

# Allelopathic and Competitive Interactions Between Native and Alien Plants

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# Abstract

The novel-weapons and homeland-security hypotheses are based on the idea that aliens and natives are not adapted to each other's allelochemicals as they did not co-evolve. However, as only a few studies have tested this, it remains unclear how important co-evolutionary history is in determining the strength of allelopathic interactions between aliens and natives. Here, we tested for potential pairwise allelopathic effects on each other of five alien and five native herbaceous species in China. We did a germination and a competition experiment. In the germination experiment, we tested whether aqueous extracts of the ten study species had allelopathic effects on each other's seed germination. In the competition experiment, we tested whether the alien and native species differed in their competitive effects and responses, and whether these were changed by the presence of activated carbon – a presumed allelopathy neutralizer – in the soil. Plant extracts had negative allelopathic effects on seed germination. This was particularly the case for extracts from the native species. Moreover, aqueous extracts had slightly stronger negative effects on germination of the aliens than on germination of the natives. In the competition experiment, on the other hand, the natives suffered more from competition than the alien species did, but we could not relate this to allelopathy. Alien plants had negative competitive and allelopathic effects on native plants, and the reverse was also true. These alien-native interactions, however, were not consistently stronger or weaker than native-native interactions or alien-alien interactions.

# Introduction

With increasing globalization, thousands of plant species have been introduced from their native areas into foreign lands. Although many of those alien plant species are found in cultivation only (van Kleunen et al. 2018a), over 13,000 alien species worldwide have established persistent wild populations (i.e., have become naturalized; van Kleunen et al. 2015). Some of those naturalized plants are considered invasive, as they spread rapidly (Richardson et al. 2000), and can have negative ecological (Vilà et al. 2011) and socioeconomic impacts (e.g. Schaffner et al. 2020). Hence, it is important to identify mechanisms that underlie naturalization and invasion success (Sakai et al. 2001; van Kleunen et al. 2018b).

It has frequently been suggested that strong competitors are more likely to become invasive (e.g. Baker, 1974). Indeed, empirical studies found evidence that many invasive plants exert stronger competitive effects or are more tolerant to competition than many native ones (Kuebbing and Nuñez, 2016; Golivets and Wallin, 2018; Zhang and van Kleunen, 2019). The high competitiveness of invasive aliens could be due to them having traits, such as high specific leaf area and low root tissue density (Pyšek and Richardson 2007; van Kleunen et al. 2010; Lachaise et al. 2020), which allow them to rapidly take up available resources, and consequently have high intrinsic growth rates (Dawson et al. 2011; Zhang and van Kleunen 2019). However, as already pointed out by Baker (1974), the competitiveness could also be due to the production of allelochemicals that suppress germination and growth of neighboring plants. Indeed, the novel-weapons hypothesis predicts that some alien plants are successful invaders because they produce allelopathic compounds to which the native plants are not adapted (Callaway and Ridenour 2004). These allelopathic compounds may be released through leaf leachates, root exudates, volatiles and decaying above- and below-ground litter (Callaway and Ridenour 2004; Callaway et al. 2005; Inderjit et al. 2011). Several studies provided support for the novel-weapons hypothesis (e.g. Ridenour and Callaway 2001; Prati and Bossdorf 2004; Abhilasha et al. 2008; Gómez-Aparicio and Canham 2008; Thorpe et al. 2009; Li and Jin 2010; Inderjit et al. 2011; Becerra et al. 2018; Irimia et al. 2019), suggesting that allelopathy can play an important role in the ability of invasive plants to suppress native plants.

If allelochemicals released by alien plants are novel to natives, the reverse should also be true. In other words, alien plant species, irrespective of whether they are invasive or not, may also be susceptible to negative effects of allelochemicals released by native species (the homeland-security hypothesis; Cummings et al. 2012). There is empirical support for the predictions of this hypothesis (Cummings et al. 2012; Christina et al. 2015; Ning et al. 2016; Mignoni et al. 2018; Adomako et al. 2019). For instance, phytochemicals released by the native legume *Sesbania virgate* reduced germination and seedling growth of the alien invader *Leucaena leucocephala* in Brazil (Mignoni et al. 2018). However, as only a few studies have tested the homeland-security hypothesis, it remains unclear how common allelopathic effects of native plants on alien plants are. Moreover, it is not known whether these effects are of similar magnitudes as the allelopathic effects of alien plants on native plants.

Here, we tested for potential pairwise allelopathic effects of five alien and five native herbaceous species on each other in China. As allelopathic effects may act at different stages of a plant's life cycle, we did both a germination and a competition experiment. In the germination experiment, we tested whether aqueous extracts of the ten study species had allelopathic effects on their seed germination. In the competition experiment, we tested whether the alien and native species differed in their competitive effects and responses, and whether these were changed by the presence of activated carbon – a presumed allelopathy neutralizer – in the soil. Specifically, we addressed the following questions: (1) Do the effects of aqueous plant extracts on germination and the germination responses to the aqueous extracts differ between the native and alien species? (2) Do the native and alien species differ in their competitive effects and responses to competition, and are their competitive interactions mediated by allelopathy?

## Materials And Methods

### Study species

We obtained seeds of five species that are native to eastern China and five species that are alien. All ten species are herbaceous. The seeds were collected from fields in Taizhou (Zhejiang province, China) or obtained from commercial seed companies (**Table 1**). The 10 species were selected on the basis that they co-occur across a wide range of habitats in China (Zhang SY and Ding BY 1993). All five alien species are naturalized in more than 18 of the 34 Chinese provinces (including municipalities and autonomous regions) (Yan et al. 2019), and have been classified as general common invasive, local invasive or serious invasive plants (Yan et al. 2014) (**Table 1**).

### Germination Experiment

To test the effects of aqueous extracts of the 10 study species on seed germination of themselves and each other, we first grew plants for the production of the extracts. On 4 January 2018, seeds of each of the ten species were sown in plastic boxes (l × w × h: 54 × 28 × 5 cm) that had been filled with an 1:1 (v:v) mixture of sand and vermiculite (both purchased from Xiaoxuan Horticulture, Hebei province, China). The boxes were then placed in a phytochamber (day-time temperature: 18–21°C, night-time temperature: 16–20°C, day length: 14 hours, relative humidity: 60%). Two weeks after emergence, the seedlings were transplanted individually into 1-L plastic pots that had been filled with an 1:1 (v:v) mixture of sand and vermiculite. For each species, we had 10 pots that were then transferred to a phytochamber with the same conditions for germination. The seedlings were fertilized once a week for a total of 15 weeks with a Woshibao® liquid fertilizer (N: 50 g/L, P: 30 g/L, K: 50 g/L, Mg: 1.8 g/L, S: 2.2 g/L, micronutrients: 0.2 g/L) (purchased from Woshibao fertilizer sales Co., Ltd, Lu'an, China), which was diluted 1:200 with distilled water. We watered the plants twice a week *ad libitum*.

Like the vast majority of alleopathy studies (Zhang et al. 2021), we used plant extracts. To produce aqueous extracts, we harvested five individual plants per species after 15 weeks of growth (i.e. on 10 April 2018). We washed the roots to remove the growth medium. We then chopped each plant into 2 cm pieces, mixed the shoot and root pieces of the five plants per species, and then took a random sample of 300 g from each species. We then transferred the sampled plant tissue into a beaker containing 900 mL of distilled water and let it soak for 24 h at room temperature. Thereafter, the extracts were filtered, first through a layer of Whatman No. 1 filter paper, and then –to remove fungal spores– through a 0.8 µm filter membrane (25 mm in diameter) into separate autoclaved Falcon tubes. A fresh filter membrane was used each time. The filtrate was stored at -20°C until use.

To test the allelopathic effects of the aqueous extracts of each of the 10 species on seed germination of the 10 species, we set up a germination experiment at Taizhou University, China. We filled a total of 660 Petri dishes (6 cm in diameter) with agar gel that contained the aqueous extracts. To make the agar gel, we first mixed 12 g agar (high strength) with 30 g sucrose and 3.225 g Murashige and Skoog culture medium in 1 L distilled water. The pH of the resulting solution was adjusted to 6.0 with HCL or NaOH. The liquid agar medium was then poured into 250 mL Schott glass bottles and autoclaved for 15 minutes at 120°C at a pressure of 100 kPa. The hot agar medium was then immediately removed from the autoclave and placed into a 40°C water bath for 10 minutes. This was done to cool down the medium while keeping it in a liquid state. The plant extracts were taken from storage at -20°C and thawed in a heating cabinet at 35°C for 30 minutes. They were filtered once again under sterile conditions through 0.8 µm filter membranes, and then 300 mL of the filtrate was mixed thoroughly with 600 mL of the liquid agar medium in separate beakers for each species. Then 10 mL of the extract-agar mixture was poured into each Petri dish, after which it cooled down and solidified. As a control treatment, we used agar medium in which we had mixed distilled water instead of plant extract.

On 15 and 16 April 2018, we sowed the seeds. Prior to sowing, the seeds were sterilized for five minutes in a 5 % sodium hypochlorite solution and rinsed with distilled water. In each Petri dish, we placed 10 seeds of one of the 10 species. The experimental set-up resulted in a total of 660 Petri dishes: 10 plant species × 11 extract types (10 plant-species extracts and 1 control of distilled water) × 6 replicates (see Fig. 1 for all species combinations). We sealed each Petri dish with parafilm to avoid evaporative loss of water. The 660 Petri dishes were randomly allocated to positions within a phytochamber (day-time temperature: 21°C, night-time temperature: 16°C, day length: 15 hours, relative humidity: 60%). We recorded the number of seeds that had germinated in each Petri dish daily. This allowed us to calculate germination success as the proportion of germinated seeds, and the days to first germination. On 16 July 2018, c. two months after the last seedling had emerged, we stopped the experiment.

## Competition Experiment

Of the 880 target plants in the competition experiment, 76 had died by the end of the experiment. Survival varied among species, and was reduced by the presence of activated carbon, particularly for the native species (89.3% survival for native species vs 93.4% for alien species; **Fig. S1**; **Table S1**).

Above-ground biomass varied among species, but on average did not differ between the native and alien species (Figs. 3 and 4; **Table 3**). Above-ground biomass was significantly reduced by activated carbon, and by the presence of competition (Figs. 3 and 4; **Table 3**). The negative effect of competition, however, was significantly stronger for native species (-59.6%) than for alien species (-37.5%; Fig. 4; **Table 3**). There was no significant effect of intra- vs interspecific competition or effect of competition with alien vs competition with native species; nor were there significant interactions of those effects with activated carbon or origin (Fig. 4; **Table 3**).

# Statistical Analyses

All analyses were done in R version 3.6.1 (R Development Core Team 2019). The proportion of germinated seeds and the number of days to first germination in the germination experiment, as well as survival and above-ground biomass in the competition experiment were analyzed with generalized linear mixed models (GLMMs) using functions of the packages *lme4* (Bates 2014) and *nlme* (Pinheiro et al. 2015).

For the proportion of germinated seeds, we used a binomial error distribution, and, for the number of days until first germination, we used a Poisson error distribution. In both models, we included 'Origin' (alien vs native) of the test species, 'Extract type' (control, self, alien species, native species) and their interaction as fixed terms. We accounted for variation among test species and the species that served as sources of extracts by including identities of the test and extract species (including the control) as random terms. To test the effects of the individual extract types in more detail, we created three dummy variables to make three planned orthogonal contrasts. First, we tested whether the effects of the aqueous plant extracts differed from the control treatment (i.e. water). Second, among the treatments with aqueous plant extracts, we tested whether the effects of the plant species on itself differed from those of other species. Third, among the aqueous extracts of other plant species, we tested whether the effects of alien species differed from those of native species.

For above-ground biomass in the competition experiment, we used a Gaussian error distribution. In the model, we included 'Activated carbon' (with vs without), 'Origin' (native vs alien) of the test species, 'Competition treatment' (intraspecific competition, interspecific with alien species, interspecific with native species, without competition) and their interactions as fixed terms. We accounted for variation among target species and the species that served as competitors by including them as random terms. To improve normality and homoscedasticity of the residuals, above-ground biomass was cubic transformed, and the *varIdent* function was used to allow for different variances per test species (Zuur et al. 2009). To test the effects of competition treatment in more detail, we create three dummy variables to make three planned orthogonal contrasts. First, we tested whether the effects of competition differed from the effects of treatment without competition. Second, among the treatments with competition, we tested whether the effects of intraspecific and interspecific competition differed. Third, among the interspecific treatments, we tested whether the effects of alien species differed from those of native species.

In all GLMMs, we assessed the significance of the fixed terms with log-likelihood-ratio tests (Zuur et al. 2009), in which a model with the term of interest was compared to a model without that term. The log-likelihood ratios are approximately  $\chi^2$ -distributed (Zuur et al. 2009).

## Results

### Germination experiment

Species varied in their germination success (i.e. proportion of germinated seeds), with the lowest and highest values for the natives *Platycodon grandiflorus* (4%) and *Achyranthes bidentata* (61%), respectively (Fig. 1a). Germination success also depended on the species used as source for the extract, with the lowest germination on extracts made of the alien *Lepidium virginicum* (6%) and the native *Achyranthes bidentata* (6%), and the highest germination on extracts made of the alien *Capsella bursa-pastoris* (49%; Fig. 1a).

Averaged across all treatments, the proportion of seeds that germinated did not differ significantly between alien and native test species (Fig. 2a, **Table 2**). The presence of a plant extract (compared to the water-control treatment)

reduced the germination, most strongly for the alien test species and only little for the native test species (Fig. 2a; significant Origin × [Extract vs control] interaction in **Table 2**). Seeds, particularly of alien species, treated with extracts of native plants had a slightly, but significantly, stronger germination reduction than seeds treated with extracts of alien plants (Fig. 2a; **Table 2**). However, germination was overall lowest for seeds treated with extracts of the same species (Fig. 2a; **Table 2**).

Among the Petri-dishes with germinated seeds, the ones with a low proportion of germinated seeds also germinated later (Pearson's  $r = -0.41$ ,  $n = 357$ ,  $P < 0.001$ ). The slowest and fastest germination were found for the natives *Platycodon grandiflorus* (16 days) and *Achyranthes bidentata* (2 days), respectively (Fig. 1b). The slowest germination was found on extracts made of the alien *Crotalaria pallida* (10 days), and the fastest germination on extracts made of the alien *Amaranthus tricolor* (5 days; Fig. 1b).

On average, the alien and native species did not differ in the number of days to first germination (Fig. 2b; **Table 2**). The presence of plant extracts delayed the germination (significant Extract vs control effect), and this was particularly the case for the alien test species (Fig. 2b; significant Origin × [Extract vs control] interaction in **Table 2**). The effect of extracts of the same plant species did not significantly differ from those of other plant species (Fig. 2b; **Table 2**). Germination of the native test species was delayed slightly more by extracts of other native species than by extracts of alien species, whereas the reverse was true for the alien test species (Fig. 2b; significant Origin × [Alien vs native] interaction in **Table 2**).

## Discussion

We tested for allelopathic and competitive interactions between alien and native plants. In our large germination experiment, we found evidence for negative allelopathic effects of plant extracts on seed germination (germination rate or days to first germination). This was particularly the case for extracts from the native species. Moreover, the extracts had slightly stronger negative effects on germination of the aliens than on germination of the natives. On the other hand, in our competition experiment, the natives suffered more from competition than the alien species did. Unfortunately, as activated carbon had negative effects on all plants, both with and without competition, the role of allelopathy in the competitive interactions remained unclear. Nevertheless, our results show that both native and alien species have negative effects on each other at different life-cycle stages, but that the strength of the effects might differ between the native and alien species.

While the novel-weapons hypothesis predicts strong allelopathic effects of some aliens on natives (Callaway and Ridenour 2004), the homeland-security hypothesis posits that the reverse should also be true (Cummings et al. 2012). Both hypotheses are based on the idea that aliens and natives are not adapted to each other's allelochemicals. While we found that the aliens had negative effects on the natives and *vice versa*, we did not find that these effects were stronger than the effects of natives on other natives. This could indicate that differences in co-evolutionary history between the species plays no major role. Another explanation could be that the alien species have been present in China for already such a long time that they and the natives have already adapted to some degree to each other's allelochemicals. This is not unlikely as the five alien species are widespread in China and have been present there for at least 100 years (Table 1). Indeed, rapid co-evolutionary responses in allelochemical interactions have been shown before (Callaway et al. 2005; Lankau 2012; but see Oduor et al. 2020). Alternatively, it could indicate that the natives that we selected also share little co-evolutionary history with each other. The latter seems unlikely as the native species occur in similar habitats. However, as the seed material did not originate from the same locations, it could be that the chosen accessions are not adapted to each other.

Although we found no evidence for the importance of co-evolutionary history in the interactions between the aliens and natives, we found that they differed in their allelopathic effects and responses. The natives had on average slightly stronger allelopathic effects, and the aliens showed stronger negative responses, in terms of germination. The latter, however, mainly reflects that the aliens had far higher germination rates than the natives in the absence of aqueous extracts, whereas the aliens and natives showed similar low germination rates with extracts. This is in line with the idea that naturalized aliens are more opportunistic and take more advantage of beneficial conditions (i.e. in this case from allelochemical-free conditions). On the other hand, in the competition experiment, the aliens appeared to take less advantage of the competition-free conditions. Therefore, it remains unclear whether overall the aliens are more opportunistic than the natives.

The species with low germination rates generally also took longer to germinate. However, there were a few differences in the effects of the aqueous extracts on germination rate and speed. For example, while for germination rate there was no significant interaction between the origins of the test and extract species, germination of native species was delayed most by extracts from other native species, whereas germination of alien species was delayed most by extracts from other alien species. These results are opposite to the predictions of the novel-weapons and homeland-security hypotheses (Callaway and Ridenour 2004; Cummings et al. 2012). Although these differences in germination speed did not coincide with differences in the proportion of germinated seeds, earlier or delayed seed germination can have fitness consequences. For example, earlier germination could give the seedlings a size advantage over the ones that emerge later (priority effects; Gioria and Osborne 2014; Godoy et al. 2009; Wainwright et al. 2012). On the other hand, delaying germination could also avoid competition if it allows seeds to remain dormant until more favourable conditions arise (Fenner and Thompson 2005).

Several previous studies found, just like our study, evidence for allelopathic effects of native plant species on alien species. For instance, adding leaf litter of the native legume tree *Diphysa americana* to soil reduced tiller survival and height growth of the invasive grass *Saccharum spontaneum* in Panama (Cummings et al. 2012). Similarly, adding litter of native plant species from monsoon evergreen broadleaf forests to soil decreased biomass and leaf area of the alien invader *Mikania micrantha* in subtropical China (Hou et al. 2011). Furthermore, the native shrub *Polygonella myriophylla* had negative allelopathic effects on the alien grass *Paspalum notatum* in Florida (Weidenhamer and Romeo 2005), and communities of native species had negative allelopathic effects on the invader *Solidago canadensis* in China (Adomako et al. 2019). This can be interpreted as support for the homeland-security hypothesis. However, while these studies showed that invasive alien plants may suffer from allelopathy of native plants, most of them did not test whether these effects differ between invasive and non-invasive alien or native species. An exception is a study by Mignoni et al. (2018) who found allelopathic effects of the native *Sesbania virgata* on the alien *Leucaena leucocephala* in Brazil, but not the other way around. This shows that allelopathic effects of species on each other can be asymmetric. Ning et al. (2016), on the other hand, also found evidence for allelopathic effects of native communities on alien species in Germany, but did not find differences in the magnitude of the effects between invasive and non-invasive alien species. In our study, we also found variation in the allelopathic effects and responses among species, but we did not find that interactions between an alien and a native species were consistently different from interactions between two native species or two alien species. More studies are needed to test whether this is a general result, and whether it depends on the degree of invasiveness of the alien species and the commonness of the native species.

Interestingly, we found that germination was most strongly inhibited if the aqueous extract was from the same species as the test seeds. In other words, we found strong evidence for auto-allelopathy (Kato-Noguchi et al. 2017; Hisashi et al. 2017; Asif et al. 2019). Particularly, if the auto-allelochemicals induce seed dormancy and do not decrease seed viability, auto-allelopathy can regulate populations over space and time to avoid severe intraspecific

competition (Singh et al. 1999). Although auto-allelopathy has been found for a large number of weeds and crops in natural and agricultural ecosystems (Singh et al. 1999; Hisashi et al. 2017; Asif et al. 2019; Zhang et al. 2019), we found that it actually exceeded hetero-allelopathy. This is surprising given that a recent meta-analysis found that the effect of allelopathy became more negative with increasing phylogenetic distance between the species (Zhang et al. 2020). This meta-analysis, however, did not distinguish explicitly between auto- and hetero-allelopathy, and testing the generality of our finding thus requires more research.

Most of the previous studies testing for potential allelopathic inhibition of seed germination also used aqueous plant extracts (i.e. leachate), and found negative effects on germination (Zhang et al. 2021). Some studies compared the effects of the aqueous extracts to effects of known allelopathic compounds, which suggest that the effect of the aqueous extract is due to chemical inhibition (e.g. Krstin et al. 2021). It has, however, also been suggested that the effect of the aqueous extracts is not always due to chemical inhibition but can be due to the high osmolality of the extracts (Inderjit and Nilsen 2003, Loydi et al. 2015, Oduor et al. 2020). Therefore, it has been suggested that the osmolality of the control treatment should be adjusted by adding for example polyethylene glycol (PEG), which consists of large molecules that change the osmolality but cannot be taken up by the plant cells. We refrained from this in our study, because in contrast to many other studies, which usually germinated the seeds on filter paper soaked with aqueous extract, we had mixed the aqueous extract into a semi-solid agar medium. A previous bachelor-thesis project, that used agar medium both with a water control and a control that had its osmolality adjusted with PEG to the average value of the aqueous extracts, did not find a difference in germination between both controls (Kern 2018). We therefore believe that the osmolality of the aqueous extracts is not responsible for the negative effects on germination in our study.

As allelopathy usually has stronger impacts on plant growth than on seed germination (Zhang et al. 2020), we also did a competition experiment. We used activated carbon as a presumed neutralizer of allelopathic compounds, as this has been successfully used in previous studies on allelopathy (Mahall and Callaway 1992; Inderjit and Callaway 2003; Mangla et al. 2008; Lankau 2010). However, other studies have pointed out that activated carbon can have undesired side-effects, and that it is therefore important to also test its effects in the absence of competition (Lau et al. 2008; Weißhuhn and Prati 2009; Kabouw et al. 2010). We did this and unfortunately found that activated carbon had strong negative effects on plant growth, even when plants were grown without competition. Therefore, we not able to infer how much of the competitive interactions between the plants were due to resource competition and how much was due to allelopathy (a form of interference competition). To gain some insights into the effects of activated carbon, we also measured several root traits on the plants in the competition-free treatment (Supplement material), and found that activated carbon addition reduced the total root length as well as the root-weight ratio (Fig. S3). This shows that plants invested less biomass in roots when activated carbon was present. Furthermore, the specific root length (i.e. root length/root biomass) and the number of root tips per unit root biomass were both higher in the presence of activated carbon, indicating that activated carbon caused the roots to elongate (i.e. become thinner) and branch (Fig. S3). So, activated carbon clearly affected the plant's root system and overall growth, and we therefore recommend against the use of activated carbon in studies on allelopathy, unless it has been shown that it does not have side effects in the particular study system.

In conclusion, we showed alien plants have negative competitive and allelopathic effects on native plants, and that the reverse is also true. These alien-native interactions, however, were not consistently stronger or weaker than native-native interactions or alien-alien interactions. So, we found no strong evidence that co-evolutionary history between the plant species played an important role in the strengths of their interactions. In other words, while some alien species may have novel weapons, and some natives may contribute to homeland security, we did not find general support for those hypotheses.

## Declarations

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## Tables

### Table 1

Information on the ten herbaceous species used in this study

Species	Family	Number of Chinese provinces	Status in China (year of first record)	Region of origin	Life cycle	Seed source
<i>Amaranthus tricolor</i> L.	Amaranthaceae	34	Alien-3 (1909)	Tropical Asia	Annual	Junjie Nursery Stock Company (Jiangsu, Suqian)
<i>Lepidium virginicum</i> L.	Brassicaceae	33	Alien-2 (1910)	North America	Annual/biennial	Field in Taizhou
<i>Trifolium repens</i> L.	Fabaceae	31	Alien-2 (1908)	Europe, and parts of Asia and Africa	Perennial	Junjie Nursery Stock Company (Jiangsu, Suqian)
<i>Capsella bursa-pastoris</i> (L.) Medik.	Brassicaceae	34	Alien-4 (1906)	Europe, and parts of Asia and Africa	Annual	Field in Taizhou
<i>Crotalaria pallida</i> Aiton	Fabaceae	19	Alien-3 (1913)	Tropical Africa and Tropical Asia	Perennial	Junjie Nursery Stock Company (Jiangsu, Suqian)
<i>Achyranthes bidentata</i> Blume	Amaranthaceae	14	Native	Eastern and Tropical Asia	Perennial	Thousand Green Seed Company (Jiangsu, Suqian)
<i>Crepidiastrum sonchifolium</i> (Maxim.) Pak & Kawano	Asteraceae	19	Native	Eastern Asia	Annual/Biennial	Thousand Green Seed Company (Jiangsu, Suqian)
<i>Platycodon grandiflorus</i> (Jacq.) A. DC.	Campanulaceae	22	Native	Eastern Asia	Perennial	Thousand Green Seed Company (Jiangsu, Suqian)
<i>Glycyrrhiza uralensis</i> Fisch	Fabaceae	11	Native	Temperate Asia	Perennial	Thousand Green Seed Company (Jiangsu, Suqian)
<i>Plantago asiatica</i> L.	Plantaginaceae	27	Native	Eastern and Tropical Asia	Biennial/perennial	Wancao Seed Industry (Hebei, Baoding)

Information on the number of Chinese provinces in which a species occurs is from Yan et al. (2019) for alien species, and from the Flora of China ([http://www.efloras.org/flora\\_page.aspx?flora\\_id=2](http://www.efloras.org/flora_page.aspx?flora_id=2)) for native species. Information on the alien status and its invasiveness grade in China is from Yan et al. (2014): Alien-2 = serious invasive plant (i.e. alien species having obvious impact on the economy or environment), Alien-3 = local invasive plant (i.e. alien species having local impacts), Alien-4 (i.e. alien species that are common but do not have obvious impacts). Information on the year of first record of alien species is from the Chinese Virtual Herbarium (<https://www.cvh.ac.cn/>). Information on the region of origin is from POWO (2019). Information on life cycle is from the Flora of China.

## Table 2

Results of generalized linear mixed models testing for effects of species origin (alien, native) and extract type (control, self, alien species, native species) on the proportion of germinated seeds (binomial distribution) and number of days to first germination (Poisson distribution). The effect of extract type was decomposed into three orthogonal contrasts: treatments with plant extracts *vs* the water control, treatments with extracts of the same species *vs* those with extracts of another species, and treatments with extracts of another species that is alien *vs* those with extracts of another species that is native

	Proportion of germinated seeds			Days to first germination	
	df	$\chi^2$	<i>P</i>	$\chi^2$	<i>P</i>
<i>Fixed terms</i>					
Origin	1	0.38	0.535	0.02	0.875
Extract type	3	30.92	<b>&lt;0.001</b>	5.95	0.114
Extract <i>vs</i> control	1	1.46	0.227	4.85	<b>0.028</b>
Self <i>vs</i> other	1	25.46	<b>&lt;0.001</b>	0.35	0.552
Alien <i>vs</i> native	1	4.00	<b>0.045</b>	0.75	0.387
Origin × Extract type	3	29.68	<b>&lt;0.001</b>	32.29	<b>&lt;0.001</b>
Origin × [Extract <i>vs</i> control]	1	27.84	<b>&lt;0.001</b>	13.91	<b>&lt;0.001</b>
Origin × [Self <i>vs</i> other]	1	1.42	0.233	0.07	0.795
Origin × [Alien <i>vs</i> native]	1	0.42	0.519	18.31	<b>&lt;0.001</b>
<i>Random terms</i>					
		Standard deviation		Standard deviation	
Test species		1.114		0.2683	
Species used as source of extracts		1.341		0.6343	

The identities of the test species and the species used as source of the extracts were included as random factors. For the proportion of germinated seeds, *n* = 626, and for days to germination, *n* = 357. *P*-values < 0.05 are highlighted in bold.

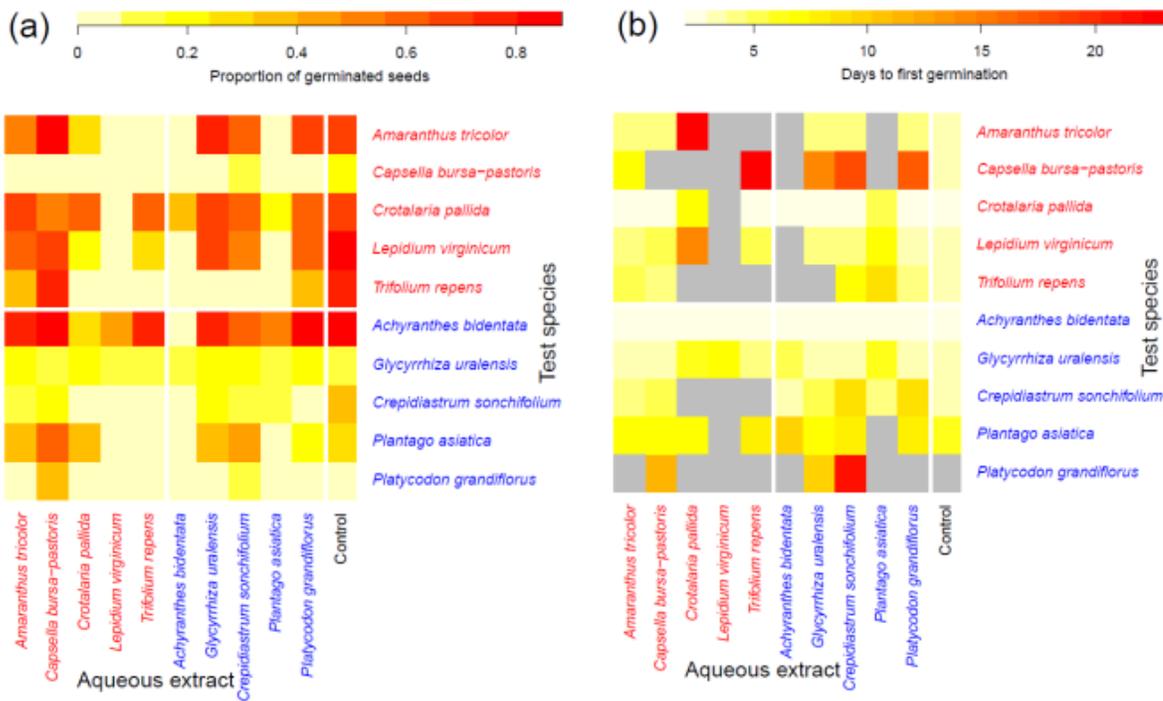
## Table 3

Results of generalized linear mixed models testing for effects of activated carbon (with, without), species origin (alien, native) and competition treatment (intraspecific competition, interspecific with alien species, interspecific with native species, without competition) on aboveground biomass. The effect of competition treatment was decomposed into three contrasts: with *vs* without competition, intra- *vs* interspecific competition, and competition with an alien *vs* with a native

	Aboveground biomass		
	df	$\chi^2$	<i>P</i>
<i>Fixed terms</i>			
Activated carbon (AC)	1	258.74	<b>&lt;0.001</b>
Origin	1	0.57	0.450
Competition treatment	3	8.10	<b>0.044</b>
<i>With vs without competition</i>	1	6.81	<b>0.009</b>
<i>Intra- vs interspecific</i>	1	0.003	0.959
<i>Alien vs native</i>	1	1.29	0.256
AC × Origin	1	0.48	0.490
AC × Competition treatment	3	2.90	0.407
<i>AC × [With vs without competition]</i>	1	0.29	0.589
<i>AC × [Intra- vs interspecific]</i>	1	1.27	0.259
<i>AC × [Alien vs native]</i>	1	1.34	0.248
Origin × Competition treatment	3	6.25	0.100
<i>Origin × [With vs without competition]</i>	1	5.61	<b>0.018</b>
<i>Origin × [Intra- vs interspecific]</i>	1	0.55	0.460
<i>Origin × [Alien vs native]</i>	1	0.09	0.763
AC × Origin × Competition treatment	3	0.07	0.995
<i>AC × Origin × [With vs without competition]</i>	1	0.07	0.798
<i>AC × Origin × [Intra- vs interspecific]</i>	1	0.008	0.930
<i>AC × Origin × [Alien vs native]</i>	1	0.0004	0.984
<i>Random terms</i>			
	Standard deviation		
Test species	0.3919 <sup>†</sup>		
Competitor species	0.0239		
Residual	0.3959		

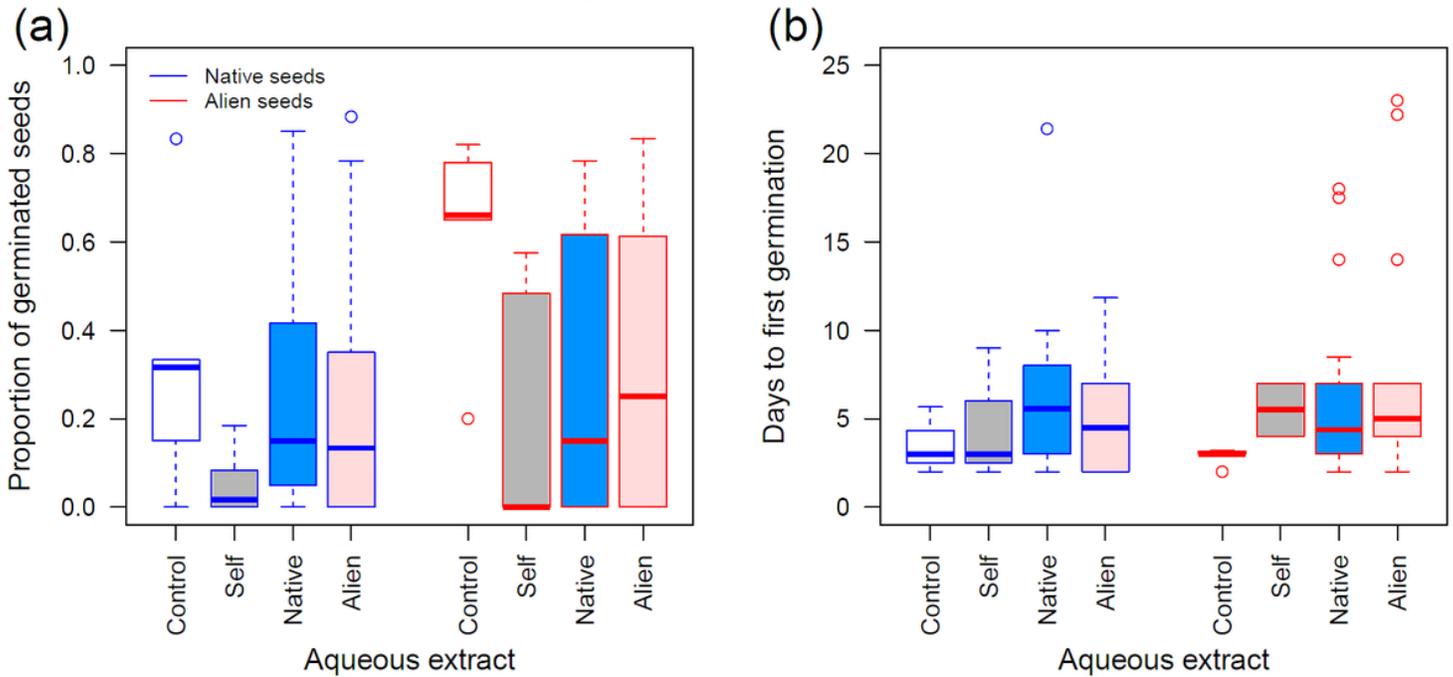
n = 797. The identities of the test species and of the species used as competitors were included as random factors. *P*-values < 0.05 are highlighted in bold. <sup>†</sup>Standard deviations were allowed to differ among test species. The one shown is for *A. bidentata*. For the other species, this value is multiplied with 1.105 (*A. tricolor*), 0.695 (*C. bursa-pastoris*), 1.165 (*C. sonchifolium*), 0.561 (*C. pallida*), 0.377 (*G. uralensis*), 0.731 (*L. virginicum*), 1.042 (*P. asiatica*), 0.534 (*P. grandiflorus*) or 0.520 (*T. repens*).

## Figures



**Figure 1**

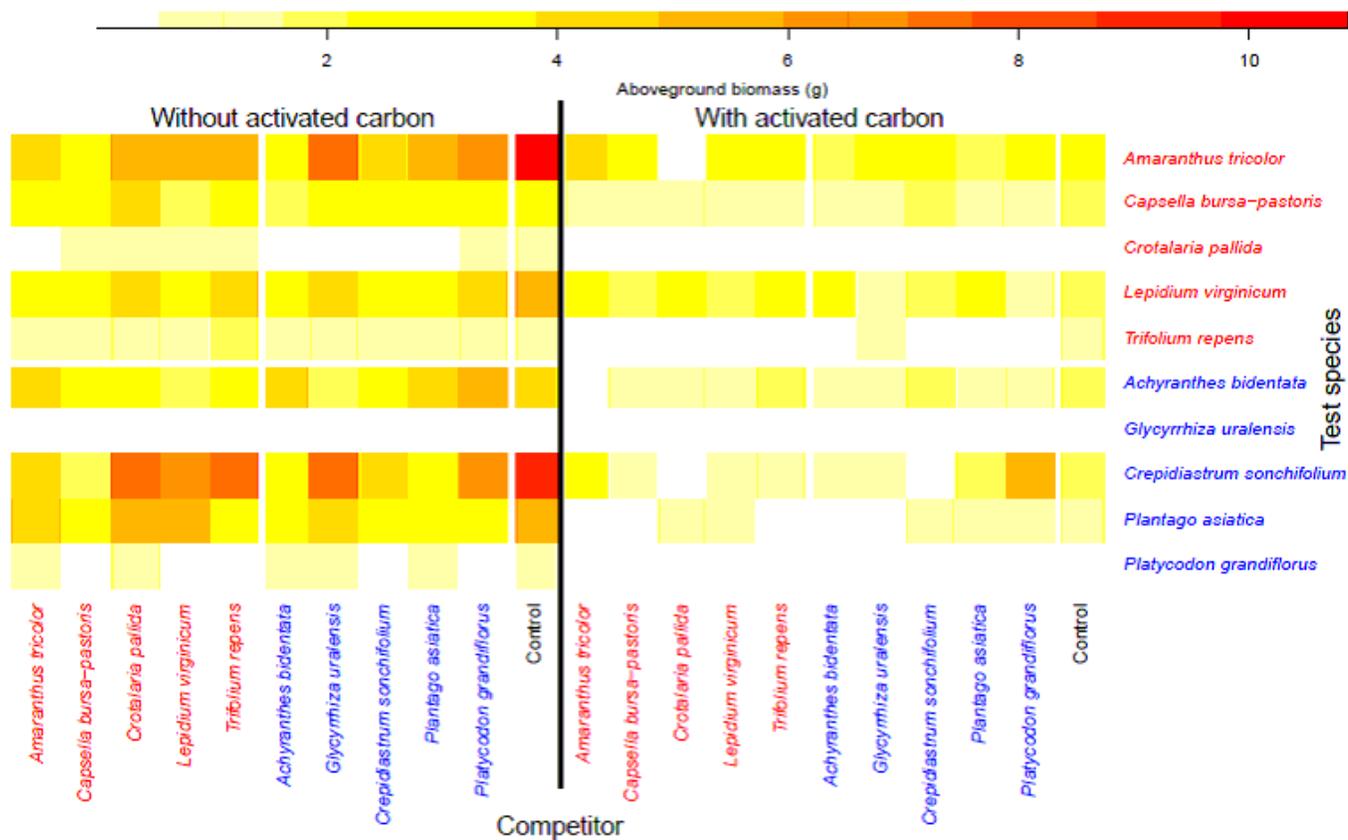
Heatplots showing the mean (a) proportion of germinated seeds and (b) days to first germination for each combination of a test species and a plant-extract species (or the water control). Alien species are in red, and native species are in blue. Combinations with missing data are indicated in grey.



**Figure 2**

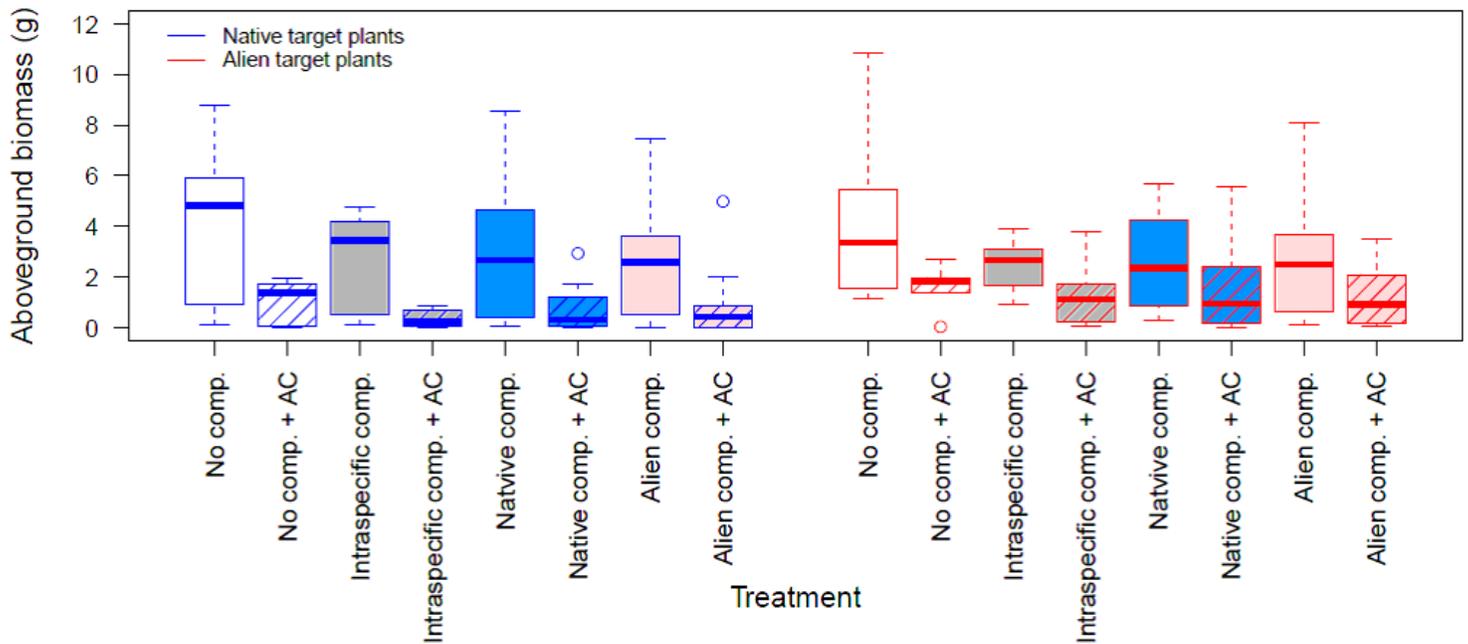
Boxplots of the (a) proportion of germinated seeds and (b) days to first germination for alien (red box lines) and native species (blue box lines) for the control treatment (white filling of boxes), and treatment with plant extracts of the species itself (grey filling), a native species (light blue filling) and an alien species (light red filling). The boxplots are

based on the means of each combination of a test species with an aqueous extract species (or control treatment). Boxes show the interquartile range around the median (thick horizontal line), whiskers extend to 1.5× the interquartile range, and circles indicate outliers.



**Figure 3**

Heatplot showing the mean aboveground biomass of a target species for each combination of a test species and competitor species (or control treatment without competition) in the absence or presence of activated carbon. Alien species are in red, and native species are in blue.



**Figure 4**

Boxplots of the aboveground biomass for native target plants (blue box lines) and alien target plants (red box lines) for the treatment without competition (white filling of boxes) with and without activated carbon (hatch filling), and the treatment with intraspecific competition (grey filling) with and without activated carbon, the treatment with native competitor (light blue filling) with and without activated carbon, and treatment with alien competitor (light red filling) with and without activated carbon. The boxplots are based on the means of each combination of a target species with a competitor species (or control treatment). Boxes show the interquartile range around the median (fat horizontal line), whiskers extend to 1.5× the interquartile range, and circles indicate outliers.

## Supplementary Files

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