ecology (NIOO-KNAW) in Wageningen. This is NIOO thesis 110.

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