Plant-microbe-herbivore interactions in invasive and non-invasive alien plant species

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Summary

1. Plants interact with many organisms, such as microbes and herbivores, and these interactions are likely to affect the establishment and spread of plants. In the context of plant invasions, mycorrhizal fungi and constitutive and induced resistance of plants against herbivores have received attention independently of each other. However, plants are frequently involved in complex multi-trophic interactions, which might differ between invasive and non-invasive alien plants.

2. In a multi-species comparative experiment, we aimed to improve our understanding of plant traits associated with invasiveness. We tested whether eight invasive alien plant species use the mycorrhizal symbiosis in a more beneficial way, and have higher levels of constitutive or induced resistance against two generalist bioassay herbivores, than nine non-invasive alien species. We further assessed whether the presence of mycorrhizal fungi altered the resistance of the plant species, and whether this differed between invasive and non-invasive alien species.

3. While invasive species produced more biomass, they did not differ in their biomass response to mycorrhizal fungi from non-invasive alien species. Invasive species also did not have higher levels of constitutive or induced resistance against the two generalist herbivores. Mycorrhizal fungi greatly affected the resistance of our plant species, however, this was also unrelated to whether the alien species were invasive or not.

4. Our study confirms the previous findings that invasive species generally grow faster and produce more biomass than non-invasive alien species. We further show that alien plant species used a variety of defence strategies, and also varied in their interactions with mycorrhizal fungi. These multi-trophic interactions were not consistently related to invasiveness of the alien plant species.

5. We suggest that awareness of the fact that alien plant species are involved in multi-trophic interactions might lead to a more complete understanding of the factors contributing to a plant’s success.

Key-words: herbivore resistance, induced and constitutive resistance, invasive plant species, mycorrhizal dependency, phenotypic plasticity, plant defence, plant defence strategies, plant dominance, plant invasions, plant-microbe-herbivore interaction

Introduction

Growing in both the above and below ground compartments, plants interact with a variety of different organisms that may affect their establishment and spread. The response of plants to one group of organisms might also affect their response to another group. Such multi-trophic interactions can have substantial consequences for the functioning of all parties involved. One such example is the multi-trophic interaction of below ground microbes, plants and above ground herbivores. To defend themselves against enemies, plants evolved a variety of resistance strategies, which can be constitutively expressed or induced following damage (Karban & Baldwin 1997). Recently, there has been growing evidence that symbiotic microbes (e.g. N fixing bacteria, mycorrhizal fungi) associated with plants might influence constitutive (Kempel, Brandl & Schädler 2009; Pineda et al. 2010) and induced resistance to herbivores (Bennett, Bever & Bowers 2009; Kempel et al. 2010), and thus may both directly and indirectly affect a plant’s success.
Although it has frequently been suggested that alien plants are released from enemies (Keane & Crawley 2002), herbivores contribute to the biotic resistance of native communities against invaders (Levine, Adler & Yelenik 2004). This might imply that alien species with high levels of constitutive resistance should have a higher chance to become invasive. However, alien species most likely interact with generalist rather than with specialist herbivores (Parker, Burkepile & Hay 2006). Therefore, plastic induction of resistance in the presence of enemies—which has been suggested to be particularly effective against generalist herbivores (Agrawal 1999a)—has recently attracted the attention as a potentially useful strategy for dominant invasive plants (Richards et al. 2006; van Zandt 2007; Verhoeven et al. 2009; Berg & Ellers 2010; Orians & Ward 2010). Moreover, induced resistance is suggested to be less costly than permanent constitutive resistance (Herms & Mattson 1992). This adaptive plastic response (Baldwin 1998; Agrawal 1999b, 2001) would allow plants to allocate more resources to growth and competitive strength in the absence of enemies but would still provide resistance in the presence of enemies. Indeed, Kempel et al. (2011) recently found a positive association between induced resistance and competitive ability of plant species.

So far, only a handful of experimental studies addressed the roles of constitutive and induced resistance in invasion success of alien species. This was done by comparing either patterns of resistance between the native and introduced range of an invasive plant species (Cipollini et al. 2005; Eigenbrode et al. 2008; Carrillo et al. 2012) or between native and invasive plant or animal species (Engelkes et al. 2008; Engel & Tollrian 2009; Zas, Moreira & Sampedro 2011). Although these approaches can reveal whether there has been evolutionary change, and whether invasive alien species have strategies that differ from the ones of natives, they cannot reveal why some alien species became invasive and others did not (Strauss, Webb & Salamin 2006; van Kleunen et al. 2010). Moreover, previous studies were restricted to one or a few species, which makes it difficult to draw more general conclusions on the importance of a certain trait for invasiveness.

The role of interactions between plants and soil microbes in general (e.g. van Grunsven et al. 2007), and especially mycorrhiza, has received increased attention in the context of plant invasions (Reinhart & Callaway 2006; van der Putten, Klironomos & Wardle 2007; Pringle et al. 2009). Although associations between plants and mycorrhizal fungi are frequently assumed to be mutualistic, plant responses to mycorrhizal fungi can range across the whole spectrum from negative to positive, depending on environment variables and the identities of the plants and fungi (Johnson, Graham & Smith 1997; van der Heijden, Wiemken & Sanders 2003; Klironomos 2003; Hoeksema et al. 2010). Many invasive plant species can form associations with mycorrhizal fungi (Fumanal et al. 2006; Smith et al. 2008; Štajerová, Smilauerova & Smilauer 2009), most likely generalistic ones (Moora et al. 2011). However, being not obligatorily dependent on mycorrhizal fungi might be more advantageous for a plant in a new range (Richardson, Williams & Hobbs 1994; Reinhart & Callaway 2006; van der Putten, Klironomos & Wardle 2007; Pringle et al. 2009). Concordantly, several studies showed that mycorrhizal dependency was lower in alien than in native species (Reeves 1979; Allen & Allen 1980; Pendleton & Smith 1983; Vogelsang & Bever 2009). However, the weaker positive response to mycorrhizal fungi of alien species does not explain why some aliens become invasive and others do not, unless the dominant invasive aliens use the mycorrhizal symbiosis in a more beneficial or less detrimental way than non invasive alien species do.

Most, if not all, studies on invasive plants and mycorrhizal fungi are focused on bitrophic interactions (Harvey, Bukovinszky & van der Putten 2010). However, mycorrhizas can also affect the herbivore community by changing plant quality, thereby affecting plant fitness. A meta analysis showed that mycorrhizal fungi usually increase plant resistance against generalist chewing herbivores, but reduce resistance against specialist chewing herbivores (Koricheva, Gange & Jones 2009). The net effect of mycorrhizal fungi on herbivores might depend on altered nutrient uptake by plants and changes in the constitutive production of secondary defence metabolites. Much less is known about the effects of mycorrhizal fungi on induced resistance of plants to herbivores. In one study, induced resistance was decreased by mycorrhizal fungi (Bennett, Bever & Bowers 2009), while in another study it was increased (Kempel et al. 2010). In addition, a growing body of evidence suggests that mycorrhizal fungi themselves may induce plant resistance, possibly involving cross talk between the salicylic acid and jasmonate dependent defence pathways (Pozo & Azcon Aguilar 2007). Given that the effect of mycorrhizal fungi on plant resistance differs between species and is ecologically relevant, one might expect that species whose resistance is increased by an association with mycorrhizal fungi might be more successful than species whose resistance is decreased or not affected.

In this study, we therefore address questions on multitrophic interactions between arbuscular mycorrhizal fungi (AMF), plants and two generalist herbivores in the framework of biological invasions. Using a multi species approach, we assessed the biomass response of eight dominant invasive alien and nine non invasive alien plant species to a short period of herbivory and to a mixture of common mycorrhizal fungi. We also assessed, in the presence and in the absence of mycorrhizal fungi, constitutive and induced resistance of the 17 plant species to the generalist bioassay caterpillars Spodoptera littoralis and Mamestra brassicae. Specifically, we addressed the following questions. (i) Are dominant invasive alien plant species more able to induce resistance against generalist herbivores than non invasive alien species are, and do they generally differ in their constitutive level of resistance? (ii) Do invasive species utilize mycorrhizal fungi in a more beneficial
way than non invasive alien species do? (iii) Do mycorrhizal fungi affect constitutive and induced resistance of alien plant species, and if yes, do mycorrhizal fungi strengthen the resistance of dominant invasive species more than the resistance of non invasive alien species?

Materials and methods

PLANT SPECIES

We used 17 herbaceous plant species that are alien to Switzerland, from three different plant families (Table 1). We considered eight of the alien plant species to be invasive and the remaining nine alien species to be non invasive in Switzerland. We compared invasive alien species with non invasive alien species because this is the most direct test of determinants of invasiveness, and allows us to contribute to answering why some alien species became invasive while others did not (van Kleunen et al. 2010). We categorized plant species as invasive if they are on the Black List or the Watch List for invasive plant species in Switzerland (http://www.cps.swiss/fileadmin/template/pdf/deutsch/Empf_Liste_IV_d.pdf, assessed January 2012), and are widespread in Switzerland (Table 1). The alien species categorized as non invasive are not on the Black List or Watch List, and are naturalized at only a few locations in Switzerland. The non invasive alien species are not known to occur in native plant species, and are always less dominant than their invasive counterparts (Landolt et al. 2010). The invasive and non invasive alien plant species were chosen in such a way that they belonged to the same genus and had similar life forms, to avoid confounding effects of invasiveness status of the species with taxonomic and growth form (Table 1). For the analyses, we considered the confamilial species pair Polygonum orientale MAKINO, Polygonum cuspidatum SIEBOLD & ZUCC.) as a congeneric pair. Due to a lack of historical introduction data, we cannot exclude the possibility that invasive and non invasive alien plant species were introduced at different times. However, among the 12 species for which we could find the exact year of first recorded naturalization in Germany, there was hardly any difference (mean for seven invasive alien species: 1867; mean for five non invasive alien species: 1876; Table 1).

We collected the seeds from wild populations in Switzerland, obtained them from botanical gardens, or ordered them from a commercial seed supplier (B & T World Seeds, Aigues Vives, France, Table 1). Congeneric pairs were mostly obtained from the same seed source. Because F. japonica in Europe does not reproduce by seeds but by rhizomes, we collected rhizome pieces for this species.

HERBIVORE SPECIES

Direct measurements of the production of plant defensive compounds and their effectiveness against herbivores for many different plant species are very laborious, especially when chemicals are not yet identified for certain species. Therefore, we used the growth response of generalist herbivores as integral indicator of constitutive and induced plant resistance (e.g. Joshi & Vrieling 2005; van Zandt 2007; Kempel et al. 2011). To assess constitutive resistance and the ability to induce resistance of our plant species, we used larvae of two generalist herbivores, the native cabbage armyworm M. brassicae (L.) (Lepidoptera: Noctuidae), and the non native Egyptian cotton leafworm S. littoralis (Boisdalav) (Lepidoptera: Noctuidae). Larvae of both species are known to feed on a wide range of plant species of more than 20 (Rojas, Wyatt & Birch 2000) and 40 plant families (Brown & Dewhurst 1975), respectively. The extreme polyphagy of both species has lead to their frequent use in bioassays (Joshi & Vrieling 2005; van Zandt 2007; Kempel et al. 2010, 2011). Their feeding response and

Table 1. List of the 17 plant species used in the experiment. LF: frequency of occurrence in Switzerland [%] D: dominance in the wild (numbers from 1 to 5 with 5 being the most dominant category), List: species listed in the Black List (B) or Watch List (W) (SKEW 2009), or not listed (-). Time: Time of first naturalization in Germany. Data were obtained from Landolt et al. (2010), and the Biolflor database (Kuhn, Durka & Klotz 2004)

<table>
<thead>
<tr>
<th>PLANT SPECIES</th>
<th>LF</th>
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<th>D</th>
<th>List</th>
<th>Time</th>
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<td>Asteraceae</td>
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<td>Solidago canadensis L.</td>
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<td>46</td>
<td>5</td>
<td>B</td>
<td>1853</td>
<td>Bot. Gard. Gottingen</td>
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<tr>
<td>Solidago gigantea AITON</td>
<td>h</td>
<td>42</td>
<td>5</td>
<td>B</td>
<td>1859</td>
<td>Bot. Gard. B&amp;T</td>
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<tr>
<td>Senecio inaequidens DC.</td>
<td>h</td>
<td>8</td>
<td>4</td>
<td>B</td>
<td>1889</td>
<td>Bot. Gard. Wild collections</td>
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<tr>
<td>Bidens frondosa L.</td>
<td>t</td>
<td>5</td>
<td>4</td>
<td>B</td>
<td>1891</td>
<td>Bot. Gard. Wild collections</td>
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<tr>
<td>Erigeron annuus (L.) DESF.</td>
<td>h,t</td>
<td>44</td>
<td>2</td>
<td>W</td>
<td>18th c.</td>
<td>Bot. Gard. Wild collections</td>
</tr>
<tr>
<td>Artemisia verlotiorum LAMOTTE</td>
<td>h</td>
<td>24</td>
<td>5</td>
<td>B</td>
<td>1920</td>
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<tr>
<td>Brassicaceae</td>
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<tr>
<td>Lepidium virginicum L.</td>
<td>h,t</td>
<td>31</td>
<td>2</td>
<td>1786</td>
<td>Wild collections</td>
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<td>Polygonaceae</td>
<td></td>
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<tr>
<td>Fallopia japonica (HOUTT.) RONSE DECR.</td>
<td>g</td>
<td>43</td>
<td>5</td>
<td>B</td>
<td>1872</td>
<td>Bot. Gard. Bern (Rhizomes)</td>
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*Note that S. graminifolia is a Synonym of Euthamia graminifolia (L.) NUTT.
Two weeks after germination, we transplanted the cia! diet to avoid adaptation of the MYCORRHIZAL FUNGI DESIGN sand and plain field soil from an agricultural field in the Bern fig. 1. Experimental design. ('Endweber four more weeks after the induction treatment of 5 em, washed them carefully and placed them on moist filter of plant resistance against generalist herbivores (Joshi & Vrieling 2005; van Zandt 2007). Mamestra brassicae and S. littoralis are both known to induce plant resistance (Anderson, Jonsen & Morte 2001; Gomez, van Dijk & Stuefer 2010; Kempel et al. 2011). Spodoptera littoralis larvae originated from a lab stock at the University of Bern (Switzerland), and M. brassicae egg batches were obtained from the Entomology Laboratory of Wageningen University (the Netherlands). Caterpillars were bred on an artificial diet to avoid adaptation of the insects to specific plant species.

Experiment

DESIGN

In August 2009, we surface sterilized seeds of 16 plant species with 1% H2O2, and germinated them in trays filled with potting soil ('Aussaat und Pikiererde 191', Ricoter, Switzerland). This soil had first been steam sterilized at 100 °C for a minimum of 4 h to eliminate AMF. For F. japonica, we cut the rhizomes into pieces of 5 cm, washed them carefully and placed them on moist filter paper. We filled 1.36 L pots with a steam sterilized 1:1 mixture of sand and plain field soil from an agricultural field in the Bern region ('Landerde', Ricoter, Switzerland, N content: 0.17%), and placed them in a greenhouse (14 30 °C, a constant day length of 14 h, and additional light). We irrigated the pots twice prior to the start of the experiment to leach excess nutrients from the soil, which might have become available during the steaming process (Endweber & Scheu 2006). The experiment was set up in a nearly full factorial design (Fig. 1) using mycorrhizal fungi, induction of resistance and herbivore species as treatments, with the restriction that each M. brassicae larva fed only on M. brassicae induced plants, and each S. littoralis larva fed only on S. littoralis induced plants. All 14 treatment combinations were replicated five times resulting in 70 pots per plant species. We randomly assigned pots to five blocks in the greenhouse and randomized pots within each block several times during the experiment. We watered all plants when needed.

MYCORRHIZAL FUNGI TREATMENT

Two weeks after germination, we transplanted, if possible, 70 plants of each species into the experimental pots. Before planting, we added 10 g of a multi species AMF inoculum, consisting of spores and hyphae of five common AMF species: Rhizophagus intraradices (Glomus intraradices), Funneliformis mosseae (Glomus mosseae), F. geosporum (Glomus geosporum) Claroideoglomus claroideum (Glomus claroideum), and C. etunicatum (Glomus etunicatum) (Symblom, Lanskroun, Czech Republic), in a layer 4 cm below the soil surface in half of the pots. We used a multi strain inoculum rather than a single fungus, since AMF generally occur as assemblages in ecosystems and in individual plant roots (Vandenkoonhuyse et al. 2002). Although all fungal species have a global distribution, the fungal strains used originate from Europe. Control plants received 10 g of a sterilized mycorrhizal inoculum to control for the substrate component of the inoculum, and 10 mL of a microbial wash, to return a portion of the potential non mycorrhizal microbial community of the mycorrhizal inoculum to the control plants (Koide & Li 1989). The microbial wash was created by mixing 150 g of the live mycorrhizal inoculum with 7 L of water and decanting the mixture three times through 20 µm filter paper (AMF spores are bigger than 20 µm and cannot pass the filter).

We did not quantify the degree of mycorrhization, but at the end of the experiment, we checked whether mycorrhizal fungal colonization occurred in the mycorrhizal fungal treatment, and was prevented in the non mycorrhizal fungal treatment. For this purpose, we randomly selected one plant per species of the non mycorrhizal fungal treatment and two plants per species of the mycorrhizal fungal treatment. Root samples of these plants were washed, cleared in 10% KOH (80 °C for 15 35 min, depending on the plant species), stained in 5% black ink (Parker Quink, black) in vinegar solution (80 °C, 5 min), and destained in 50% glycerol. We assessed the presence or absence of mycorrhizal structures (hyphae, arbuscules and vesicles) under a microscope. This examination showed AMF structures in the mycorrhizal fungal treatment for all species except Artemisia annua, while we found no such structures in plants of the non mycorrhizal fungal treatment.

INDUCTION AND BIOASSAY OF RESISTANCE

Induced plant resistance is defined as a reduced preference or performance of herbivores in response to previous stress or injury of the plant (Karban & Myers 1989). Therefore, we used the performance response of the caterpillars to quantify the effects of induction on plant resistance. Because AMF may need up to several weeks to colonize roots, and it may require another several weeks

Fig. 1. Experimental design. Plants grew with or without a mixture of mycorrhizal fungi (AMF treatment) and were exposed for 2 days to herbivory (induction) by either Mamestra brassicae (Ia) or Spodoptera littoralis (Is), or not induced at all (x). To assess the constitutive and induced resistance, we added the two generalist herbivores as bioassay caterpillars to a set of those plants and determined their growth. To assess the growth response of plants to induction and arbuscular mycorrhizal fungi, we grew a second set of those plants for four more weeks after the induction treatment.
of root colonization to have an effect on plant growth and herbivores, we started the induction treatment to assess herbivore performance on undamaged and damaged (induced) plants after 10 weeks of growth.

We enclosed all plants with a nylon gauze. To induce possible resistance mechanisms, we added two third to fourth instar larvae of *M. brassicae* and *S. littoralis* (hereafter called induction caterpillars) to each of 10 plants per species per mycorrhizal fungal treatment, and let them feed for 2 days. The remaining plants were used as non induced control plants. One day after removal of the induction caterpillars, we added one third instar *M. brassicae* larva as a bioassay caterpillar to each of the five *M. brassicae* induced and five non induced plants of each mycorrhizal fungal treatment. Similarly, we added one third instar *S. littoralis* larva to each of five *S. littoralis* induced and five non induced plants per species and mycorrhizal fungal treatment. We allowed the larvae to feed on the plants for 5 days (Fig. 1). We assessed the increase in biomass of the bioassay caterpillars by recording fresh mass of the caterpillars before and after feeding. We calculated their relative growth rate as ln (larval mass after feeding/ larval mass before feeding) (Kogan & Cope 1974). The remaining plants per species (five *M. brassicae* induced, five *S. littoralis* induced and five undamaged plants per species and mycorrhizal fungal treatment) were allowed to grow for four more weeks without herbivores to investigate their growth responses to induction and mycorrhizal fungi (Fig. 1). For those plants above and below ground biomass was harvested, dried at 70 °C for at least 3 days and weighed.

For some non invasive alien plant species, we had low germination rates, and as a consequence, we did not have enough plants for both mycorrhizal fungal treatments. In those cases, we used most plants for the mycorrhizal fungal treatment because this treatment is more likely to reflect natural field conditions. In some analyses, we therefore had to exclude those species for which we did not have data on all treatment combinations (*A. annua, Eriogonum karvinskianum, P. orientale, Solidago graminifolia and Senecio vernalis*) as well as their congeneric counterparts.

### STATISTICAL ANALYSIS

We used linear mixed effects models with the function *lmer* of the *lme4* package (Bates & Maechler 2009) in R (R Development Core Team 2010). For the analysis of total plant biomass, we used mycorrhizal fungal treatment (yes, no), induction treatment (not induced, induced by *M. brassicae*, induced by *S. littoralis*), invasive status (non invasive, invasive) and their interactions as fixed factors. We included block, genus and species nested within genus (and invasive status) as random factors. We also ran models with random slopes for genus and species with respect to induction, herbivore, invasive status and mycorrhizal fungi, and induction, but because this increased the AIC values (i.e. decreased model fit), we did not further consider these models. We simplified the full model by removing non significant terms to produce a minimum adequate model. Significance was assessed using likelihood ratio tests comparing models with and without the terms of interest (Zuur *et al*. 2009). In this analysis, we had to exclude the plant species for which we had too few replicates in the non mycorrhizal fungal treatment, as well as their congeneric invasive counterparts. To be better able to test for the effect of invasive status and its interaction with induction of resistance, we also did a separate analysis for the mycorrhizal fungal treatment only, where we only had missing data for the two non invasive alien plant species *P. orientale* and *Senecio vernalis*.

We calculated the biomass response to the presence of mycorrhizal fungi for each plant species as ln (mean dry biomass growing with mycorrhizal fungi/mean dry biomass growing without mycorrhizal fungi). A positive value would indicate that mycorrhizal fungi acted parasitically, and a negative value would indicate that mycorrhizal fungi acted mutualistically.

Also tested whether invasive or non invasive alien plant species responded more or less strongly to mycorrhizal fungi, comparing their absolute (i.e. unsigned) values of response to mycorrhizal fungi by means of an ANOVA.

For the analysis of the relative growth rate of the bioassay caterpillars, we used induction treatment (yes, no), herbivore (*M. brassicae, S. littoralis*), invasive status, mycorrhizal fungi and their interactions as fixed factors, and block, genus and plant species nested within genus (and invasive status) as random factors.

Again, this analysis missed several plant species, and we therefore did a separate analysis for plants in the mycorrhizal fungal treatment only, for which we had complete data. We also ran models with random slopes for genus and species with respect to induction, herbivore, invasive status and mycorrhizal fungi, and kept the random slopes when the model had a lower AIC value (i.e. when there was an increased model fit).

### Results

#### PLANT BIOMASS

In all congeneric species pairs, invasive alien plants produced more biomass than non invasive alien plants (Fig. 2). This effect was only marginally significant in the overall analysis, but was significant when we did the analysis for a larger number of species by analysing mycorrhized plants only (Table 2). Overall, inoculation with mycorrhizal fungi tended to reduce total plant biomass (marginally significant mycorrhiza effect in Table 2), both for invasive and non invasive alien plant species (Fig. 3, no significant invasive status × mycorrhizal fungal interaction in Table 2). However, invasive species tended to have less extreme positive and negative responses to mycorrhizal fungi (unsigned values differed marginally significantly between non invasive and invasive plant species, estimate of invasives ± SE: −0.311 ± 0.165, *P* = 0.084, Fig. 3) suggesting lower sensitivities of invasive species to mycorrhizal fungi. Potential induction of resistance by the two bioassay caterpillars had no effect on total plant biomass, and did neither differ between mycorrhized and non mycorrhized plants nor between invasive and non invasive alien species (no significant induction effect and induction × invasive status interaction in Table 2).

#### HERBIVORE PERFORMANCE

Overall, herbivore performance did not differ significantly between invasive and non invasive alien plant species (*P* = 0.49, see Table S1 in Supporting information, Fig. 4). Caterpillars feeding on previously induced plants had a lower growth rate than caterpillars feeding on non induced plants (*P* = 0.018, Table S1, Supporting information), indicating that overall, our plant species were able to induce resistance. However, induced resistance did not differ between invasive and non invasive alien plant species (no significant induction × invasive status interaction) and also not between mycorrhized and non mycorrhized plants (no significant induction × mycorrhizal fungi interaction in Table S1, Supporting information). Furthermore, the effect of mycorrhizal fungi on the ability to induce...
Table 2. Results of a linear mixed effect model using total plant biomass of invasive and non invasive alien plant species as dependent variable for plants of both mycorrhizal fungal treatments (here we had to remove eight of the 17 plant species) and of the mycorrhizal fungal treatment only (here we had to remove four of the 17 plant species).

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<th>Both AMF treatments</th>
<th>AMF treatment only</th>
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<td>Fixed factors</td>
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<tr>
<td>Mycorrhiza</td>
<td>3·356</td>
<td>1</td>
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<tr>
<td>(M) Induction</td>
<td>2·857</td>
<td>2</td>
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<td>(I) Status</td>
<td>2·931</td>
<td>1</td>
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<td>M x S</td>
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<td>S x I</td>
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<td>S x M</td>
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<td>Block</td>
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<td>Genus / species</td>
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<td>224·630</td>
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Analysis of the subset of plants in the mycorrhizal treatment confirmed the negative effect of induction on the relative growth rate of the caterpillars \( (P = 0·048) \), and of the AMF treatment only \( (P = 0·048) \). Furthermore, *S. littoralis* caterpillars had a higher growth rate than *M. brassicae* caterpillars \( (P = 0·048) \). *Spodoptera littoralis* caterpillars tended to have lower growth rates on invasive species (invasive status x herbivore interaction: \( P = 0·062) \). However, herbivore performance as well as the ability to induce resistance did not differ significantly between invasive and non invasive alien plant species, and thus varied without consistent pattern among species (Table S1 and S2, Supporting information; Fig. 4). This was confirmed when analysing species pairs separately. In some species pairs, the invasive alien species was significantly more able to induce resistance than the non invasive alien one, whereas in other species pairs the opposite was the case, and in yet others, the induction response did not differ between the invasive and non invasive alien species (Table S3 and Fig. S1, Supporting information).

Discussion

**Constitutive and induced resistance valuable for some but not for all**

In recent years, general strategies and traits associated with plant invasiveness have gained more and more research attention, and results from database and experimental studies suggest that invasions are not just random events. For instance, several studies report a higher performance and fecundity of invasive alien compared with native or non invasive alien species (Pyšek & Richardson 2007; van
Biomass response to mycorrhiza of invasive (grey) and non invasive (white) alien plant species in increasing order. Response to mycorrhiza is calculated as the log response ratio of total plant biomass growing with and without mycorrhiza, separately for each species. Error bars are based on the variance of the log response ratio (Hedges et al. 1999). Due to low numbers of replication we have no error bars for Solidago graminifolia. We had no complete data for Polygonum orientale, Eriogon karvinlikianus and Artemisia annua. (a) Asteraceae, (b) Brassicaceae, (p) Polygonaceae.

Fig. 4. The interacting effects of arbuscular mycorrhizal fungi (without AMF: M-, with AMF: M+) and induction on larval relative growth rates of Spodoptera littoralis and Mamestra brassicace feeding on the eight invasive (species name in bold) and the nine non invasive alien plant species. Values are means ± standard errors. Significant effects of induction (I), mycorrhizal fungi (M) and their interactions (I x M) on larval growth are indicated (see also Supporting Information Table S3).
multiple pairs of related invasive and non invasive alien or native species, complex results are quite common (e.g., Dostal 2011).

Our results are in line with recent findings that species capable of high biomass production appear to be pre adapted for invasion (van Kleunen et al. 2010; Schlaepfer et al. 2010; Godoy, Valladares & Castro Diez 2011). However, our results indicate that different invaders use different sets of defence related traits, and a trait being advantageous for a particular alien species might not be so for another. For instance, one of the world’s most noxious invaders, the Japanese knotweed F. japonica, strongly induced resistance against both generalist herbivores (Table S2, Supporting information), and this was stronger than in the non invasive alien confamiliar species P. orientale (Table S3, Supporting information). As F. japonica is a strong competitor, it is indeed likely that the ability to plastically increase the defence against herbivores represents a key adaptation for invasion success in this particular species. On the other hand, Solidago gigantea, another dominant and successful invader, showed no induction response at all to any of the two herbivore species (Table S2, Supporting information), whereas its non invasive alien congeners did (Table S3, Supporting information). However, herbivores generally performed very poorly on S. gigantea, and a further increase of resistance would probably not add any further benefit to the already high level of constitutive resistance (Fig. S1, Supporting information; Herms & Mattson 1992; Kempel et al. 2011). Herbivores performed best on the invasive Artemisia verlotiorum, which also had the highest biomass production. This could indicate that tolerance, investment in growth rather than resistance, may play a role in the invasion success of this alien species. Future studies should test explicitly, whether invasive and non invasive alien species differ consistently in their tolerance to herbivory.

Our results thus suggest that different species have different solutions to the same problem, and that consequently, no general pattern appeared. From our multi species study, we therefore conclude that the ability to plastically increase defences upon herbivore attack could be valuable for certain plant species, but is not a general key trait associated with invasion success. Invasive species are also not generally less or more defended against generalist herbivores than non invasive alien species are, and show as the non invasive alien species do the whole spectrum of different levels of constitutive and induced resistance.

**LOW MYCORRHIZAL DEPENDENCY OF BOTH INVASIVE AND NON INVASIVE ALIEN SPECIES**

Mycorrhizal fungi have been suggested to play an important role in plant invasions (Richardson et al. 2000; Pringle et al. 2009; Stajerová, Smilauerova & Smilauer 2009). One might thus expect that invasive species use the mycorrhizal symbiosis in a more beneficial way than non invasive alien species or that they are less dependent on them. In our study, plant growth responses to arbuscular mycorrhizal fungi ranged from mainly negative to slightly positive, indicating that mycorrhizal fungi mainly acted parasitically. Such negative effects of mycorrhizal fungi are not rare, and are more common when nutrient rich instead of nutrient poor soil is used (Smith & Read 2008).

Although there are examples of invasive plant species that are obligately mycorrhizal (Richardson, Williams & Hobbs 1994; Fumanal et al. 2006; Smith et al. 2008), low mycorrhizal dependency has been suggested to be advantageous for species colonizing a new range (Reinhart & Callaway 2006; van der Putten, Klironomos & Wardle 2007). Our results showed that the invasive and non invasive alien plant species did not differ consistently in their average response to mycorrhizal fungi. A glance at the response to mycorrhizal fungi of all species, however, suggests that the range of positive and negative responses to mycorrhizal fungi was greatest for non invasive alien species, and that invasive species were not or only slightly negatively affected by mycorrhizal fungi. Although this difference was only marginally significant, a reduced response of invasive species to mycorrhizal fungi would not only reduce the potentially beneficial, but also the detrimental parasitic aspects of the symbiosis. The latter are usually strongest in nutrient rich conditions (van der Putten, Klironomos & Wardle 2007; Smith et al. 2008), which are also the type of conditions preferred by many invasive plant species (Dostal et al. 2012). If this difference in sensitivity holds true, future studies should address the question whether this reflects pre adaptation or evolutionary change in the new range. Our results only allow us to conclude that, although a mismatch of the local mycorrhizal community with an exotic plant might in some cases be a barrier to invasion (Richardson, Williams & Hobbs 1994), a more beneficial use of the mycorrhizal symbiosis does not appear to necessarily lead to invasion success of alien species.

**MYCORRHIZAL FUNGI AFFECT INTERACTIONS BETWEEN ALIEN PLANT SPECIES AND HERBIVORES BUT NOT IN A CONSISTENT WAY**

It has been frequently suggested that generalizations in invasion biology are rare due to context dependency and interactions among traits (Pysek & Richardson 2007), but surprisingly few studies have tested for such interactions (but see Küster et al. 2008). Mycorrhizal fungi are known to affect levels of constitutive and induced resistance in plants (Bennett, Bever & Bowers 2009; Koricheva, Gange & Jones 2009; Kempel et al. 2010). We found that the way in which mycorrhizal fungi affected the outcome of the plant herbivore interactions did not differ consistently between invasive and non invasive alien plant species. Our results are thus in line with the current literature exhibiting the whole spectrum from positive to negative effects of
mycorrhizal fungi on constitutive resistance and the ability to induce resistance against generalist herbivores, but with an emerging general pattern.

Eight of the 13 alien plant species altered their constitutive or induced resistance significantly in the presence of mycorrhizal fungi (Fig. 3, Table S2, Supporting information), with some being more resistant or more able to induce resistance in the presence of mycorrhizal fungi, and others in their absence. The effect of mycorrhizal fungi on resistance against herbivores does not appear to be related to their effect on plant biomass. For instance, *Lepidium virginicum* showed as expected among the Brassicaceae almost no change in plant biomass to the presence of the fungi, but its ability to induce resistance was significantly reduced. Although we cannot exclude that this significant result is a type I error due to multiplex testing, a biological explanation could be that upon recognition of the mycorrhizal fungi, cross talk between defense related pathways has modulated the plant’s defense system (Pozó & Azcon Aguilar 2007). So, even when direct effects of mycorrhizal fungi on plant performance are absent, mycorrhizal fungi can have far reaching consequences for herbivores, and possibly, also for other community members such as pollinators and natural enemies of herbivores, and thereby ultimately on the performance of plants. The diversity of our results illustrates the spectrum of such multi trophic interactions, and demonstrates their presence even within alien plant species that have no or only a short sympatric evolution history with these fungi.

**Conclusions**

Our study demonstrates that high biomass production is a general characteristic of invasive alien plants, whereas induced resistance and constitutive resistance are not. Invasive and non invasive alien species both exhibited a variety of strategies to cope with herbivores. In addition, invasive alien species neither used the mycorrhizal symbiosis in a more beneficial nor in a less detrimental way than non invasive alien species did. However, a reduced sensitivity of invasive species to the local AMF community appears to be common, and might be a potential mechanism to avoid the parasitic nature of the fungi in nutrient rich habitats. Generally, it appears as if ‘simple traits’, such as biomass production, show more consistent differences between invasive and non invasive alien species than do ‘complex traits’ such as resistance and mycorrhization.

The great variability of mycorrhizal effects on constitutive and induced resistance of our species illustrates that mycorrhizal fungi can have substantial effects on non co-evolved plant species and their associated herbivores although its contribution to a plant’s performance is less clear. Nevertheless, bearing in mind the multi trophic nature of biotic interactions of alien plant species might help us to better understand their effect on the composition and functioning of recipient communities and ecosystems.

**Acknowledgements**

We thank Andreas Gygax for assembling the species pairs and information on invasive or non-invasive status of the alien plant species, and Matthias Rillig for advice on the mycorrhizal fungal treatment. We further thank Jake Alexander for providing seeds of *E. annua*, Madalin Parepa for providing thistles of *F. japonica*, Andreas Gygax for collecting seeds of *B. frondosa*, *B. bipinnata*, *L. virginicum*, *L. neglectum*, *S. inequidens*, and *R. salzburgeri*, and the Botanical Gardens of Gottingen, Salzburg, Konstanz and Bern for providing seed material. We thank Prof. Beate Lanzrein for providing eggs of *S. littoralis* and Riea Gols for providing eggs of *M. brassicae*. We also thank the ‘Nähr-Atelier’ in Bern for sowing gauze-bags, Christine Giele for help with mycorrhiza scoring, and Andreas Gygax, Moritz Jöst, Lediwje Keser, Madalin Parepa and Yuan-Ye Zhang for help in the greenhouse. We thank Wim van der Putten, Matthias Rillig, Eric Allan and five anonymous reviewers for comments on previous versions of the manuscript.

**References**


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**Supporting Information**

Fig. S1. Relative growth rate of *Spodoptera littoralis* and *Mamestra brassicae* feeding on undamaged and damaged plants (mycorrhized plants only).

Table S1. Results of a linear mixed effects model using larval growth rate as dependent variable.

Table S2. Results of an ANOVA using either the mean larval growth rate of both herbivore species, of *Spodoptera littoralis* only and of *Mamestra brassicae* only, as dependent variable.

Table S3. Results of linear mixed effect models using the relative growth rate of the caterpillars as dependent variable.

Data S1 Details on the effects on herbivore growth rate, separately for each plant species.