

Article

Effect of Competition from Native on Alien Species in a Multi-Trophic Setting

Eva M. Malecore ¹ and Mark van Kleunen ^{1,2}

¹ Ecology, Department of Biology, University of Konstanz, Universitätsstrasse 10, D-78457 Konstanz, Germany

² Zhejiang Provincial Key Laboratory of Plant Evolutionary Ecology and Conservation, Taizhou University, Taizhou 318000, China

* Correspondence: eva.malecore@uni-konstanz.de

Abstract:

1. Darwin’s naturalization hypothesis predicts that alien species closely related to native species are less likely to naturalize because of strong competition due to niche overlap. Closely related species are likely to attract similar herbivores and to release similar plant volatiles following herbivore attack, thus could attract the same predators. However, the importance of phylogenetic relatedness on the interaction between alien and native plants has never been tested in a multitrophic context.
2. In a mesocosm experiment we grew six alien target plant species alone and in competition with nine native plant species of varying phylogenetic relatedness. To test the effects of multitrophic interactions on the performance of alien target species, we used enclosure cages to expose plants to the presence and absence of herbivorous arthropods, predatory arthropods and nematodes.
3. Surprisingly, biomass and number of flowering structures increased with presence of competitors for some of the alien species, but overall there was no consistent competition effect. Similarly, we found that none of the multitrophic-interaction treatments affected survival, biomass or number of flowering structures of the alien species.
4. We conclude there was no significant relationship between performance measures of the alien species and their phylogenetic relatedness to the native competitors.

Keywords: enemy release hypothesis; multitrophic interactions; Darwin’s naturalization hypothesis; competition; alien species; exotic species; mesocosm experiment.

1. Introduction

In the field of invasion biology, one of the major goals is to understand the factors that allow an alien species to establish in a new range and become invasive [1, 2, 3]. Elucidating these factors is also important for improving our understanding of fundamental ecological processes, such as community assembly. Whenever an alien species manages to invade a native community, the latter is altered, new interactions are established and over time a new balance is reached. However, interactions with the different community members might be key to the establishment success of the alien in the first place.

In this context, two major hypotheses, Darwin’s naturalization hypothesis [4] and the enemy release hypothesis [5] have received much attention, however few studies have tried to address them simultaneously and disentangle them. Darwin’s naturalization hypothesis states that closer evolutionary relatedness of the alien to native species hampers its naturalization due to increased niche overlap and thus increased competition. The enemy release hypothesis states that an alien

invader will be more successful in the introduced range because it will be released from its co-evolved enemies, like herbivores and pathogens in the case of plants. Studies addressing the enemy release hypothesis have reported inconsistent findings [6], with some showing a lower number of enemies for native species compared to aliens (e.g. [7]), and others showing reduced damage for aliens in the introduced range (e.g. [8]). While this inconsistency has been attributed among others to differences in spatial scale among studies [6], evolutionary relatedness of the alien species to the natives could be another factor explaining inconsistencies, and thus deserves attention. As host switching by specialized enemies can occur between closely related species, enemy release is predicted to be strongest for alien species distantly related to the natives [9]. The relatedness between the introduced species and the resident community reconnects to Darwin's naturalization hypothesis. Despite the conceptual link, so far, no studies have addressed whether relationships, if any, between establishment success of alien plants and their phylogenetic relatedness to natives are primarily caused by competition or enemy release (but see [10]).

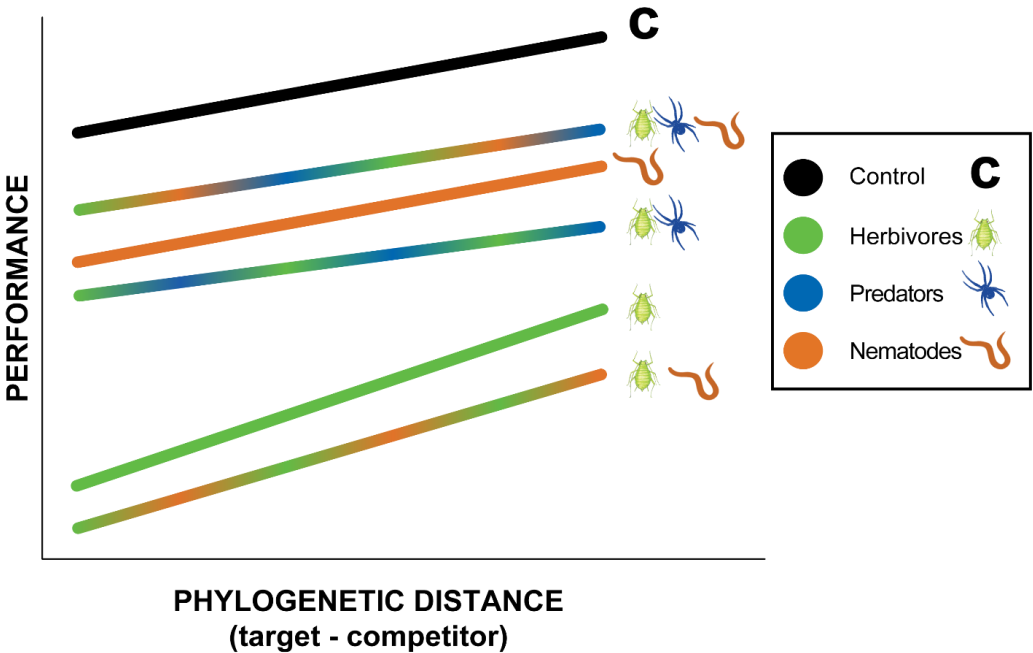
Following attack or egg deposition by herbivores, plants often release secondary metabolites in the air, also known as herbivore-induced plant volatiles [11]. Those signals can attract predators and parasitoids of the herbivores [12], and can also "warn" other plants in the community for the presence of herbivores [13]. By preying on the herbivores, the predators and parasitoids attracted by the herbivore-induced volatiles could counterbalance the negative effects of herbivore-host switching between native and alien plants. This could also depend on the phylogenetic relatedness of the alien and native plant species. If closely related natives and alien species are likely to be attacked by similar herbivores, they are also likely to produce similar volatiles able to attract the predators and parasitoids of those herbivores. Moreover, the volatiles released after a herbivore attack might directly induce defences in neighbouring conspecific or heterospecific plants [14,15]. For conspecifics, it has been shown that this effect is stronger for closely related genotypes [13], and it is therefore not unlikely that these volatile-induced defences will similarly be stronger for related species. Phylogenetic relatedness could thus decrease fitness of alien plants through more intense competition and reduced enemy release, but the latter could partly be compensated by the increased attraction of predators.

These potential multitrophic interactions are valid not only for plants and above-ground organisms but also for plants and below-ground organisms. Indeed, Ali, Alborn and Stelinski [16] showed that entomopathogenic nematodes were attracted to herbivore-induced plant volatiles from *Citrus paradisi* × *Poncirus trifoliata* when fed upon by a root weevil. Similarly, maize attacked by the Western corn rootworm emits chemicals attracting entomopathogenic nematodes [17]. Nematodes cover a variety of trophic levels, and can be both harmful and beneficial for plants. In the context of an alien plant colonizing a new range, new below-ground interactions might contribute to its success or failure, and again the phylogenetic distance to the native community might influence the outcome of these interactions.

The importance of evolutionary relatedness can be evaluated by assessing how alien species perform in relation to their phylogenetic distance to the native species in the recipient community. The relationship between success and relatedness is thought to be linear or curvilinear, depending on geographical scale, on type of phylogenetic distance measure used, and on life stage [18–20]. The relationship can thus be complex and linked to a multitude of factors, including new multitrophic interactions of the alien species with herbivores and their predators. The role of these multitrophic interactions in the relationship between alien plant performance and phylogenetic relatedness to the native plants has rarely been studied.

To address how phylogenetic relatedness can affect the success of an introduced species and to assess the role of potentially complex multitrophic interactions therein, we performed a large outdoor mesocosm experiment. We grew six alien target species alone and in pairwise competition with nine native species of varying phylogenetic relatedness in mesh enclosure cages. We exposed the plants to a factorial combination of a nematode-addition treatment and an insect-herbivore-addition treatment, and plants in the insect-herbivore treatment were either grown with or without arthropod predators (resulting in six treatment combinations). We determined survival, biomass and number

95 of flowering structures of the alien plants as performance measures. We tested the following
96 expectations (Figure 1): (1) Without any enemies, competitor presence will be the only factor to affect
97 performance of the alien targets, and we expect closely related targets to suffer more from
98 competition than distantly related ones (i.e. a positive relationship between target performance and
99 phylogenetic distance). (2) With herbivores, we expect target performance to be further reduced, and
100 that this reduction is stronger when in competition with a closely related native plant, due to host
101 switch of native herbivores. Thus the relationship between alien target performance and phylogenetic
102 distance in the presence of herbivores is expected to be positive and stronger compared when in the
103 absence of herbivores. (3) When there are also predators of the herbivores present, we expect the
104 negative effect of herbivores to be reduced, and the relationship between alien target performance
105 and phylogenetic distance to be similar to when herbivores are absent. (4) We expect nematodes –
106 which include both herbivores and predators - to have a similar effect as the combined herbivore and
107 predator treatment, unless interactions between below-ground and above-ground happen.



108
109 **Figure 1.** Expected outcomes for the performance of alien target plants grown in competition in
110 relation to its phylogenetic distance to the competitor species for each of the six different combinations
111 of insect-herbivore, predator and nematode addition

112 **2. Results**

113 Survival of the alien target species ranged from 0.57 for *Papaver somniferum* to 0.99 for
114 *Ambrosia artemisiifolia*. Aboveground biomass of the alien target plants ranged from a minimum of
115 0.002 for *Papaver somniferum* to a maximum of 116.697 g for *Bidens frondosa*. The number of
116 flowering structures of the alien target plants ranged from 0 to 2166, with *Veronica persica* having
117 the highest number of flowering structures. Mean competitor biomass per pot ranged from 0.008 for
118 *Geum rivale* to 56.447 for *Lathyrus pratensis*.

120 **2.1 Effects of competition on target performance**

121 The survival, aboveground biomass and number of flowering structures of the alien target plants
122 were not significantly affected by the presence of competitors (Figure 2) and also not by the presence
123 of herbivores, predators or nematodes (Table 1). Nevertheless, the responses to competition varied
124 among the different target species (Figure 3). Aboveground biomass and number of flowering
125 structures increased with competitor presence for the four smallest target species (*Salvia verticillata*,

Veronica Persica, Eschscholzia californica and Papaver somniferum), and slightly decreased for the two largest target species (Ambrosia artemisiifolia and Bidens frondosa (Figure 3).

Table 1. Results of GLMMs for survival (binomial), aboveground biomass (Gaussian) and number of flowering structures (negative binomial), of the alien target plants with the presence of competitors, herbivores, predators and nematodes as explanatory variables. Target biomass was cube-root transformed. P-values were calculated by comparing models with and without the term in question.

Competitor presence	Survival		Biomass		# flowering structures	
Parameter	Estimate	P	Estimate	P	Estimate	P
<i>Fixed terms</i>	(SE*)	(>X ²)	(SE)	(>X ²)	(SE)	(>X ²)
Intercept	1.6387 (0.847)	0.0552	1.9990 (0.620)	0.0082	3.0896 (1.107)	0.01825
CP	1.0337 (0.924)	0.4168	0.3863 (0.410)	0.4439	0.6888 (0.691)	0.3285
Herbivores	0.6981 (0.851)	0.0775	0.0800 (0.150)	0.7114	-0.0103 (0.125)	0.8278
Predators	0.5010 (1.003)	0.2725	-0.0969 (0.150)	0.5267	0.1151 (0.125)	0.3133
Nematodes	-0.7679 (0.735)	0.2152	0.0099 (0.156)	0.2609	0.0394 (0.125)	0.8897
Herbivores×Nematodes	0.7669 (1.177)	0.0648	-0.0900 (0.220)	0.3096	-0.0180 (0.177)	0.9187
Predators×Nematodes	-0.8793 (1.332)	0.2505	0.1927 (0.217)	0.2553	-0.0519 (0.176)	0.7687
CP×Herbivores	-0.7820 (0.884)	0.2318	-0.1407 (0.158)	0.6156		
CP×Predators	-0.5393 (1.030)	0.6428	0.1266 (0.158)	0.8783		
CP×Nematodes	0.1614 (0.769)	0.5357	-0.0684 (0.164)	0.5802		
CP×Herbivores×Nematodes	0.0921 (1.24)	0.9413	0.1761 (0.231)	0.4459		
CP× Predators×Nematodes	0.3920 (1.374)	0.7781	-0.2989 (0.228)	0.1901		
<i>Random terms</i>	Standard deviation		Standard deviation		Standard deviation	
Cage	0.2228		0.0000		0.0000	
Target Species intercept	1.0496		1.2379		1.2379	
Target Species CP	1.1769		0.3829		0.3829	
Competitor Species	0.4617		0.3430		0.3430	

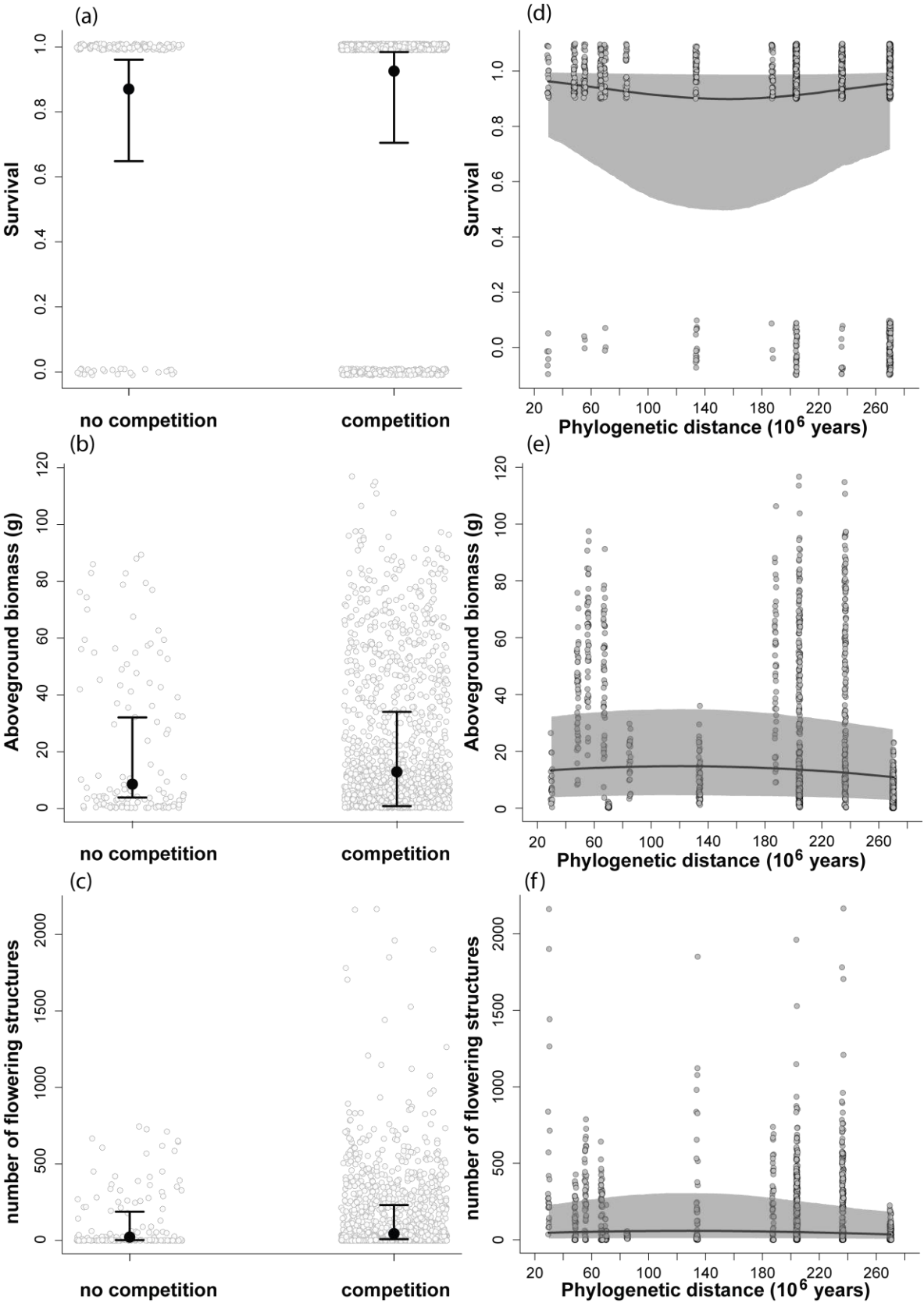


Figure 2. Fitted values with 95% credible intervals and data points (jittered) for survival (a), above-ground biomass (b) and number of flowering structures (c) of alien target plants grown with and without competition. Fitted lines with 95% credible intervals and data points (jittered) of the relationship between survival (d), above-ground biomass (e) and number of flowering structures (f) of alien target plants grown in competition and phylogenetic distance to its native competitor.

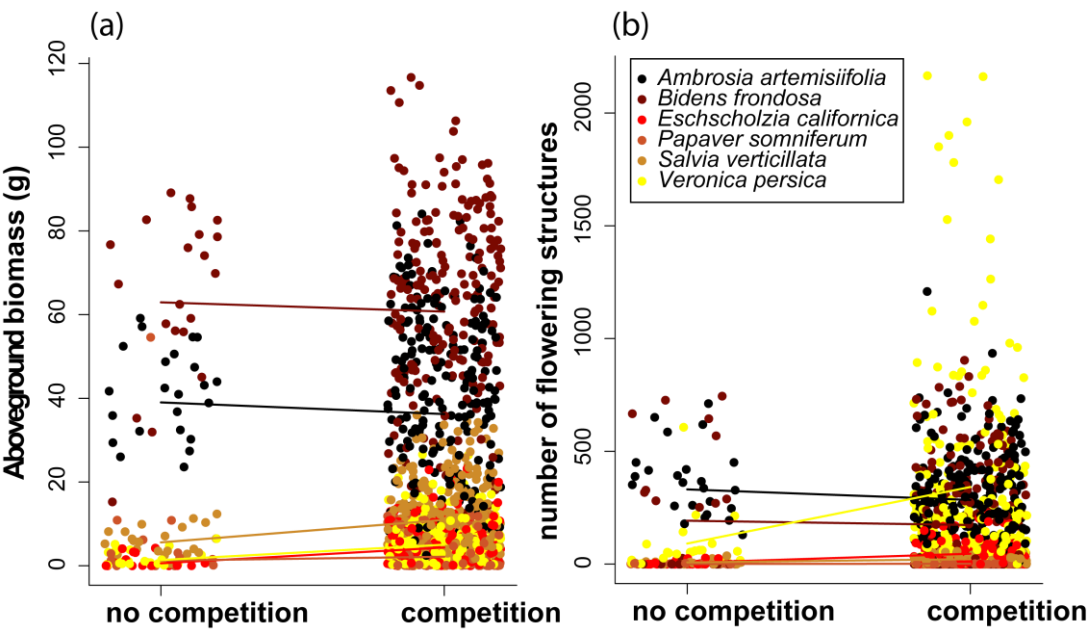


Figure 3. Fitted values and data points comparing above-ground biomass (a) and number of flowering structures (b) of each of the six alien target plants grown with and without competition.

2.1 Effect of phylogenetic distance on target performance

For the plants grown with competitors, phylogenetic distance had neither a linear nor a quadratic effect on survival, aboveground biomass or number of flowering structures of the alien target plants (Figure 2). There were also no significant effects of the presence of herbivores, predators or nematodes, or their interactions with phylogenetic distance (Table 2)

Table 2. Results of GLMMs for survival (binomial), aboveground biomass (Gaussian) and number of flowering structures (negative binomial) of the alien target plants with phylogenetic distance, both as linear and quadratic terms, herbivores, predators and nematodes as explanatory variables. Target biomass was cube-root transformed. P-values were calculated by comparing models with and without the term in question.

Phylogenetic distance (PD)	Survival		Biomass		#flowering structures	
	Estimate	P	Estimate	P	Estimate	P
Parameter	(SE*)	(>X ²)	(SE)	(>X ²)	(SE)	(>X ²)
Fixed terms						
Intercept	2.2747 (1.073)	0.06746	2.438 (0.405)	0.0005	3.9377 (0.810)	0.0017
PD	0.4221 (0.549)	0.1543	-0.1336 (0.079)	0.2940	-0.2756 (0.205)	0.6122
PD ²	0.3820 (0.278)	0.1741	-0.0567 (0.044)	0.2023	-0.1366 (0.109)	0.2072
Herbivores	0.4079 (0.267)	0.1646	-0.0187 (0.036)	0.5998	-0.0399 (0.091)	0.6600
Predators	-0.3687 (0.267)	0.2713	-0.0227 (0.035)	0.5206	0.1198 (0.090)	0.1855
Nematodes	-0.2453 (0.213)	0.3065	-0.0357 (0.029)	0.2189	-0.0044 (0.073)	0.9518
PD × Herbivores	-0.1281 (0.243)	0.6024	0.0227 (0.036)	0.5263		
PD × Predators	0.2258 (0.241)	0.3504	0.0076 (0.035)	0.8294		
PD × Nematodes	0.0926 (0.189)	0.6280	-0.0004 (0.029)	0.9903		
Random terms						
	Standard deviation		Standard deviation		Standard deviation	
Cage	0.2856		0.0000		0.0000	
Target Species	2.4218		0.9327		0.9327	
Competitor Species	0.4606		0.3754		0.3754	

3. Discussion

In our study, none of the performance measures of the alien target species were affected by the presence of native competitors, herbivores, predators or nematodes. Among the plants grown with competition, there was also no significant effect of phylogenetic distance between target and competitor species on performance of the target plant. Thus, in our study, phylogenetic relatedness played no important role in survival, growth and flower production of the alien plants grown in mesocosms with native plants.

3.1 Effect of competition on target performance

We found, averaged across the six alien target species, no significant effect of the presence of native competitors on any of the performance measures. Although recent studies have shown that invasive alien plants are usually less strongly affected by competition than non-invasive alien or native plants [27–29], our finding of no significant competitor-presence effect is nevertheless surprising. A closer look at the individual alien target species, however, showed variation in their responses to the presence of competitors. Two of the six target species – *Ambrosia artemisiifolia* and *Bidens frondosa* – showed a slight decrease in performance (aboveground biomass and number of flowering structures) in response to the presence of competitors, whereas the four others – *Salvia verticillata*, *Veronica Persica*, *Eschscholzia californica* and *Papaver somniferum* – showed an increased performance (Figure 3). The latter four species also had the smallest statures overall (Figure A1), which suggests that smaller target species benefited from competitor presence. This suggests that the small alien target plants were facilitated by the presence of the native plants.

This facilitation of small alien target species by the native plants could be due to the very warm weather conditions during the experiment (Figure A2). Under these conditions, a competitor could have a beneficial effect on the target plant by shading it and by creating a more humid micro-climate around it. Indeed, facilitation is generally more frequently observed under stressful than under benign conditions as predicted by the stress-gradient hypothesis [30, 31], and canopy shading has been shown to benefit seedling performance in dry climates [32, 33]. While the smaller species in our study could take advantage of the protection by native competitors, this was not the case for the taller species, and resource competition would have become more important, resulting in a negative effect of competition. The hot temperature during our experiments is likely to have had an even harsher effect for several reasons. First, our mesh-cages used to enclose the arthropods reduced air flow and thus prevented cooling. Second, the plants were planted in black pots, thus affecting the soil temperature more than in normal field condition, where the soil is completely covered by vegetation. Third, although the floor of the area was covered with a light coloured fabric, the solar irradiance would warm it up quickly. Under these extreme temperature conditions, it is reasonable to expect competitor plants to have a nurse effect on the smaller sized target species.

3.2 Effect of phylogenetic distance on target performance

Phylogenetic distance between the alien target and native competitor species had neither a linear nor a non-linear effect on any of the performance measures of the alien targets. Thus, in our experiment, phylogenetic relatedness did not affect alien species success. Previous experimental studies have shown that phylogenetic distance between native and introduced species can affect performance of the latter [34] and that this relationship may depend on several factors like life stage, disturbance, fungal pathogen presence and herbivore presence [20]. Here, we could find no relationship between alien target performance and phylogenetic distance to the native competitor. This could mean that phylogenetic distance is not important at all, but it could also be the result of two opposing effects of phylogenetic distance. On the one hand, competition between closely related species may benefit alien species that are distantly related to the species, because of the competitive exclusion from niche overlap among close relatives. On the other hand, environmental filtering may benefit alien species that are closely related to the native species. These two processes may result in a hump-shaped relationship [20] or, if they fully cancel each other out, result in a flat relationship between performance of the alien species and their phylogenetic distance to the native species.

Another possible explanation for the absence of an effect of phylogenetic relatedness could be that facilitation, as observed in our study, might not depend as much on relatedness as competition does. Instead, facilitation may depend more on the environmental conditions, as its importance increases with increasing abiotic stress. If traits relevant to dealing with stress, in particular heat, are less correlated evolutionarily as compared to those relevant for direct biotic interactions, the assumptions of phylogenetic distance as a proxy of functional trait distance becomes invalid. Future studies should explicitly test how the relationship between performance of invaders and phylogenetic distance to the native residents changes with environmental stress.

The absence of an effect of phylogenetic distance was independent of the herbivore, predator and nematode presence. The nematode, herbivore and predator treatments, as well as their interactions with the competition treatment, did not cause any significant effects on the performance of the alien target species. However, we cannot exclude the possibility that the numbers of individuals of nematodes, herbivores and predators that we added were too low to cause any impact on performance. We repeated the collection and release of arthropods and nematodes three times during the experimental period of 2.5 months. Although this resulted in hundreds of individuals of each group (herbivores, predators, nematodes) in each enclosure cage, we did not achieve arthropod and nematode densities similar to those in the habitat of collection (Eva Malecore, personal observation). This might be partially due to low numbers of individuals released, but also due to mortality and failure to reproduce in the enclosures. The latter might have been exacerbated by the hot temperatures in the cages, and the lack of sufficient vegetation. Although the approach of catching different trophic groups from the wild and releasing them into enclosures could in principle allow us to experimentally address major questions in community ecology, we are not aware of any other studies that have attempted this. Future studies should try to optimize experimental protocols for this approach.

4. Materials and Methods

4.1 Study species and seed material

To assess the effects of phylogenetic distance on performance of introduced species, we chose six alien target species (*Ambrosia artemisiifolia*, *Bidens frondosa*, *Eschscholzia californica*, *Papaver somniferum*, *Salvia verticillata* and *Veronica persica*) and nine native competitor species (*Achillea millefolium*, *Centaurea jacea*, *Daucus carota*, *Geum rivale*, *Leontodon hispidus*, *Lotus corniculatus*, *Plantago major*, *Prunella vulgaris* and *Veronica chamaedrys*). All species are forbs. The native competitor species are all species present in the Hockgraben, a semi-natural grassland close to the University of Konstanz [20], where we collected nematodes, herbivores and predators for the experiment. The seed material of all competitor species was ordered from Rieger-Hofmann GmbH (Blaufelden-Raboldshausen, Germany), while the seed material for the alien target species came from the seed collection of the Botanical Garden of the University of Konstanz or had been ordered from other botanical gardens. We sowed each species in trays with standard potting soil (Standard soil, Gebr. Patzer GmbH & Co. KG, Sinntal, Germany) in the first week of May 2015, and we put them in a growing chamber with a 12 h photoperiod until germination.

4.2 Experimental setup

We transplanted the seedlings at the end of May 2015 in circular 2.5 litre pots (diameter= 18 cm, height = 15 cm) using a 1:1 mix of potting soil (Standard soil, Gebr. Patzer GmbH & Co. KG, Sinntal, Germany) and sand. To provide the plants with sufficient nutrients throughout the experiment, we mixed 6.25 g slow-release fertilizer (Osmocote Pro; Everris GmbH, Nordhorn, Germany) into the substrate of each pot. For each pot, we transplanted a single seedling of an alien target species in the centre, and surrounded it with five seedlings of one of the native competitor species. To control for the effect of competition, we also grew each alien target species without competitors. In total, we had 60 target-competitor combinations (6 target species × 9 competitor species + 6 target species without competitor), and each combination was replicated 24 times. The 1440 pots were distributed over 24

enclosure cages, so that each cage contained one replicate of each of the 60 target-competitor combinations. The cages, located outside in the Botanical Garden of the University of Konstanz (47.69°N, 9.18°E), were 2.5 m × 2.5 m × 2 m (L × W × H) in size and covered with a fine mesh to prevent the passage of arthropods. The cage sides were lined with a white nylon fabric (mesh size: 0.2 × 0.2 mm; McStoff. de), the cage-tops with a netting material (mesh size: 0.8 × 0.8 mm; FA.BIO 02, Hartmann-Brockhaus, Egenburg, Germany), and the cage-floors with plastic sheets and a white fabric to prevent overheating [21]. In each cage, the pots were randomly assigned to fixed positions and watered ad libitum.

Each cage was randomly assigned to one of six treatment combinations: control, nematodes, herbivores, nematodes-herbivores, herbivores-predators, nematodes-herbivores-predators, with each treatment combination replicated four times. We had a factorial combination of a nematode-addition treatment and an insect-herbivore-addition treatment, and plants in the herbivore treatment were grown either with or without predators. Each treatment (herbivore, predator and nematode application) was applied three times during the experiment (Table A1).

4.2.1 Nematode extraction and application

We extracted nematodes from soil collected in four locations in Hockgraben, a semi-natural grassland close to the University of Konstanz, where each of the nine native competitor plants occur. At each of the four locations, we dug up a total of two litres of soil from two separate spots (one litre from each) until a depth of ~10 cm. We extracted nematodes from the soil using the Whitehead-tray method [22] (see appendix for more details), obtaining 800 ml of nematode solution with a concentration of ca. 15 nematodes per ml. We applied 1 ml of the solution (ca. 15 nematodes) to each pot in the nematode-treatment using a syringe on 20 June 2015. We repeated the nematode addition two more times, on 20 July 2015, and 3 August 2015 (Table A1).

4.2.2 Arthropod collection and application

We collected arthropods using a modified leaf vacuum (LS 1.0 by ECON-Handel, 0.75 kW / 1HP, engine speed: 2500 rotations/min, max. air speed: 85 m/s). We modified the leaf vacuum by inserting a wide tube (30 cm diameter) between the vacuum tube and the fan, and put a fine meshed fabric (0.2 × 0.2 mm) collecting bag in it. The wide tube was inserted to reduce the airflow and minimize damage and mortality of the arthropods in the collecting bag (see appendix for more details).

We collected the arthropods in the Hockgraben area by vacuuming the vegetation. Every five minutes, we replaced the collecting bag, and we collected one bag for each cage. After collection, we stored the bags with the arthropods at 5° C until further processing, minimizing the storage time as much as possible. To apply the herbivore and the predator treatments, we first sorted the content of each bag using an insect aspirator. We separated the herbivorous arthropods and predatory arthropods. We excluded all Diptera, because categorizing them according to their feeding guild often requires identification at the species level, which was not feasible for such a large number of individuals. We also excluded species of which the feeding guild was not clear or that were omnivorous. Among the herbivores, we included all caterpillars (Lepidoptera), herbivorous beetles (Coleoptera), herbivorous grasshoppers (Caelifera), aphids (Aphidoidea, Hemiptera), leafhoppers (Cicadellidae, Hemiptera), leaf bugs (Miridae, Heteroptera) and all other herbivorous Hemiptera. Aphids and leafhoppers were the most abundant herbivores. Among the predators, we included predatory mites (Acari), spiders (Araneae), lacewings (Neuroptera, both larvae and adults), ladybirds (Coccinellidae, Coleoptera, both larvae and adults), earwigs (Dermaptera), assassin bugs (Reduviidae, Hemiptera), damsel bugs (Nabidae, Hemiptera), and all other predatory Hemiptera. Spiders were the most abundant predators.

The number of herbivores in each collecting bag was always in the order of hundreds, while the predators were always in lower number, never exceeding one hundred. Between 8 and 11 July 2015, we released the herbivorous and predatory arthropods into the respective cages. To maintain the populations of herbivores and predators, and to cover a potential shift in herbivores and predators

through the season, we repeated the sampling and release of herbivores and predators between 23 and 25 July 2015 and between 6 and 7 August 2015 (Table A1).

4.3 Harvest and measurements

We harvested the experiment from the middle of August until the middle of-September. The harvest took one month, because harvesting was ordered by alien target species according to the life stage of the species. All pots with *Papaver somniferum* as target species were harvested first, because it was the first species to flower and set seed. For each alien target species, we recorded survival at harvest (alive/dead). Further, we counted the number of flowering structures (single flowers or fruits for *Papaver somniferum*, *Eschscholzia californica* and *Veronica persica*, inflorescences or infructescences for *Salvia verticillata*, and capitula for *Bidens frondosa*). We did not distinguish between buds, flowers and fruits. For each target and competitor species in a pot, we collected the above-ground biomass, dried it for three days in a drying oven at 70° C, and weighed it with an accuracy of 0.001 g, pooling competitor individuals per pot.

4.4 Statistical Analyses

To test whether herbivore, predator and nematode presence, as well as competitor presence and phylogenetic distance, affected the performance of the alien target species, we used generalized linear mixed models (GLMMs) implemented with functions of the “lme4” package (Bates et al. 2015) in R [23]. We used survival, above-ground biomass and number of flowering structures as measures of alien target performance. For target survival (0/1), we used binomial GLMMs implemented in the glmer function. For target plants that had survived, we used Gaussian LMMs implemented in the lmer function to analyse above-ground biomass, and negative binomial GLMMs implemented in the glmer.nb function to analyse the number of flowering structures. The negative binomial models were used to reduce overdispersion. In a first set of models, we tested the effect of the presence of competitors, and of the presence of herbivores, predators and nematodes. In a second set of models, we analysed the subset of plants with competitors to test the effect of phylogenetic distance.

4.4.1 Competitor presence, herbivores, predators and nematodes

As above-ground biomass varied greatly among the alien target species, we cube-root transformed the data. For each of the three response variables (survival, above-ground biomass and flowering structure number), we used the same model structure. We first performed analyses including only competitor presence as explanatory factor in the fixed part of the random model. In a second step, besides competitor presence (0/1), we included herbivore presence (0/1), predator presence (0/1), nematode presence (0/1), the nematode × herbivore presence interaction, the nematode × predator presence interaction, as well as the interaction of competitor presence with all other factors (hereafter “full model”). The interactions of competitor presence with all other factors were excluded from the model of the number of flowering structures due to non-convergence of the model. To account for non-independence among data points from the same target species and the same cage and from plants grown with the same competitor species, we included target species, cage and competitor species as random factors (i.e. random intercepts). In addition, as each target species might respond differently to competition, we also included random slopes for the response to competition of the target species. We used a Bayesian approach to plot the 95% credible intervals in the figures using the sim function of the “arm” package [24].

4.4.2 Phylogenetic distance, herbivores, predators and nematodes

To test how phylogenetic distance between alien target and native competitor species affected survival, above-ground biomass and number of flowering structures of the target plants, we used a subset of data that excluded the plants grown without competition. The phylogenetic tree of target and competitor species was constructed by pruning a modified version (Malecore et al. 2018) of the dated DaPhnE supertree of Central European plant species [25]. For each target-competitor

combination, we calculated the phylogenetic distance using the cophenetic function of the “ape” package [23, 26] We first ran a model including the phylogenetic distance only, both as linear and quadratic terms. The latter was included to test for potential nonlinear effects. In a second step, we added herbivore presence (0/1), predator presence (0/1), nematode presence (0/1), as well as the interaction of phylogenetic distance with all those factors (hereafter “full model”). The interaction of phylogenetic distance with all other factors was excluded from the model of the number of flowering structures due to non-convergence of the model. To account for non-independence among data points from the same target species and the same cage and from plants grown with the same competitor species, we included target species, cage and competitor species as random factors (i.e. random intercepts). We used a Bayesian approach to plot the 95 % credible intervals in the figures using the sim function of the “arm” package [24].

5. Conclusions

We aimed to test whether the positive relationship between performance of alien invaders and their phylogenetic distance to the native residents, as predicted by Darwin (1856) and experimentally confirmed in several studies [20, 34, 35] (but see [36] and citations within), changes if one considers multitrophic interactions (Figure 1). We found, however, no significant overall effects of competition, phylogenetic distance or the addition of herbivores, predators and nematodes. In other words, we did not find any support for our hypotheses. Reasons for this could be the hot temperatures during the experiment, which may have turned the competitors into facilitators, and that we did not manage to build up large communities of the herbivores, predators and nematodes. Therefore, we call for studies that test the roles of multitrophic interactions under less stressful environmental conditions, and with larger numbers of each of the multitrophic actors.

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377 **Conflicts of Interest:** The authors declare no conflict of interest.

378

Appendix

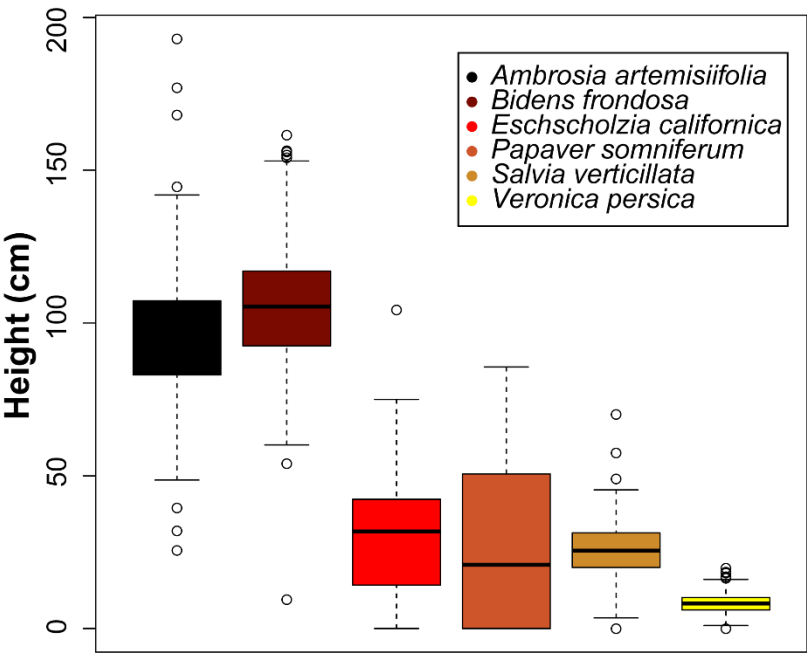


Figure A1 Height measurements of the nine alien target species. Height was measured from soil surface up to the last leaf, without stretching the plant.

Temperature measurements

In each of the 24 cages, temperature was measured using temperature loggers (Maxim Integrated Products, DS1922L-F5# Thermochron iButton 8K -40 to 85°C). Each temperature logger was sealed within a piece of tin foil and put in a small plastic bag to avoid dampness, and stapled at a height of 20 cm from the soil on the inside of the wooden structure of the cage. The loggers took measurements every hour throughout the experiment.

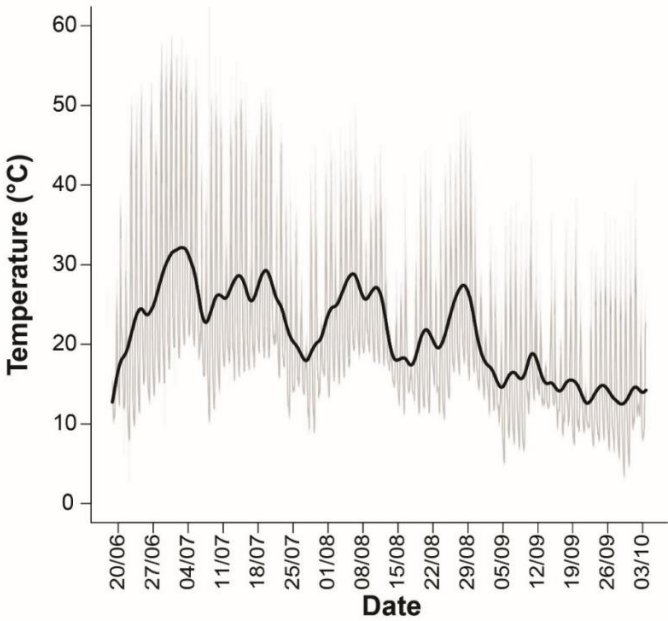


Figure A2 Temperature data for each cage for the time period of the common garden experiment (year 2015). In grey, the overlapping data for each of the 24 cages. In black, a smoother spline obtained using the smooth.spline function (spar=0.5) in R (R Core Tea, 2017).

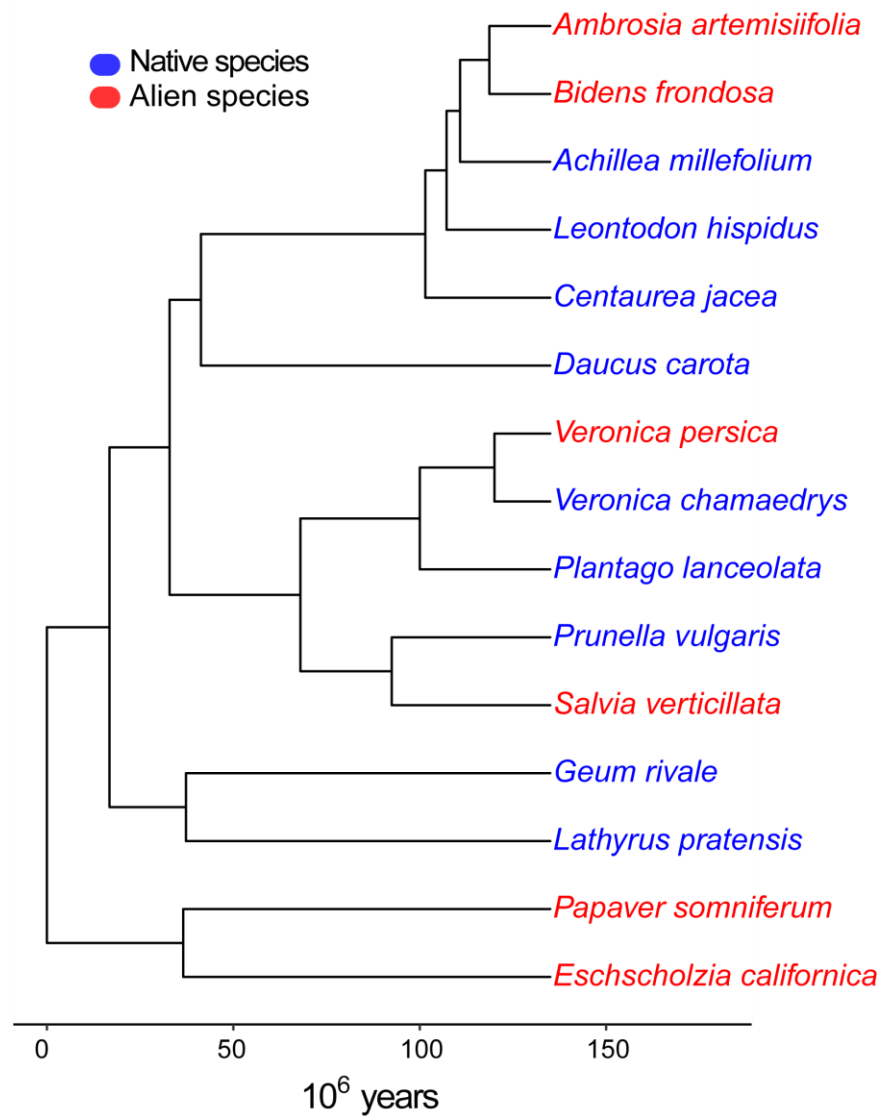


Figure A3 Phylogenetic tree of the species used as alien targets and native competitors.

Nematode extraction

For the nematode extraction, we used the Whitehead-tray method (Whitehead & Hemming, 1965). We put the collected soil (two liters) in two large sieves above a layer of fine meshed fabric and a layer of kitchen paper tissue. We put the sieves in two trays and filled them with water until the soil was almost covered by the water. In this way, the nematodes present in the soil will move downwards, go through the fine meshed fabric and the kitchen tissue, and will end up in the water on the bottom of the tray. Since soil nematodes are not able to swim actively, they will not go back up into the soil.

After at least 30 h, the sieves with the soil were removed and the water was filtered using sieves with mesh widths of 40 µm and 20 µm. The sieves were then washed out and the extract was collected in a cup and diluted in ca. 800 ml of water. To check for the effective presence of nematodes in the solution, we took three samples of 1 ml of the solution, and counted the number of nematodes under a microscope. We counted between 15 and 20 nematodes each time. The extraction of nematodes was repeated three times (middle of June, middle of July and end of July).



Figure A4 Soil during nematode extraction. The soil was placed in a sieve, above a layer of kitchen paper and a layer of fine meshed fabric, and then covered with water. Nematodes will reach the bottom of the tray going through the fine meshed fabric and the other layers.

Technical sheet for the LS 1.0 by ECON-Handel Leaf Vacuum

- Model: LS 1.0
- Engine Size: 25.4 cc
- Fuel: Mixture 1: 40
- Net Weight: 4.5 kg
- Dimensions: 410 x 260 x 350 mm
- Fuel Tank Capacity: 0.5 litre
- Certificate number: S50248934
- Motor power: 0.75 kW/1PS
- Rotation speed: 2500 rpm.
- Max. AIR SPEED RATING: 85 m/s
- Leaf bag volume: approx. 50 litres.
- Noise Level: 95 dB (a)



Figure A5 The leaf vacuum, after modification, used to collect arthropods in the Hockgraben grassland area. Arthropods are collected in a mesh bag positioned in the bucket, where airflow is reduced due to the larger volume

Table A1 Dates of treatment applications throughout the experiment

Treatment	1. application	2. application	3. application
Herbivores and predators	8-11.7.2015	23-25.7.2015	6-7.8.2015
Nematodes	20.06.2015	20.07.2015	3.08.2015

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