Chemical Defenses (Glucosinolates) of Native and Invasive Populations of the Range Expanding Invasive Plant *Rorippa austriaca*

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Abstract Due to global warming, species are expanding their range to higher latitudes. Some range expanding plants have become invasive in their new range. The Evolution of Increased Competitive Ability (EICA) hypothesis and the Shifting Defense Hypothesis (SDH) predict altered selection on plant defenses in the introduced range of invasive plants due to changes in herbivore pressures and communities. Here, we investigated chemical defenses (glucosinolates) of five native and seven invasive populations of the Eurasian invasive range expanding plant, *Rorippa austriaca*. Further, we studied feeding preferences of a generalist and a specialist herbivore among the populations. We detected eight glucosinolates in the leaves of *R. austriaca*. 8-Methylsulfinyloctyl glucosinolate was the most abundant glucosinolate in all plants. There were no overall differences between native and invasive plants in concentrations of glucosinolates. However, concentrations among populations within each range differed significantly. Feeding preference between the populations by a generalist herbivore was negatively correlated with glucosinolate concentrations. Feeding by a specialist did not differ between the populations and was not correlated with glucosinolates. Possibly, local differences in herbivore communities within each range may explain the differences in concentrations of glucosinolates among populations. Little support for the predictions of the EICA hypothesis or the SDH was found for the glucosinolate defenses of the studied native and invasive *R. austriaca* populations.

Keywords Biological invasions · Brassicaceae · *Mamestra brassicae* · *Pieris rapae* · Evolution · Global change

Introduction

Due to globalization and climate change, species distributions are changing, leading to range expansions or range shifts. Consequently, the biotic environments of plants and their interacting communities are altered either by leaving species-specific interactions behind or by meeting new communities in the new range (Parmesan and Yohe 2003; Van der Putten et al. 2010). Some plants that are currently expanding their range toward the poles are becoming invasive in the new environments (Chen et al. 2011; Engelkes et al. 2008). The reason why some plant species become invasive whereas most others do not is still the subject of considerable debate. Several factors are thought to be important, and many hypotheses about mechanisms of biological invasions have been proposed (Catford et al. 2009). These theories were developed mostly for intercontinental invasive plants. Thus far, few studies have tested whether the same hypotheses also apply to intracontinental range-expanding plant species (Lakeman-Fraser and Ewers 2013). One of the prominent hypotheses is the "Enemy Release Hypothesis" (ERH). It assumes that exotic species are freed from their specialist herbivores in the new range and therefore have an advantage over native species (Keane and Crawley 2002). These advantages could be either direct and/or indirect via altered allocation patterns. For example, according to the "Evolution of Increased Competitive Ability" (EICA) hypothesis the loss of enemies will lead to lower defense levels because plants that allocate fewer resources to defenses and more into growth are favored by

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natural selection in the absence of enemies (Blossey and Nötzold 1995).

To study the role of herbivory and plant defenses in plant invasions it is important to incorporate also the function of chemical defenses against specialists and/or generalists. High concentrations of plant secondary compounds can protect plants from non-adapted generalists, but at the same time specialist herbivores may use these compounds as oviposition or feeding cues (Renwick et al. 1992). Such contrasting selection pressures of generalist and specialist herbivores are thought to maintain genetic variation of defenses within and between populations (Van der Meijden 1996). The "Shifting Defense Hypothesis" (SDH) assumes that exotic plants evolve towards shifts in chemical defenses against generalists in the new range, i.e., higher levels of toxins (Doorduin and Vrieling 2011; Müller-Schärer et al. 2004). Fewer specialist enemies may be present in the introduced range that use the metabolites as host finding cues, but generalist herbivores still will be present in the new range. In addition, the composition of toxins also may be under selection by changes in biotic communities. For example, some defense compounds may be active against one generalist species but may not be effective against another generalist species (Macel et al. 2005). If a specific generalist is abundant in a population, selection by herbivores may change the chemical profile towards compounds that are effective against that particular herbivore. Both hypotheses, the EICA and SDH, are based on the assumptions that evolutionary changes take place rapidly in the new range. Support for both is variable. Some studies support the EICA hypothesis, showing lower levels of defenses in introduced populations (e.g., Wolfe et al. 2004; Zangerl and Berenbaum 2005; Zou et al. 2008). However, a recent meta-analysis showed in general that there is little support for the EICA hypothesis (Felker-Quinn et al. 2013). Other studies support the SDH, showing higher levels of defenses in invasive populations (e.g., Cano et al. 2009; Cipollini et al. 2005; Joshi and Vrieling 2005; Müller and Martens 2005). Most of these studies, however, have focused on plants that are invading new continents.

With range expanding invasive plants, the differences between exotic and native populations might not be as pronounced as in exotic invasive plants from other continents. Gene-flow between the native and exotic plant populations might still be present with range expansion, while this is largely absent in intercontinental invaders. Therefore, evolution of the exotic populations, e.g., drift and/or adaptive differentiation, may be happening at a different rate or magnitude (Morrien et al. 2010). Furthermore, some specialist insects may move with the plants or are already present in the new range. Nevertheless, it is likely that the plants will lose part of their enemies from the native range and might encounter new ones. Here, we present one of the first studies that addresses shifts in chemical defenses of intra-continental range expanding plants.

We investigated the concentrations and composition of glucosinolates in the range-expanding Austrian yellowcress, *Rorippa austriaca* (Crantz) Besser (Brassicaceae). This species is native in Central South-Eastern Europe, and has recently been expanding its range to North West Europe and has become invasive along riverine areas in its new range (e.g., Germany, Netherlands) (Haeupler and Schönfelder 1989; Dietz et al. 2002; Bleeker 2003). *Rorippa austriaca* is a perennial species and like most Brassicaceae, it contains glucosinolates as defense compounds. Hydrolysis products of glucosinolates can be repellent and toxic to generalist herbivores (Hopkins et al. 2009). In contrast, they also are used as oviposition cues by some specialist insects (Renwick et al. 1992). How herbivores react to glucosinolates depends on the concentration and the side chain of the glucosinolate e.g., indol, aliphatic, or aromatic (Textor and Gershenzon 2009). Small differences in glucosinolate structure can have a significant impact on the behavior and performance of (generalist) herbivores (van Leur et al. 2008). If *R. austriaca* has escaped from even a small proportion of its enemies in the new range, we might expect an increase in glucosinolates of populations from the new range according to the SDH. Furthermore, we might also expect changes in the composition of the glucosinolates between plants of the native and invasive ranges. As a first step to determine changes in chemical defenses of this range expanding plant, we compared the glucosinolate composition and concentrations in invasive and native *R. austriaca* populations, grown under common greenhouse conditions. We also conducted feeding choice experiments with a Brassicaceae specialist and a generalist herbivore (lab organisms) to test feeding preferences of these herbivores among the native and invasive populations and whether this was related to chemical defenses.

Methods and Materials

Plants and Herbivores Plants of *R. austriaca* from 12 populations of the invasive and the native range were collected in the field between May and October 2011 by digging out parts of the roots. Of each population, ten plants were collected. Five populations were sampled in the Netherlands, two populations in Germany (invasive range), and five populations in the Czech Republic (western edge of native range (Bleeker 2003)) (see Table 1). All field-collected plants were first grown in the same soil, 1:1 mixture of potting soil and sand, in 3 L pots in a common garden in Tübingen (Germany) for 7 months. Each plant then was propagated again via root cuttings at the end of April 2012. Two root pieces of 6 cm length and 3 mm diam of each plant were cut. Each root piece was potted individually in 9×9×10 cm pots with a mix of potting soil and sand (1:1). One set of plant genotypes (120 plants) was grown in a greenhouse under controlled

Table 1 Geographical position, code, and habitat of the analyzed native and invasive *Rorippa austriaca* populations

Region	Country	Population	Code	Latitude	Longitude	Habitat
Native	Czech Republic	Moravské Budějovice	CMB	49°02′47″N	15°48′01″E	ditch
	Czech Republic	Modřany - Na Cikorce	CMC	50°00′35″N	14°24′53″E	grassland
	Czech Republic	Modřany - U jezu	CMJ	50°00′17″N	14°24′07″E	river bank
	Czech Republic	České Budějovice	CCB	48°58′41″N	14°26′41″E	road side
	Czech Republic	Hluboká nad Vltavou	CHV	49°03′24″N	14°26′44″E	grassland
Invasive	Germany	Rastatt	GRS	48°52′15″N	8°12′32″E	grassland
	Germany	Plittersdorf	GPL	48°53′40″N	8°09.08″E	grassland
	Netherlands	Rotterdam	NRD	51°57′58″ N	4°27′15″E	road side
	Netherlands	Meinerswijk	NMW	51°58′56″N	5°52.08″E	river bank
	Netherlands	Millingerwaard	NML	51°51′52″N	5°59′15″E	river bank
	Netherlands	Maassluis	NMS	51°55′49″N	4°13′37″E	river bank
	Netherlands	Wageningen	NWG	51°57′15″N	5°39′24″E	river bank

conditions (20 °C, 16 h L / 8 h D, 30 % humidity) for 6 weeks and used for the chemical analysis. Another 120 plants, the same clones that were used in the chemical analysis, were grown for 15 weeks (April to July) in a greenhouse with variable conditions (15–40 °C, natural light condition) and used for the herbivore preference experiment.

As a specialist herbivore, we used *Pieris rapae* (L.) (Lepidoptera, Pieridae). This species is found in North America and all over Eurasia (Kudrna et al. 2011). Larvae of *P. rapae* feed on many species of the Brassicaceae family (Johansson 1951) and are adapted to glucosinolates (Schoonhoven et al. 2005; Wittstock et al. 2004). As a generalist, we used *Mamestra brassicae* (L.) (Lepidoptera, Noctuidae). *Mamestra brassicae* feeds on 70 different plants of 22 families including Brassicaceae and Chenopodiaceae (Popova 1993). Eggs of both species were obtained from the Laboratory of Entomology of Wageningen University, The Netherlands. The hatched caterpillars were fed organic white cabbage (*Brassica oleracea*) for 9 days before the feeding trials.

Glucosinolate Composition and Concentrations The first two fully grown leaves below the inflorescence of 6-wk-old plants were cut off and put in a paper bag. Because some plants did not grow, we only used 99 plants. The bags were flash-frozen in liquid nitrogen to stop enzymatic activity. Samples were stored at −80 °C until freeze drying. After drying, the dried leaves were ground, and 20–30 mg of each sample were weighed for glucosinolate extraction. Samples were extracted in 80 % methanol, adding sinigrin (2-propenyl glucosinolate, Phytoplan) as internal standard, and glucosinolates were converted to desulfoglucosinolates by a sulfatase according to Agerbirk et al. (2001). Samples were analyzed by high performance liquid chromatography (HPLC 1200 Series, Agilent Technologies, Santa Clara, CA, USA) with a quaternary pump and a diode array detector, using a flow rate of 0.25 ml/min on a Supelcosil LC 18 column (15 cm×3 mm, 3 µm, reverse phase, at an oven temperature of 25 °C. Samples were eluted

with a gradient from water (solvent A) to methanol (solvent B), increasing from 5 to 40 % B within 7 min, to 60 % in 2 min, to 95 % in 4 min, with a hold at 95 % for 4 min and a cleaning cycle.

Peak areas were integrated at 229 nm, and glucosinolates were quantified in relation to the area of the internal standard and using the response factors as described in Brown et al. (2003). Identities were confirmed by the exact masses revealed from ultra-HPLC coupled with a time of flight mass spectrometer (1290 Infinity UHPLC and 6210 TOF-MS Agilent, Technologies, Santa Clara, CA, USA).

Herbivore Preference Tests We tested for herbivore preferences in a choice experiment. Leaf discs (diam: 80 mm^2) were cut from the youngest leaves of six 15-wk-old plants of every population. In a dish (diam: 28 cm^2) 12 leaf discs, one of each population, were put at random positions in a circle on wet filter paper (diam: 26 mm^2) to prevent the leaf discs from drying out. One larva (2nd instar) of the generalist *M. brassicae* or one caterpillar (4th instar) specialist *P. rapae* was placed in the middle of the dish. The dish was covered with a nylon mesh to keep the caterpillars in the dish. This was replicated 15 times for each herbivore. The amount of leaf tissue consumed by the herbivores was observed after 12 hr by quantifying the percentage leaf area removed for each disc.

Statistical Analyses For the data analysis, we used IBM SPSS Statistics 20. The glucosinolate concentration data were log-transformed, to meet the requirements for normality and homoscedasticity, and tested with a Univariate Analysis of Variance (ANOVA). Origin (native vs. invasive) was added to the model as fixed factors, population nested within origin was added as a random factor. *Post-hoc* Bonferroni tests were performed to analyze which populations differed from each other. A PCA (Principal Component Analysis) was performed to analyze differentiation in the glucosinolate profiles. Feeding preference of the herbivores was analyzed with a

Friedman Analysis of Variance on ranks. A Spearman rank correlation test was used to analyze the relation between the averaged percentage feeding and the averaged total glucosinolate concentration of a population for each herbivore.

Results

Glucosinolate Composition and Concentrations Eight glucosinolates were identified in the sampled *R. austriaca* populations (Table 2). Five of them were aliphatic glucosinolate; 6-methylsulfinylhexyl glucosinolate (6MSOH), 7-methylsulfinylhexyl glucosinolate (7MSOH), 8-methylsulfinyloctyl glucosinolate (8MSOO), 5-methylthio-pentenyl glucosinolate (5MPT), and one unidentified. Three compounds were indol glucosinolates; indol-3-ylmethyl glucosinolate (I3M), 1-methoxy-indol-3-ylmethyl glucosinolate (1MOI3M), and 4-methoxy-indol-3-ylmethyl glucosinolate (4MOI3M). The most abundant glucosinolate in all plants was 8MSOO, which represented more than 50 % of the total glucosinolate concentration in most plants, except in plants of one population in the invasive range (NRD). All plants in this population had low total glucosinolate concentrations (Table 2). The individual concentrations varied considerably among the plants within a population, sometimes even 10 fold.

The highest total concentrations were found in native population CMC and the lowest in invasive population NRD (Table 2). Invasive and native populations, however, did not differ significantly in their total or individual glucosinolate concentrations (Table 2). Most variation in the data was due to population and not to origin, i.e., native vs. invasive (Table 2). For two glucosinolates (8MSOO and 1MOI3M), the difference between native and invasive plants was significant when excluding population from the model ($P=0.049$ and $P=0.034$, respectively). However, with population nested within origin in the statistical model, the effect of origin (native vs. invasive) was not significant anymore. Population within origin was almost always significant (Table 2). For most glucosinolates, the invasive population NRD, with low concentrations, was different from the other populations (*post-hoc* tests $P<0.05$). The PCA showed no clustering, indicating that the relative abundance of the individual glucosinolates in the mixture did not differ between populations (data not shown).

Generalist and Specialist Feeding Preference The generalist herbivore *M. brassicae* tended to have a feeding preference for some populations over others (Friedman ANOVA $P=0.057$, Fig. 1). Leaves of the invasive population NRD were consumed more intensively by *M. brassicae* caterpillars, whereas leaves of the native population CMB were consumed the least (Fig. 1). The specialist *P. rapae* did not distinguish between the populations ($P=0.603$, Fig. 2). At the time of the experiment, caterpillars of *P. rapae* were larger than *M. brassicae* and ate overall more of the leaf discs after 12 hr compared to *M. brassicae*. There was a negative correlation between mean total glucosinolate concentrations and percentage feeding by *M. brassicae* ($R_s=-0.70$, $N=12$, $P=0.011$, Fig. 3a). There was no correlation between percentage

Table 2 Mean glucosinolate (GLS) concentrations (µMOL/G DW ± SE) of native and invasive *Rorippa austriaca* populations

Region	Population	N	Total GLS	6MSOH	7MSOH	8MSOO	5MTP	unknown	I3M	1MOI3M	4MOI3M
N	CMB	10	6.02 (±1.33)	0.06 (±0.02)	0.21 (±0.05)	4.84 (±1.05)	0.23 (±0.05)	0.01 (±0.01)	0.16 (±0.08)	0.50 (±0.18)	n.d.
N	CMC	8	10.10 (±2.63)	0.18 (±0.07)	0.18 (±0.06)	8.44 (±2.27)	0.50 (±0.15)	0.13 (±0.07)	0.40 (±0.12)	0.28 (±0.11)	<0.01
N	CMJ	6	5.53 (±1.74)	0.11 (±0.01)	0.26 (±0.09)	4.62 (±1.53)	0.20 (±0.07)	0.03 (±0.001)	0.21 (±0.06)	0.10 (±0.04)	<0.01
N	CCB	9	3.67 (±1.40)	0.01 (±0.01)	0.11 (±0.50)	3.11 (±1.21)	0.19 (±0.06)	0.01 (±0.01)	0.14 (±0.06)	0.10 (±0.04)	n.d.
N	CHV	8	4.31 (±1.46)	0.05 (±0.02)	0.07 (±0.02)	3.80 (±1.32)	0.20 (±0.06)	0.02 (±0.001)	0.10 (±0.04)	0.07 (±0.03)	n.d.
I	GRS	10	6.21 (±1.61)	0.02 (±0.01)	0.15 (±0.04)	4.19 (±1.19)	0.12 (±0.03)	0.04 (±0.01)	0.22 (±0.05)	0.90 (±0.30)	<0.01
I	GPL	10	4.01 (±2.10)	0.05 (±0.03)	0.13 (±0.07)	3.04 (±1.58)	0.22 (±0.16)	0.09 (±0.03)	0.12 (±0.06)	0.36 (±0.24)	<0.01
I	NRD	5	0.59 (±0.15)	0.18 (±0.08)	n.d.	0.29 (±0.08)	0.03 (±0.01)	0.04 (±0.01)	0.02 (±0.001)	0.04 (±0.02)	<0.01
I	NMW	8	6.58 (±2.25)	0.19 (±0.04)	0.12 (±0.08)	5.04 (±1.74)	0.32 (±0.17)	0.05 (±0.01)	0.18 (±0.06)	0.67 (±0.25)	<0.01
I	NML	9	3.02 (±0.93)	0.02 (±0.01)	0.13 (±0.04)	2.13 (±0.71)	0.19 (±0.06)	0.02 (±0.01)	0.19 (±0.07)	0.34 (±0.17)	<0.01
I	NMS	6	4.09 (±1.50)	0.02 (±0.01)	0.32 (±0.13)	3.30 (±1.10)	0.07 (±0.03)	0.09 (±0.04)	0.20 (±0.09)	0.47 (±0.22)	<0.01
I	NWG	9	5.04 (±1.31)	0.07 (±0.02)	0.21 (±0.06)	3.85 (±1.03)	0.20 (±0.04)	0.11 (±0.06)	0.15 (±0.05)	0.46 (±0.17)	n.d.
	F origin		1.21	0.02	0.01	0.21	2.88	1.16	0.24	1.64	0.58
	F populations within origin		**3.73*** **	**7.16*** **	1.91	**3.50***	0.37	1.75	**2.17***	**3.22** **	0.33

Region indicates whether populations where from the native range (N) or invasive range (I). For population codes see Table 1. Codes are given of individual glucosinolates, see methods section for full names. I3M, 1MOI3M, and 4MOI3M are indol glucosinolates, all other aliphates. n.d. = not detected. The two last rows are F values of the factors origin (native vs. invasive) and populations nested in origin (ANOVA, *$P<0.05$, **$P<0.005$, ***$P<0.001$). Population codes as in Table 1

Fig. 1 Feeding preference of the generalist *Mamestra brassicae* on *Rorippa austriaca* populations. Bars indicate the mean amount eaten (%) ± SE of leaf discs of different *R. austriaca* populations by larvae of *M. brassicae* after 12 hs in choice experiments. The solid light grey bars show values of populations from the Netherlands (invasive range), German populations are marked with grey stripes (invasive), and the solid dark grey bars show values of populations from the Czech Republic (native). (Friedman ANOVA *P*=0.057, *N*=15, 12 choices per replicate). Population codes as in Table 1
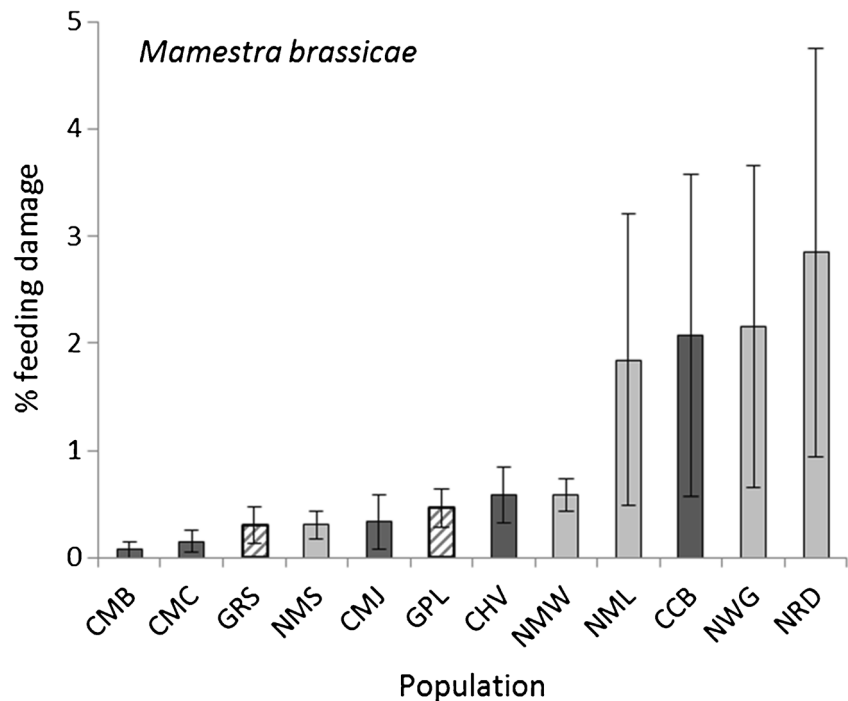
feeding and glucosinolate concentrations for *P. rapae* (R_s=−0.29, *N*=12, *P*=0.35, Fig. 3b).

Discussion

We found no significant overall difference in concentrations of glucosinolates, characteristic defense compounds in the

Brassicaceae, between native and invasive populations of *R. austriaca*. Thus, neither the EICA hypothesis nor the SDH were supported by the results. Variation in glucosinolate concentrations among populations within a range was higher than between invasive and native plants in general. The glucosinolate composition also was similar between populations from the native and invasive range. Of the eight glucosinolates detected, 8MSOO was always the most abundant. Therefore, we found no indication that there is strong divergent selection

Fig. 2 Feeding preference of the specialist *Pieris rapae* on *Rorippa austriaca* populations. Bars indicate the mean amount eaten (%) ± SE of leaf discs of different *R. austriaca* populations by larvae of *P. rapae* after 12 hr in choice experiments. The solid light grey bars show values of populations from the Netherlands (invasive range), German populations are marked with grey stripes (invasive) and the solid dark grey bars show values of populations from the Czech Republic (native). (Friedman ANOVA *P*=0.603, *N*=15, 12 choices per replicate). Population codes as in Table 1
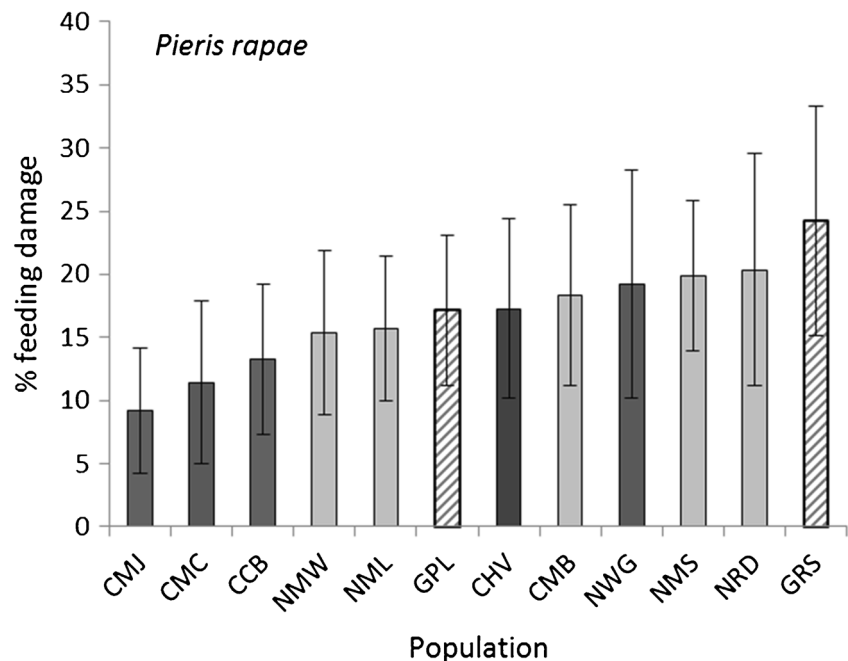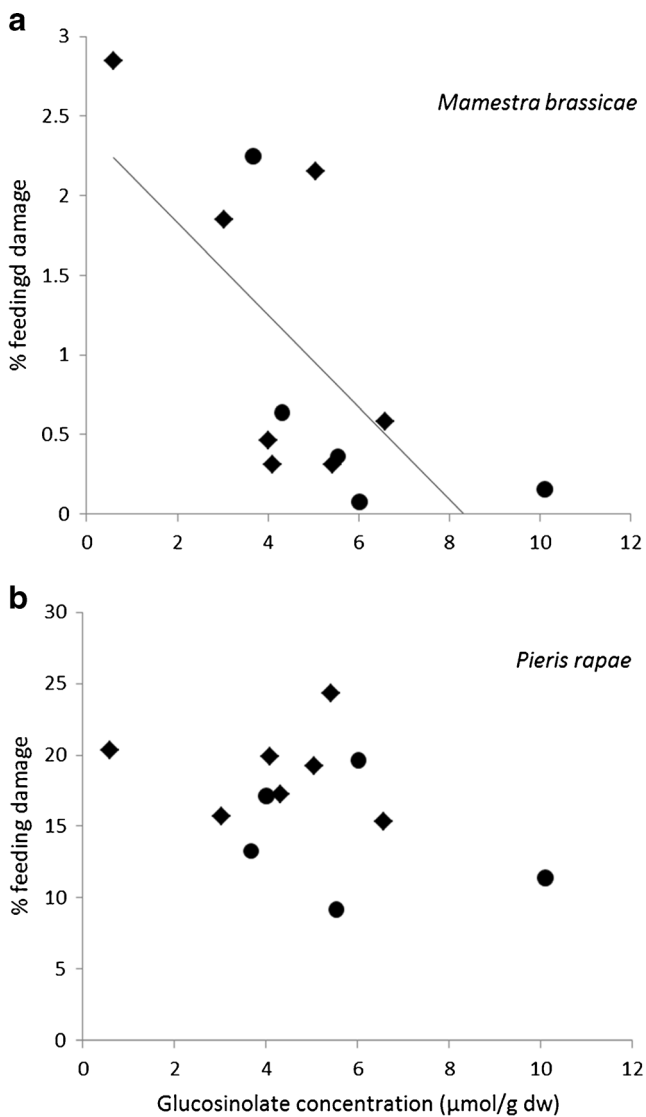
Fig. 3 Relations between glucosinolate levels and herbivore feeding damage. **a** Relation between the average total glucosinolate concentration (μmol/ g DW) and the average percentage eaten of leaves by *Mamestra brassicae* per *Rorippa austriaca* population ($R_s=-0.70$, $N=12$, $P=0.011$). **b** Relation between the average total glucosinolate concentration and the average percentage eaten of leaves by *Pieris rapae* ($R_s=-0.29$, $N=12$, $P=0.35$). Round symbols indicate native populations, squares indicate invasive populations

on glucosinolate composition between the native and the invasive populations of this range expanding plant. We analyzed young leaves, which in general have a relatively high concentrations of defenses, and glucosinolate composition and concentrations in other plants parts could be different (Hopkins et al. 2009).

The differences in glucosinolate concentrations among populations within a range could be due to random processes such as genetic drift and/or founder effects, or due to natural selection (Linhart and Grant 1996). Glucosinolates and their breakdown products play an important role in biotic interactions such as plant – plant and plant – insect/pathogen

interactions (Hopkins et al. 2009). Herbivore composition in the populations also may have an influence on the concentrations of glucosinolates (Mauricio and Rausher 1997; Zust et al. 2012). Therefore, it is probable that the differences in concentrations between populations are due to differential selection on a local level. Here, we showed that there is a negative correlation between glucosinolate concentration in *R. austriaca* and generalist herbivore feeding, while there is no such relationship with the specialist. Thus, high concentrations of glucosinolates that are effective against generalists could be favored by selection in populations where the abundance of generalists is high (Lankau 2007). An overall low glucosinolate concentration, such as in the population NRD, may be due to a lack of generalist herbivores. An investigation of the herbivore communities in the different populations could show whether there are indeed large differences in herbivore presence and abundance between the plant populations. It also is striking that there is a large variation in concentrations of defense compounds among plants within populations. Similar variation also was found in other studies (Harvey et al. 2011; Wolf et al. 2011). Genetic variation of defense levels within a populations is thought to be maintained by contrasting selection of different herbivores, e.g., generalists vs. specialists (Lankau 2007; Van der Meijden 1996) but other biotic factors such as competing plants and/ or microorganisms also may maintain variation of the defense levels (Lankau and Strauss 2007).

The overall lack of difference between populations of the native and the extended invasive range in glucosinolate composition and concentrations could be due to several factors. No differences in defenses between native and invasive populations has been found in various other invasive species (Felker-Quinn et al. 2013), but below we highlight those factors that differ between range expanding invasive plants and invaders from other continents. First, continuing gene flow between the populations in the expansion zone and the source populations may inhibit differentiation between the expanded and the native range. Unlike intercontinental invaders, range expanders are not completely cut off from source populations, which can hamper further differentiation by diluting any effect of local selection (Morrien et al. 2010; Sakai et al. 2001). Second, herbivore communities between the two ranges may not be consistently different, but rather differ on a local scale. Although it is generally assumed that herbivore load and diversity are lower with increasing latitude (Salazar and Marquis 2012), not only plants but also herbivores and other trophic levels may be shifting their range, and thus some of the herbivores may be present in the new range as well (Van der Putten et al. 2010). This also is essentially different from intercontinental invaders where the herbivore community in the introduced range is usually completely different compared to the native range. With range expanders, the shifts in herbivore communities could be more subtle.

Potentially, some of the herbivores in the new range also are adapted to native species of the same genus or to chemically closely related species within the plant family. Native herbivores could be partially adapted to the chemicals that are shared within the genus (family) and that are also present in the exotic species (Strauss et al. 2006). Additionally, many specialist herbivores of Brassicaceae adapted to glucosinolates are relatively cosmopolitan, such as *Brevicoryne brassicae, Pieris rapae, Plutella xylostella* (CABI Invasive Species Compendium www.cabi.org/isc/; Kudrna et al. 2011). Not all specialists, however, necessarily feed on all Brassicaceae species. For example, gall midgets (Cecidomyiidae) were found only on the native *R. sylvestris* and not on *R. austriaca* in its new range (Engelkes et al. 2012), thus indicating species-specific interactions. Finally, it might be due to the fact that the plant has only recently expanded its range northwards (e.g., since the 1970s) and time for evolution to occur has been relatively short. Although *R. austriaca* is perennial, it can produce seeds yearly from the first year onwards, and rapid evolution in invasive plants has been shown to occur within 25 generations (Dlugosch and Parker 2008).

We cannot exclude that other toxins or repellents besides glucosinolates were higher in invasive plants. These were not measured. For example, flavonoids are known to have negative effects also on specialist insects and can be found in plants of the Brassicaceae family (Haribal and Renwick 2001). Furthermore, although glucosinolates themselves are not toxic, their breakdown products can be (Schoonhoven et al. 2005). The enzyme used for this purpose, myrosinase, is stored separately from the glucosinolates. If plant tissue is damaged, glucosinolates and myrosinase come into contact, and the toxic breakdown products are formed. Myrosinase concentrations can have an influence on toxicity of plants for herbivores. If a plant has very high concentrations of myrosinase, glucosinolates are broken down faster than with low amounts of myrosinase (Siemens and Mitchell-Olds 1998). In plants of *Lepidium draba* (Brassicaceae), myrosinase activities were significantly higher in mature plants of the invasive range compared to plants of the native range (Müller and Martens 2005). The breakdown products of the glucosinolates (isothiocyanates, thiocyanates, nitriles), which have less (nitriles) or more (isothiocyanates) toxic effects on herbivores (Bones and Rossiter 1996), should be analyzed in *R. austriaca*.

In an earlier study on herbivore resistance of different *R. austriaca* populations, invasive *R. austriaca* genotypes that were introduced to North America (intercontinental invaders) were compared to plants of the native range. The common slug (*Arion lusitanicus*) did not show any preferences for either native European or invasive American *R. austriaca* genotypes (Buschmann et al. 2005). The authors of this study concluded that although the invasive plants grew bigger, this was not due to a trade-off between defense and growth, although no chemical defenses were measured. Future studies should compare traits between native populations, invasive range expanding European populations, and invasive North American populations to understand the role of evolution in invasion processes, specifically in light of climate change driven range expansions.

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