Little evidence for release from herbivores as a driver of plant invasiveness from a multi-species herbivore-removal experiment

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Enemy release is frequently posed as a main driver of invasiveness of alien species. However, an experimental multi-species test examining performance and herbivory of invasive alien, non-invasive alien and native plant species in the presence and absence of natural enemies is lacking. In a common garden experiment in Switzerland, we manipulated exposure of seven alien invasive, eight alien non-invasive and fourteen native species from six taxonomic groups to natural enemies (invertebrate herbivores), by applying a pesticide treatment under two different nutrient levels. We assessed biomass production, herbivore damage and the major herbivore taxa on plants. Across all species, plants gained significantly greater biomass under pesticide treatment. However, invasive, non-invasive and native species did not differ in their biomass response to pesticide treatment at either nutrient level. The proportion of leaves damaged on invasive species was significantly lower compared to native species, but not when compared to non-invasive species. However, the difference was lost when plant size was accounted for. There were no differences between invasive, non-invasive and native species in herbivore abundance. Our study offers little support for invertebrate herbivore release as a driver of plant invasiveness, but suggests that future enemy release studies should account for differences in plant size among species.

The enemy release hypothesis (ERH) is one of the most widely invoked mechanisms used to explain why some alien plant species become invasive in their introduced ranges reviewed by (Colautti et al. 2004, Liu and Stiling 2006, Chun et al. 2010). Plant species introduced to a new range may escape natural enemies, such as pathogens and herbivores (Keane and Crawley 2002). The absence of natural enemies may increase plant performance compared to the native range, and also relative to native plant species in the new range as the latter should still suffer from their full suite of natural enemies (Colautti et al. 2004). Under the ERH, alien plant species that are sufficiently released from enemy damage to increase performance and fitness, may greatly increase their populations and become invasive. In contrast, aliens that undergo less release from enemy damage do not have the same performance advantage, and are less likely to outcompete native species and become invasive (Keane and Crawley 2002). Thus, for the ERH to be a general rule, only invasive alien, but not non-invasive alien species should benefit through a reduction in enemy damage, which results in greater performance, relative to native species.

A number of studies have compared damage from herbivory on alien and native plant species in the introduced range (Agrawal and Kotanen 2003, Colautti et al. 2004, Agrawal et al. 2005, Chun et al. 2010, Funk and Throop 2010). Others have compared the level of damage by

natural enemies on native, non-invasive alien and invasive alien species (Liu et al. 2007, Parker and Gilbert 2007), or investigated the relationship between viral/fungal pathogen release and invasiveness of alien plant species (Mitchell and Power 2003, van Kleunen and Fischer 2009). These approaches test the ERH partially, in that reduced attack and damage of invasive alien species does not necessarily result in increased plant performance relative to native species, or non-invasive aliens. To fully test the ERH, plant performance in the absence and presence of enemies has to be assessed, which only few studies did so far (Parker and Gilbert 2007, Chun et al. 2010).

Further, the identity of the herbivores may determine the outcome of the ERH (Cripps et al. 2006, Ando et al. 2010, Alba et al. 2012), as it assumes release from specialist herbivores (Keane and Crawley 2002, Mitchell et al. 2006). However, many alien species occur in urban environments (Pyšek 1998), where it is likely that most herbivores are generalists (Niemelä et al. 2011). Whether release from generalist herbivores also contributes to plant invasion remains open: while some studies show that also generalist herbivores avoid alien and alien invasive plant species (Jogesh et al. 2008, Tallamy et al. 2010, Schaffner et al. 2011), others did not find this pattern (Parker and Hay 2005).

Plants growing under higher nutrient levels are likely to be more susceptible to herbivory due to greater tissue

nutrient content (Mattson 1980, Butler et al. 2012). Therefore, plant species that grow in high-nutrient environments and benefit most from increased nutrient availability may suffer greater levels of herbivory than species in low nutrient environments (Coley et al. 1985, Dostal et al. 2013, Lind et al. 2013). The resource–enemy release hypothesis (Blumenthal 2005, 2006) states that alien plants from nutrient-rich environments will benefit more from enemy release. A prediction made by this hypothesis is that if invasive alien species already benefit from enemy release in terms of performance, they may do so to a greater extent under higher nutrient levels compared to more susceptible natives that are prevented from realizing the benefits of the increased nutrient availability by natural enemies.

We tested the ERH by manipulating exposure of native, alien invasive and alien non-invasive herbaceous plant species in Switzerland to invertebrate herbivores. If invasive species already benefit from natural enemy release, they should benefit less strongly from experimentally reduced herbivore exposure compared to native and non-invasive alien species. We recorded 1) the abundance and identity of major invertebrate herbivores, which we subsequently classified according to their feeding preferences as generalists or specialists; 2) leaf damage, and 3) plant biomass in response to enemy exclusion. For biomass, we tested the effect of nutrient availability on the level of enemy release experienced by the plants by exposing plants to low and high nutrient levels. Specifically our hypotheses were that invertebrate herbivore damage and herbivore abundance should be lower in invasive alien than non-invasive alien and native species, and invasive species should show no or little decrease in herbivore damage and abundance when treated with pesticide, while non-invasive and native species should exhibit significantly lower herbivore damage/abundance when treated with pesticide. In addition, plant performance (biomass) of invasive species should show no or little increase when treated with pesticide, while non-invasive and native species should show a larger increase in biomass. We also expect the differences in effects of pesticide treatment between native and non-invasive species, and invasive species to be more pronounced with the addition of nutrients.

Material and methods

Study species

Seeds of 29 species were collected in 2008 and 2009 from plants in wild populations throughout Switzerland (Table 1). These 29 species included fourteen native, eight non-invasive alien and seven invasive alien species within Switzerland, and represent six taxonomic confamilial groups (Table 1). Species belonging to the Plantaginaceae, Phrymaceae and Scrophulariaceae, which until recently all belonged to the Scrophulariaceae, were considered one group, and the Asteraceae species were split into two groups (*Bidens* genus and non-*Bidens* species). Except for five native species, all other species occur in nitrophilous plant communities (Landolt et al. 2010; Supplementary material Appendix 1 Table A1). For the non-*Bidens* Asteraceae group, the native *Solidago*

Table 1. The 29 species used in this study, their status, and the corresponding code per species shown in the figures. Numbers in parentheses for Asteraceae species are sub-block groupings for this family.

Species	Family	Status
Eryngium giganteum*†	Apiaceae	alien non-invasive
Oenanthe lachenalii*†	Apiaceae	native
Heracleum mantegazzianum*†	Apiaceae	alien invasive
Artemisia borealis*†	Asteraceae (1)	native
Artemisia vulgaris	Asteraceae (1)	native
Aster lanceolatus*†	Asteraceae (2)	alien non-invasive
Cirsium montanum*†	Asteraceae (2)	native
Conyza canadensis*†	Asteraceae (2)	alien invasive
Gnaphalium luteo-album	Asteraceae (1)	native
Inula helvetica	Asteraceae (2)	native
Rudbeckia hirta*†	Asteraceae (1)	alien non-invasive
Senecio inaequidens*†	Asteraceae (1)	alien invasive
Solidago canadensis*†	Asteraceae (2)	alien invasive
Solidago virgaurea*†	Asteraceae (2)	native
Bidens radiata*†	Asteraceae (*Bidens*)	native
Bidens tripartita*†	Asteraceae (*Bidens*)	native
Bidens bipinnata*†	Asteraceae (*Bidens*)	alien non-invasive
Bidens frondosa*†	Asteraceae (*Bidens*)	alien invasive
Brachypodium sylvaticum†	Poaceae	native
Eleusine indica†	Poaceae	alien non-invasive
Panicum capillare†	Poaceae	alien invasive
Rumex maritimus*†	Polygonaceae	native
Rumex obtusifolius*†	Polygonaceae	native
Persicaria orientalis*†	Polygonaceae	alien non-invasive
Mimulus guttatus*†	Phrymaceae	alien non-invasive
Scrophularia nodosa*†	Scrophulariaceae	native
Veronica anagallis-aquatica*†	Plantaginaceae	native
Veronica peregrina*†	Plantaginaceae	alien non-invasive
Veronica persica*†	Plantaginaceae	alien invasive

*Species used in leaf damage analyses. †Species used in analysis of herbivore presence/abundance. The Phrymaceae, Plantaginaceae and Scrophulariaceae species were considered as one taxonomic group.

virgaurea was used to assess herbivory damage, while the natives *Gnaphalium luteoalbum* and *Artemisia vulgaris* were used to assess biomass responses to pesticide and nutrient addition treatments, due to limited numbers of plants available. Thus, for native non-*Bidens* Asteraceae species, herbivory damage and biomass responses to pesticide and nutrient treatments are not directly comparable. Expert opinion from the National Data and Information Centre of the Swiss Flora, and the associated Info Flora website (<www.infoflora.ch/de/flora/art-abfragen.html>) were used in order to assign species status as native, alien invasive or alien non-invasive, with invasive species generally being more widespread in Switzerland according to Atlas records (Supplementary material Appendix 1 Table A1).

Experiment set-up

Seeds were planted as individual seed families collected from mother plants, in a 1:1 mixture of seedling compost and sharp sand in the third week of April 2010 in a greenhouse. After germination, individual seedlings were transplanted to

separated compartments within trays, filled with a 1:1 mixture of alluvial soil and sharp sand. These seedlings were then grown for a further 4–5 weeks until the end of June 2010.

We set up a common-garden experiment in a 1000 m² field in Bern, Switzerland, surrounded by short grassland and gardens (i.e. the type of habitat where many plant invasions have started). Eight experimental blocks were set up (in a four by two configuration), and each block was split into two halves (Fig. 1). Plants in each block were represented by one seed family per species, to minimise the contribution of genetic differences to variation among plants in different treatments within blocks. One half of each block was later randomly assigned to the herbivore exclusion treatment (see below), and the block-halves were separated by 2 m. Each block-half contained seven sub-blocks (Fig. 1), and sub-blocks were paired according to their position across the block-halves. One pair of sub-blocks was randomly assigned a taxonomic group per block (Fig. 1; the non-*Bidens* Asteraceae group was split into two plots, giving seven instead of six sub-blocks in total, Table 1). Two plants per species per sub-block were planted individually in 3-l pots, with the same soil as the seedling trays. Pots within each sub-block had either no nutrient addition or nutrient addition applied once prior to planting (12 g of slow-release NPK fertiliser pellets; N:P:K 16:9:12 + 2MgO + trace elements). The soil had a nitrogen content of 1.79 g kg⁻¹ of dry weight substrate (5.83 g N pot⁻¹), and nutrient addition resulted in a 33% increase in nitrogen concentration. These plants were used for assessment of plant performance in response to herbivore exclusion and nutrient addition (hereafter referred to as the performance set). An additional plant per species in each taxonomic group per sub-block was planted in a 3-l pot containing the same soil as other plants, and without nutrient addition. These plants were subsequently used to record rates of herbivore damage and invertebrate herbivores (hereafter referred to as the herbivory set). The total numbers of performance plants without nutrient addition and with nutrient addition, and plants used for measuring herbivory
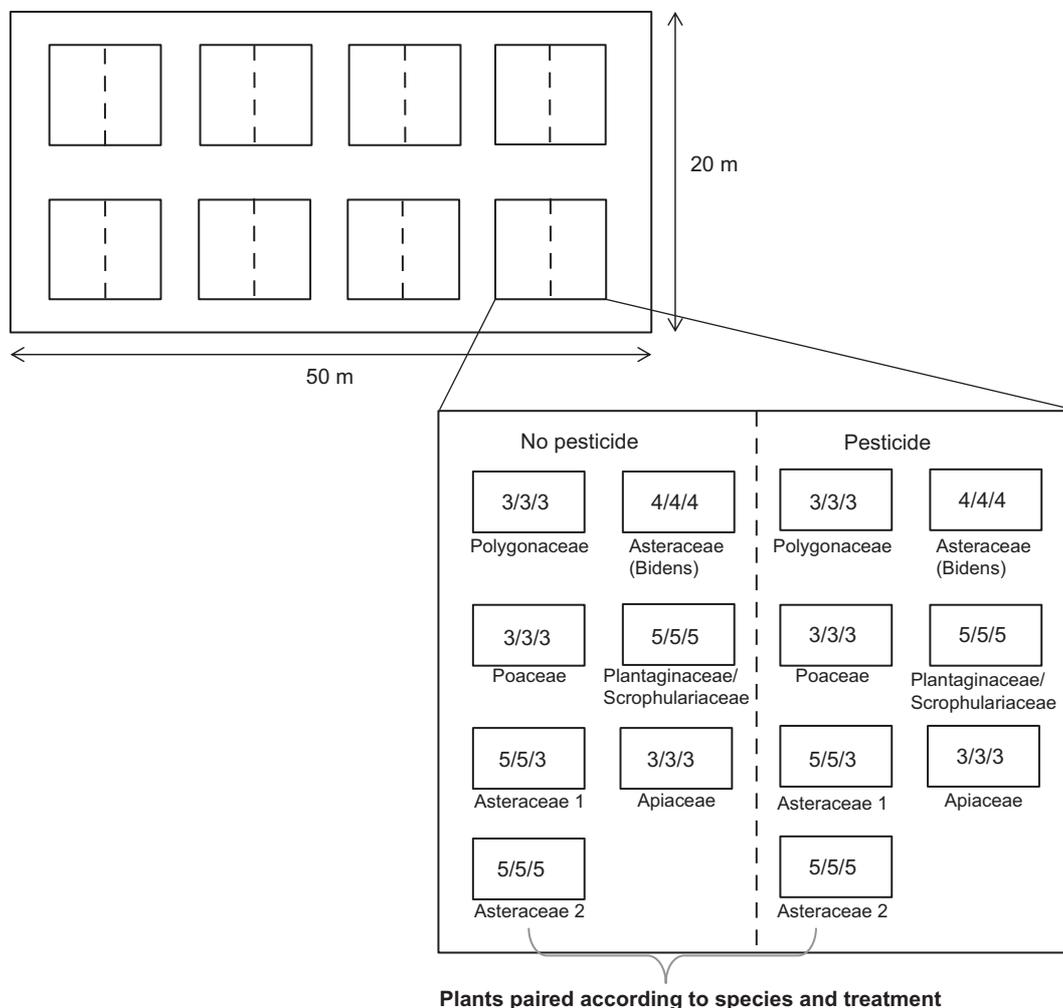

Figure 1. Schematic plan of the experimental set-up. Each of the eight blocks was split in half, and one half was randomly assigned the pesticide application treatment. Each half-block contained seven sub-blocks, with one of the seven taxonomic groups randomly assigned to each of them. Each sub-block contained plants belonging to the designated taxonomic group. The numbers shown in each sub-block represent the total number of plants (across species) per sub-block without nutrients added/with nutrients added/used for assessing herbivory levels. As one plant per treatment per species is in each sub-block, the numbers also represent the number of species, which varies among the taxonomic groups.

per sub-block, are indicated in Fig. 1. Pot positions within each sub-block were randomised.

In summary, 11 native, eight non-invasive alien and seven invasive alien species were used to assess herbivory damage (Table 1), with initially eight plants per pesticide treatment per species (giving 416 plants in total). For measuring plant biomass in response to pesticide and nutrient addition treatments, there were also eight plants per species for each pesticide/nutrient treatment combination (giving a total of 896 plants). During the experiment, some plants died, and others were lost due to complete consumption by molluscs before the enemy exclusion experimental treatment started. Thus, 200 complete pairs of pesticide/non-pesticide treated plants remained of those for measuring herbivory (paired according to block, 400 in total), and 382 pairs remained for measuring nutrient/pesticide effects on biomass (764 in total). The Supplementary material Appendix 1 Table A1 shows final sample sizes (numbers of plants) per species per treatment.

Two weeks after planting (to allow plants to overcome transplant stress), one block-half per block was randomly assigned to the herbivore-exclusion treatment. A below-ground pesticide was applied in pellet form (Cortilan, 1.5% Chlorpyrifos) once to each pot individually, at a rate of 5 g per m^2 (0.16 g per pot). An above-ground pesticide (Per-fekthion, 500 g per l dimethoate) was applied as a fine mist spray with a concentration of 1 ml per l of water, and was repeated two weeks later at the beginning of August, and in the last week of August. Spray treatments were applied on calm, non-windy days. As a control, a fine mist spray of water was applied to the plants in the non-pesticide treatment. To contain the application of pesticide and procedural controls to the target plants, the treatments were carefully applied at a constant rate to each plant individually at close range, and not above the plants using a pump-action canister. Anti-mollusc pellets (Mioplant, active ingredient: Metaldehyde) were applied liberally on the ground surrounding the pots in the enemy exclusion block-halves at the end of July and in the third week of August. Whilst it is unlikely that all herbivores were excluded by the pesticide treatment, the abundance of invertebrate herbivores on plants was reduced compared with plants with no pesticide applied. To encourage colonisation by invertebrate herbivores, grassland was allowed to grow in strips separating and bordering the blocks, with care taken to avoid shading of the experimental plants. The plants were grown for 12 weeks until 22 September 2010, giving a period of 10 weeks for the herbivore exclusion treatment.

Herbivory and performance measurements

On the herbivory set of plants, we collected herbivores once every two weeks, for a total of six times throughout the experimental period between 9 a.m. and 4 p.m. (6–9 a.m. for molluscs), under sunny and calm weather conditions. Most insects were sampled through removal. For aphids, however, we counted the number of individuals on the plants, and we removed only a few individuals for identification. This was done to avoid artificially decreasing aphid abundance through harvesting over time. Molluscs and aphids were identified to species level, while other invertebrates were identified to

family or order level. Aphids, molluscs, orthopterans and thysanopterans were the most abundant herbivore groups, and thus further analysed. On a species level, aphids and molluscs were classified as generalists or specialists according to host plants cited in Lampel and Meier (2007) for aphids, and in Frömming (1954) and Boschi (2011) for molluscs. Species were judged to be generalist if they were known to feed on host plants from ≥ two families/recorded as strongly polyphagous. The abundance of aphids on each plant was summed across all survey times. Damage sustained by the herbivory set of plants was recorded as the proportion of leaves on plants that were damaged, at the end of the experiment. Leaf chewing, gall formation and leaf mining were all considered forms of damage, but only leaf chewing was present. For plants with few leaves, all leaves were surveyed for signs of damage. For branched plants with many leaves, one branch per plant was randomly chosen and surveyed. Mean damage and aphid abundance values are given in the Supplementary material Appendix 1 Table A2.

For the performance set of plants, we harvested aboveground and belowground biomass of surviving plants after 12 weeks, and dried it at 80–85°C for at least 72 h prior to weighing. After 12 weeks, we also harvested, dried and weighed the aboveground biomass of the herbivory set of plants, for use as a covariate in subsequent analyses.

Analysis

Mixed effects models were used for all analyses, with species nested in taxonomic group, and block added as random effects throughout. An identity variance structure modelling different variances per species was included in all linear mixed effects models using the 'varIdent' function in the R package 'nlme' (plus an additional identity variance structure according to nutrient treatment for biomass models) to meet error normality and homoscedasticity assumptions. For non-pesticide treated plants from the herbivory set, we analysed aphid abundance (ln(x + 1) transformed) with a linear mixed model, and the proportion of leaves damaged and the presence of other herbivores using binomial generalized linear mixed models. In each case, species status was a fixed effect. We also re-analysed the proportion of leaves damaged with aboveground biomass (square-root transformed) and the number of leaves per plant (natural-log transformed) as covariates, to account for differences in plant size (both centred to the mean and scaled to one standard deviation, to allow estimates of differences among invasive, non-invasive and native species to be calculated for the average-sized plant). The three Poaceae species had no plants with damaged leaves and were excluded from analyses of leaves damaged to avoid zero-inflation.

As measures of experimental enemy release, we calculated the difference in the proportion of leaves damaged and the difference in aphid abundance between pairs of plants of a species treated and not treated with pesticide for each block (giving up to eight values per species, one per block). Linear mixed models were used to analyse these two variables, with ln(x + 22) transformation for the difference in aphid abundance, because the most negative difference between paired plants was –21 (21 aphids fewer on the non-pesticide plant than the pesticide-treated plant). Species status was a fixed

effect. The difference in proportion of leaves damaged was reanalysed with average biomass (natural-log transformed) and the difference in the number of leaves between plants in each pesticide/non-pesticide pair added as covariates (centred and scaled). In addition, in order to assess whether or not biomass actually correlated with proportion of leaves damaged, we analysed the aboveground biomass (square-root transformed) as a function of the proportion of leaves damaged for non-pesticide and pesticide-treated plants separately. In these analyses, the intercept and slope were allowed to vary according to taxonomic group and species nested within taxonomic group. A fixed variance structure (variance increasing with increasing biomass) was used to account for variance heterogeneity.

To quantify the effect of experimental enemy release on plant performance, we analysed total biomass (square-root transformed) of the performance set of plants using a linear mixed effects model. Only data points representing complete pairs were used, where both the 'enemy-excluded' and 'enemy-exposed' plants were present and surviving in a block per nutrient treatment. Species status, nutrient treatment and pesticide treatment were fixed effects. We used likelihood ratio tests (χ^2-values) to assess the significance of interactions and main effects of these three factors throughout, and in order to obtain a minimum adequate model explaining plant performance. All analyses were conducted in R ver. 3.0.2. We used the function 'lme' in the package 'nlme' (Pinheiro et al. 2013) for linear mixed effects models, and the function 'glmer' in the package 'lme4' (Bates et al. 2013), for generalised linear mixed effects models.

Results

Herbivore damage

In the treatment without pesticides, 20% of leaves on native species (n = 10) were damaged on average, compared to 7% and 13% on invasive alien species (n = 6) and non-invasive alien species (n = 7), respectively; the proportion of leaves damaged on invasive species was significantly lower compared to native species, but not to non-invasive species (Fig. 2a, Table 2). However, the significant difference was lost when plant biomass and total number of leaves per plant were accounted for (likelihood ratio test: $\chi^2 = 2.37$, DF = 2, p = 0.306; Table 2). There was a significant reduction in proportion of leaves damaged as both biomass ($\chi^2 = 16.10$, DF = 1, p < 0.001) and the number of leaves increased ($\chi^2 = 19.34$, DF = 1, p < 0.001; Table 2).

The proportion of leaves damaged on non-pesticide-treated plants was significantly greater than on pesticide-treated plants across all species, on average (mean increase in proportion of leaves damaged on non-pesticide treated plants = + 0.041, SE = 0.008, t = 5.238, p < 0.001). The difference in proportion of leaves damaged between treatments was significantly smaller for invasive species (+ 0.0095 ± 0.026%) compared to non-invasive alien species (+ 0.077 ± 0.021%;), but only marginally compared to native species (+ 0.056 ± 0.026%; Fig. 2b, Table 2). When plant-size covariates were included, differences according to status were no longer significant ($\chi^2 = 2.119$, DF = 2,
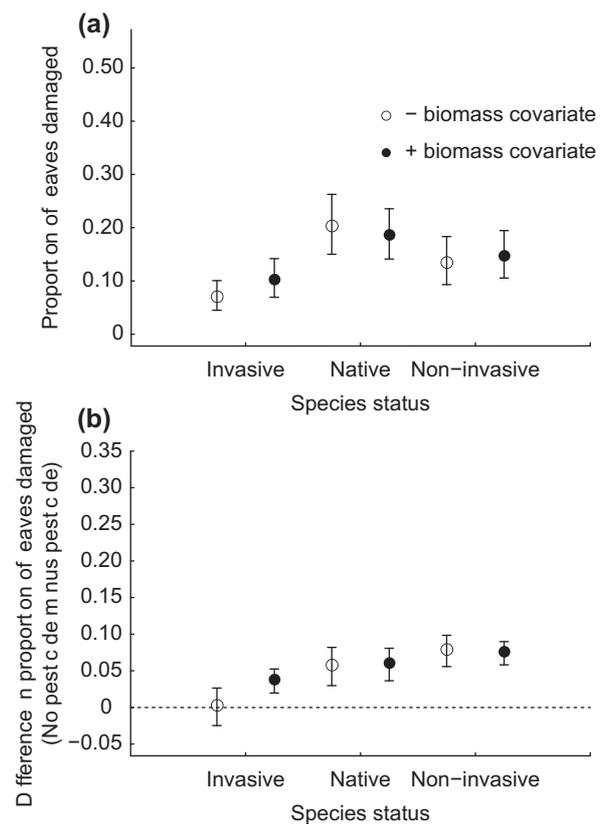

Figure 2. (a) Mean proportion of leaves damaged on pesticide-treated plants and (b) mean differences in proportion of leaves damaged on pesticide versus non-pesticide treated plants, for native, invasive alien and non-invasive alien plant species. Error bars represent ± 1 SE. In both (a) and (b), means are shown from models excluding and including plant-size covariates. The dashed line in b) signifies zero difference in proportion of leaves damaged between non-pesticide and pesticide-treated plants.

p = 0.347; Fig. 1b, Table 2). The difference in proportion of leaves damaged between non-pesticide plants and pesticide-treated plants decreased significantly with increasing average biomass ($\chi^2 = 7.01$, DF = 1, p = 0.008) and difference in the number of leaves for each plant pair ($\chi^2 = 9.16$, DF = 1, p = 0.003; Table 2).

Among non-pesticide-treated plants, aboveground biomass of plants used to survey herbivory decreased significantly with increasing proportion of leaves damaged (Supplementary material Appendix 1 Table A3, Fig. A1). In contrast, aboveground biomass was not significantly related to proportion of leaves damaged on pesticide-treated plants (Supplementary material Appendix 1 Table A3, Fig. A1). In both cases, models with slopes and intercepts varying according to taxonomic group and species explained significantly more variation than random intercept models (non-pesticide plants $\chi^2 = 40.06$, DF = 1, p < 0.001; pesticide plants $\chi^2 = 34.71$, DF = 1, p < 0.001). This indicated that the effect of proportion of leaves damaged on biomass was variable among species (see Supplementary material Appendix 1 Table A4 for intercepts and slopes per species).

Table 2. Parameter estimates (and standard errors in parentheses) from models of differences in proportion of leaves damaged between pesticide and non-pesticide treated plants, and the proportion of leaves damaged on non-pesticide treated plants only. Models were analysed with and without covariates of plant size, and with species status as fixed effects. Values given for random effects are standard deviations. Significant estimates (p < 0.05) are shown in bold.

Variable	Proportion of leaves damaged on non-pesticide plants	
	Excluding covariates	Including covariates
sqrt (biomass)		**−0.628 (0.141)**
ln (no. of leaves)		**−0.492 (0.104)**
Intercept	**−2.623 (0.432)**	**−2.198 (0.399)**
Non-invasive	0.738 (0.476)	0.419 (0.472)
Native	**1.240 (0.443)**	0.707 (0.440)
Random effects		
Block	0.445	0.382
Family	0.168	0.267
Species	0.592	0.760

Variable	Difference in proportion of leaves damaged	
	Excluding covariates	Including covariates
ln (biomass)		**−0.030 (0.012)**
Difference in number of leaves		**−0.021 (0.001)**
Intercept	0.001 (0.026)	**0.036 (0.016)**
Non-invasive	**0.077 (0.021)**	0.023 (0.026)
Native	0.056 (0.026)	0.038 (0.021)
Random effects		
Block	2.97×10^{-9}	4.97×10^{-5}
Family	0.033	0.014
Species	2.24×10^{-6}	3.096×10^{-8}
Residual	0.201	0.176

Table 3. Parameter estimates (and standard errors in parentheses) from models of differences in aphid abundance between pesticide and non-pesticide treated plants, and the number of aphids on non-pesticide treated plants only. Fixed effects included species status. Values given for random effects are standard deviations. Significant estimates (p < 0.05) are shown in bold.

Variable	Aphid abundance on non-pesticide plant [ln(x + 1) transformed]	Difference in aphid abundance [ln(x + 22)-transformed]
Intercept	**3.820 (0.227)**	**3.129 (0.618)**
Native	−0.054 (0.138)	0.341 (0.696)
Non-invasive	0.002 (0.155)	0.463 (0.754)
Random effects		
Block	6.596×10^{-6}	8.099×10^{-6}
Family	0.511	0.586
Species	0.209	1.252
Residual	1.562	1.170

Herbivore abundance

All eight species of aphid found on the plants were considered generalists (Supplementary material Appendix 1 Table A5); *Aphis frangulae*, *Aphis fabae*, *Aulocorthum solani*, *Macrosiphum euphorbiae*, *Myzus persicae*, *Rhophalosiphum nymphaeae*, *R. padi* and *Sitobion avenae*. According to Wittenberg, R. and Schweiz Bundesamt Für Umwelt (2006), *Myzus persicae* and *Macrosiphum euphorbiae* are alien to Switzerland. On average, 21 aphids were found per plant on native plant species (n = 11) without pesticide treatment, compared with 24 and 21 for invasive (n = 7) and non-invasive (n = 8) species, respectively; these differences were not significant (Table 3). There were significantly more aphids on non-pesticide treated plants than on pesticide-treated plants, across all species (mean difference in aphid abundance = + 22.34, 95% CI = 7.63–44.37). However, the difference in aphid abundance between treatments was similar for invasive, non-invasive and native plants (Table 3).

Four species of mollusc were found; *Arion vulgaris*, *Deroceras reticulatum*, *Succinea putris* and *Xerolenta obvia*. Wittenberg, R. and Schweiz Bundesamt Für Umwelt (2006) list *Arion vulgaris* as alien to Switzerland. Molluscs and orthopterans were no more likely to be present on native species, than on invasive or non-invasive alien species, while thysanopterans were marginally (but not significantly) less likely to occur on native than on invasive species (Supplementary material Appendix 1 Table A6).

Effect of pesticide and nutrient treatments on biomass

There was an overall significant effect of pesticide treatment ($\chi^2 = 14.55$, DF = 1, p < 0.001), with significantly more biomass on average for plants treated with pesticide than compared to plants not treated with pesticide (mean difference in biomass = 1.092 g, 95% CI = 0.210–1.974). Responses of individual species were variable (Supplementary material Appendix 1 Fig. A2). Invasive, non-invasive and native species did not significantly differ from one another in their responses to pesticide treatment (two-way interaction: ($\chi^2 = 2.97$, DF = 2, p = 0.227; Fig. 3a). The effects of pesticide treatment on biomass also did not significantly differ according to nutrient treatment (two-way interaction: ($\chi^2 = 0.88$, DF = 1, p = 0.348). Species of different status responded differently to nutrient addition (two-way interaction: $\chi^2 = 29.257$, DF = 2, p < 0.001), with a greater relative increase in biomass for native (n = 13) and invasive (n = 7) species compared to non-invasive species (n = 8), and native compared to invasive species (Fig. 3b, Table 4). The invasive species increased their biomass from 25.44 g to 48.54 g with nutrient addition, compared to 14.57 and 36.34 g for native species, and 17.82 and 28.92 g for non-invasive species without and with nutrient addition, respectively. There was no significant three-way interaction between pesticide treatment, nutrient treatment and species status affecting plant biomass ($\chi^2 = 0.69$, DF = 2, p = 0.708).

Discussion

Evidence for the enemy release hypothesis (ERH) involving herbivores would require a smaller response to experimental herbivore exclusion for invasive alien species than for native and non-invasive alien species (Keane and Crawley 2002). In our study, invasive species suffered less foliar herbivory from invertebrates than native, but not than non-invasive species, and there was a positive effect of pesticide treatment in terms of reduced herbivory for native and non-invasive species, but not for invasive species, which would partially support the ERH. However, the differences between invasive and other species were lost after accounting for plant
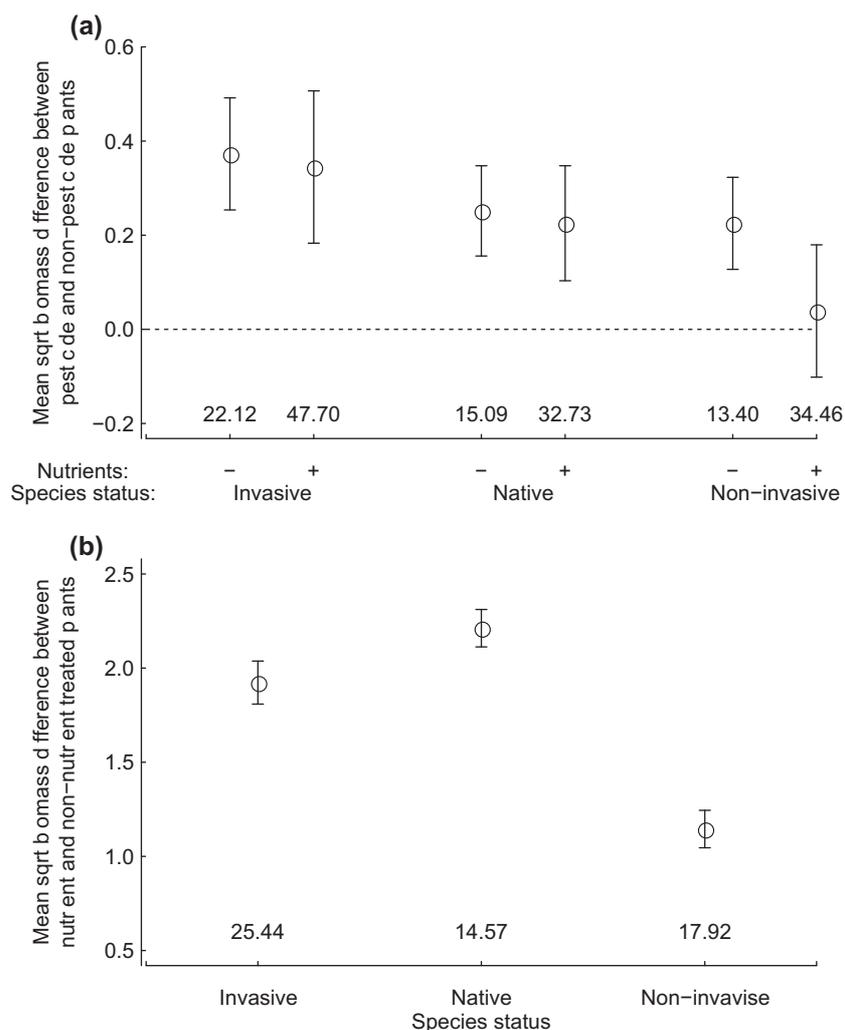
Figure 3. (a) Mean difference in square-root transformed total biomass between pesticide and non-pesticide treated plants, for native, invasive alien and non-invasive alien species without (−) and with (+) nutrient addition. The dashed line signifies zero difference in biomass. (b) Mean difference in square-root transformed total biomass between nutrient-treated and non-nutrient treated plants, for native, invasive alien and non-invasive alien species (averaged across pesticide-treatments). Error bars in (a) and (b) represent ± 1 SE. For reference, numbers above the x axis represent mean biomass without pesticide treatment in (a), and without nutrient addition in (b).

size, indicating that differences in enemy attack may be confounded by plant size (i.e. larger plants have a tendency to suffer invertebrate herbivory on a smaller proportion of their leaves). The change in response for invasive but not native/non-invasive species to pesticide treatment when accounting for size might reflect the influence of larger invasive species, such as *Bidens frondosa*, suffering less herbivory due to their size. Unfortunately, tests of the ERH involving invertebrate herbivores rarely consider the effects of variation in plant size among compared species on enemy attack, and the apparent partial support for the ERH from our results indicate that it should be taken into account in such studies. In addition, there were no differences among invasive, non-invasive and native species in herbivore presence and abundance, or in their biomass responses to pesticide treatment. This is similar to a recent review of the few studies comparing herbivore abundance and damage in introduced and native ranges of invasive species, showing that plants in the introduced range suffer less herbivory overall, but the magnitude of difference

was small (Liu and Stiling 2006). When using a phylogenetically controlled multi-species approach the enemy release hypothesis involving invertebrate herbivores does not appear to be a general mechanism explaining the invasiveness of plant species. The levels of foliar herbivory observed in the experiment were generally low, and it may be that this form of natural enemy attack does not have a major impact on the performance and population dynamics of the species studied. We acknowledge, however, that release from other guilds of natural enemies, such as fungal pathogens, soil-borne enemies (Engelkes et al. 2008) and seed predators could potentially still play a role in the success of the invasive, although evidence for release from these types of enemies in general is not conclusive (Parker and Gilbert 2007, van Kleunen and Fischer 2009, Hill and Kotanen 2011).

Measures of herbivory and enemy damage commonly used to test the ERH may not always translate into plant performance effects. Even though Parker and Gilbert (2007) found that invertebrate herbivore damage and pathogen

Table 4. Parameter estimates (and standard errors in parentheses) from full and minimum models explaining total biomass of native, non-invasive and invasive plant species, under pesticide and non-pesticide treatments, and with or without nutrient addition. Values given for random effects are standard deviations. Significant estimates (p < 0.05) are shown in bold.

	Model	
Variable	Full	Minimum adequate
Intercept	**5.479 (0.965)**	**5.412 (0.963)**
Native	−1.757 (0.918)	−1.722 (0.912)
Non-invasive	−1.183 (1.010)	−1.106 (1.004)
Nutrient addition	**1.871 (0.158)**	**1.911 (0.111)**
Pesticide absent	**−0.364 (0.157)**	**−0.231 (0.059)**
Native : Nutrient addition	0.385 (0.209)	**0.352 (0.147)**
Non-invasive : Nutrient addition	**−0.839 (0.211)**	**−0.758 (0.150)**
Native : Pesticide absent	0.068 (0.207)	
Non-invasive : Pesticide absent	0.152 (0.209)	
Nutrient addition : Pesticide absent	0.075 (0.223)	
Native : Nutrient addition : Pesticide absent	−0.058 (0.295)	
Non-invasive : Nutrient addition : Pesticide absent	0.165 (0.298)	
Random effects		
Block	1.727×10^{-17}	0.0008
Family	1.372	1.372
Species	1.884	1.884
Residual	0.911	0.914

infection were lower on alien compared to native plant species in North America, this did not translate into greater survival of alien compared to native species, or of invasive alien compared to non-invasive alien species. Also, a recent study on *Artemisia ambrosiifolia*, in its native range, found that enemy exclusion reduced damage on adult leaves, but did not result in increased growth or reproduction (MacDonald and Kotanen 2010). Moreover, a meta-analysis of enemy-release studies that manipulated the presence of natural enemies found no consistent differences in plant-performance responses to enemy exclusion between invasive species and native comparators (Chun et al. 2010). The lack of clear evidence for reduced plant damage resulting in greater plant performance may be a consequence of differing abilities among plant species to tolerate herbivory. We found that for non-pesticide treated plants, those suffering invertebrate herbivory on more leaves had a lower biomass overall, but there was a significant amount of variation in the relationship according to species. Understanding how herbivory (and natural enemy impacts in general) actually relate to plant performance is essential if we want to adequately assess the relevance of enemy release to plant invasions. Other effects of defoliation on plant performance can include reduced over-winter survival and reproduction (Rose et al. 2009). Moreover, the plants used in the experiment were already 4–5 weeks old, and any important differences in herbivory (and subsequently survival) of younger plants among invasive, non-invasive and native species would have been missed. Nonetheless, our results and others mentioned suggest it is unlikely that release from invertebrate herbivory

alone can increase growth performance of invasive compared to native plants.

Nutrient addition did not affect the differences in total biomass between pesticide and non-pesticide treated plants, and also did not affect the differences among native, non-invasive and invasive species. This result appears to suggest that while plants obviously increased biomass with nutrient addition, overall, the absolute difference in biomass with enemy exclusion did not vary greatly, which runs counter to the prediction of the resource–enemy release hypothesis (Blumenthal 2006). As we only assessed herbivore damage and herbivores on a subset of plants that did not include a nutrient-addition treatment, we were not able to verify that the number of leaves damaged by herbivores or the abundance of herbivores found on nutrient-treated plants remained the same as on non-nutrient treated plants. Notwithstanding this, while invasive species are able to increase biomass more than non-invasive alien species under nutrient addition (in line with theory on fluctuating resources; Davis et al. 2000), our results suggest that nutrient levels may not mediate enemy release differences between invasive and non-invasive alien species in terms of performance. Moreover, native species increased biomass significantly more than invasive species in response to nutrient addition. This could be due to, in part, the inclusion of common, fast-growing native species, which may perform at least as well as invasive aliens under higer nutrient levels (Dawson et al. 2012). However, invasive alien species were also already ~ 1.7 times larger on average than native species without nutrient addition, which may have limited their potential to increase biomass with nutrient addition.

A potential limitation of our study (and garden experiments in general), could be that plants were not exposed to the whole suite of potential herbivores of the species used, especially those that would occur belowground. Thus, whether the ERH in relation to invertebrate herbivores explains the invasion success of a plant species might not only depend on the species involved but also the herbivore community present. We only found species of the two most abundant herbivore groups known to feed on multiple genera and plant families (Supplementary material Appendix Table A5). If present, specialist species of the herbivore groups not identified to species level (because they were mostly still larvae) may have played a minor role. However, the meaning of generalism versus specialism is not clearly dichotomous; even among species considered 'generalist', there may be some preference shown for certain food plants, and this could be mediated by the choice of plant species available in the community. Notwithstanding this, if invertebrate herbivores show preferences, then such preferences did not result in enemy release differences of invasive compared to native and non-invasive species. This may not be surprising if preferences are expressed at higher (e.g. familial) taxonomic levels; differences in herbivory may be greater among families than according to species status within families. A further potential caveat is that the pesticides used in the experiment were organophosphates. We cannot rule out entirely the possibility that additional phosphorus from the pesticide treatment could have increased plant biomass; however we estimate that only ~ 4 mg of P in total was added to plants receiving pesticide treatment.

The soil used was a relatively nutrient-rich agricultural soil, and we therefore think a confounding fertilisation effect of pesticide use is unlikely. However, we cannot rule out other potential non-target effects of pesticides, such as impacts on soil microbiota.

Conclusions

To the best of our knowledge, this is the first multi-species experiment that assessed invertebrate herbivore loads and herbivore damage as well as performance with and without herbivore suppression of invasive alien, non-invasive alien and native species. We found equivocal support for enemy release involving invertebrate herbivores as a mechanism explaining invasion success of alien plant species in our study. The species of herbivores identified were considered generalists, and plant size was an important variable explaining variation in herbivory in our multi-species approach. In addition, our study suggests that increased resource availability may not necessarily increase the extent to which species benefit from enemy release. The degree of herbivore release experienced by alien plant species under varying resource availability would be better considered relative to plant size, and under a plant community context with manipulation of different herbivore guilds.

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