Doctoral Thesis

Studies of herbivory in native and invasive plant populations

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Studies of Herbivory
in Native and Invasive Plant Populations

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presented by

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Table of contents

Summary 1

Zusammenfassung 3

Chapter 1
  General introduction 7

Chapter 2
  Variation in herbivore damage on invasive and native woody plant species in open forest vegetation on Mahé, Seychelles
  DIETZ, H., WIRTH, L.R. & BUSCHMANN, H. 13

Chapter 3
  The effect of slug grazing on vegetation development and plant species diversity in experimental clover-ryegrass swards
  BUSCHMANN, H., KELLER, M., PORRET, N., DIETZ, H. & EDWARDS, P.J. 31

Chapter 4
  Responses of invasive and native Brassicaceae species to slug herbivory
  BUSCHMANN, H., EDWARDS, P.J. & DIETZ, H. 55

Chapter 5
  Variation in growth pattern and response to slug damage among native and invasive provenances of four perennial Brassicaceae species
  BUSCHMANN, H., EDWARDS, P.J. & DIETZ, H. 83

Chapter 6
  Overall conclusions 113

Acknowledgements 117

Curriculum vitae 119
Summary

1 Variation in herbivore damage and response patterns between native and invasive plant species may be important for plant invasiveness or habitat invasibility. In this thesis several hypotheses that suggest how invasive plants may profit from herbivory relative to native plants were investigated. Invasive plant species (i) may profit from lower herbivore damage than co-occurring native species (ERH hypothesis), particularly in less diverse vegetation that supports lower herbivore diversity, (ii) may evolve increased competitive ability under enemy release (EICA hypothesis), (iii) may be rather unpalatable to herbivores or better able to compensate herbivore damage than native species or (iv) may be superior in tolerating herbivory at particular life stages. These hypotheses were investigated in a more general approach, looking at patterns and consequences of herbivory in the vegetation and in a more specific approach, concentrating on the role of the important generalist slug herbivores in the invasiveness of Brassicaceae forbs.

2 In a survey study to evaluate the enemy release hypothesis (ERH) the median percentage of leaves affected by herbivores was significantly higher on native trees (50%) than on invasive trees (27%) in tropical open forest on Mahé, Seychelles. In addition, the species with the highest leaf area loss were all native to the Seychelles. These results support the enemy release hypothesis. While herbivore damage varied greatly between invasive species there was a significant increase in damage with increasing tree diversity in the forest, suggesting that higher community diversity entails increased herbivore load on invasive plants.

3 In experimental clover-ryegrass swards slugs reduced total aboveground biomass by more than 25%. Vegetation cover was significantly lower in the slug plots in the first year while there were only small differences between treatments in the third year. Slugs had a negative impact on plant species diversity in the first year, reducing particularly forb species. However, after three years plant species diversity was higher in the slug plots than in the control plots due to a higher number of forb species than in the control plots. In grazed plots annual and palatable species were reduced in terms of biomass and cover but not in species number. The results suggest that slug herbivory can reduce species diversity and the dominance of competitively superior species in herbaceous vegetation.
4 In a comparative study evaluating how slug herbivores affect invasive vs. native Brassicaceae forbs variation in leaf damage or plant responses were high among species but mostly low among the two groups. However, the invasive species, particularly those with clonal reproduction, showed higher compensatory growth after slug herbivory than the native species. Results were relatively consistent between field experiments using established plants and seedling assays. While the proportions of seedlings damaged or killed by slug herbivory did not differ between the two groups of plants, rootlings (young root fragment regenerates) that were only produced in the invasive species, had generally higher survival rates than the seedlings.

5 In a common garden experiment that compared native (NP) and invasive (IP) provenances of four Brassicaceae species all but one species (*Bunias orientalis*) showed significant differences in growth and reproductive characteristics between IP and NP plants. *Barbarea vulgaris* and *Rorippa austriaca* from the introduced range had a considerable higher growth rate than those from the native range. While IP plants of the non-clonal *B. vulgaris* allocated more resources to seed production than NP plants, IP plants of the clonal *R. austriaca* showed a decreased number of seeds compared to the NP. Contrary to the EICA hypothesis, there were no differences between NP and IP plants in the number of damaged leaves and leaf area consumed by slugs, nor in the proportion of seedlings damaged and killed.

6 The results of this thesis provide some support for the enemy release hypothesis but it obtained no clear evidence for the evolution of increased competitive ability due to herbivore release in invasive Brassicaceae. While some invasive plant species profit from enemy release that may increase their competitive ability compared to native, co-occurring species, no indication was found that slugs, in their function of generalist and main herbivores that can affect community structure and composition, consistently prefer native species or provenances over invasive ones or *vice versa*. However, the results suggest that higher compensatory growth in invasive Brassicaceae or (re-)colonization by regenerates that are less susceptible to herbivores than seedlings allow part of the invasive species to profit from herbivory relative to co-occurring and competing native plants.
Zusammenfassung

1 Der unterschiedliche Herbivorendruck bzw. die unterschiedliche Reaktion von nativen und invasiven Pflanzenarten auf Herbivorenfraß könnten eine wichtige Rolle für das Invasionspotenzial von Neophyten und für die Invasibilität von Habitaten spielen. In der vorliegenden Arbeit wurden mehrere Hypothesen untersucht, die Anregungen liefern, wie invasive Pflanzen von der Herbivorie profitieren könnten. Invasive Pflanzen könnten (i) von einem gerin- geren Herbivorendruck profitieren (ERH-Hypothese) und das besonders in weniger diverser Vegetation, die eine geringere Diversität an Herbivoren beinhaltet, (ii) ein erhöhtes Wachstumspotenzial und damit eine höhere Konkurrenzfähigkeit entwickeln, wenn sie sich im neuen Areal nicht mehr gegen ihre Feinde verteidigen müssen (EICA-Hypothese), (iii) für Herbivore weniger palatibel sein oder ein erhöhtes Kompensationswachstum bzw. eine flexi- bliere Ressourcenallocation nach Herbivorie aufweisen oder (iv) den nativen Arten während bestimmter Lebensstadien durch das Tolerieren von Herbivorenfraß überlegen sein. Diese Hypothesen wurden sowohl in einem breiten Ansatz betrachtet, der die Muster und Konse- quenzen von Herbivorie in der Vegetation ermittelt, als auch in spezifischeren Untersuchungen, die sich mit der Rolle beschäftigten, die Schnecken als wichtige Herbivoren für das Invasionspotenzial von perenn Brassicaceen-Arten spielen können.

2 In einer Untersuchung der ERH-Hypothese im offenen tropischen Wald der Insel Mahé (Seychellen), war der Median des prozentualen Anteils durch Herbivore angefressener Blätter signifikant höher bei nativen (50%) als bei invasiven Baumarten (27%). Zusätzlich waren alle Arten mit dem höchsten Blattflächenverlust nativ. Diese Ergebnisse stützen die ERH- Hypothese. Während das Ausmaß der Blattschäden zwischen den invasiven Baumarten sehr unterschiedlich war, gab es einen signifikanten Anstieg der Schäden mit ansteigender Baumdiversität im Wald. Dies lässt vermuten, dass eine höhere Pflanzendiversität einen stärkeren Herbivorendruck für invasive Arten zur Folge hat.

3 In experimentellen Klee-Raygrass-Wiesen verringerten Nacktschnecken die absolute ober-irdische Biomasse um mehr als 25%. Der Deckungsgrad der Vegetation war im ersten Jahr signifikant niedriger in Plots, die Schnecken beinhalteten, während im dritten Jahr nur geringe Unterschiede zwischen den Behandlungsweisen vorhanden waren. Schnecken hatten im ers- ten Jahr einen Einfluss auf die Pflanzenartendiversität, in dem sie die Anzahl Arten um Kräu-
Zusammenfassung

Im Gegensatz dazu war die Diversität im dritten Jahr durch eine größere Anzahl Kräuter in den “Schneckenplots” höher als in den “Kontrollplots”. Annuelle and palatable Arten wurden durch die Schnecken in ihrer Biomasse und ihrem Deckungsgrad, aber nicht in ihrer Anzahl verringert. Diese Ergebnisse legen nahe, dass Schnecken sowohl die Artendiversität insgesamt als auch die Dominanz von konkurrenzkräftigen Arten verringern können.


6 Die Ergebnisse der vorliegenden Arbeit stützen zwar die ERH-Hypothese, aber es gab keine eindeutige Bestätigung der EICA-Hypothese in Bezug auf einen verringerten Herbivorenfraß bei verschiedenen Brassicaceen-Arten. Einige invasive Pflanzenarten profitieren anscheinend davon, dass sie im Vergleich zu nativen, im gleichen Habitat vorkommenden Arten einen geringeren Herbivorendruck haben, der vermutlich ihre Konkurrenzfähigkeit erhöht. Dennoch gab es keine Hinweise darauf, dass Schnecken als Generalisten und Hauptherbivore, die...
sowohl die Struktur als auch die Zusammensetzung von Pflanzengesellschaften verändern können, generell native Arten oder Populationen gegenüber nativen bevorzugen. Die Ergebnisse zeigten aber, dass ein stärkeres Kompensationswachstum und das Regenerieren aus Pflanzenteilen einem Teil der invasiven Brassicaceen-Arten erlaubt, im Vergleich zu konkurrierenden, in den gleichen Habitaten auftretenden nativen Arten von der Herbivorie zu profitieren.
Chapter 1

General introduction

Biological invasions are very complex phenomena that defy simple causes and explanations in most cases (Radford & Cousens 2000; Dietz & Steinlein 2003). This may explain why general factors underlying species invasiveness (i.e. species-related traits that contribute to their successful spreading in the introduced area) are still largely unknown despite the strongly increasing number of studies on biological invasions in recent time (Dietz & Steinlein 2003, and references therein).

Herbivory may play an important role in plant invasions but has not been thoroughly studied so far in this respect (see e.g. Daehler 2003). There are several plausible hypotheses that suggest how variation in herbivory between co-occurring native and introduced plants may affect the invasiveness of the latter. For example, it has been hypothesised that enemy release of plant species introduced to a new area will give them a competitive advantage over native species (ERH hypothesis, e.g. Keane & Crawley 2002; Wolfe 2002). In particular, it has been suggested that release from natural enemies and pathogens results in higher plant vigour due to greater allocation of resources to growth and reproduction than to defence mechanisms (e.g. Fowler et al. 1996). Plants with this ability may have an advantage in the absence of herbivores because of the evolution of increased competitive ability (EICA hypothesis, Blossey & Nötzold 1995). Herbivory could also benefit invasive plants compared to native plants if the invasives are better able to cope with herbivore damage, e.g. due to higher compensatory growth (Schierenbeck et al. 1994). In addition, the herbivore load on a given invasive plant species may be influenced by characteristics of the invaded plant community (Prieur-Richard et al. 2002). It appears that a richer plant community offers more herbivore niches and may thus support a higher diversity of herbivores on introduced plants.

Several recent studies in invasion ecology have focussed on these hypotheses by comparing native and invasive congeners or native and invasive provenances of the same species in terms of growth, fecundity and herbivory (e.g. Agrawal & Kotanen 2003; Daehler 2003, and references therein; Bossdorf et al. 2004b). But there is still no sufficient body of studies that tested ERH, EICA and the other assumptions in comparative assays and common garden
experiments using related native and invasive species or native and invasive plant provenances of the same species (Sakai et al. 2001; Buschmann et al. 2002). Of many different ecosystems and herbivore groups only very few have been investigated to date. In addition, differences in herbivore effects between native and invasive plants may vary with life stage of the plants, and may depend on particular habitat conditions. For example, the patterns and consequences of herbivory observed in successional vegetation with established plants may not be the same that are present in seedling communities after disturbance (cf. Hanley et al. 1995; Fenner et al. 1999). So, the robustness of the results reported in previous studies that mostly did not test herbivore effects under a range of environmental conditions, is rather unclear. The results reported so far are also not unequivocal (e.g. Mitchell & Power 2003, and references therein; Siemann & Rogers 2003a, b; van Kleunen & Schmid 2003; Bossdorf et al. 2004a) and, hence, we are still far from reaching general conclusions on the role of herbivory in plant invasions.

THESES OUTLINE
The main motivation of this thesis was to contribute to the knowledge of the role that herbivores can play in plant invasions. As there is often confusion about denoting a species as invasive (Daehler 2001) I use this term for alien species or provenances that are introduced in a new area where they are in spread (see Richardson et al. 2000). I first investigate whether herbivore attack to invasive plants is lower than to native plants in open forest vegetation in the tropical Seychelles and whether herbivore damage to invasive plants increases with diversity in these forests. Then I analyze the more general effects of generalist (slug) herbivory on plant species diversity and community development in secondary succession in herbaceous vegetation. This part provides the context for the following parts of the thesis. In these I test whether there is evidence for ERH and EICA, in invasive compared to native provenances and species of Brassicaceae forbs. I also test whether the invasive Brassicaceae are better able to compensate herbivory than the native species and whether they have advantages with respect to herbivory at particular stages.
First feature

In chapter 2 Variation in herbivore damage on invasive and native woody plant species in open forest vegetation on Mahé, Seychelles I present comparative investigations on leaf herbivore damage to invasive and native woody plants in open forests on Mahé, the main island of the tropical Seychelles. In this project we took advantage of pronounced environmental gradients on Mahé. We studied the variation in leaf herbivory on three abundant invasive woody plant species in response to differences in site conditions, particularly in response to woody plant diversity. The main objective in this project was to evaluate the degree of enemy release in a larger set of invasive plant species compared to a similar set of co-occurring native plant species and to assess how variations in environmental conditions influence patterns of enemy release.

Second feature

In chapter 3 The effect of slug grazing on vegetation development and plant species diversity in experimental clover-ryegrass swards I present a more general study on the impact of slug grazing upon vegetation development in early secondary succession of arable land. In this study we intitiated a clover-ryegrass sward on soils with a large seed bank of mainly ruderal species and subjected the developing sward to slug herbivory. The main objective was to evaluate the influence of slug herbivory on plant species diversity in arable vegetation. We were particularly interested in possible changes in the response of the sward to persistent slug herbivory over the course of succession, in comparison to ungrazed swards.

Third feature

In chapter 4 Responses of perennial Brassicaceae to slug herbivory at the seedling/rootling and adult stage: a comparison of native and invasive species I narrow the focus on the role of slug herbivory in the invasiveness of native and invasive perennial Brassicaceae species. In different experimental setups the responses to herbivory were compared between three invasive and three native species that are similar in life form. The main objective was to test whether the invasive species suffer less from herbivory, show higher compensatory growth following herbivory or benefit at particular life stages from herbivory relative to native conspecifics.
Introduction

Fourth feature

In chapter 5 Variation in growth pattern and response to slug damage among native and invasive provenances of four perennial Brassicaceae species I complement the results presented in chapter 4 by experimentally comparing the impact of slug herbivory on native and invasive provenances of four Brassicaceae species. The main objective of this study was to test whether invasive provenances of Brassicaceae forbs have more vigorous growth and are more attractive to slugs than native provenances, as postulated by the EICA hypothesis.

References


Chapter 2

Variation in herbivore damage to invasive and native woody plant species in open forest vegetation on Mahé, Seychelles

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**Summary**

1. Enemy release of introduced plants and variation in herbivore pressure in relation to community diversity are presently discussed as factors that affect plant species invasiveness or habitat invasibility. So far few data are available on this topic and the results are inconclusive.

2. We compared leaf herbivory between native and invasive woody plants on Mahé, the main island of the tropical Seychelles. We further investigated variation in leaf herbivory on three abundant invasive species along an altitudinal gradient (50–550 m a.s.l.). The median percentage of leaves affected by herbivores was significantly higher on native species (50%) than on invasive species (27%). In addition, the species suffering from the highest leaf area loss were native to the Seychelles.

3. These results are consistent with the enemy release hypothesis (ERH). While the invasive species showed significant and mostly consistent variation in the amount of leaf damage between sites, this variation was not related to general altitudinal trends in diversity but rather to local variation in habitat structure and diversity.

4. Our results indicate that in the Seychelles invasive woody plants profit from herbivore release relative to the native species and that the amount of herbivory and, therefore, its effect on species invasiveness or habitat invasibility may be dependent on local community structure and composition.

**Introduction**

Introduced plants often lack many or all of their native herbivores and have lower herbivore loads than native plants in the area of introduction (e.g. DeBach & Rosen 1991; Keane & Crawley 2002). This enemy release can have several important consequences for the invasion potential of the plant species. For example, reduced herbivore pressure may result in a plastic or evolutionary shift from a defence-oriented to a more competitive growth strategy (e.g. Blossey & Nötzold 1995; Fowler et al. 1996). Introduced plants may profit in terms of overall increased population growth as a result of decreased enemy attack in the new area (Keane & Crawley 2002; Wolfe 2002; Torchin et al. 2003); or may be able to shift their environmental tolerance (e.g. shade tolerance, see DeWalt 2003). This ‘enemy release hypothesis’ (ERH) assumes that enemies that are specialists for the native plants do not normally switch to potential invasive hosts and that generalist enemies have a greater impact on the native competitors (Keane & Crawley 2002), resulting in competitive release for the invaders.
Herbivory on invasive and native woody plant species

The ERH is supported by several studies. For example, *Clidemia hirta* (Melastomataceae) suffered less mortality in forest understory due to insects and pathogens in its invasive range in Hawaii than in its native range in Costa Rica (DeWalt *et al.* 2004). In a comparative study of 473 plants naturalized in the United States, Mitchell & Power (2003) showed that naturalized species have fewer pathogen species in their introduced than native range. In addition, naturalized species with fewer pathogen species were found to be more widely listed as problematic invaders of agricultural and natural ecosystems. Further indicative for the importance of enemy release is the often rapid reduction in population size of an invasive plant once a specialist enemy from the native range of the plant is introduced (Fowler *et al.* 1996; Mack *et al.* 2000; Keane & Crawley 2002). Nevertheless, evidence for ERH is not unequivocal (Mitchell and Power 2003 and references therein; see also Siemann & Rogers 2003). For example, in a recent experimental study comparing 30 old-field plant species, Agrawal & Kotanen (2003) found equal or higher levels of herbivory on introduced species compared to their native congeners.

The amount of herbivory or the diversity of herbivores on a given invasive plant species may be influenced by characteristics of the invaded plant community. For example, in a study on the two annual invasives *Conyza bonariensis* and *C. canadensis* in Mediterranean communities of annual plants, herbivory of the exotics increased with increasing plant species richness and was also dependent on the presence of specific functional plant groups (Prieur-Richard *et al.* 2002). It appears that a richer plant community offers more herbivore niches and may thus support a higher diversity of herbivores on exotic plants.

Overall, our knowledge is still insufficient for a sound evaluation of the role of enemy release in plant invasions. In particular, data that test the enemy release hypothesis by comparing the amount of herbivory between invasive and co-occurring native plants are still largely lacking (Agrawal & Kotanen 2003). Likewise, it has scarcely been tested so far whether and how herbivore pressure on invasive plants varies along environmental gradients or, more specifically, in relation to variation in community diversity.

We studied these questions by comparing leaf herbivore damage on invasive and native woody plants on Mahé, the main mountainous island (142 km²) of the Seychelles archipelago in the Indian Ocean. Like most small islands, the vegetation has been drastically altered by human activities. Almost all lowland forest was felled by the early 19th century (Kueffer *et al.* 2003). Today, the afforested or regenerating intermediate forests represent a mixture of native and invasive tree and shrub species. The number of introduced plant species now far exceeds the number of native species, and some of these are invading (semi)-natural habitats, including highly invasive trees and shrubs such as *Alstonia macrophylla, Chrysobalanus icaco, Cinnamomum verum, Paraserianthes falcatoria, Psidium cattleianum, Syzygium jambos* and *Tabebuia pallida*. The prominent and well-recognized problem of woody plant invasions into the Seychelles forests
(Gerlach 1984, 1996; Fleischmann 1997; Kueffer et al. 2003) and the pronounced environmental gradients that occur in the rough mountainous terrain of Mahé provide a system well-suited for a comparative assay of herbivore damage on native and invasive plants under varying environmental conditions.

Our study had two objectives. The first was to test whether leaf herbivore damage is reduced on invasive woody plants compared to the most abundant co-occurring natives, as predicted by the ERH. To this end we selected ten of the most frequently encountered species each of invasive and native woody plants of open forest areas and compared the levels of leaf damage between both groups at different sites.

The second objective of the study was to evaluate variation in leaf herbivory on three abundant invasive woody plants in response to differences in site conditions. Although there are no strong climatic differences between 0 and 500 m a.s.l. on Mahé (cf. Walsh 1984), it has been hypothesized that general community diversity increases with altitude and reaches a peak in the intermediate forest zone between 200 and 500 m a.s.l. before it declines again towards the mountain mist zone above 700 m a.s.l. (Carlstroem 1996). Hence, to maximise variation among sites, open forest areas were chosen along an altitudinal gradient (50–550 m a.s.l.). We asked to what degree herbivore damage varied in response to forest diversity and density and whether this response was similar among the invasive species.

**Material and methods**

**Species**

We chose ten native and ten invasive (introduced) species that we encountered most frequently in open forest areas on Mahé (see Table 1). The three most frequently encountered invasive species, *C. icaco*, *C. verum* and *P. cattleianum* were chosen for the investigation of variation in leaf herbivory related to environmental gradients.

Within groups, with two exceptions, all species belong to different families, thereby preventing bias due to close phylogenetic relatedness (cf. Silvertown & Dodd 1997). For the two within-group confamiliar species pairs (*P. cattleianum* and *S. jambos* in the invasive group and *Canthium bibracteatum* and *Paragenipa wrightii* in the native group), the difference in the mean percentage of damaged leaves was slightly or even considerably higher than was the average for all the other possible species pairs within the respective groups (excluding the confamiliar species). We therefore maintained the confamiliar species as independent data units for the analysis.
Herbivory on invasive and native woody plant species

Sites
Open forest sites were chosen for study because they were common and accessible and offered higher woody plant species diversity than forest understory sites. These included mainly glacis sites with naturally open vegetation in areas of granite rock outcrops (see Fleischmann et al. 1996; Biedinger & Fleischmann 2000), and sites with open forest due to management and forest edges along mountain roads and carriage ways. In these sites trees and shrubs grew mostly under unshaded or partly shaded conditions. Tree and shrub density was very variable among sites as was canopy height ranging from 3 to 10 m. For the comparative investigation of leaf herbivory each of the 20 species was sampled at three different sites (Aphloia theiformis and Ochna ciliata were only sampled at two sites). As only a fraction of the species co-occurred at a given site a total of 18 sites was needed for the whole sample (see Figure 1a for the distribution of sampling sites). Suitable sites were chosen arbitrarily but care was taken to include only sites of the intermediate forest that extends from c. 200 to 550 m a.s.l.. This most dominant forest zone is intermediate in altitude between the coastal and lowland forests below 200 m and the mountain forests at high altitudes. The intermediate forests harbour the highest number of invasive woody species (Kueffer et al. 2003). We did not include coastal, lowland or mountain forest because of the different conditions and species composition found there (cf. Kueffer et al. 2003). We also avoided bias in the choice of sampling sites with respect to the presence of native and invasive species and, if possible, avoided sampling individuals of the same species too closely.

For the investigation of variation in leaf damage within the three invasive species the same types of sites were chosen but in this case sites were selected so as to represent an altitudinal gradient from 50 to 550 m a.s.l. (n = 10 sites; see Figure 1b). At each site all three species were present except for the highest altitude site (550 m a.s.l.), where C. icaco was missing.

Data collection
For comparative investigation of leaf herbivory in the 20 species five randomly chosen individuals per species and site were sampled. For each individual a random outer branch was selected and for the first 100 leaves of the branch we assessed whether the leaf experienced herbivore damage and, if so, how severe herbivory was. We assigned each individual to one of four herbivore damage categories (based on the average leaf damage of the 100 leaves in steps of 25% loss of leaf area. In most cases it was possible to sample branches at comparable heights (1 to 2.5 m above ground). If the selected branches had fewer than 100 leaves, leaves of a neighbouring branch were sampled until the sample size reached 100. Young leaves, i.e. leaves that were not fully expanded or whose pigmentation was not yet fully developed, were excluded. Nine mature leaves (three each from three individuals) of each species were collected, the area of the
leaves was determined (see below) and the leaves were dried to constant mass for determination of specific leaf area (SLA, see Table 1).

<table>
<thead>
<tr>
<th>Species</th>
<th>Family</th>
<th>Life-form</th>
<th>Status</th>
<th>Abundance</th>
<th>Site No</th>
<th>SLA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alstonia macrophylla</td>
<td>Apocynaceae</td>
<td>tree</td>
<td>invasive</td>
<td>common</td>
<td>1; 3; 5</td>
<td>15.5</td>
</tr>
<tr>
<td>Aphloia theiformis</td>
<td>Flacouriaceae</td>
<td>shrub</td>
<td>native</td>
<td>common</td>
<td>4; 13</td>
<td>19.2</td>
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<tr>
<td>Calophyllum inophyllum</td>
<td>Guttiferae</td>
<td>tree</td>
<td>native</td>
<td>common</td>
<td>5; 9; 11</td>
<td>6.7</td>
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<tr>
<td>Canthium bibracteatum</td>
<td>Rubiaceae</td>
<td>shrub</td>
<td>native</td>
<td>common</td>
<td>1; 3; 7</td>
<td>8.0</td>
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<tr>
<td>Chrysobalanus icaco</td>
<td>Chrysobalanaceae</td>
<td>shrub</td>
<td>invasive</td>
<td>very common</td>
<td>1; 5; 10</td>
<td>8.4</td>
</tr>
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<td>Cinnamomum verum</td>
<td>Lauraceae</td>
<td>tree</td>
<td>invasive</td>
<td>very common</td>
<td>1; 3; 5</td>
<td>6.1</td>
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<td>Dillenia ferruginea</td>
<td>Dilleniaceae</td>
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<td>native</td>
<td>common</td>
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<td>scattered</td>
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<td>locally common</td>
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<td>less common</td>
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<td>common</td>
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<td>Sapotaceae</td>
<td>tree</td>
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<td>common</td>
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</tr>
<tr>
<td>Paragenipa wrightii</td>
<td>Rubiaceae</td>
<td>small tree</td>
<td>native</td>
<td>common</td>
<td>4; 5; 10</td>
<td>4.7</td>
</tr>
<tr>
<td>Premna serratifolia</td>
<td>Lamiaceae</td>
<td>shrub</td>
<td>native</td>
<td>less common</td>
<td>3; 10; 12</td>
<td>10.7</td>
</tr>
<tr>
<td>Psidium cattleianum</td>
<td>Myrtaceae</td>
<td>small tree</td>
<td>invasive</td>
<td>common</td>
<td>1; 3; 5</td>
<td>10.7</td>
</tr>
<tr>
<td>Soulamea terminaloides</td>
<td>Simaroubaceae</td>
<td>small tree</td>
<td>native</td>
<td>less common</td>
<td>5; 12; 14</td>
<td>5.6</td>
</tr>
<tr>
<td>Swietenia macrophylla</td>
<td>Meliaceae</td>
<td>tree</td>
<td>introduced (non-invasive)</td>
<td>less common</td>
<td>2; 5; 8</td>
<td>18.1</td>
</tr>
<tr>
<td>Syzygium jambos</td>
<td>Myrtaceae</td>
<td>tree</td>
<td>invasive</td>
<td>common</td>
<td>2; 11; 13</td>
<td>11.8</td>
</tr>
<tr>
<td>Tabebuia pallida</td>
<td>Bignoniaceae</td>
<td>tree</td>
<td>invasive</td>
<td>common</td>
<td>4; 7; 9</td>
<td>16.5</td>
</tr>
</tbody>
</table>

For the investigation of variation in leaf damage within the three invasive species three individuals each of C. icaco, C. verum and P. cattleianum were sampled at each site. Individuals and branches were selected and leaves sampled as described above. Fifty leaves per individual were sampled. Of these the damaged leaves were collected in plastic bags for quantitative analysis of leaf loss. For each of the sites canopy cover was estimated to the nearest 10% and woody plant diversity was categorized as low, low to inter-mediate, intermediate or intermediate to high, respectively.
DATA ANALYSIS

The area of the collected leaves was determined using image analysis. For that the leaves were pressed flat beneath a glass plate and were photographed vertically from above using a digital camera (Minolta Diimage 7i). The images were transferred to a computer and were processed and leaf area was analysed in Adobe Photoshop™ 7.0. Leaf area loss was calculated by subtracting the green leaf area from total leaf area (methodological details cf. Dietz & Steinlein 1996).

![Figure 1](image.png)

Figure 1. Distribution of sampling sites on Mahé, the main island of the Seychelles. In (a) the symbols denote the location of sites used for comparing leaf damage between native and invasive woody plants. In (b) the sites used for the evaluation of variation in leaf damage within and between three invasive species are shown (the numbers in the boxes indicate altitude in m a.s.l.).

The mean percentage of damaged leaves was calculated for each site and species. For each species these values were used to calculate the coefficient of variation corrected for small sample size ($CV_{corr}$, Sokal & Rohlf 1981). $CV_{corr}$ was used to test whether within-species variation in herbivore damage is significantly different between the invasive and the native species (Mann-Whitney $U$-test). In addition, using the species means, it was tested whether the percentage of damaged leaves is significantly different between both groups ($U$-test).

For analysis of the variation in leaf damage within and among the three invasive plants along the altitudinal gradient the median percent leaf area loss of the whole leaf sample per site and species was calculated. Using these data, for each species separately, it was tested whether between-site variation in leaf damage contributed significantly to overall variation in leaf damage (ANOVA using STATISTICA 6.0, Statsoft Inc. 2002). It was further tested whether variation in
leaf damage among sites could be attributed to differences among species. Across all sites, for each species, the median percent leaf area loss values were standardized to make values comparable between species. For each site the standardized values of the three species were summed and the absolute values of these sums were added to produce a measure of the degree of covariation among species (total sum). A permutation test was used to test whether the degree of covariation was significantly higher than expected under the null model (no relationship among species in the variation of leaf damage). For that, within species, the standardized leaf loss values were randomly assigned to the different sites and the total sums were calculated as described above. This procedure was repeated to yield a distribution of 999 total sums (null distribution). Significant co-variation between species was tested by comparison of the observed score (total sum) with the null distribution (Manly 1999).

Results

Differences in leaf damage between native and invasive species

For the native species the percentage of leaves affected by herbivores was significantly higher than for the invasive species ($U = 20; P < 0.05$). The native species had a median of 50% damaged leaves whereas in the invasive species only 27% of the leaves were affected (Fig. 2a). Furthermore, among-species variation in leaf damage was considerably higher in the native species (Fig. 2a). Among native species, the amount of damaged leaves varied between 5% and 83% whereas in the invasive species this range was only 7% to 42% with one outlier species (C. icaco) at 70%. There was also high within-species variation in herbivore damage, but this was not significantly different between the invasive and the native species ($U = 38; P = 0.83$; Fig. 2b).

Mean leaf area loss due to herbivory was less than 25% (damage category 1) in most cases and for most species (data not shown). Only in a few species were there exceptional individuals with leaf damage between 25% and 50% (category 2), and only Premna serratifolia and C. bibracteatum were set apart by increased leaf loss in a third or half of the individuals, respectively (with leaf area losses 25% to 50% or even 50% to 75%). As both species are natives this supports the finding that the natives suffer more from herbivory than the invasives.

The median specific leaf area (SLA) of the invasive plants was twice as high (12.5 cm$^2$ g$^{-1}$ dry mass) as that of the native species (6.7 cm$^2$ g$^{-1}$ dry mass; $U = 23; P < 0.05$; Table 1). However, overall there was only a non-significant trend of decreasing leaf damage with increasing SLA ($P = 0.12$), i.e. differences in SLA explained only 12% of the variance in leaf damage (linear regression analysis).
Figure 2. Box plots of the variation in (a) percentage of damaged leaves between species within groups (native and invasive species) and (b) within species within groups (native and invasive species).
Chapter 2

VARIATION IN LEAF DAMAGE WITHIN AND AMONG INVASIVE SPECIES

In the three invasive species (C. icaco, C. verum and P. cattleianum) that were investigated along an altitudinal gradient the median percentage of leaf area consumed ranged between 0% and 4% in C. verum and P. cattleianum and between 2% and 14% in C. icaco (Fig. 3). The amount of leaf damage differed significantly among sites for all three species (d.f. = 8 or 9; F ≥ 2.8; P < 0.03), but there was no trend in the variation related to altitude (P ≥ 0.2, regression analysis; see Fig. 3). However, there was a significant covariation among species (P = 0.036, one-tailed test); all species showed relatively high leaf damage at the 110 and 380 m a.s.l. sites, whereas herbivore damage was comparatively low at 170, 220 and 300 m a.s.l. (Fig. 3). The among-site variation in leaf damage, which was moderately consistent among species, was correlated with a vegetation index calculated by multiplying a diversity rank number (1 = low to 4 = intermediate – high) with estimated canopy cover (30 to 80%) of the site ($R_s = 0.70; P < 0.05$; Spearman rank correlation). On average, at sites with a denser canopy cover and/or higher plant species diversity the invasive species suffered from higher herbivore damage.

![Figure 3](image_url)

*Figure 3.* Variation in leaf damage in three abundant invasive woody species among sites representing an altitudinal gradient and different habitat conditions. Squares/solid line, *Chrysobalanus icaco*; diamonds/long dashed line, *Cinnamomum verum*; circles/short dashed line, *Psidium cattleianum*.

In the three invasive species sampled along the altitudinal gradient there was only a weak, non-significant trend for a positive correlation between the percentage of leaves damaged and the mean area consumed per damaged leaf ($P ≥ 0.11$; data not shown).
Discussion

There are only few, rather limited studies on the invertebrate fauna of the Seychelles (Matyot 1998). It appears that the insect fauna is still mainly composed of native or endemic species many of which rely on the endemic plants as food source (Scott 1933; Matyot 1998). Although there are almost no data available on the relative importance of different invertebrate groups for leaf herbivory in the Seychelles, from the damage patterns and animal sightings during the study Coleoptera, Lepidoptera, Orthoptera and Phasmatodea appear to be the main herbivores (see also Matyot 1998). Folivorous insects are diverse taxonomically and physically, and they are the most important consumers in tropical forests (Coley & Barone 1996). In the Seychelles insect herbivores may be even more dominant because of the low number of vertebrate herbivore species present. Even large tortoises that were or partly still are important herbivores on some islands in the Indian Ocean (Hansen et al. 2003) are today not present in the wild on Mahé.

Although there were considerable differences in leaf damage between the native and invasive plants, leaf loss was about 10% or lower in most species, which is relatively low compared to mean figures for wet tropical forests (cf. Coley & Barone 1996). This is probably a result of the small size and remoteness of the island (cf. Arnold & Asquith 2002, and references therein). Therefore, it could be questioned whether differences at this rather low herbivory level could favour the invasive plants relative to the natives. However, an annual leaf loss of 10% is reported to be sufficient to reduce plant fitness (Dirzo 1984) and growth (Poorter 2001). For example, 10% experimental defoliation of Piper arietanum, an understory shrub, reduced growth, seed production and seed viability (Marquis 1984, 1992). In addition, herbivore damage may be higher on shade leaves and in the understory than in more open sites such as those studied here (see Lowman 1985), so that our results on the degree of herbivory on Mahé are probably rather conservative.

While data from the Aldabra Atoll (Seychelles) suggest that insect abundance is higher in wet periods (Frith 1979), there are no distinct rainy or dry seasons in the Seychelles (see Walsh 1984). Seasonal fluctuations in herbivory that have been reported for other tropical regions (see Coley & Barone 1996; Arnold & Asquith 2002) are therefore probably less pronounced on Mahé. There was neither a particularly wet nor dry period preceding our field study so that our data on herbivore damage probably do not deviate much from average values.

The results of our study are not conclusive with respect to the role of SLA/leaf longevity in determining the amount of herbivory. As expected, SLA was much greater for the invasive plants, which indicates that this group consists mainly of rapidly growing species with comparatively high leaf turnover (cf. Reich et al. 1997; Baruch & Goldstein 1999; Yamashita et al. 2000) whereas the native plants appear to develop thicker and longer-lasting leaves. It
Chapter 2

therefore seems, at first, as if the higher damage of the leaves of the native species results from the longer time the leaves were on display for potential herbivores. This may present a methodological problem because from single measurements herbivore damage rates cannot be easily compared between species that differ considerably in leaf longevity (Newbery & de Foresta 1985). On the other hand, across all species, there was no clear relationship between SLA and the degree of leaf damage. We also had no indication that older leaves were more damaged than younger leaves (except for leaves that have just unfolded – but these were not sampled). Actually, for tropical shade-tolerant species that have a leaf life span of two to four years, 68% of the lifetime damage was found to occur during the short period of time of leaf expansion (Lowman 1992). Even if, due to higher leaf turnover, the rate of consumed leaf area by herbivory were higher in the invasive species than suggested by our data, this would not invalidate our results that suggest lower herbivory on the invasive species. This is because (i) biomass removal per consumed leaf area was twice as high in the natives, resulting from their low SLA and (ii) independent of the rate of leaf area consumed, the differences remain between species groups in the proportion of photosynthetically active area removed at any one point in time.

**DIFFERENCES IN LEAF DAMAGE BETWEEN NATIVE AND INVASIVE SPECIES**

Although in both groups leaf damage varied greatly among species and, in part, within species between sites, less than 50% of the leaves on invasive species were damaged (except for C. icaco, see below). In contrast, well over 50% of the leaves were damaged on several native species. This result is opposite to what would be expected from leaf characteristics alone. As leaf life-span tends to be longer in species with low SLA (e.g. Reich et al. 1997; Williams-Linera 2000; Wright et al. 2002), the proposed longer leaf life span of the natives may be related to higher concentrations of secondary defensive compounds against herbivory (Coley 1988). This and the greater toughness of the leaves should reduce the attractiveness of the natives’ leaves for the herbivores (Coley 1983; Cunningham et al. 1999). The invasive plants belong mainly to the rapidly growing gap species (see e.g. Fine 2002). In tropical forests these species are viewed to be poorly defended and should therefore suffer higher rates of herbivory (Coley 1983). Hence, for woody plants in open forest situations on Mahé, Seychelles, our results support the assumption of the enemy release hypothesis that the invasive plants are released from their specialist herbivores or that generalist herbivores have a greater impact on the native plants. It is difficult to discriminate between these two effects because little is known about the relative proportion of damage caused by specialist and generalist herbivores in tropical forests (Coley & Barone 1996). Our results are consistent with earlier observations that found native invertebrate diversity and abundance to be pronouncedly higher on native than exotic trees in coastal areas of the Seychelles (Currie 2002).
Herbivory on invasive and native woody plant species

Our results differ from those obtained for Canadian old-field forbs where folivorous insects produced higher damage in the exotic species (Agrawal & Kotanen 2003). Although insects were the main herbivores in both cases, the studies differ in the habitat, plant life forms and the climatic zone studied. This suggests that the extent of enemy release may depend on the system studied and the ERH may not explain all successful plant invasions. For example, in tropical forests, predominant host-specificity of folivorous insects (cf. Barone 1998) may result in a lower degree of herbivory on the invasive species. A further example of habitat-dependent enemy release is given in DeWalt et al. (2004).

**Variation in leaf damage among invasive species**

If generally higher diversity also supports more diverse herbivore communities, we would expect an increase in herbivore damage of the invasive species with increasing altitude, up to c. 500 m because forest diversity is hypothesized to increase over this altitudinal gradient on Mahé (Carlstroem 1996). However, our results instead indicated strong site-specificity of herbivore damage. It appears that the local diversity and density of the vegetation was the most important determinant of herbivore damage, compared to general altitudinal trends or interactions between site conditions and species specificity. Therefore, our results suggest that the role herbivory plays for plant species invasions may be modified in relation to the invaded community, i.e. herbivore damage or release from herbivory appears to be an attribute not only of species-specificity but also habitat-specificity.

Furthermore, the fairly consistent variation in herbivore damage on the three invasive species among sites indicates non-specific fluctuations in the presence of (generalist) herbivores rather than specific interactions of particular herbivore species with specific invaders, depending on the habitat. Thus, our results agree with the suggestion that richer plant communities support a higher herbivore diversity resulting in higher herbivory levels on invasive plants (Prieur-Richard 2002).

For native tropical trees it has been reported that the most abundant species had the highest herbivore damage (Lowman 1985; Gross 2000), which has been discussed as a possible high resource concentration effect. This pattern does not seem to hold for the main invasive species on Mahé; although *C. icaco* is less abundant than *C. verum* it shows far higher herbivore damage (cf. Fig. 3). However, where it is present, *C. icaco* usually forms very dense stands, whereas the two invasives *C. verum* and *Psidium cattleianum* are less patchily distributed. Hence, if resource concentration effects at the species level contribute to the amount of herbivory on invasives it may act on a patch scale rather than in relation to total species abundance.
Our study provides evidence that there is lower herbivore pressure on invasive woody species than on native ones in open forest vegetation on the tropical island Mahé, Seychelles. Furthermore, local habitat structure and composition seem to have considerable influence on the amount of herbivory found on invasive trees. Whether these patterns in herbivory vary with forest structure and composition and how strong they contribute to the invasiveness of the species and the invasibility of the habitats awaits further study. One promising approach for further investigations would be to compare other islands in the Seychelles of different sizes to examine differences in herbivore pressure and correlate invasive species abundance with herbivore damage.

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References


Herbivory on invasive and native woody plant species


Chapter 3

The effect of slug grazing on vegetation development and plant species diversity in experimental clover-ryegrass swards

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Summary

1 Generalist herbivores such as slugs have the potential not only to reduce plant density and biomass but also to alter species diversity within vegetation. Their impact on species diversity may be either negative, which would occur if they concentrate feeding on the most palatable plant species, or positive, if they feed on the most abundant species, thereby reducing their vigour. In this study we investigate the influence of slugs on plant species diversity during early stages of secondary succession on arable land.

2 In a common garden experiment, clover-ryegrass swards were subjected to slug herbivory by Arion lusitanicus. Using the natural local seed bank complemented by sowing Lolium perenne and Trifolium repens the number of species, plant cover and aboveground dry mass of yield were measured over three years to calculate diversity indices.

3 Slugs had a highly negative effect on total aboveground biomass, which was reduced by more than 25%. Vegetation cover increased in the control plots from 50% in the first year to 90% in the third year. Cover was significantly lower in the slug plots in the first year (more than 22%) while there were only small differences between treatments in the third year.

4 Slugs had a negative impact on plant species diversity in the first year, reducing particularly forb species, whereas after three years plant species diversity was higher in the slug plots than in the control plots due to higher number of forb species. Annual and palatable species were reduced in biomass and cover but not in species number under slug grazing.

5 Our results suggest that slugs can have a significant effect upon plant species diversity in plant communities, but that their impact changes during the course of succession. In early secondary succession, when seedlings or juveniles of all species are present, this effect leads to reduced species diversity because favoured species are eliminated. In closed vegetation, in which competitive interactions are important, slugs can reduce the dominance of the more competitive species and thus provide gaps in which plants can establish from seed. As a consequence, slugs cause an increase in plant species diversity and may also reduce the rate of successional change by promoting the persistence of annual species.
Effect of slugs on vegetation development and species diversity

Introduction

By feeding on plant material, herbivores may not only reduce the biomass and productivity of vegetation, but also alter plant species composition. The processes by which they do so are complex, and many factors may play a role (e.g. Rees & Brown 1992; Crawley 1989, 1997). If the main herbivores select only one or a few plant species, then the abundance of these species is likely to be reduced and they may even be eliminated from the vegetation. However, this does not necessarily lead to a loss of plant species diversity; if the selected plants are highly competitive species, herbivory could promote species diversity by allowing other, less vigorous species to persist in the vegetation (e.g. Brown & Gange 1989; Bach 1994; Olff & Ritchie 1998, and references therein). The plant life stage selected by the herbivore is another important factor. Grazing damage to a seedling is often fatal, and selective feeding on the seedlings of certain species may therefore have a major effect upon the species composition of the vegetation (Edwards & Gillman 1987; Hanley 1998). In contrast, an established plant is more likely to survive grazing, though its vigour and fitness may be reduced (e.g. Ehrlén 1995). A third factor is the tolerance of particular plant species to defoliation; even unselective herbivory may have selective effects upon the vegetation composition because some species are better able to recover from grazing damage (Fraser & Grime 1999; Chapter 4). Finally, an important effect of many herbivores is to create small-scale heterogeneity in the vegetation. This could arise through the formation of gaps as a result, for example, of trampling or removal of plant material, or through the creation of nutrient rich patches associated with excreta (e.g. Grubb 1977; Huntly & Reichman 1994; Lavorel et al. 1994). The relative importance of these factors varies according to the types of herbivore present in an ecosystem and their abundance (Edwards and Gillman 1987).

Vertebrate herbivores such as cattle, sheep and rabbits have long been the subject of ecological studies (e.g. Jones 1933; Watt 1981; Crawley 1990) and their effects on vegetation, through selective grazing as well as through creating habitat heterogeneity at various spatial scales, are well known (see Davidson 1993; Crawley 1997, and references therein). The possible impact of invertebrate herbivores on vegetation is less well understood (e.g. Hulme 1994, 1996; Fenner et al. 1999); furthermore, given the enormous diversity of invertebrate herbivores, generalisations are difficult. One essential distinction in considering their impact
upon vegetation is between highly specialised invertebrate herbivores that feed on one or few related plant species, and polyphagous herbivores that feed on a wide range of different host plants. While the first are limited by the abundance of their host plants (e.g. Kéry et al. 2001), the latter have the potential to turn to more common species after eliminating the preferred species from a community. However, the relative impact of specialist and generalist herbivores upon vegetation remains poorly understood (cf. Fraser & Grime 1999; Maron & Vila 2003).

Slugs are among the most important herbivores in low herbaceous vegetation in the cool-temperate zone (Rees & Brown 1992; Hulme 1996; Rodríguez & Brown 1998; Hanley et al. 2003) and can influence both the biomass and species composition of plant communities (Oliveira Silva 1992; Hanley et al. 1995a; Hulme 1996; Bruelheide & Scheidel 1999). Many slug species are more or less polyphagous herbivores though they show distinct food preferences, with plant species varying greatly in their acceptability to slugs (e.g. South 1992; Briner & Frank 1998; Frank & Friedli 1999). Previous work has shown that leaf palatability is affected by a wide range of factors including secondary chemistry (Dirzo & Harper 1982; Gouyon et al. 1983; Mølgaard 1986; Moens 1989; Glen et al. 1990; Newman et al. 1992), physical defence (Grime et al. 1968; Dirzo 1980; Jennings & Barkham 1975; Westerbergh & Nyberg 1995), nutrient quality (Schädler et al. 2003) and morphological structures that make foliage more or less inaccessible to slugs (Dirzo 1980; Rathcke 1985). Plants are particularly vulnerable to slug damage at the seedling stage (Frank 1998a, b; Hanley 1998; Hanley et al. 1995a, 2003), which may explain why seedlings of some species have higher levels of chemical defences than mature plants (Fenner et al. 1999). Thus, slugs should have highest impacts on vegetation composition in early successional stages when most plants are present as seedlings (cf. Dirzo 1980; Edwards & Gillman, 1987, Hanley et al. 1995a, b; Wardle & Barker 1997).

It has been shown that slugs may reduce species diversity and may increase the rate of secondary plant succession by selecting more palatable species, which are, in general, annual and early successional perennial species (e.g. Cates & Orians 1975; Reader & Southwood 1981; Fraser & Grime 1999). It has also been shown that food selection of slugs is affected by the relative abundance of plant species as slugs feed preferentially on less abundant species; such “antiapostatic” selection also leads to reduced species diversity in vegetation (Cottam
1985; Hanley et al. 1995a; Bruelheide & Scheidel 1999; Frank 2003). In contrast, other studies have shown that slug grazing reduces the abundance of common species in grasslands (Cottam 1986; Hanley et al. 1996), thus enhancing species diversity (cf. Olff & Ritchie 1998).

Most experiments to investigate the impact of slug grazing on plant species composition have been short term, lasting from a few weeks up to two years, and have focussed upon the seedling phase rather than on established vegetation (e.g. Hulme 1994, 1996; Hanley et al. 1995a, b, 1996; Frank 1998a, b). One exception comes from the work of Fenner et al. (1999), who observed effects of slugs on the species composition of a herbaceous community due to the differential survival of seedlings in vegetation gaps created 5 years earlier by Hanley et al. (1996). However, our knowledge of the influence of slugs upon the course of secondary succession is still limited.

In this study we investigated the impact of slug grazing upon biomass and species composition during the first three years of a succession on arable land. We hypothesised that slugs have different impacts at the beginning of the succession, when most plants are present as seedlings, and after closed vegetation has established. Specifically, we expected slugs to have a negative effect on plant species diversity at the beginning of the succession due to selection of palatable seedlings, and a positive effect on plant diversity in later stages of vegetation as a result of their reducing the competitive ability of the more abundant species. The questions of this study were: (i) how do slugs influence vegetation development and the diversity of plant species establishing from the seed bank? (ii) does the influence of slug herbivory upon vegetation development change during the course of succession?, and (iii) are particular ecological groups of plants, i.e. annual vs. perennial and highly palatable vs. less palatable plant species, differentially affected by slug herbivory?

We investigated these questions by sowing a clover (Trifolium repens)- ryegrass (Lolium perenne) sward in a soil with a large seedbank of mainly ruderal species. This mixture was chosen because these species are commonly sown as part of an arable rotation, and it is known that the development of the sward leads to reduced slug populations (Frank 1998a, b). As slug species we chose Arion lusitanicus Mabille, a common agricultural pest in central Europe (Reischütz 1986; Frank 1998a, b). The experimental design allowed us to investigate...
how slugs influence the establishment of weed species in a sward initially dominated by the sown species.

**Material and methods**

The experiment was established in April 1997 on former arable land in a flat, open site (105 m²) on base-rich, loamy soil in the experimental garden at ETH Hönggerberg in Zurich (523 m a.s.l., Switzerland); it was run until September 1999. In order to reduce patchiness of the natural seed bank, the top soil (about 15 cm) was removed, mixed thoroughly and returned to the site. Fourteen plots were established, each surrounded by commercial slug-proof frames (2 x 2 m², width 34 cm). The frames were buried 10 cm into the soil and were arranged in a regular grid with a spacing of 75 cm between neighbouring frames (Fig. 1).

The plant varieties sown in the experiment were *Lolium perenne* var Bastion (13 kg ha⁻¹) and *Trifolium repens* var Ladino Regal (4 kg ha⁻¹); nomenclature of plant species follows Oberdorfer (1990). *Lolium perenne* is reported to be rather unpalatable to slugs (Dirzo 1980) while the chosen variety of *Trifolium repens* has a low content of cyanogenic glycosides and is, therefore, attractive to slugs (cf. Dirzo & Harper 1982; Horrill & Richards 1986). The seeds were sown on 14 April 1997. There was vigorous regeneration from fragments of various clonal species, notably of *Vicia* spp., *Ranunculus* spp., *Achillea millefolium*, *Taraxacum officinale* agg. and *Cirsium arvense*) and during the first 2 month it was necessary to remove these species by weeding. Thereafter it was no longer possible to distinguish between vegetative regrowth and seedlings (which were the subject of this study) and so weeding was discontinued.

The slugs for the experiment were collected from semi-natural vegetation in the surroundings of the experimental area at Hönggerberg. Five weeks after sowing (12 May 1997) half of the plots, chosen at random, received 22 slugs in the first and 10 slugs in consecutive years, while slugs were excluded from the rest of the plots. The numbers of slugs per plot were chosen to represent high natural slug densities (cf. Frank 1998a), thus conforming to the recommendations by Hanley *et al.* (2003) for mollusc feeding experiments. Four wooden shelters were placed in each plot at 10 cm from each side to protect the slugs during drought
Effect of slugs on vegetation development and species diversity

(cf. Keller et al. 1999; Fig. 1). From March to November the number of slugs per plot was monitored on a weekly basis. Missing slugs were replaced and surplus individuals and other mollusc species were removed. A molluscicide (metaldehyde pellets) was applied twice to prevent slugs from entering the slug-exclosure control plots. All plots were mown twice in the first and second year and three times in the third year with a motor-seythe.

![Diagram of experimental setup]

**Figure 1.** Arrangement of the experimental area and design of experimental plots (slug frames) with sub-plots used to measure plant cover in 1997. 0, slugs absent; 1, slugs added.

**Data collection**

The early development of the vegetation was monitored closely by taking photographs of two sub-plots per plot (30 x 45 cm²) every second day from 24 May to 9 June 1997. These sub-plots were located at 30 cm from the fence in the centre of the long side of each half-plot (Fig. 1). Using image analysis (cf. Dietz & Steinlein 1996) the cover of vegetation was calculated from the photographs. In the third year, when the vegetation cover was nearly 100%, this technique was no longer feasible, and thus the percent cover of each plant species was estimated visually.

37
In the first year, the aboveground yield of each plant species was measured in eight randomly placed subplots (20 x 20 cm²). A slightly different procedure was used in the third year, when vegetation composition in all plots was recorded by cutting samples within a randomly placed ring of 0.125 m² (four samples per plot). In contrast to the first year, it was not longer possible to separate the harvested material into individual species; instead it was divided into five categories (see above). All material was oven-dried at 80 °C and weighed. The number of species present in the plots was recorded each year.

Information about palatability of the plant species was obtained from the literature (Grime et al. 1968; Duval 1971; Jennings & Barkham 1975; Dirzo 1980; Rathecke 1985; Mølgaard 1986; Cook et al. 1996; Wardle & Barker 1997; Briner & Frank 1998; Scheidel & Bruelheide 1999; Peters et al. 2000; Schädler et al. 2003). Life history information (annual and biennial vs. perennial) was obtained from Oberdorfer (1990).

**DATA ANALYSIS**

Cumulative dry mass of the different harvests in one year was used for data analysis (two harvests in 1997, three harvests in 1999). The Shannon-Wiener index and Shannon evenness (Magurran 1996) were calculated based on the biomass data of the species in the first year and on the cover values obtained for the third year (see Table 1). We used the following equations:

\[
H' = - \sum_{i=1}^{S} p_i \ln p_i
\]

Shannon evenness:

\[
E' = H/\ln S
\]

where \( p \) is the biomass of species (first year) or cover (third year) and \( S \) is the number of species.

JMP 5.0 (SAS Institute Inc. 2002) was used for all statistical analyses; values of \( P < 0.05 \) accepted as significant.

T-tests were used separately for each year to test for treatment differences in diversity indices, total dry mass and total vegetation cover. If the data deviated significantly from a normal distribution (Shapiro-Wilks-test) we used the Wilcoxon-test.
Effect of slugs on vegetation development and species diversity

Table 1. The vegetation cover-abundance code adapted from Braun-Blanquet (cf. Westhoff & van der Maarel 1973, and references therein) used for plant cover analysis in 1999. For clonal plants ramets were counted as individuals.

<table>
<thead>
<tr>
<th>Category</th>
<th>Cover (%)</th>
<th>Abundance (indiv.)</th>
<th>Weights</th>
</tr>
</thead>
<tbody>
<tr>
<td>R</td>
<td>1 or 2</td>
<td></td>
<td>0.1</td>
</tr>
<tr>
<td>+</td>
<td>&lt; 1</td>
<td>&gt; 2</td>
<td>0.5</td>
</tr>
<tr>
<td>1</td>
<td>1-5</td>
<td>or &gt; 10</td>
<td>3</td>
</tr>
<tr>
<td>2</td>
<td>5-10</td>
<td>or &gt; 50</td>
<td>7.5</td>
</tr>
<tr>
<td>3</td>
<td>10-20</td>
<td>or &gt; 100</td>
<td>15</td>
</tr>
<tr>
<td>4</td>
<td>20-30</td>
<td></td>
<td>25</td>
</tr>
<tr>
<td>5</td>
<td>30-50</td>
<td></td>
<td>40</td>
</tr>
</tbody>
</table>

Log-linear analysis was used to test for differences in the number of plant species per life form (annual vs. perennial) and per palatability category (palatable vs. unpalatable) between the years and between the slug treatments. Separate analyses were run for life form and palatability category.

MANOVA was used for the analysis of harvest data using five categories of living and dead plant material (herbs, legumes, grasses, litter, mosses) as dependent variables and two harvest times (1997 and 1999). To look for differences in the mean biomass of the different categories between slug treatments slug density was tested as between subject against plot as random factor. To test for differences between the two years year was used as within subject for repeated measure analysis. Also, to investigate possible shifts in dominance of annual (including biennial) versus perennial species and palatable versus unpalatable species over time in relation to the slug treatments repeated measures MANOVA was used. Separate analyses were run for life form and palatability category. For 1997 information was available on the dry mass of individual species while in 1999 only cover data were available. To investigate changes in species abundance between the two years the species data for each year and treatment were ranked and mean rank values were compared. As total cover in 1999 was correlated with total biomass of the same year (Spearman rank correlation: $R_s = 0.63$, $P < 0.001$) we assume that the ranking of these two parameters was a valid way to compare the data from 1997 and 1999. In addition, Röttgermann et al. (2000) showed that biomass of several species in open herbaceous vegetation increases linearly with vegetation cover.
Results

Development of the vegetation

The two sown species, *Lolium perenne* and *Trifolium repens*, germinated and established well. As had been intended, large numbers of seedlings of weed species also established from the seed bank. Amongst the most abundant were *Stellaria media*, *Euphorbia helioscopia*, *Cichorium intybus* and *Silene alba*.

Even at the first recording on 24 May 1997, when the sward was 7 weeks old and slugs had been present for just two weeks, there was a clear reduction in plant cover in the plots with slugs (grazed 12%, control 19%; Fig. 2, Table 2). The difference was still evident in the last recording on 9 June 1997, when the total plant cover in grazed plots was 40% compared with 52% in controls ($P < 0.05$; Table 2).

![Figure 2](image_url)

*Figure 2.* Development of vegetation cover in 1997 and cover-abundance of the vegetation at harvest time in 1999 (plot means ± SE; $n = 7$ replicate plots). Open circles, slug plots; filled circles, control plots.

There was a large effect of slug grazing on aboveground plant yield after half a year (13 August 1997, summed data for two harvests). The total yield in the control plots was 130 g m$^{-2}$, while it was around 25% lower in the grazed plots ($P < 0.01$; Table 2). The yield of both sown species was reduced by slug grazing; by 22% for *L. perenne* and by 36% for *T. repens*. 
In both the control and the grazed plots, weed species derived from the seed bank accounted for about 50% of the yield (Fig. 3).

In the third year the mean percentage cover of vegetation was 83% in the slug plots and 90% in the control plots (Fig. 2, Table 2). The sown species accounted for only a low proportion of the total cover, and there were marked differences in the relative proportions between the treatments: thus *L. perenne* had a cover of 10.7% in the control plots but only 3.9% in the grazed plots; the corresponding values for *T. repens* were 7.3% and 2.2%. The yield recorded at the end of the experiment (August 1999, summed data for three harvests) was 28% lower in the grazed plots than in control plots (198 g m⁻² and 278 g m⁻², respectively, *P* < 0.001; Table 2). Weed species excluding legumes made up 46% of total yield in control plots and 59% in slug plots (Fig. 3); the equivalent values for grasses (mainly *L. perenne*) were 36% and 31%, respectively, and 18% and 10% for leguminous species (of which *T. repens* contributed < 50%). In addition to vascular species, there were also some mosses, which were significantly more abundant in the slug plots (*P* < 0.01; Fig. 3, Table 3).

**Table 2.** Percentage cover and aboveground yield of vegetation, species number, species diversity and evenness in plots with and without slug grazing (mean values ± SE). The significance of differences between the treatments (t-test except for total yield in 1997 which was tested using the Wilcoxon-test) are indicated as: ***, *P* < 0.001; **, *P* < 0.01; *, *P* < 0.05.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>1997</th>
<th>1999</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control plots</td>
<td>Slug plots</td>
</tr>
<tr>
<td>Date</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cover</td>
<td>May 19±2 *</td>
<td>12±3 *</td>
</tr>
<tr>
<td></td>
<td>Jun 52±3 **</td>
<td>40±6 *</td>
</tr>
<tr>
<td>Mean dry mass of yield per harvest (g/m²)</td>
<td>Aug 65±4**</td>
<td>49±6**</td>
</tr>
<tr>
<td>Total dry mass of yield per year (g/m²)</td>
<td>Aug 130±2***</td>
<td>96±10***</td>
</tr>
<tr>
<td>Number of species (total)</td>
<td>Aug 50</td>
<td>54</td>
</tr>
<tr>
<td>Number of species (mean)</td>
<td>Aug 29±1*</td>
<td>24±3*</td>
</tr>
<tr>
<td>Shannon-Wiener index</td>
<td>Aug 1.5±0.1***</td>
<td>1.2±0.2**</td>
</tr>
<tr>
<td>Shannon-evenness</td>
<td>Aug 0.05±0.01</td>
<td>0.05±0.01</td>
</tr>
</tbody>
</table>

**Species diversity**

A total of 92 plant species were recorded during the course of the experiment. Many of these had low biomass and cover values, were represented by only a few individuals, and occurred in only one year (21 species only in 1997; 34 species only in 1999). Species observed only in
the first year were annual species (with one exception) while species observed only in the third year were mostly perennials. Twenty-one (control plots) and 32 (slug plots) species were present in both years. The mean number of species per plot ranged from 27 in 1997 to 29 in 1999, but the differences between years were not significant ($t$-test: d.f. = 26, $t = -1.4$, $P = 0.17$).

![Figure 3](image-url)  
*Figure 3.* Mean yield (dry mass) at harvest time (1997 two harvests and 1999 three harvests) of the different functional groups in control and slug plots (plot means ± SE; $n = 7$ replicate plots). Black bars, 1997; grey bars, 1999; filled bars, control plots; hatched bars, slug plots.

The presence of slugs significantly affected the mean number of species per plot, though the direction of the effect changed with time. In the first two years, mean number of species per plot was lower in the slug plots than in controls (15% and 10% lower in years one and two, respectively; Table 2). However, in the third year species numbers were 23% higher in the slug plots. These differences were also reflected by the mean value of the Shannon-Wiener diversity index, which was lower in the slug plots than in the controls in 1997 but higher in 1999 (Table 2). As Shannon evenness was similar in the two years in both treatment and control plots (Table 2), it is clear that the differences in the Shannon-Wiener index were caused mainly by the number of species.
Table 3. Results of repeated measures MANOVA of harvest data (mean dry mass). Treatment factors are slug density (slug vs. control plots) and year (1997 vs. 1999). Shown are F values of main and interaction effects on overall test and on five categories of living and dead plant material (herbs, legumes, grasses, litter, mosses) (***, P < 0.001; ***, P < 0.01; *, P < 0.05).

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>Overall test</th>
<th>Test for individual response variables</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>d.f.</td>
<td>Herbs</td>
</tr>
<tr>
<td>Between plots</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Slug density</td>
<td>5</td>
<td>28***</td>
</tr>
<tr>
<td>Plot[slug density]</td>
<td>60</td>
<td>0.9</td>
</tr>
<tr>
<td>Within plots</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Year</td>
<td>5</td>
<td>141***</td>
</tr>
<tr>
<td>Year x slug density</td>
<td>5</td>
<td>18***</td>
</tr>
</tbody>
</table>

Table 4. Results of log-linear analysis. Treatment factors are slug density (slug vs. control plots) and year (1997 vs. 1999). Shown are Chi² values of main and interaction effects on the number of species per life strategy (annuals vs. perennials) and palatability (palatable vs. unpalatable) (d.f. = 1 in each case; other explanations as in Table 3).

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>Life form</th>
<th>Palatability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Slug density</td>
<td>1.4</td>
<td>0.2</td>
</tr>
<tr>
<td>Year</td>
<td>21***</td>
<td>4.4*</td>
</tr>
<tr>
<td>Year x slug density</td>
<td>0.1</td>
<td>&lt;0.1</td>
</tr>
</tbody>
</table>

The proportion of annual species declined strongly between 1997 and 1999 (Fig. 4, Table 4; P < 0.001), especially in grazed plots. The proportion of unpalatable species also increased between 1997 and 1999; although this trend was slightly greater in the slug plots than in the controls, there was no significant treatment effect. The occurrence of species with contrasting life history traits was investigated in more detail by calculating the mean dominance ranks for the paired categories annual/perennial and palatable/unpalatable. This analysis confirmed the declining prominence of annual species with time (Fig. 5, Table 5; P < 0.001). It also revealed that annual species had a lower ranking in grazed than in control plots (42 ± 3 (slugs), 35 ± 2 (control); P < 0.05), while the perennials showed no differences in rank between treatments, particularly in 1999 (37 ± 2 (slugs), 39 ± 3 (control); P = 0.52). Similar results were obtained for palatability categories. While the mean rank of palatable species decreased over the course of the experiment the mean dominance rank of unpalatable species increased (P < 0.001).
Palatable species had a higher ranking in both years in the control plots while unpalatable species had a higher ranking in the slug plots in 1999 (Fig. 5, Table 5; \( P < 0.05 \)).

![Figure 4](image)

*Figure 4.* Changes in total species number over time for annual vs. perennial and palatable vs. unpalatable plant species. Open circles, annual; filled circles, perennial; open squares, palatable; filled squares, unpalatable.

*Table 5.* Results of repeated measures MANOVA of possible shifts in dominance of annual (including biennial) vs. perennial species and palatable vs. unpalatable species. Treatment factors are slug density (slug vs. control plots) and years (1997 vs. 1999). Shown are \( F \) values of main and interaction effects on the species rank in biomass or cover, respectively. Tests were run separately for life form and palatability category (d.f. = 2 for overall tests; d.f. = 1 for individual tests; other explanations as in Table 3).
Effect of slugs on vegetation development and species diversity

Discussion

The aim of this experiment was to study the impact of slugs upon early-successional plant species on arable land. We chose *Arion lusitanicus* as the herbivore because this is an abundant species which commonly occurs at the edges of arable fields and in hedgerows, and may invade in large numbers nearby arable fields up to a distance of about 5 m (Frank 1998a). The smaller *Deroceras reticulatum* - a serious agricultural pest in this habitat type in central Europe - has similar food preferences to *A. lusitanicus* (see Chapter 4) but tends to move further into arable fields (Frank 1998a, b). We believe that our results provide a realistic illustration of the impact of slugs in agricultural clover-ryegrass swards, though the natural slug community would usually be more diverse.

Our experiment demonstrated that slugs can have a major impact on the development of vegetation by reducing the aboveground biomass and production of vegetation and thus delaying the development of complete plant cover. Reduced plant cover due to slug grazing was
evident as early as two weeks after the onset of the treatments, and was still detectable in the third year. Perhaps more surprising was the fact that slugs reduced yield by around 25%, not only in the first year when the plants were mainly small, but also in the third year. In this respect our results contrast with those of Hulme (1996) and Frank (2003) who found differences in plant density due to slug grazing but no effects upon plant biomass.

Slug grazing had a considerable effect upon plant species composition. At the beginning of the experiment the vegetation was dominated by the sown species, and the yield of these was reduced by slugs. In the case of Lolium perenne this reduction was approximately in proportion to the overall yield reduction, while Trifolium repens was affected disproportionately. In the third year the negative impact of grazing upon the two species was even greater, and they were much less abundant in grazed plots than in controls (3% cover of L. perenne, 2% cover T. repens). Although Trifolium repens is often found to be rather unpalatable (e.g. Dirzo & Harper 1982; Hanley et al. 2003), we deliberately chose a variety with low cyanide content, which may explain why this species was strongly reduced by grazing. L. perenne is also rather unpalatable to slugs (e.g. Dirzo 1980), and the reduction in its abundance in the grazing plots could be because slugs “felled” seedlings and young shoots without feeding on them, as observed by Hatto & Harper (1969) and Dirzo & Harper (1980).

In the first year the mean species number and diversity were lower in the slug plots than in the controls. Species which were almost absent from the plots include Matricaria inodora, Malva silvestris and Geranium pusillum. The loss of certain plant species in these plots is probably a direct reflection of the feeding preferences of slugs, and possibly also of differences between seedlings in their tolerance of grazing damage. At this early stage in succession, with a high proportion of bare ground, competitive interactions between plants were probably relatively unimportant in limiting some species.

In the third year slug grazing promoted plant species richness and diversity. At this stage, the biomass of vegetation was considerably greater and the plant cover was nearly 100%; thus, competition probably became a more significant factor than in the first year (cf. Schädler et al. 2003). It was noticeable that three of the most common perennial species, Daucus carota, T. repens and L. perenne, were vigorous in the control plots but substantially reduced in the slug plots. This observation suggests that an important effect of slug grazing was to reduce the vigour of the abundant species and allow less competitive species to persist.
Effect of slugs on vegetation development and species diversity

(cf. Cottam 1986). We also observed that lug grazing also helped to maintain a more heterogeneous environment, with a higher proportion of small gaps (cf. Dirzo 1980; Cottam 1985), which may have been important in allowing weedy species to establish from seed (Crawley 1989, 1997). In addition, the reduced quantity of litter in the slug plots may have provided more favourable conditions for seed germination (see Wilby & Brown 2001). Mosses were more abundant in the slug plots, probably because they are highly unpalatable to most slugs (South 1992, and references therein) and could benefit from the more open conditions in the grazed plots.

Contrary to our results, Wilby & Brown (2001) found higher species richness in plots without slugs in their study of an early old-field succession. However, in their three-year experiment the vegetation reached a maximum cover of 70% and thus more closely resembled conditions during the first year of our experiment. The study of Hanley et al. (1996) confirms our findings that slugs are able to enhance plant species diversity. However, their results were for seedling communities in small gaps in grassland, and from our experiments we would predict less diversity at this stage. This contradiction may be because the seed community of grasslands is very different from that of the ruderal vegetation we studied, as grasslands contain more mid-successional species (see Hanley et al. 1996).

The analysis of species composition in terms of ecological groups of plants revealed relatively minor changes due to slug grazing. Although the palatable species were reduced in biomass by slugs, we found neither fewer palatable species in the slug plots than in the control plots nor more unpalatable species. This discrepancy could reflect a methodological problem, because we used data from earlier leaf disc assays to determine the palatability of species. Results of these palatability assays should be used cautiously because they may not reflect palatability of the species under field conditions (Hanley 1998; Fenner et al. 1999; Chapter 4). There was also no significant reduction of annual plant species in the grazed plots, although these tend to be more palatable than perennial species (e.g. Grime et al. 1968; Cates & Orians 1975; Reader & Southwood 1981). Between the years 1997 and 1999 annual species that were dominant in the control plots in 1999 were reduced in dominance in the grazed plots. This could explain why, although the overall biomass of annual species in the grazed plots was reduced, the number of species tended to be higher in 1999 than in 1997. Seemingly herbivory by slugs had different influences at the population level and at the community level.
At the population level herbivory had negative effects for most individuals, leading to reduced dominance of annual species (perhaps because annuals tend to be less able to compensate for leaf loss than perennial species; cf. Grime 2001). This effect may have been stronger in 1999 than in 1997 because the annuals were less tolerant of grazing under the greater pressure of competition in 1999 (cf. Bazzaz 1996). In contrast, at the community level annuals may profit from slug herbivory because grazing may reduce local extinction of competitively subordinate species and provide favourable conditions for germination and establishment (cf. Olff & Ritchie 1998).

**Conclusions**

We conclude that slugs can have a significant effect upon plant species diversity in early-successional plant communities, but that their impact changes during the course of succession. As shown by Hanley et al. (1996), slugs can shift the relative proportions of species in a community in favour of those which are more resistant to slug herbivory. Our results indicate that this effect is stronger in early secondary succession when most species are represented as seedlings, which leads to reduced species diversity as favoured species are eliminated first. In closed vegetation, in which competitive interactions are more important, slugs can reduce the dominance of competitive species and provide gaps in which plants can establish from seed. As a consequence, they tend to cause an increase in plant species diversity and may also reduce the rate of successional change by promoting the persistence of annual species.

**Acknowledgements**

We are grateful to T. Pfau for donating the slug fences. Johannes Kollmann and Sabine Güsewell provided helpful comments on the manuscript. We thank Bettina R. Kahlert for her assistance with the field work. The study was supported financially by the Geobotanical Institute ETH Zurich.
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Chapter 3

Chapter 4

Responses of native and invasive Brassicaceae species to slug herbivory

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Summary

1 It has been proposed that invasive plants are often less palatable or better able to compensate for biomass losses by herbivory than related, non-invasive species growing in the same area.

2 We hypothesised that low palatability to slugs and/or increased ability to compensate for grazing damage are traits contributing to the invasiveness of perennial Brassicaceae forb species introduced to western and northern central Europe.

3 In common garden and glasshouse experiments we compared life-history and fitness parameters of three native and three invasive Brassicaceae species (Barbarea vulgaris, Bunias orientalis, Cardaria draba, Diplotaxis tenuifolia, Isatis tinctoria, Rorippa austriaca) of central European provenances that were subjected to slug herbivory by Arion lusitanicus and Deroceras reticulatum. Using the same species we performed leaf disc palatability assays and investigated the effects of slug herbivory on small plants regenerated from root fragments (rootlings) and seedlings in field and glasshouse experiments.

4 We found high between-species variation in susceptibility to slug herbivory (proportions of leaves damaged and leaf area consumed) but these were not related to the native or invasive status of the species. While the proportions of seedlings damaged or killed by slug herbivory did not differ between the two groups of species, the survival of damaged rootlings was higher than that of seedlings. Between the two groups of species, the survival of damaged rootlings was higher than that of seedlings. Consistent with our hypothesis, the invasive species, particularly those with clonal reproduction, showed higher compensation growth after slug herbivory.

5 Our results suggest that a high ability for compensation growth in invasive Brassicaceae species makes them more tolerant of slug damage than native congeners. The potential to regenerate from root fragments, which are less vulnerable than seedlings to herbivory, appears to be another important factor contributing to the invasiveness of some clonal species. Since many invasive plant species share these traits (though regeneration may be from plant parts other than roots), we suggest that tolerance of herbivory may be one of the characteristics of many successful invaders.
Introduction

The success of many invasive plant species is at least partly attributable to their vigorous growth in the introduced area (e.g. Noble 1989, Williamson 1996, Jakobs et al. 2004). It has been suggested that the release of introduced plants from specialist natural enemies (e.g. Keane & Crawley 2002, Wolfe 2002) is often directly responsible for this higher plant vigour, and may also lead to the evolution of increased competitive ability in some species (Evolution of Increased Competitive Ability (EICA) hypothesis, Blossey & Nötzold 1995). Several recent studies have investigated these hypotheses by comparing native and invasive provenances of the same species in terms of growth, fecundity and levels of herbivory. Very often, however, no differences have been found in the responses of plants of different provenance to herbivory, indicating that in these cases the EICA hypothesis does not explain the observed differences in growth (e.g. Willis et al. 1999, 2000; van Kleunen & Schmid 2003; Bossdorf et al. 2004; Chapter 5).

However, herbivory could benefit invasive plants relative to native plants in ways other than suggested by the EICA hypothesis. Plants may thrive in the presence of generalist herbivores not only because they are protected physically or chemically to different degrees, but also because their growth form makes their meristems or foliage inaccessible to herbivores (Dirzo 1980, Rathcke 1985, Moens 1989, Glen et al. 1990, Westerbergh & Nyberg 1995). Furthermore, grazing-tolerant plants may exhibit a higher capacity for compensatory growth in response to defoliation (Edwards & Gillman 1987, Louda et al. 1990, Oliveira Silva 1992, Mutikainen & Walls 1995). Successful invasive species could have a particularly high capacity for compensatory growth and flexible resource allocation (e.g. Schierenbeck et al. 1994). Hence, if invasive plants profit from lower susceptibility to herbivory or higher compensatory growth, they could have a competitive advantage over co-occurring native plants that suffer more severely from shared herbivores. The importance of tolerance of grazing has received less attention than anti-herbivore defences, and more experimental work is needed to understand how altered patterns of selection might influence the evolution of plant tolerance during the invasion process (Müller-Schärer et al., in press).

A further factor that is often not adequately considered is that the sensitivity of plants to herbivore damage varies with growth stage. Young plants are generally more palatable than
adult plants (Hanley et al. 1995; Fenner et al. 1999), and tolerance or resistance to grazing at this stage could determine the ability of a species to invade. For clonal species, survival of a young plant may even depend upon whether it regenerated from a root or rhizome fragment or from seeds (Buschmann et al., submitted). However, given the few comparative studies on these aspects of herbivory, we can only speculate about their importance in plant invasions (cf. Daehler 2003, and references therein).

In north-western and central Europe, slugs are among the most important herbivores of low herbaceous vegetation (Rathcke 1985, Edwards & Gillman 1987, Rees & Brown 1992, South 1992, Hulme 1994, 1996, Rodríguez & Brown 1998) and can influence both biomass and species composition of plant communities, as shown in many studies in grasslands, arable fields and mountain meadows (Oliveira Silva 1992, Hanley et al. 1995, Hulme 1996, Hanley 1998, Bruelheide & Scheidel 1999, Frank 2003). Several Brassicaceae species that grow in this type of vegetation are highly palatable to slugs; comparative studies have even demonstrated a preference by some slug species for Brassicaceae over other families, despite the fact that the mustard oils they contain are known to be feeding deterrents for slugs (e.g. Cates & Orians 1975, Dirzo 1980, Rathcke 1985, Rees & Brown 1992, Briner & Frank 1998).

In addition, the Brassicaceae is a family with a high proportion of invasive species (e.g. Sebald et al. 1993), including perennial forbs, and several species native to south-eastern Europe have increased their range into northern and western Europe (cf. Sebald et al. 1993, Jalas & Suominen 1994, Jalas et al. 1996). Hence, comparative investigations of the effects of slug herbivory on native and invasive Brassicaceae species in central Europe may improve our understanding of the role played by herbivory in plant invasions (Buschmann et al. 2002).

The aim of this study was to compare the impact of slug herbivory upon native and invasive species of Brassicaceae in central Europe. The specific research questions posed were: (i) are invasive Brassicaceae forbs less susceptible to slug herbivory? (ii) do they show higher compensatory growth following slug herbivory than related species that are native to this area? and (iii) do the young stages of the native species suffer more from herbivory than those of the invasive species? We compared growth parameters and responses to herbivory between sets of three invasive and three non-invasive species. In a common garden (mesocosm) experiment, established plants were exposed to slug herbivory under field-like conditions. In
microcosm experiments, the susceptibility of seedlings or juvenile plants to slug herbivory was compared, and leaf palatability was tested in food-choice bioassays.

**Methods**

**Plant Species**

We studied six herbaceous Brassicaceae: *Barbarea vulgaris* R.Br., *Bunias orientalis* L., *Cardaria draba* (L.) Desv., *Diplotaxis tenuifolia* (L.) DC., *Isatis tinctoria* L. and *Rorippa austriaca* (Crantz) Besser. *Barbarea vulgaris, D. tenuifolia* and *I. tinctoria* are native or old introductions in central Europe while *B. orientalis, C. draba* and *R. austriaca* have been introduced to at least the western parts of central Europe and are invasive there (Jalas & Suominen 1994, Jalas et al. 1996). The species possess the characteristic traits of this rather homogeneous family (Hedge 1976) and share similar growth forms and habitat preferences; thus, all are polycarpic herbaceous perennials with a semi-rosette growth form and mostly deep storage roots, and all have a preference for sun-exposed, moderately disturbed sites on nutrient rich, calcareous soils (Oberdorfer 1990). However, the species differ in certain life-history characteristics including life-span and the relative importance of vegetative and sexual reproduction (Hegi 1986, Dietz & Ullmann 1998, Dietz et al. 2002). *Rorippa austriaca* and *C. draba* show low seedling recruitment but can spread clonally by lateral root growth and can also regenerate from dispersed root fragments. In contrast, the other four species produce abundant seedlings but do not spread clonally (although *B. orientalis* also has these potential to regenerate from root fragments after mowing or soil disturbance; Dietz & Steinlein 1998). Most species are rather long-lived (Dietz & Ullmann 1998, Wojtke 2001) but *B. vulgaris* and *I. tinctoria* are exceptions that live for only a few years and mostly die after flowering (H. Dietz, unpubl. data).

**Collection of Plant Material**

Plants were collected near Würzburg in Germany, in an region where several invasive and native Brassicaceae species coexist in the same habitats. Except for *I. tinctoria*, which was collected in spring 2000, populations of all species were sampled in 1998. For each species, three populations (*I. tinctoria* two populations) that were at least 10 km apart (in...
Randersacker, Sommerhausen, Thüngersheim, Winterhausen) from each other were sampled at typical ruderal sites. Adult plants or seeds from natural populations were not available for *D. tenuifolia*. Thus, for this species we obtained seeds from a commercial supplier (Blausiegel, Würzburg). Sixty individuals per species and population were grown either from seeds (*B. vulgaris, D. tenuifolia, I. tinctoria*) or, when these were not available in sufficient numbers, regenerated from adult plants (*B. orientalis*) or plant fragments (*C. draba, R. austriaca*). They were grown in pots of 10 cm diameter filled with commercial potting soil and kept in unregulated glasshouses, initially in Würzburg (for 16 months) and later in Zurich (for 6 months).

**Choice and Collection of Slug Species**

We chose two of the most common European slug species, *Arion lusitanicus* Mabille and *Deroceras reticulatum* Müller (Runham & Hunter 1970, South 1992, Frank 1998), both of which are agricultural pests in some regions. Preliminary leaf disc palatability assays revealed no differences in feeding preferences between slugs from Germany and Switzerland, and for practical reasons we used slugs from Switzerland for the experiments. These were collected as required from the surroundings of the experimental garden at Hönggerberg in Zurich (532 m a.s.l., Switzerland). For each experiment and each experimental run newly collected slugs were used.

**Common Garden Experiments**

The common garden experiment was established in October 2000 in the experimental garden and ran for two years, until October 2002. The site was a homogeneous, unshaded area with a base-rich, loamy soil.

The randomly arranged 2 x 2 m² plots were arranged in a regular grid with a spacing of 75 cm. (Fig. 1). Each plot was surrounded by a slug-proof metal frames 30 cm high and buried 5 cm into the soil; in those plots from which slugs were excluded, the upper rim of the frame was bent outwards, while the rim bent inwards in plots maintaining an increased number of slugs. The area enclosed by each plot was subdivided into a grid of 12 equally sized cells (Fig. 1). Five plants per species (randomly chosen but stratified for comparable sizes from the three populations per species) were planted into one cell within each plot with
an inter-individual spacing of 25 cm. After an establishment phase of four weeks during which dead plants were replaced by new ones the slugs were introduced to the plots. The mixture of species offered to slugs may be important and can influence consumption rates (Peters et al. 2000). Thus, presenting slugs with a choice of species seems to be important in investigating their natural feeding behaviour. To make conditions as natural as possible, every second cell was left unplanted and vegetation was allowed to develop spontaneously (Fig. 1).

Figure 1. Design of the common garden experiment and experimental plots (slug frames) used to investigate interspecific differences in slug damage and compensatory growth between invasive and native species. Ba, Barbarea vulgaris; Bu, Bunias vulgaris; Ca, Cardaria draba; Di, Diplotaxis tenuifolia; Is, Isatis tinctoria; Ro, Rorippa austriaca; 0, slugs absent; 1, spontaneous slugs; 2, Deroceras reticulatum; 3, Arion lusitanicus; a, added in autumn 2000; b, added in spring 2001.

There were six treatments, achieved by varying the slug species present and the timing of grazing. These treatments were: 1) no grazing by slugs at any time, 2) natural development of the slug populations (i.e. removal of the surrounding frame in spring 2001), 3) Deroceras introduced in autumn 2000, 4) Deroceras introduced in spring 2001, 5) Arion introduced in autumn 2000, 6) Arion introduced in spring 2001. There were three replicates of each
treatment combination. The numbers of slugs per plot (40 Deroceras or 20 Arion) were chosen to represent high natural slug densities (cf. Frank 1998) and conformed to the recommendations for mollusc feeding experiments given by Hanley et al. (2003). From March to November the number of slugs in the plots was monitored on a weekly basis. Missing slugs were replaced in the ‘slug plots’ and surplus individuals randomly removed. Other slugs and snail species were removed as well. Six wooden shelters placed into each plot protected the slugs from drought and helped to maintain the number of individuals (cf. Keller et al. 1999). In September 2001 molluscicide was applied to prevent the slug Deroceras reticulatum from entering the slug-exclosure plots.

For each plant we measured the following life-history characters: number of leaves, number of damaged leaves (measured twice a year in spring and autumn), number of shoots per plant, number of flowering plants (measured once a year in summer), root diameter and above-ground biomass (measured once at the end of the experiment after 2 years). Since there were usually rather few leaves present in autumn, we present only spring data for leaf number.

**SEEDLING AND ROOTLING BIOASSAYS**

The susceptibility of seedlings and rootlings (i.e. small plants regenerated from root fragments) to grazing by slugs was tested in garden and climate chamber experiments. The seedlings and rootlings of the clonal species (C. draba, R. austriaca) were grown in a greenhouse in Zurich from seeds and root fragments, respectively.

In May and September 2002, plastic tray (45 x 28 x 5 cm³) filled with potting soil were sunk into the soil in an area adjacent to the experimental plots. Fifty seedlings (all species) or rootlings (C. draba, R. austriaca) of each species were planted into one randomly chosen cell of each tray which was subdivided into a grid of six equally sized cells. After 2 days, each tray was subjected randomly to one grazing treatment: (1) exposure to ambient grazing pressure by slugs, and (2) no slugs (molluscicide treatment). The numbers of slugs and the slug species observed in the trays or in the surroundings were noted at intervals during the experiment, which lasted for 4 days. There were five replicate trays per treatment.

In climate chamber experiments, seedlings and rootlings were offered to D. reticulatum in September 2001 and to A. lusitanicus in May 2002 in plastic boxes (20 x 10 x 5
Slug herbivory on native and invasive Brassicaceae

3 cm³; 17 °C, 12 h light : 12 h dark). The bottom of each box was covered with 2 cm of commercial potting soil. Forty-eight hours after transplanting the seedlings into the boxes, one slug was introduced and allowed to feed for 24 h. The number of damaged and dead seedlings/rootlings was scored at the end of both experiments.

In previous common garden and climate chamber experiments we tested the effect of using either the same number or the same mass of seedlings per species. Both arrangements produced similar results about slug feeding preferences (H. Buschmann, unpubl. data) and we chose to use an equal number of seedlings/rootlings in subsequent experiments.

LEAF DISC PALATABILITY ASSAYS
Leaf discs were cut from fresh, mature leaves of all species and offered to D. reticulatum and A. lusitanicus in Petri dishes (Ø = 13.5 cm). In each dish, two (Ø = 6 mm, D. reticulatum) or three (Ø = 11 mm, A. lusitanicus) leaf discs of each plant species were randomly arranged in a circular pattern on a moist blotting paper. In addition, three larger discs of Taraxacum officinale, known to be a highly palatable species to slugs (Dirzo 1980, Rathcke 1985, Frank & Friedli 1999), were placed in the centre of each Petri dish to ensure that the experimental material was not totally consumed.

Prior to the experiments each slug was placed in a separate plastic box and fed ad libitum with T. officinale for 24 h in a climate chamber (17 °C, 12 h light : 12 h dark). Subsequently, one slug was introduced to the centre of each dish and was allowed to feed for 12 h in the dark (17 °C) in a climate chamber.

The experimental trials with A. lusitanicus were conducted in August 2001 and those with D. reticulatum in September 2002 (20 replicate Petri dishes). Death or inactivity of some slugs reduced the effective number of replicates.

At the end of the experiments the amount of leaf area consumed was determined using image analysis (cf. Dietz & Steinlein 1996). Ten surplus leaf discs per species were collected and oven-dried at 70 °C for 24 h to measure the dry mass. The live weight of each slug was measured before and after the experiment. Following Briner & Frank (1998) we transformed the fresh body mass of the slugs into dry mass using the dry mass calibration curves of Bullock & Smith (1971). Palatability is expressed as the consumption index CI (Waldbauer 1968):
Chapter 4

\[ CI = 1000 \times \left( \frac{F}{T \times W} \right) \]

where \( F \) is the amount of food eaten (mg), \( T \) is the duration of the feeding period (h), and \( W \) is the mean body weight of the test slug during the feeding period (mg).

**Data analysis**

JMP 5.0 (SAS Institute Inc. 2002) was used for all analyses. Nested two-way ANOVAs were calculated to test for effects of status (i.e. invasive vs. native plant species), and slug density (slugs absent, slugs present, spontaneous slug immigration) on the plant parameters (common garden experiment). Slug density was used as between-subject factor and status as within-subject factor. The effect of species identity was nested within status. As time of introduction of the slugs to the plots and slug species had no significant effect on the test plants the corresponding data were pooled for analysis in the common garden and the outdoor seedling bioassay experiment.

One-way ANOVA was used to test for effects of species status or species identity on the percentage of plants damaged and killed in the seedling/rootling bioassays, and on the consumption index in the leaf disc bioassays. Percentage data were arc-sin-transformed and some raw data were log- or square root-transformed to remove heterogeneity of variances.

**Results**

**Common garden experiment**

In plots containing slugs (slugs added, natural slug density) grazing damage varied widely (affecting 2-100% of leaves) according to time of grazing and plant species (Fig. 2). Differences in damage between plots with slugs added and those with natural slug densities were rather small, but there was more damage in autumn, when the slugs were larger, than in spring. Grazing damage was negatively correlated with dry mass of the species (Spearman rank correlation: \( R_s = -0.49, P < 0.01 \)).
Table 1. Results of two-way ANOVA (common garden experiment). Treatment factors are status of the species (invasive vs. native species with nested species factor) and slug density (slugs absent, natural slug density, slugs added). Shown are F-values of main and interaction effects on vegetative plant traits. The results were analysed separately for the different study years (***, P < 0.001; **, P < 0.01; *, P < 0.05; m, P < 0.10; d.f. = 1 for status; d.f. = 4 for species[status]; d.f. = 2 for slug density and status x slug density; d.f. = 8 for species[status] x slug density).

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<td></td>
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<td>2001</td>
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<td>2002</td>
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<td>9.8***</td>
<td>84***</td>
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<td>9.3***</td>
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</tr>
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</tr>
<tr>
<td>Species[status] x slug density</td>
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<td>1.5</td>
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</table>

Figure 2. Development of leaf damage between spring 2001 and autumn 2002 in the common garden experiment (plot means ± SE; n = 3 replicate plots). Open bars, slugs absent; grey bars, spontaneous slugs; black bars, slugs added.
There was no significant difference in grazing damage associated with plant status (i.e. whether invasive and native) but there were highly significant differences between species (Table 1). Four species (two invasive, two native) showed high levels of damage (60 - 100%), while D. tenuifolia (native) and C. draba (invasive) were much less grazed (5 and 30%, respectively; Fig. 2). Plant mortality ranged from 0% (R. austriaca) to 19% (C. draba) (Fig. 3), and was related positively to the percentage of damaged leaves ($R_s = 0.43$, $P < 0.01$) and negatively to the percentage of plants in flower ($R_s = -0.56$, $P < 0.001$). However, in the ANOVA there was no significant effect of the slug treatments on either mortality. (Table 2). The proportion of plants flowering varied greatly between species but there were no significant effects of either status or treatment. While the invasive species showed better performance than natives in all growth parameters in 2002 (number of leaves and stems, root diameter, dry mass; $P < 0.05$) there was no effect of slug treatments ($P > 0.34$; Fig. 3, Tables 1 and 2).

The relative increase in stem number (first to the second year) was higher in the invasive than in the native species (Fig. 4). This effect was stronger in the plots with slugs than in the slug exclosure plots, especially for the clonal species (R. austriaca, C. draba; Fig. 4, Table 2). The relative increase in leaf number showed a similar but non-significant trend ($P > 0.12$; Fig. 4, Table 2).

**Table 2. Results of two-way ANOVA (common garden experiment).** Treatment factors are status of the species (invasive vs. native species with nested species factor) and slug density (slugs absent, natural slug density, slugs added). Shown are $F$-values of main and interaction effects on vegetative plant traits. Other parameters as in Table 1.

<table>
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<td>8.9*</td>
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<td>0.6</td>
<td>0.7</td>
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Figure 3. Effects of slug treatments on number of leaves and stems developed in the first and in the second year and on mortality, number of flowering plants, dry mass and root diameter in the second year (common garden experiment; plot means ± SE; n = 3 replicate plots). Ba, Barbarea vulgaris; Bu, Bunias orientalis; Ca, Cardaria draba; Di, Diplotaxis tenuifolia; Is, Isatis tinctoria; Ro, Rorippa austriaca. Other parameters as in Fig. 2.
Chapter 4

Seedling and rootling bioassays

The dominant slug species recorded in and around the trays exposed to ambient slug herbivory (common garden) were *A. lusitanicus* (Mabille) and *D. reticulatum* (Müller). Other species found in small numbers included *D. leave* (Müller), *A. distinctus* (Mabille) and *Boettgerilla pallens* (Simroth). The impact of mollusc grazing was high, with between 20 and 95% of seedlings of the plant species showing some damage or death (Fig. 5). However, there were no significant differences between native and invasive species in the incidence of damage and death (*P* > 0.31; Table 3). Both slug species fed most *Bunias orientalis* and *R. austriaca* seedlings and avoided seedlings of *C. draba*.

![Figure 4. Effects of slug treatments on relative increase of number of stems and number of leaves between July 2001 and July 2002. Other parameters as in Fig. 3.](image-url)
In all climate chamber experiments slug herbivory caused considerable damage to the seedlings. Although the experimental conditions were different from those of the seedling experiment in the common garden, the results were largely consistent (Figs. 5 and 6); however, *I. tinctoria* and *D. tenuifolia* received much less damage in the garden than in the climate chamber and the numbers of seedlings killed were lower for all species (Figs. 5 and 6). There were also some differences in slug feeding preferences between the common garden experiments, which used adult plants, and the bioassays, which used young plants. Once again, there was no evidence for a general preference of invasive species over native, or vice versa (*P* > 0.29; Table 3).

In the invasive clonal species *C. draba* and *R. austriaca* the percentage of damaged rootlings was higher in all experiments than the percentage of damaged seedlings. However, mortality of rootlings of both species was much lower than that of seedlings (Figs. 5 and 6).
Table 3. Results of one-way ANOVA (seedling bioassays). Treatment factors are status of the species (invasive vs. native species) or species, respectively (d.f. = 1 for status; d.f. = 5 for species). There were no interaction effects. Other parameters as in Table 1.

<table>
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<td></td>
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<tr>
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<td>13*</td>
</tr>
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<tr>
<td>Climate chamber</td>
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<td>15***</td>
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</tr>
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</tr>
<tr>
<td>Species</td>
<td></td>
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</tbody>
</table>

Figure 6. Percentage of damaged or killed seedlings in the climate chamber herbivory experiments with seedlings of all species and rootlings of C. draba and R. austriaca, respectively (means ± SE; n = 19). Black bars, A. lusitanicus; grey bars, D. reticulatum; filled bars, killed seedlings; hatched bars, damaged seedlings. Other parameters as in Fig. 3.
LEAF DISC PALATABILITY ASSAYS

The consumption index varied according to plant species between 5 and 25 for *A. lusitanicus* and between 40 and 180 for *D. reticulatum* (Fig. 7). *Deroceras reticulatum* showed greater selectivity between plant species than did *A. lusitanicus*. The species rankings differed somewhat from the other experiments, with *D. tenuifolia* being much more palatable. However, the results are consistent in showing no differences in palatability between native and invasive groups of species (*A. lusitanicus: F*₁,₃ = 0.4, *P* = 0.55; *D. reticulatum: F*₁,₃ < 0.1, *P* > 0.92).

![Figure 7. Leaf palatability of the study species (leaf disc bioassay) based on the consumption index of *A. lusitanicus* and *D. reticulatum* (means ± SE; *n* = 10). Other parameters as in Fig. 3.](image)

**Discussion**

In most palatability experiments with slugs, individual test animals have been offered only one plant species (as leaves or leaf discs or leaf extracts), and palatability has been calculated relative to a highly acceptable reference species (e.g. Grime *et al.* 1968, Whelan 1982, Briner & Frank 1998). Such bioassays have the advantage that conditions can be precisely controlled, but they are highly artificial and may tell us rather little about the role of herbivory under field conditions (Hanley 1998, Fenner *et al.* 1999). For example, the leaf discs may be
more palatable to herbivores because attractive odours are released from the cut edges or because physical barriers such as spines or tough leaf edges are removed (Grime et al. 1968). This may explain why D. tenuifolia, which has rather small, hard leaves, was one of the preferred plant species in the leaf disc experiment (cf. Grime et al. 1968, Coley 1987) while it was not heavily damaged in the common garden and seedling experiments. The advantage of leaf disc experiments linked with garden experiments is that leaf palatability can be evaluated isolated from other influencing factors such as plant morphology.

The choice of slug species is also important, and considerable differences have been reported in feeding preferences, especially between slugs from the genera Deroceras and Arion (Cates & Orians 1975, Jennings & Barkham 1975). However, these differences between species appear to be less clear under more natural conditions with established plants. In our common garden experiment, different proportions of slug species had little impact upon the relative performance of native versus invasive species. The highly consistent results between plots with natural slug densities and plots with added slugs also indicate that the chosen slug densities produced levels of grazing activity comparable to those occurring naturally.

**Susceptibility to Slug Herbivory**

Although we found large differences in the attractiveness of different Brassicaceae species to slugs, there was no evidence that slugs consistently preferred native Brassicaceae species over invasive ones or *vice versa*. These results correspond well with the results of a parallel study that revealed no differences in slug herbivory between native and invasive provenances of four Brassicaceae species (see Chapter 5). In addition, Frenzel & Brandl (2003) showed that insect communities attacking alien and native Brassicaceae in Europe are more or less similar in respect to abundance, species richness, guild structure and community similarity across host species. That suggests that these results not only apply for slugs but also for arthropods.

What factors could explain variation in palatability between the species studied? Leaf hairiness appears to be unimportant, since both the invasive *B. orientalis* and the native *I. tinctoria* have hairy leaves but were highly acceptable to slugs. This is consistent with previous studies that showed no reduction of mollusc herbivory in species with hairy leaves (Grime et al. 1968, Dirzo 1980, Cook et al. 1996). However, damage was related to plant
height: the tallest species *B. orientalis*, *I. tinctoria* and *R. austriaca* (stem height < 120 cm; Lauber & Wagner 2001) received more damage than *B. vulgaris* (stem height < 90 cm), and this in turn was grazed more than the shortest species, *D. tenuifolia* and *C. draba* (stem height < 60 cm). That larger plants/ramets tend to be grazed more than smaller plants has also been shown by Hulme (1994) and Piqueras (1999).

Most Brassicaceae produce a range of glucosinolate compounds (Fahey et al. 2001), some of which are known to function as feeding deterrents to snails (Newman et al. 1992) and slugs (Moens 1989, Giamoustaris & Mithen 1995, Byrne & Jones 1996). Allocation of resources to chemical defence is widely seen as a trade-off associated with reduced growth rates for mature plants (Herms & Mattson 1992, Vrieling & van Wijk 1994). Our results suggest that there may be a lower investment in chemical defence in taller growing species, possibly as the result of such a trade-off.

**Compensatory Growth**

Compared to the native species, the invasive species showed stronger compensatory growth responses (and even overcompensation) after slug herbivory. This may result from their higher growth capacity and, especially in the case of the clonal species, their drawing upon resources stored in lateral roots (cf. Dietz & Steinlein 2001, and references therein). *Cardaria draba* was the least palatable species among the invasives. Although this species has lower growth rates and is less invasive than *R. austriaca* and *B. orientalis* in western central Europe (cf. Woitke 2001) it may, like many other species with low RGR (Coley et al. 1985, Jing & Coley 1990, Westoby et al. 1997), allocate more resources to anti-herbivore defence; under high herbivore pressure this could compensate, at least in part, for its comparatively weak growth.

In previous studies it was shown that without occasional disturbance *B. orientalis* and *R. austriaca* tend to be outcompeted by native ruderal forbs (Dietz et al. 1998, 1999, Woitke 2001, Woitke & Dietz 2002). Thus, high slug populations could promote the survival and establishment of invasive Brassicaceae by suppressing native species which are less able to compensate for tissue losses due to grazing.
The role of seedling versus rootling recruitment

In seedlings, secondary chemistry may particularly important in protecting plants against herbivores, since physical defences are likely to be less effective at this stage (cf. Moens 1989, Glen et al. 1990). We found large differences between species in the levels of damage to seedlings but these were not related to the status of the species. This suggests that there are no consistent differences in the effective chemical defences present in seedlings of native and invasive species.

The species grown from root fragments (C. draba and R. austriaca) were more heavily damaged than those grown from seed. It may be that the seedlings were less nutritious than rootlings, or they contained higher concentrations of defensive compounds, or both may have been true (cf. Fenner 1999, Wallace & Eigenbrode 2002). However, despite greater damage, mortality of rootlings was lower, suggesting that species with the ability to regenerate from plant fragments are at an advantage under strong pressure from generalist herbivores. A high ability to disperse as plant fragments may be one reason for the success of many invasive species (e.g. C. draba, R. austriaca, Elodea canadensis and Reynoutria spp.; Vogt Andersen 1995, Dietz et al. 2002).

Conclusions

The herbivory effects obtained in our study were relatively consistent across life stages and different experimental approaches, and the results can be regarded as robust. We found no indication that species of Brassicaceae invasive in central Europe are less attacked by slugs than related native species. However, as there was strong within-species variation, low herbivory levels on some invasive species may still contribute to their invasiveness. Some invasive species may also benefit from a high capacity to regenerate from plant fragments, since the young plants produced in this way are less vulnerable to generalist herbivores than are seedlings. Strong compensatory growth after herbivory may be yet another trait that enhances the invasiveness of introduced Brassicaceae. Both an ability to regenerate from plant fragments and high compensatory growth may be especially advantageous traits in
Slug herbivory on native and invasive Brassicaceae

disturbed ruderal habitats where there is strong grazing pressure from molluscs (cf. Frank 1998).

**Acknowledgements**

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plant species to the slug *Agriolimax caruanae*. *Journal of Ecology, 68,* 981-998.
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reticulatum*, in oilseed rape grown beside sown wildflower strips. *Agriculture, Ecosystems & Environment, 67,* 67-78.
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palatability using the snail *Cepaea nemoralis* L. *Journal of Ecology, 56,* 405-420.


Chapter 5

Variation in growth pattern and response to slug damage among native and invasive provenances of four perennial Brassicaceae species

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Summary

1 It is often assumed that invasive introduced plants escape control by herbivores and pathogens in the new area. The EICA- (Evolution of Increased Competitive Ability) hypothesis suggests that this release from natural enemies and pathogens results in higher vigour of invasive plants by selection of less defended but rapidly growing genotypes.

2 Because slug diversity and abundance appear to be low in North America compared to Europe, especially in the continental region, we hypothesised that release from slug herbivory contributes to the invasiveness of European Brassicaceae species in North America.

3 In common garden and glasshouse experiments we compared life history and fitness parameters in native (NP) and introduced (IP) provenances of four invasive Brassicaceae species (Barbarea vulgaris, Bunias orientalis, Cardaria draba, Rorippa austriaca) that were subjected to slug herbivory by Arion lusitanicus. In climate chamber bioassays we investigated slug damage to seedlings and leaf discs using the same sources of plant material.

4 In all species except B. orientalis we found significant but not always consistent differences in growth and reproductive characteristics between IP and NP plants. Plants of B. vulgaris and R. austriaca from the introduced range had a higher growth rate than those from the native range. While IP plants of the non-clonal B. vulgaris allocated more resources to seed production than NP plants, the IP plants of the clonal R. austriaca showed a decreased number of seeds.

5 Contrary to our initial hypothesis, there were no differences between NP plants and IP plants in the number of damaged leaves and leaf area consumed by slugs, nor in the proportion of seedlings damaged and killed. There were also no interaction effects between slug treatments and provenance.

6 The results suggest that there are genetically based differences in growth and reproductive parameters between NP and IP plants which could be due either to founder effects or to post-introduction evolution. As there were no differences in herbivore damage between the provenances we conclude that this genetic differentiation is probably not due to differences in levels of herbivory but rather to other factors such as competition.
Introduction

It has often been observed that plants grow more vigorously and attain larger size in introduced areas than conspecifics growing in the native range (Noble 1989; Blossey & Nötzold 1995; Crawley et al. 1996; Rees & Paynter 1997; Leger & Rice 2003; Jakobs et al. 2004; but see Thebaud & Simberloff 2001; Simons 2003). Amongst hypotheses proposed to explain this phenomenon are, first, that release from natural enemies and pathogens results in higher plant vigour (e.g. Coley et al. 1985; Herms & Mattson 1992; Fowler et al. 1996; Keane & Crawley 2002; Wolfe 2002) and, second, that poorly defended but rapidly growing genotypes are selectively favoured in the absence of herbivores (Evolution of Increased Competitive Ability (EICA) hypothesis, Blossey & Nötzold 1995). To investigate whether differences in selection pressures have resulted in genetic differentiation between plants in the native and introduced range requires comparative experiments under standardised conditions (Wolfe et al. 2004).

There have been rather few such common garden experiments of invasive and native plant provenances (Blossey & Nötzold 1995; Willis et al. 2000; Siemann & Rogers 2001; Leger & Rice 2003; Wolfe et al. 2004). In particular, the EICA-hypothesis has been tested using real or simulated herbivore pressure for only a few invasive plant species (Sakai et al. 2001) and the results are contradictory (e.g. Siemann & Rogers 2003; van Kleunen & Schmid 2003; Bossdorf et al. 2004a, b). Most investigations have either focused on differences in herbivore damage or on differences in growth patterns between provenances, and few have considered both aspects together (but see Wolfe et al. 2004). However, differences in growth between provenances might be unrelated to herbivory or, conversely, differences in herbivore pressure may not be reflected in changes in plant growth, as postulated by the EICA hypothesis.

Plants differ in their palatability to generalist herbivores and in their ability to withstand herbivore damage (Louda et al. 1990; Oliveira Silva 1992; Mutikainen & Walls 1995). Hence, the impact of herbivory on plant growth and fitness may differ between species. It can also differ between plants of the same species but of different ages (for example, due to age dependent accumulation of secondary compounds; Moens 1989). In general, successful invasive species are likely to have a particularly high capacity for compensatory growth and flexible resource allocation (e.g. Schierenbeck et al. 1994). In Europe, and especially in north-western parts where winters are wetter and milder (South 1992), slugs are
among the most important herbivores of low herbaceous vegetation (Rathcke 1985; Rees & Brown 1992; Hulme 1996; Rodríguez & Brown 1998) and can influence both the biomass and the species composition of plant communities (Oliveira Silva 1992; Hanley et al. 1995a; Hulme 1996; Bruelheide & Scheidel 1999; Scheidel & Bruelheide 1999). For plants invading such vegetation, resistance or tolerance of slug herbivory may therefore be important.

In this study we compare growth characteristics and effects of herbivores on four species of Brassicaceae. Several perennial species of Brassicaceae native to south-east or central Europe have increased their range into northern and western Europe, and some are also strongly invasive in North America (cf. Sebald et al. 1993; Jalas & Suominen 1994; Jalas et al. 1996; USDA 2002). Slugs are known to be important herbivores of at least some Brassicaceae species and comparative studies have even shown a preference by some slug species for Brassicaceae over other families, despite the presence of mustard oils (Duval 1973; Cates & Orians 1975; Dirzo 1980; Dirzo & Harper 1980; Rathcke 1985; Rees & Brown 1992; Briner & Frank 1998). Whereas several voracious slug species have long been abundant in Europe, high levels of slug herbivory appear to be a more recent phenomenon in some regions of the US and are mainly related to just one species, Deroceras reticulatum (McCracken & Selander 1980). This slug was introduced approximately 150 years ago and is now expanding its range. In contrast, because of their relatively low abundance slug species native to North America are not usually considered to be serious pests (South 1992).

Against this background we hypothesize that the lower intensity of slug herbivory in the US has contributed to an increased invasiveness of certain European species of Brassicaceae in this new area. In addition, we assume that young plants are not only more vulnerable to slug attack than adult established plants (cf. Dirzo 1980; Hanley et al. 1995b; Frank 1998) but also that the preferences for native and invasive populations vary according to life stage or plant morphology. For example, if there is a trade-off between clonal propagation and seed production in clonal plant populations (Eriksson 1997 and references therein) the seedling and adult stage of clonal species could differ in their palatability due to the relative importance of the one or other stage for invading a new area.

The main questions posed in this study were: (i) are the introduced plants from the US more severely attacked by slugs than their native conspecifics from Europe? (ii) to what degree do
the populations from the introduced area differ from the native ones with respect to life-history characters and fitness? (iii) do any differences between provenances in the impact of herbivores depend upon plant morphology and life stage or upon leaf palatability?

Three types of experiments were performed to investigate these questions. (i) In a common garden ("mesocosm") experiment, established plants were exposed to slug herbivory under field-like conditions, (ii) in microcosm experiments, the susceptibility of seedlings or juvenile plants to slug herbivory was compared, and (iii) in food-choice bioassays, the palatability to slugs of leaf discs was investigated. An important feature of our study was the use of several taxonomically related species differing in their invasiveness. By using this approach we hoped to obtain more general conclusions about the interactions between slug herbivory and plant invasion than could be obtained from the study of a single species (Buschmann et al. 2002).

Materials and methods

Plant species characteristics

We studied four herbaceous species of Brassicaceae - Barbarea vulgaris R.Br., Bunias orientalis L., Cardaria draba (L.) Desv. and Rorippa austriaca (Crantz). Besides sharing the characteristic traits of this rather homogeneous family (Hedge 1976), the species are relatively similar in life-form and habitat preference. All are polycarpic herbaceous perennials with a semi-rosette growth form and relatively deep storage roots, and all have a preference for moderately disturbed, open sites on nutrient-rich calcareous soils (Oberdorfer 1990). However, they differ in the relative importance of vegetative and sexual reproduction (Table 1), the longevity of individuals and the type of the seed bank (Hegi 1986; Dietz & Ullmann 1998; Dietz et al. 2002). Rorippa austriaca and C. draba show low seedling recruitment but can spread clonally by lateral root growth and can also regenerate from dispersed root fragments. In contrast, B. orientalis and B. vulgaris produce abundant seedlings but do not spread clonally. Barbarea vulgaris lives for only a few years and mostly dies after flowering (H. Dietz, unpub. data), while the other species are longer lived (Dietz & Ullmann 1998; Woitke 2001).

All species are native in south-eastern Europe (Table 1). Barbarea vulgaris is also native - or perhaps archaeophytic - in central Europe while B. orientalis, C. draba and R.
*austriaca* have been introduced to at least the western parts of central Europe (Jalas & Suominen 1994; Jalas et al. 1996). All species were introduced to North America in the 19th century or earlier (cf. Voss 1985) where they are invasive to different degrees. However, *B. orientalis* seems to be virtually extinct in the US now (cf. USDA 2002).

**Table 1.** Main regeneration strategy, status of the study species (NP, native provenance; IP, invasive provenance) and collection scheme of plant material of three populations per provenance and species with year of sampling given in parentheses. SE-Eur, southeastern central Europe (SE of Vienna (Austria) in Achau, Laxenburg and Fischamend Markt); W-Eur, western central Europe (S of Würzburg (Germany) in Winterhausen, Thüngersheim and Randersacker); N-Am, North America (SE Michigan (Ann Arbor and Ypsilanti), Illinois (Peoria) and Wisconsin (Madison), all (USA)). The number of plant fragments collected in each population is given in parentheses.

<table>
<thead>
<tr>
<th>Provenance</th>
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<th>Regeneration</th>
<th>Status</th>
<th>Collection scheme</th>
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<td></td>
<td></td>
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<td>adult plants (60 per pop)</td>
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<tr>
<td></td>
<td></td>
<td>Cardaria draba</td>
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<td>fragments of clones (90 per pop)</td>
</tr>
<tr>
<td></td>
<td></td>
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<td>fragments of clones (90 per pop)</td>
</tr>
<tr>
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<td></td>
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<td></td>
<td>Rorippa austriaca</td>
<td>-</td>
<td>IP</td>
</tr>
</tbody>
</table>

**Collection of Plant Material**

Three populations per species and provenance were sampled in typical ruderal sites between 1998 and 2000. Native populations (NP) of *B. orientalis*, *C. draba* and *R. austriaca* were sampled in the Vienna area in Austria (south-eastern central Europe) and of *B. vulgaris* in northern Bavaria (western central Europe). Invasive populations (IP) were sampled in the southern Great Lakes region of the US and in northern Bavaria (*B. orientalis*; Table 1). For each species populations at least 10 km apart were sampled. The detailed sampling scheme is given in Table 1. Sixty to ninety individuals per species and population were grown, either from seed (*B. vulgaris*) or from plant fragments (*B. orientalis*, *C. draba*, *R. austriaca*). The plants were raised in pots of 10 cm diameter filled with commercial potting soil and were kept in an unregulated glasshouse in Würzburg and later in Zurich.
Native and invasive provenances of Brassicaceae

Choice and collection of slug species

For our herbivory experiments we chose to use *Arion lusitanicus* Mabille, one of the most common European slugs and an agricultural pest in some regions (Reischütz 1986; Fechter & Falkner 1989; Frank 1998). This species was found for the first time in North America in 1999 (R. Hammond, *pers. comm.* ) and is not abundant there.

We first used leaf disc palatability assays to test for variation in feeding preference among slug populations sampled at ruderal sites in northern Bavaria and Zurich. There were no differences in the consumption index between slugs from Germany and Switzerland (t-test: \( t = -0.44, \) d.f. = 38, \( P > 0.66 \)). Based on these results, and for practical reasons, only slugs from Switzerland were used for the experiments. These were collected as required from the surroundings of the experimental garden at Hönggerberg in Zurich (Switzerland).

Common garden experiments

The common garden experiment was established in October 2000 and ran for two years. The site was a homogeneous, sunny area in the experimental garden with a base-rich, loamy soil.

'Plant origin' and 'slug presence/absence' were the treatment factors. There were three replicate plots per treatment. Using a split-plot design five plants chosen at random from the three populations of each provenance were planted 25 cm apart in each half of a plot. Care was taken to ensure that the plants from the different provenances were similar in size. The plots were surrounded by slug-proof frames (1 x 1 m², height 30 cm and buried 5 cm into the soil) and were arranged in a regular grid with a spacing of 75 cm between neighbouring frames. After an establishing period of 5 weeks, during which dead individuals were replaced by new ones, five slugs each were introduced to half of the plots, chosen at random, in autumn 2000. A wooden shelter was placed in the middle of each plot to provide a refuge for the slugs and help to maintain their numbers (cf. Keller et al. 1999).

For each plant we measured the following characters related to life-history, phenology and fitness: total number of leaves, number of grazed leaves (measured twice a year in spring and autumn), number of shoots, starting date of flowering, number of fruits formed, number of seeds per fruit, and seed mass (measured once a year in summer). The start of flowering was related to the main flowering period of *Taraxacum officinale* in the area so as to take
account of climatic differences between the years. Twenty fruits of each plant were sampled randomly to measure the mean number of seeds and seed weight.

From March to November the numbers of *Arion lusitanicus* in the slug plots were monitored on a weekly basis and adjusted to five per plot by adding or removing animals as necessary. Any individuals of other mollusce species were removed. In September 2001 molluscicde (metaldehyde pellets) was applied to prevent slugs from entering the slug-enclosure plots.

**SEEDLING AND ROOTLING BIOASSAYS**

The susceptibility to grazing of seedlings and rootlings (i.e. small plants regenerating from root fragments) was tested in garden and climate chamber experiments. The seedlings and rootlings of the clonal species (*C. draba, R. austriaca*) were grown in a greenhouse in Zurich from seeds and root fragments collected from surplus individuals of the plants that were cultured separately. For these plants care was taken to avoid gene flow between individuals obtained from different areas.

The garden experiments were performed in May and September 2002. Plastic flats (45 x 28 x 5 cm³) filled with potting soil were buried into the soil to ground level in the area of the common garden experiments. For each species, 100 seedlings (cotyledon stage) or rootlings from each provenance were planted into one randomly chosen half of each flat. After two days, each flat was assigned randomly to one of two grazing treatments: exposure to ambient grazing pressure by slugs (the numbers of slugs observed in the flats or in the surroundings the species were noted at intervals during the experiment) and a control with no slugs (molluscicide treatment). There were ten flats per treatment and for each plant species. The numbers of damaged and dead seedlings/rootlings were scored after 4 days.

In the climate chamber experiment, seedlings and rootlings were offered to the slugs in plastic boxes (20 x 10 x 5 cm³; 17°C, 12 h light : 12 h dark) in spring 2002. The bottom of each box was covered with 2 cm of commercial potting soil. Forty-eight hours after transplanting the seedlings into the boxes, one slug was introduced to each box and allowed to feed for 24 h. The numbers of damaged and dead seedlings/rootlings were scored at the end of the experiment.
LEAF DISC PALATABILITY BIOASSAYS

A bioassay experiment was conducted in which individuals of *A. lusitanicus* in Petri dishes (Ø = 13.5 cm) were presented with leaf discs from plants of native and invasive origin. The leaf discs were 11 mm in diameter and were cut from mature leaves from all populations of each species. In each Petri dish six leaf discs from both native and invasive populations of the same species were placed alternately in a circular pattern on a moist filter paper. In addition, three larger discs of *Taraxacum officinale*, one of the most palatable plants for slugs (Dirzo 1980; Rathcke 1985; Frank & Friedli 1999), were placed in the centre of each Petri dish as an additional source of food intended to ensure that the experimental material was not totally consumed.

In a previous feeding experiment we found no significant differences in feeding behaviour between fed and unfed slugs, but fed slugs showed a lower variation in the amount of consumed leaf material and a tendency to discriminate more strongly between plant species (cf. Briner & Frank 1998). Therefore, prior to the experiments each slug was placed in a separate plastic box and fed *ad libitum* with *T. officinale* for 24 h (climate chamber; 17°C, 12 h light : 12 h dark). Subsequently, one slug was introduced to the centre of each dish and was allowed to feed for 12 h in the dark (17°C) in a climate chamber. The bioassays were conducted once in autumn and once in spring using adult and juvenile slugs, respectively. There were 20 replicate Petri dishes of each species though death or inactivity of some slugs sometimes reduced the effective number of replicates.

At the end of the experiments the amount of leaf area consumed was determined by image analysis (cf. Dietz & Steinlein 1996). Ten surplus leaf discs per species and provenance were collected and oven-dried at 70°C for 24 h to measure the dry weight. The fresh body weight of slugs was measured before and after the experiment and the data were transformed to dry weight using the calibration of Bullock & Smith (1971). Palatability was expressed as the consumption index *CI* (Waldbauer 1968):

\[
CI = \frac{F}{(T \times W)} \times 1000
\]

where *F* is the amount of food eaten (mg), *T* is the duration of the feeding period (h) and *W* is the mean body weight of the test slug during the feeding period (mg).
DATA ANALYSIS

JMP 5.0 (SAS Institute Inc. 2002) was used for all analyses. For the common garden experiment, ANOVA was used to test the effects of plant provenance and slug density on the plant parameters. Separate tests were run for each species.

For the bioassays t-tests were used to compare differences in the consumption of leaf disc material and the percentage of damaged and killed seedlings between native and introduced plants.

Prior to parametric tests the percentage values were arc sin-transformed.

Results

COMMON GARDEN EXPERIMENT

General differences between the native (NP) and invasive (IP) provenances

In general, the data were highly consistent between years and we present here only results from the second year.

Mortality was low in all species, ranging from 0 to 13% over the course of the experiment. There were also no significant differences in survivorship between IP and NP plants for any species or treatment (P > 0.44, data not shown).

There were no significant differences in the percentage of damaged leaves between NP and IP plants for all species (Fig. 1, Tables 2 and 3, P > 0.5) although Rorippa austriaca showed at one time in point a significant higher leaf damage in the NP plants. However, for all species except Bunias orientalis, plants originating from the introduced area grew more vigorously, i.e. they produced a higher number of leaves or a higher number of stems or both than those originating from the native area (Fig. 2, Tables 2 and 3).
Native and invasive provenances of Brassicaceae

Figure 1. Damaged leaves (%) in spring 2002 (plot means ± SE; n = 3 replicate plots). Also shown are results of ANOVA for the common garden experiment. Treatment factors are provenance of the plants (invasive vs. native plants) and slug density (slug presence or absence). Shown are significant main effects on vegetative plant traits. There were no interaction effects for all species. Grey bars, native populations; black bars, invasive populations; filled bars, slugs absent; hatched bars, slugs present; P, provenance; S, slug density; ***, P < 0.001; **, P < 0.01; *, P < 0.05.

Table 2. Summary table of significant main effects (ANOVA) of provenance of the plants (invasive vs. native plants) and slug density (slugs present vs. slugs absent in plots) on plant performance and fitness parameters. There were no interaction effects. P, provenance; S, slug density ; +, better performance/higher leaf damage of invasive plants/in slug plots, no effect; -, better performance/higher leaf damage of native plants/in slug exclosure plots; double symbols, strong effects.

<table>
<thead>
<tr>
<th></th>
<th>Barbarea vulgaris</th>
<th>Bunias orientalis</th>
<th>Cardaria draba</th>
<th>Rorippa austriaca</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leaf damage (%)</td>
<td>o</td>
<td>++</td>
<td>o</td>
<td>+</td>
</tr>
<tr>
<td>No. of leaves</td>
<td>++</td>
<td>++</td>
<td>o</td>
<td>++</td>
</tr>
<tr>
<td>No. of stems</td>
<td>+</td>
<td>o</td>
<td>o</td>
<td>o</td>
</tr>
<tr>
<td>No. of fruits</td>
<td>++</td>
<td>--</td>
<td>o</td>
<td>o</td>
</tr>
<tr>
<td>No. of seeds/fruit</td>
<td>++</td>
<td>o</td>
<td>o</td>
<td>o</td>
</tr>
<tr>
<td>Seed mass</td>
<td>o</td>
<td>o</td>
<td>o</td>
<td>o</td>
</tr>
<tr>
<td>Date of flowering (days)</td>
<td>+</td>
<td>o</td>
<td>o</td>
<td>o</td>
</tr>
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</table>

In both years the numbers of leaves present were generally low in autumn and differences between the provenances and slug treatments were small. Therefore, we present here only the spring data. In all species the highest numbers of leaves and stems were recorded in the second year. In Barbarea vulgaris the numbers of leaves and stems were significantly higher for IP than NP plants (Fig. 2, Tables 2 and 3). Plants from IP of Cardaria draba had approximately 40% more leaves than the plants from NP but there were no differences in the number of stems. In R. austriaca IP plants had significantly more leaves and stems than NP plants. There were no significant differences between provenances in B. orientalis.
Differences in reproductive parameters between NP and IP plants were more pronounced than those between growth parameters, but the differences were not consistent between species (Fig. 3, Tables 2 and 3). In *R. austriaca* NP plants produced significantly more fruits than IP plants (NP 4100; IP 2530), seed number per fruit did not differ, and seed mass was significantly lower in NP plants (IP 0.016 mg; NP 0.008 mg). In contrast, in *C. draba* seed mass was significantly lower in IP plants. *Barbarea vulgaris* showed significantly higher fruit production in IP than in NP plants; in the ungrazed plots IP plants produced twice as many and in the grazed plots 10 times as many fruits as did the corresponding NP plants. In addition, IP plants produced more seeds per fruit than NP plants in both the grazed and ungrazed plots (Fig. 3, Tables 2 and 3). In *B. orientalis* there were no consistent differences in the reproductive parameters between provenances or treatments.

IP and NP plants did not differ significantly in the percentage of flowering in any species (data not shown) and there were also no consistent differences in flowering phenology between provenances. IP plants of *B. vulgaris* flowered 4 to 10 days earlier than NP plants, while *R. austriaca* showed a difference in the opposite direction. (Fig. 3, Tables 2 and 3).

**Effects of slug treatments**

There were strong differences between the four study species in the level of slug damage. As the plants had a relatively low number of leaves in autumn and because of highly consistent results in spring and autumn (except for *R. austriaca* in which up to 95% of the leaves were affected by slug grazing in autumn 2001) we present only the spring data on leaf damage.

![Figure 2](image-url)
B. vulgaris up to 60% of the leaves were damaged by slugs in spring 2002 while in B. orientalis about 30% and in R. austriaca and C. draba less than 20% of the leaves were damaged (Fig. 1).

Barbarea vulgaris produced more leaves in the ungrazed than in the grazed plots whereas R. austriaca had a significantly higher leaf number in grazed plots (Fig. 2, Tables 2 and 3). Slug herbivory had no significant effect on leaf number in the other two species. The number of stems was mostly not affected by slug damage, though grazed plants of B. vulgaris tended to produce more stems than ungrazed plants (Fig. 2, P < 0.1).

Table 3. Results of ANOVA for Common Garden Experiment. Treatment factors are provenance of the plants (invasive vs. native plants) and slug density (slugs presence or absence). Shown are F-ratios of main effects on vegetative and reproductive plant traits. There were no interaction effects. The results were analysed separately for different points in time and for each species. (***, P < 0.001; **, P < 0.01; *, P < 0.05; d.f. = 1 in each case).

<table>
<thead>
<tr>
<th>Source of variation</th>
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<th>Bunias orientalis</th>
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<tr>
<td><strong>Leaf damage (%)</strong></td>
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<td>Provenance</td>
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<td>0.3</td>
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</tr>
<tr>
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</tr>
<tr>
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<td>1.4</td>
<td>34.7***</td>
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<tr>
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<tr>
<td>Provenance</td>
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<tr>
<td><strong>No. of seeds/fruit</strong></td>
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<td></td>
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</tr>
<tr>
<td>Provenance</td>
<td>12.7**</td>
<td>&lt;0.1</td>
<td>0.1</td>
<td>0.6</td>
</tr>
<tr>
<td>Slug density</td>
<td>0.2</td>
<td>0.5</td>
<td>1.1</td>
<td>0.2</td>
</tr>
<tr>
<td><strong>Seed mass</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Provenance</td>
<td>0.5</td>
<td>0.8</td>
<td>10.1*</td>
<td>9.6*</td>
</tr>
<tr>
<td>Slug density</td>
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<td>0.1</td>
<td>2.1</td>
<td>0.9</td>
</tr>
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<td><strong>Date of flowering (days)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Provenance</td>
<td>5.7*</td>
<td>2.8</td>
<td>&lt;0.1</td>
<td>29.0***</td>
</tr>
<tr>
<td>Slug density</td>
<td>0.6</td>
<td>4.0</td>
<td>5.5*</td>
<td>8.3*</td>
</tr>
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</table>
In some species slug herbivory had significant effects on flowering date and the number of fruits produced. Ungrazed plants of *B. vulgaris* produced significantly more fruits, while ungrazed plants of *R. austriaca* produced fewer fruits (Fig. 3, Tables 2 and 3). Furthermore, in the plots with slugs, *R. austriaca* flowered significantly earlier (49 and 56 days after main flowering of *T. officinale* for NP and IP, respectively) than under slug exclosure (51 and 67 days after main flowering of *T. officinale* for NP and IP, respectively) while the other species showed a tendency for later flowering (1-5 days) in the slug plots (Fig. 3, Tables 2 and 3).

**Figure 3.** Reproductive parameters of the study species in the second year (plot means ± SE; n = 3 replicate plots). Other explanations as in Fig. 1.

**SEEDLING AND ROOTLING BIOASSAYS**

In the climate chamber experiment in May 2002, slug herbivory caused considerable damage to seedlings. Both the percentage of damaged seedlings and the percentage of killed seedlings were similar between provenances (*P > 0.15* for all species) but varied strongly among species (Fig. 4).

In the common garden experiment the commonest slug species in the flats with ambient slug herbivory were *Arion lusitanicus* (Mabille) and *Deroceras reticulatum* (Müller).
Other species found in smaller numbers included *D. leaf* (Müller), *A. distinctus* (Mabille), *Boettgerilla pallens* (Simroth) and *Limax cinereoniger* (Wolf).

Slugs caused considerable damage to, and death of, seedlings in the ambient flats whereas no plants died in the ungrazed controls. Due to the slugs being larger, seedling damage rates were higher in autumn than in spring (IP 30 and 91%; NP 32 and 82%: Fig. 4), but the differences between species were generally consistent not only between the spring and autumn bioassays (data not shown) but also between the common garden and climate chamber experiments. However, *R. austriaca*, a species known to be preferred by the slug *D. reticulatum* (H. Buschmann, unpubl. data), was attacked more in the common garden experiment. Again, there were no significant differences in grazing damage between plants (whether seedlings or rootlings) from IP and NP (*P* > 0.12 for all species).

While in *C. draba* the percentage of damaged seedlings was much higher than the percentage of damaged rootlings, *R. austriaca* showed the opposite pattern. In both species, mortality of rootlings was lower than that of seedlings (Fig. 4).

![Figure 4](image-url)

*Figure 4.* Percentage of killed and damaged seedlings in the common garden seedling bioassay conducted in spring 2002 and in the climate chamber seedling bioassay (means ± SE; n = 10). Grey bars, native populations; black bars, invasive populations; filled bars, killed seedlings; hatched bars, damaged seedlings; *Ba*, *Barbarea vulgaris*; *Bu*, *Bunias orientalis*; *Ca*, *Cardaria draba*; *Di*, *Diplotaxis tenuifolia*; *Is*, *Isatis tinctoria*; *Ro*, *Rorippa austriaca*. 

97
LEAF DISC PALATIBILITY BIOASSAYS

The percentage of leaf-disc area consumed ranged from 18% for *C. draba* to 63% for *B. orientalis*. The consumption index was higher in autumn 2001 (NP 11 and 68; IP 8 and 45) than in spring 2002 (NP 8 and 39; IP 8 and 32; Fig. 5) for all species, and especially for *R. austriaca*. As in the experiments with seedlings, the slugs showed no preference for either NP or IP plants (*P > 0.4* for all species). Differences in the palatability of the various species were consistent with the results of the other experiments.

![Figure 5](image)

*Figure 5.* Leaf palatability of the study species calculated as Consumption index (CI) from the results of the leaf disc bioassays conducted in autumn 2001 and spring 2002 (*means ± SE; n = 15*). Grey bars, native populations; black bars, invasive populations.

**Discussion**

**Main and interaction effects of provenance**

Contrary to our expectations, there were no general differences in slug damage between provenances of the four Brassicaceae species in any of our experiments. Furthermore, for all species and all parameters the interaction between provenance and grazing intensity was not significant. In contrast, there were differences in growth, reproductive effort and/or phenology between provenances and in some cases between slug treatments.

With the exception of *B. orientalis*, plants from IP showed higher performance than conspecifics from NP, with the strongest differences being found in *B. vulgaris*. This species was introduced first in the North-West of the USA and is most invasive there, while *C. draba*
was introduced later and is widespread but not very abundant (see e.g. Voss 1985; USDA 2002). *Rorippa austriaca* was not known before 1941 and is now expanding its range at least in this part of North America (USDA 2002; H. Dietz, *pers. observ.*). It could, therefore, be that *B. vulgaris* has had more opportunity than the three other species to evolve in response to the new conditions in North America, especially since *C. draba* and *R. austriaca* mostly regenerate vegetatively.

Comparative data on growth performance of plants of native and invasive provenance are available for at least 12 other plant species (Blossey & Nötzold 1995; Daehler & Strong 1997; Willis *et al.* 2000; Siemann & Rogers 2001, 2003; Leger & Rice 2003; van Kleunen & Schmid 2003; Vilà *et al.* 2003; Wolfe *et al.* 2004). In five species plants from invasive populations grew larger than those from native provenances, whereas in six species there were no differences, and in one case the growth rate was higher for native plants. Hence, it is clear that no simple generalizations can be made about invasive plants having a higher growth rate, particularly between species that vary strongly in respect to their life history traits.

It is also not possible to generalise about the relative impact of pathogens or herbivores upon plants from native and invasive populations. Siemann & Rogers (2003) found that seedlings from invasive provenances of *Sapium sebiferum* lost a higher amount of leaf area to grasshoppers than native seedlings. In the experiments of Wolfe *et al.* (2004) plants from the introduced range of *Silene latifolia* had a higher susceptibility to fungal infection, fruit predation, and aphid infestation than plants from the native range. Similarly, IP plants of *Spartina alterniflora* showed lower resistance to a specialist herbivore with associated reduced growth and higher mortality (Daehler & Strong 1997); however, the NP plants with higher resistance also showed a higher intrinsic growth rate. Bossdorf *et al.* (2004a) demonstrated a specialist herbivore to cause higher herbivore damage in invasive than in native provenances of *Alliaria petiolata*, but no differences between provenances emerged with respect to plant fitness after simulated herbivory. In contrast, in a study by van Kleunen & Schmid (2003) using the same simulated herbivory regime for both provenances, native *Solidago canadensis* plants did not grow or reproduce more strongly than IP plants. In addition, Willis *et al.* (1999) could not find significant intra-specific variation in herbivore resistance between NP and IP plants of *Lythrum salicaria*. These diverse results suggest that the
responses of NP and IP plants to herbivory are species-specific and may also depend on the life stages of plants and on the herbivores used.

**What is the basis of the differences between provenances?**

It is unlikely that the observed differences in life history traits between provenances were due to climatic differences (see e.g. Modrzynski & Eriksson 2002; Olsson & Agren 2002; Jakobs et al. 2004) because the plants not only grow in similar habitats but also are found under similar climatic conditions. It is also unlikely that they are the result of maternal effects (Willis et al. 2000) because plants were raised from seeds or root fragments and cultivated for more than one year under the same conditions before being planted into the experimental plots. Furthermore, we only used plants with a similar rosette diameter. For seedling experiments only seeds from the F2-generation were used.

For all these reasons we suppose that there is a genetic basis to the differences we observed between NP and IP plants, as proposed by the EICA hypothesis. Only *B. orientalis* showed almost no differences between NP and IP plants, probably because of the relatively small separation between the two study provenances in Europe. Furthermore, both sets of plants occur in very similar environmental conditions and are exposed to a similar suite of herbivores. For the other species, our results also suggest that slug herbivory is not one of the causal factors underlying the genetic differentiation, though this could be because the time since introduction has been too short for any differences in the level of herbivory to be reflected in genetic differences between the native and introduced provenances (cf. Janzen & Martin 1982; Williamson 1996). While the genetic differences may reflect a post-introduction evolutionary change another possibility is that the genetic differences are the result of founder effects (e.g. Sakai et al. 2001).

We suggest that other factors, perhaps different competition conditions in the native and invasive ranges, were responsible for the differences between IP and NP plants in our set of species (cf. Vila et al. 2003; Müller-Schärer & Steinger 2003). For instance, *B. orientalis* and *R. austriaca* are rather weak competitors, but show highly plastic growth which helps them to escape from competition with neighbouring plants in Europe and which may be advantageous in unpredictable habitats (Dietz & Steinlein 1998; Dietz et al. 1999, 2002; Woitke & Dietz 2000).
If increased plasticity causes costs (Schlichting 1986; Via 1994), one possibility is that it could be advantageous to reduce plasticity under lower competition pressure as probably present in human-disturbed habitats of North America (cf. Mack 1989) and to reallocate the resources to other favourable plant traits. This hypothesis is contradictory to the suggestions that increased phenotypic plasticity in general should be advantageous for the invasiveness of plants in the introduced range (e.g. Sakai et al. 2001) but is supported by some studies. For example, Bossdorf et al. (2004b) showed that native plants of Alliaria petiolata (Brassicaceae) from Europe outcompeted invasive ones from North America. They attributed this result to reduced competition pressure in the new range of the species (evolution of reduced competitive ability, ERCA).

**Effects of herbivores**

A further reason for the lack of differences in grazing damage between provenances may be the choice of slugs as herbivores. Different species and types of enemies can have different impacts on host plants and lead to variable responses (Wolfe 2002), particularly due to feeding on other parts of the plant (e.g. Coleman & Leonard 1995). Bossdorf et al. (2004a) showed that feeding damage to Alliaria petiolata by a specialist weevil (Coleoptera: Curculionidae) was higher on plants of invasive provenance. However, when the authors used a generalist herbivore (caterpillar) there were no differences between plants of native and invasive provenance. In contrast, other studies showed that generalists including slugs and snails can have different impacts on plants taken from the native and introduced range (Cates 1975; Wolfe 2002) and can be suitable herbivores to test the ElCA hypothesis (Siemann & Rogers 2003). In our study, we have some indication that we would found differences due to provenance even if we had used a specialist herbivore. Adults of two species of herbivore specialists on Brassicaceae occurred spontaneously in the common garden experiment; one was the flea beetle *Phyllotreta atra* (F.), found on *B. vulgaris*, and the other was *P. undulata* (Kutsch.), found on *R. austriaca*. In neither case, however, did the percentage of damaged leaves due to flea beetle herbivory vary between the NP and IP plants (in both cases between 20 and 40%) though there were differences between species.
Although there were no differences in slug damage between provenances the amount of slug herbivory varied considerably among species. Furthermore, the differences were fairly consistent between the various experiments showing the robustness of the results. These results correspond well with those of a parallel study that revealed high variation in slug herbivory among six Brassicaceae species but no significant differences between invasive and native species in central Europe (H. Buschmann, unpubl. manuscript). Most Brassicaceae produce a range of glucosinolate compounds (Fahey et al. 2001), some of which are known to function as feeding deterrents to slugs (e.g. Giamoustaris & Mithen 1995; Byrne & Jones 1996). Allocation of resources to chemical defence is widely seen as a trade-off associated with reduced growth rates for mature plants (Herms & Mattson 1992; Vrieling & van Wijk 1994). Our results suggest that there may be a lower investment in chemical defence in faster growing species as R. austriaca, possibly as the result of such a trade-off.

There was also considerable variation between species in their response to slug herbivory. Barbarea vulgaris showed a decreased number of seeds and delayed flowering after slug damage and was obviously not able to compensate for the loss of resources due to leaf area removal. Decreasing fecundity and later flowering after removal of leaf material, have been reported in several other studies with non-clonal species, including some using slugs as herbivores (e.g. Cates 1975; Dirzo & Harper 1980; Crawley 1989, and references therein; Sheppard & Vitou 2000; Prieur-Richard et al. 2002; Strauss et al. 2002). In contrast, the clonal species C. draba and R. austriaca, had the possibility to compensate for slug damage, probably drawing on stored resources in their lateral roots (cf. Dietz & Steinlein 2001, and references therein).

THE ROLE OF SEXUAL VERSUS CLONAL PROPAGATION

Our data provide support for the idea of a trade-off between clonal propagation and seed production in clonal plant populations, as suggested by Eriksson (1997). In R. austriaca and C. draba, which propagate mainly by lateral roots, higher growth in the invasive populations was associated with lower fecundity or lower seed mass, suggesting that IP plants of these species tend to allocate more resources to growth rather than to sexual reproduction. Although the growth of lateral roots was not explicitly measured in our study we assume that higher vegetative growth indicates a higher investment to clonal propagation (cf. Konvalinková
Native and invasive provenances of Brassicaceae

2003). Similarly, the clonal grass Poa bulbosa exhibits mainly sexual reproduction in the native range, whereas it reproduces predominantly vegetatively in its introduced range (Novak & Welfley 1997). Pysek (1997) listed a number of factors promoting the invasion process of clonal plants compared to non-clonal plants. Among other things he pointed out that clonal spread is not delayed, as is reproduction by seed, by a more or less protracted pre-reproductive phase. A further argument that is supported by our results could be that rootlings are less vulnerable than seedlings to predation. Although a higher proportion of rootlings was damaged, at least in C. draba, we could show for both species that their survival after herbivory was higher than that of seedlings. It may be generally the case for invasive plants that root propagules are favoured over seedlings, especially in the early phase of an invasion and where there is strong pressure from generalist herbivores. Seedling recruitment may become more important later in the invasion process because it enables long-distance dispersal and the occupation of new habitats, for example, to escape from local crowding (e.g. Nishitani et al. 1999). In contrast to our results, Auge & Brandl (1997) showed a high level of seedling recruitment in Mahonia aquifolium, a clonal shrub invasive in Europe; in this study up to 50% of new ramets in an invaded area originated from sexual reproduction. In addition, Jakobs et al. (2004) demonstrated that populations of the clonal Solidago gigantea invading Europe had both a higher seed production and a higher vegetative growth. A possible explanation would be that the clonal Brassicaceae species (C. draba, R. austriaca) are rather at the beginning of their invasion in America (e.g. Voss 1985; USDA 2002) while S. gigantea, for example, was introduced to Europe 250 years ago and started spreading after 100 years (Jakobs et al. 2001). Because there has been little time for microevolution to occur, C. draba and R. austriaca rely on dispersal through root fragments rather than on dispersal through seeds, as described by Dietz et al. (2002) for R. austriaca. Alternatively, competition pressure that is possibly reduced in North America compared to Europe in habitats where these species mainly occur (cf. Ratcliffe 1984; Baker 1986; Mack 1989; Bossdorf et al. 2004) could favour clonal propagation of invasive plants in North America due to diminished need for escaping local crowding.
Conclusions

Our results show that for three out of the four species studied, the vegetative performance of plants from invasive provenances is higher than that of plants from native provenance. While these findings are consistent with the EICA hypothesis, there were no indications in any of the bioassay experiments of a higher attractiveness of the IP plants to herbivores as postulated by that hypothesis. These highly consistent findings between the various experiments show the robustness of the results.

Possibly, the time since introduction has been too short for differences in herbivore pressure to have resulted in a loss of defensive traits. Alternatively, other factors like different pressures from competition in the native and invasive range could be responsible for an evolutionary adjustment which is unrelated to herbivory. Reduced competition pressure could favour vegetative propagation in clonal plants at least during the early phase of invasion.

It is not yet possible to generalise about the importance of release from herbivory as a factor promoting invasion. To get a better understanding we need investigations of more plant species and families subjected to a variety of types of herbivory. There is a need for more comparative experimental studies to investigate the influence of antagonists (generalist and specialist herbivores and pathogens) on growth and reproduction of plants at different life stages and exhibiting contrasting reproductive strategies.

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References


Native and invasive provenances of Brassicaceae


Chapter 6

Overall conclusions

The results of the thesis demonstrate the complex role of herbivores in plant community dynamics and, in particular, plant invasions. Straightforward results on first sight, such as the strikingly lower leaf damage in invasive alien trees on the Seychelles as compared to native trees, represent just a first indication of the possible role enemy release (ER) could have for invasion success. Contrasting results from other studies (e.g. Agrawal & Kotanen 2003; Mitchell & Power 2003) suggest that the presence or absence of ER is highly system-specific. Moreover, species as Rorippa austriaca that show comparatively high herbivore damage may not suffer more than other species because of a particularly high capacity of regrowth. Hence, higher herbivore damage does not necessarily translate into reduced fitness. Conversely, the presence of effects predicted by the EICA hypothesis such as higher growth in invasive provenances of plants may be unrelated to variation in herbivore pressure between the native and introduced area as indicated by the results of this thesis. The data suggest that herbivores may have indirect effects on plant invasions in developing plant communities if the invasive plants are comparatively weak competitors such as the Brassicaceae forbs (e.g. Woitke & Dietz 2002). In herbaceous vegetation, due to increased feeding by slugs on highly competitive species during the course of succession, herbivory may favour those species that are weaker competitors but are better able to opportunistically benefit from increased resource levels.

In contrast to the complicated responses of the different Brassicaceae species or provenances to slug herbivory there was high within-species consistence in the responses of different life stages. This consistence was unexpected because seedlings and young plants are most vulnerable to herbivores and especially to slugs while mature plants have the ability to compensate for leaf loss (e.g. Hanley 1998). Hence, established plants should differ from young plants in the level of defence (c.f. Fenner et al. 1999), which could lead to different herbivory levels between different life stages.

Two hypotheses may explain why this thesis showed support for the enemy release hypothesis although there were no differences in the amount of herbivory between invasive alien and native provenances of the same species. First, the time since introduction of the in-
Conclusions

Invasive plants has been too short for differences in herbivore pressure to have resulted in a loss of defensive traits; and second, other factors that differ in the native and invasive range might have a stronger influence in the new range of the plants than herbivory. The second hypothesis seems to be more convincing because the provenances showed genetically based differences in other plant traits such as plant growth and fecundity. Thus, the findings of this thesis are only partly consistent with the EICA hypothesis. Nonetheless, invasive alien species may have an advantage over natives in the presence of herbivores due to their high compensatory ability and their regeneration from small plant fragments that are less susceptible to herbivores than seedlings.

Despite the robust and consistent findings in the experimental parts of the thesis on the role of herbivory in native vs. alien Brassicaceae it is not yet possible to generalise about the importance of release from herbivory as a factor promoting invasion. To obtain a better understanding we need investigations of more plant species and families subjected to a variety of herbivore effects. There is a need for more comparative experimental studies to investigate the influence of antagonists (generalist and specialist herbivores and pathogens) on growth and reproduction of plants at different life stages and exhibiting contrasting reproductive strategies. Moreover, investigations that combine factors possibly contributing to the invasiveness of plants may broaden our knowledge about the phenomenon of invasion. For example, a promising approach would be to study herbivore effects on invasive plants under different competition regimes. As the results of the study suggest, it may be also important to investigate the role of different community contexts on herbivore effects in particular plant invasions.
Conclusions

References


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