Fine-tuned ability to predict future competitive environment in Ambrosia artemisiifolia seeds

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Summary
Competition from native species is a key mechanism for biotic resistance to invasion. Accelerated germination to pre-empt resources or delayed germination and induced dormancy until the next growing season are two alternative strategies for annual invasive plants to avoid the drawbacks of competition at the seed stage. In Ambrosia artemisiifolia, both of these tactics could theoretically increase its long-term fitness. However, their relative importance has never been tested. We studied the germination pattern of A. artemisiifolia seeds in various competitive environments by experimentally modifying the life stage (seed, seedling, adult), density (low, high) and also the identity (intraspecific and 3 interspecific competitors) of neighbours in controlled conditions. When facing competition of seeds and seedlings at high densities or of particular identity, A. artemisiifolia accelerated its germination. In contrast, A. artemisiifolia followed a competition avoidance strategy in the presence of established adult heterospecific neighbours by delaying germination and reducing the germination fraction through induction of secondary dormancy. By testing the seedlings’ performance in the same competition situations as those of seeds, we showed that the germination responses were beneficial in the case of heterospecific, but not of conspecific neighbours.

Keywords: common ragweed, competition, plant–plant interaction, environmental cueing, perception, density dependence.


Introduction
Many plant communities are now facing invasion of non-native species due to the international transport of organisms outside their native range. The most often cited negative effects of native species on invasives are caused by competition (Levine et al., 2004). Although competition from resident species takes place at all plant life-cycle stages (ten Brink & Bruun, 2011), the vast majority of studies have studied adult plant biomass or fecundity as response variables (Leger & Espeland, 2010). However, factors affecting seed germination and recruitment also have the potential to affect plant demography and long-term survival of populations (Tielbörger & Prasse, 2009; Orrock & Christopher, 2010; ten Brink & Bruun, 2011). It has been reported that germination and initial root and shoot development may be particularly sensitive to competition (Foster & Gross, 1998).

Seed germination is known to be influenced by several abiotic factors (temperature, soil humidity, light, nitrate concentrations etc; Baskin & Baskin, 1998). However, plant individuals in the close vicinity of seeds might also have an impact on this process,
directly by influencing the chemical properties of the environment (e.g. allelopathy, Prati & Bossdorf, 2004) and indirectly by modifying the physical microenvironment of seeds. Therefore, the density, the life-history stage (seed, seedling, adult) and the identity (different life forms, strategies, allelopathic exudates) of neighbours might stimulate or hinder the germination of seeds. Several studies report density-dependent seed germination responses in which high seed densities either accelerate or inhibit germination (Callaway, 2007 and literature therein). Further, there might be differences in the reaction of seeds to conspecific or heterospecific neighbours, as previous studies reported that germination is accelerated in interspecific, but not heterospecific neighbours, as previous studies reported (Dyer et al., 2000; but Tielböger & Prasse, 2009). It is therefore understood that plants have evolved mechanisms that allow their seeds to evaluate and respond to their environment.

Seed responses to neighbouring conditions might be achieved by two alternative strategies of accelerated germination or delayed germination. Under accelerated germination, seedlings capitalise on resources and grow larger (Martin & Field, 1988; Orrock & Christopher, 2010) and achieve competitive dominance in community (Turkington et al., 2005), that is, the so-called 'priority effect'. Even a very short delay in emergence can be translated into a large decrease in final fitness (Dyer et al., 2000 and literature therein).

Under delayed germination, seeds do not germinate until subsequent growing seasons by entering secondary dormancy (Verdú & Traveset, 2005). There are two alternative, but not exclusive theories that may explain mechanisms for dormancy release. The first proposes that plants can spread the risk of recruitment failure over time in a temporally unpredictable environment ensuring that only a fraction of viable seeds germinate in any given year (diversifying bet-hedging, Childs et al., 2010). By decreasing the mean fitness, the individual might experience a reduction in temporal fitness variation in the long term (Venable & Lawlor, 1980). The second theory predicts that the number of germinating seeds depends on the ability of the species to react to the cues indicating environmental quality (Venable & Lawlor, 1980; Baskin & Baskin, 1998). In this case, the fitness can be maximised if the seeds correctly respond to the environmental cues. While the bet-hedging germination strategy is a deterministic response based on a long-term adaptation of the plant to the stochasticity of the environment, this latter strategy is a plastic response based on the perception of environmental cues.

We tested the strategies, (i) accelerated germination within season and/or (ii) delayed germination between seasons via environmental perception of increasingly competitive environments using an experimental study on invasive Ambrosia artemisiifolia L. (common ragweed). A. artemisiifolia is an ideal model organism being an annual species with long-term persistent seed bank (Fumanal et al., 2008). It is assumed that semelparous species can gain advantage from dormancy mechanisms that spread dormancy release across years (Evans et al., 2007). Due to its broad amplitude in germination temperature (Brandes & Nitzsche, 2006), seeds can modify their germination time as a function of other environmental factors. Therefore, we studied the germination pattern of A. artemisiifolia by modifying the competitive environment of the seeds in a common garden pot experiment.

Our main hypothesis was that A. artemisiifolia seeds can evaluate the neighbouring environment prior to emergence and can respond by adjusting germination within and/or between seasons. We aimed to answer the following particular questions:

1 Can A. artemisiifolia seeds modify the time of their germination with the environment becoming more and more competitive? We hypothesised that high density rather than low density of neighbours, adult competitors rather than seeds or seedlings, and intraspecific neighbours rather than interspecific competitors, would accelerate the germination of A. artemisiifolia seeds.

2 Is the germination fraction of seeds correlated with the severity of the competitive environment (plastic germination) or is it independent of it (bet-hedging germination)? We assumed that A. artemisiifolia would show plastic germination by delaying germination until subsequent growing seasons in highly competitive environments.

Materials and methods

The target species

The A. artemisiifolia is an erect annual herb of the Asteraceae family and native to North America. It is a pioneer species thriving in disturbed habitats, such as roadsides, railways, waste and construction sites, abandoned or actively managed agricultural fields (Fumanal et al., 2008; Kazinczi et al., 2008). It has invaded a considerable part of Europe due to its tolerance to abiotic conditions, dense stands and rapid spread (Kiss & Béres, 2006; Pinke et al., 2011). It has high reproductive output, producing up to 10 billion pollen grains and up to 6000 seeds per individual plant (Fumanal et al., 2007). Moreover, it has relatively big seeds with high viability forming a persistent seed bank with seeds
remaining viable for more than 30 years (Bassett & Crompton, 1975). Due to its highly allergenic pollen (Gerber et al., 2011), the invasion of Ambrosia artemisiifolia has raised the awareness of biological invasions in Europe and highlighted the need for effective monitoring (Dammer et al., 2013) and control systems (Gauvrit & Chauvel, 2010).

**Common garden pot experiment**

We established a full factorial experiment with three factors to create various competitive environments for the germinating Ambrosia artemisiifolia seeds. The three treatment factors were the identity (four species), life stage (seed, seedling, adult plant) and density (low and high) of the competitor species. All treatment combinations (identity × life stage × density) and the controls had eight replicates. We used a completely randomised design, and pots were rerandomised at every census to avoid any possible edge effect.

We tested the effect of both intraspecific (one of the competitors was the Ambrosia artemisiifolia itself) and interspecific competition. The three heterospecific competitor species were chosen to be among the most abundant species in a recently abandoned old field in Baciu, Romania (46°47'34"N, 23°31'30"E) invaded by Ambrosia artemisiifolia. These species were Erigeron annuus (L.) Pers. (eastern daisy fleabane, annual), Daucus carota L. (wild carrot, biennial) and Epilobium tetragonum L. (square-stalked willowherb, perennial). Seeds of all four species were collected from at least 50 mature individuals from this old field site during October 2010 (Ambrosia artemisiifolia and Erigeron annuus) or January 2011 (D. carota and E. tetragonum). Seeds collected in autumn were kept at room temperature during the winter months, and all seeds were stratified at 4°C for 10 weeks from the beginning of February 2011.

A total of 208 L (c. 10 × 10 × 10 cm) pots were filled with commercial potting soil (pH 7.7, 0.032 mg N g⁻¹) and placed in an outside garden (University Botanical Garden from Cluj-Napoca, Romania) in a wooden frame covered with a transparent polyethylene sheet to exclude precipitation. Twenty achenes (hereafter seeds) of Ambrosia artemisiifolia were evenly distributed on the soil surface of each pot on 23 April 2011. This density was chosen to approximate those likely to occur in natural conditions (for details see Fumanal et al., 2008). To synchronise the germination of Ambrosia artemisiifolia and heterospecific competitors, seeds of Erigeron annuus and E. tetragonum were sown 5 days after Ambrosia artemisiifolia and D. carota seeds (according to a preliminary test, details and results not shown). Without synchronisation, we would have failed to create a seed neighbourhood of Erigeron annuus and E. tetragonum for the target Ambrosia artemisiifolia seeds, as the majority of these small-sized seeds germinated during the first week after sowing.

The life stage of the four competitor species was manipulated as follows: (i) competitive environment of seeds by adding seeds of four species, which were continually removed when showing the first sign of germination; (ii) competitive environment of seedlings by adding seeds of the same four species, but leaving the germinated seeds to develop; (iii) competitive environment of adult plants by transplanting individuals of the four species 10 days before broadcasting of Ambrosia artemisiifolia seeds. In each case, target seeds (Ambrosia artemisiifolia) were left in pots after germination and allowed to develop until the end of the experiment.

For the density treatment, we applied two densities (low- and high-density neighbourhoods) of all species and life stages. To establish the competitive environment of seeds and seedlings, we used 20 seeds in the low-density treatment and 40 seeds in the high-density treatment for Ambrosia artemisiifolia and D. carota, but we doubled the number of seeds for E. tetragonum and Erigeron annuus (40 in the low and 80 in the high-density treatment) due to their very small seed sizes. In case of the intraspecific competitive environment, to identify the target seeds from the competitor seeds, the 20 target seeds were marked by placing thin wires in the soil in their near vicinity. To establish the competitive environment of adult plants 1 (low density) or 3 (high density) seedlings of D. carota, E. tetragonum, Erigeron annuus or Ambrosia artemisiifolia were transplanted into experimental pots. The plants were collected from the same old field in Baciu, gently washed to remove all the soil particles from the roots and immediately transplanted into the pots. We collected same-sized overwintering rosettes of Erigeron annuus and E. tetragonum (4–5 cm width), 5–8 cm seedlings of D. carota, and Ambrosia artemisiifolia plants at two-leaf stages. All collected plants started to grow rapidly and reached considerable height by the time Ambrosia artemisiifolia seeds started to germinate. The majority of these plants were in their juvenile life stage (before reproduction) during the study period. However, as Erigeron annuus started to flower at the beginning of June, we termed this life stage, for simplicity of terminology, as adults for all neighbouring plants.

To have a control situation for comparison, we installed pots containing 20 Ambrosia artemisiifolia seeds, where the germinated seeds were not removed from the pots and seedlings left to develop until the end of the experiment (control, included in the analyses). We also installed pots containing 20 Ambrosia artemisiifolia seeds, where those seeds presenting the first signs of being germinated were immediately removed (no competitive environment, not included in the analyses but used for
quantifying the rate of germinated, dormant and dead seeds in optimum conditions.

All pots received fertilisers (N-P-K) at the beginning of the experiment and were watered every 3–4 days to field capacity. Seed germination for both target and neighbouring seeds was recorded every second day. Seeds were considered to be germinated when the radicle reached a minimum length of 1 mm. Pots were monitored through the entire period of active emergence and we concluded the study on 30 June 2011, 69 days after A. artemisiifolia sowing (1 week after the last germination event). We harvested and dried the aboveground biomass of all established A. artemisiifolia seedlings at 80°C for 48 h and thereafter measured their biomass. Post-emergence mortality was very low (on average 1.22 seedlings died per pot) and uniform across treatments. As germination experiments usually end before all seeds germinate, we did not know whether non-germinating seeds are dormant or dead (Fox, 2001). To classify each seed correctly, all non-germinating seeds were recovered from the soil by means of a film of gauze placed in every pot at 5 cm depth, and their dormancy and viability were tested by physical examination and by dissecting and soaking them in 1% tetrazolium chloride (2,3,5-triphenyltetrazolium chloride) solution for 24 h at room temperature. Seeds that were stained red were considered viable.

Statistical analyses

The germination response of A. artemisiifolia seeds was described by two variables:

1. The timing of germination. Because it is time-to-event data, it presents special statistical problems. As measurements are repeated over time, they are right-censored data and not normally distributed. Therefore, accelerated failure-time models were used to analyse the timing of germination of A. artemisiifolia seeds under different treatments (Fox, 2001; Onofri et al., 2010). Those non-germinated seeds that were viable were treated as censored data points, with the end of the study as the censoring date. The explanatory variables were the competitor identity with four levels, density with two levels and a covariate describing the competitive neighbourhood of seeds just before germinating. The use of this covariate was necessary, because by removing germinated seeds (competitive environment of seeds), and by the increasing number of competitors as seedlings emerged (competitive environment of seedlings), the remaining and later-germinating seeds experience environments that are different from those experienced by the earlier-germinating seeds. Therefore, a covariate describing the number of competitor seeds present was taken into account in the case of each A. artemisiifolia seed when competitors were in seed stage (neighbour effect of seeds), and the number of seedlings present when competitors were in seedling stage (neighbour effect of seedlings). Due to these different covariates, the germination time could not be analysed within a single model. Therefore, separate models were run for set-ups with different life stages of the competitors. As the number of adult competitors did not change during the experiment, no covariate was included when analysing the competitive neighbourhood of adult plants. To comply with the problems posed by the clustering of seeds within individual pots, a frailty approach was adopted (the random effect in the frame of survival analysis). We wanted to compare treatment levels of each factor with the control as well, therefore separate analyses were run on low and high densities, and all life stages including the control, but without the inclusion of the above-mentioned covariates. The best failure-time distributions were chosen for the data sets based on the comparison of possible distributions using the AIC and it was always log-normal. Computations were performed in R statistical environment using the survreg function in the ‘survival’ package.

2. Germination fraction was calculated by dividing the number of germinating seeds by the number of supplemented seeds in each pot. This variable was used as a dependent variable in a full factorial general linear model (LM) to test the effect of three treatment factors (life stage of the neighbour with three levels, density with two levels and species identity of the neighbour with four levels) and their interactions. To select the minimum adequate models, non-significant effects were removed by backward stepwise selection based on AIC using the step function in R. Then, Tukey’s HSD tests were used to reveal the differences between treatment levels for each factor. Because we wanted to compare treatment levels of each factor with the control as well, but control could not be included in the full factorial LM as only one absolute control was employed (control to all three treatment factors), we used planned pairwise contrasts within one-way ANOVAs. In this set of ANOVAS, we used the same dependent variable as above for low- and high-density set-ups separately, while the factor was a composite variable created by combining life stage and species identity of neighbours (i.e. 3 life stages × 4 species = 12 levels). As these analyses did not take into account the fate of ungerminated seeds,
additional analysis were carried out on the number of dormant seeds retrieved from the soil at the end of the experiment. This is an alternative (and sometimes necessary) approach to failure-time methods in cases of right-censored germination data (Fox, 2001). The effect of treatment factors and their interaction on the number of dormant seeds was tested with a generalized linear model (GLM) with negative binomial error distribution. The contrasts between levels were extracted using the glht function (multcomp package).

The performance of *A. artemisiifolia* seedlings was characterised by mean biomass by dividing the total aboveground biomass per pot by the number of harvested seedlings. This variable was also tested by LM built exactly in the same way as in the case of germination fraction. Analyses were carried out using R version 2.12.1 (R Development Core Team, 2010).

**Results**

**Timing of germination**

Based on the results of the failure-time analysis, density of neighbours significantly influenced the speed of germination of *A. artemisiifolia* seeds in the case of each life stage of the competitors (low- vs. high-density competitive environment of seeds $\chi^2 = 22.84$, $P < 0.001$, of seedlings $\chi^2 = 27.20$, $P < 0.001$, and of adult plants $\chi^2 = 32.65$, $P < 0.001$). Thus, in further step, analyses were repeated separately on low and high-density data sets (Table 1).

The neighbourhood of seeds accelerated the germination of *A. artemisiifolia* seeds in three of four competitor species in the high compared with low-density set-ups (*A. artemisiifolia* low vs. high densities: $\chi^2 = 17.39$, $P < 0.001$, *D. carota* low vs. high densities: $\chi^2 = 13.80$, $P < 0.001$, *E. tetragonum* low vs. high densities: $\chi^2 = 3.88$, $P = 0.049$, *E. annuus* low vs. high densities: $\chi^2 = 0.70$, NS = not significant). *Erigeron annuus* seeds provoked accelerated germination in *A. artemisiifolia* seeds both at low and high densities compared with the other competitors, and at high densities compared with the control as well (Table 1, Fig. 1).

Similarly, the neighbourhood of *E. annuus* seedlings at both densities accelerated the most germination of *A. artemisiifolia* seeds, compared with the other competitors and control (Table 1, Fig. 2). Generally, a high density of three competitors at the seedling stage (except *D. carota*) significantly accelerated the germination of *A. artemisiifolia* seeds compared with low-density situations (*A. artemisiifolia* low vs. high densities: $\chi^2 = 6.23$, $P = 0.01$, *D. carota* low vs. high densities: $\chi^2 = 5.60$, $P = 0.02$, *E. annuus* low vs. high densities: $\chi^2 = 5.83$, $P = 0.02$).

<table>
<thead>
<tr>
<th>Variable</th>
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<th>High density</th>
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<td>Intercept</td>
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<td><em>E. tetragonum</em> vs. <em>E. annuus</em></td>
<td>0.97</td>
<td>0.89</td>
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</tbody>
</table>

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Contrary to what was found for seed and seedling neighbours, a high density of adult neighbours significantly delayed the germination of *Ambrosia artemisiifolia* seeds compared with low density in three of four species, the only exception being the intraspecific competitive environment (*Ambrosia artemisiifolia* low vs. high densities: $\chi^2 = 1.18$, NS, *D. carota* low vs. high densities: $\chi^2 = 4.64$, $P = 0.03$, *E. tetragonum* low vs. high densities: $\chi^2 = 20.95$, $P < 0.001$, *E. annuus* low vs. high densities: $\chi^2 = 13.46$, $P < 0.001$). Regarding the identity of adult competitors, *E. annuus*, *E. tetragonum* and *A. artemisiifolia* delayed the germination of *A. artemisiifolia* seeds in comparison with the control (Fig. 3), but only in the high-density treatments. *Ambrosia artemisiifolia* seeds postponed their germination in the neighbourhood of *E. annuus* and *E. tetragonum* compared with *D. carota* in low density: $\chi^2 = 7.07$, $P = 0.007$, *E. tetragonum* low vs. high densities: $\chi^2 = 21.37$, $P < 0.001$, *E. annuus* low vs. high densities: $\chi^2 = 13.46$, $P < 0.001$). In the insert, the competitors’ germination probabilities are shown using the same procedure.
densities and with *D. carota* and *A. artemisiifolia* at high density (Table 1, Fig. 3).

**Germination fraction**

The germination fraction of *A. artemisiifolia* seeds was significantly reduced by the competitive environment of adult plants, but not of seeds or seedlings (Tukey’s *post hoc* test; seed vs. seedling: diff = 0.06, *P* = 0.99; seed vs. adult: diff = 10.33, *P* < 0.001; seedling vs. adult: diff = 10.40, *P* < 0.001, Table 2). Moreover, high-density treatment significantly reduced the germination fraction compared with the less crowded neighbourhood (Table 2). The significant density × life stage interaction highlighted that the competitive environments of high-density adult individuals were responsible for the above results, significantly reducing the germination fraction compared with all other combinations of density and life stage (Table 2, Fig. 4). The germination fraction of the target species was significantly influenced by the identity of neighbours, with *E. tetragonum* significantly and *E. annuus* marginally decreasing the germination fraction compared with *A. artemisiifolia* (intraspecific competitive environment; Fig. 4). Compared with the control, the neighbourhood of high-density *E. tetragonum* and *E. annuus* significantly reduced the germination fraction of *A. artemisiifolia* seeds (Fig. 4).

Similarly, the number of dormant seeds retrieved from soil at the end of the experiment was significantly influenced by all factors (Table 2). Adult plant neighbourhood increased the ratio of dormant seeds compared with the competitive environment of seeds, while a high density of neighbours (irrespective of life stage or identity) also increased the ratio of dormant seeds (Table 2). The dormant fraction of *A. artemisiifolia* seeds was influenced by the identity of neighbours as well; *E. tetragonum* and *E. annuus* decreased this ratio compared with *A. artemisiifolia* and *D. carota* as neighbours (Tukey’s *post hoc* test; *A. artemisiifolia* vs. *D. carota*: diff = −0.06, NS; *A. artemisiifolia* vs. *Epilobium*: diff = −0.85, *P* = 0.03; *A. artemisiifolia* vs. *E. annuus*: diff = −0.97, *P* = 0.01; *D. carota* vs. *E. tetragonum*: diff = 0.78, *P* = 0.05; *D. carota* vs. *E. annuus*: diff = −0.90, *P* = 0.01; *E. tetragonum* vs. *E. annuus*: diff = 0.11, NS). The species × life stage interaction highlighted that competitive environment of adult *E. tetragonum* and *E. annuus* individuals had the most influence on the dormant fraction of *A. artemisiifolia* seeds (contrasts not shown, Fig. 5). In no competitive environment situation the germinated seeds represented 82.5 ± 14.88% (mean ± SD), dead seeds 13.75 ± 9.91%, while only a small fraction of seeds remained alive but dormant in the soil (3.75 ± 5.82%, Fig. 5).

**Mean biomass of seedlings**

The mean biomass of *A. artemisiifolia* seedlings was influenced significantly by all studied factors (Table 2, Fig. 6). Adult plants significantly lowered the biomass of seedlings compared with seeds or seedlings (Tukey’s *post hoc* test; seed vs. adult: diff = 0.01, *P* < 0.001; seedling vs. adult: diff = 0.01, *P* < 0.001), while there were no differences between the effects of seed and seedling neighbourhoods (Tukey’s *post hoc* test; seed vs. seedling;
Table 2 Results of minimum adequate general linear models (values of $F$ statistics, degrees of freedom, mean sum of squares and probabilities are shown) constructed to test the effect of neighbour species’ identity, density and life stages on germination fraction of *Ambrosia artemisiifolia* seeds and mean biomass of seedlings. The effect of the same predictors on dormant fraction of seeds was tested by generalised linear model (GLM) with negative binomial error distribution ($\chi^2$ and $P$). Species × density interaction was left out because it was not part of the minimum adequate models in any of the cases.

<table>
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<th>Predictors</th>
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<th>Dormant fraction</th>
<th>Mean biomass</th>
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<td></td>
<td>$F_{(df)}$</td>
<td>MS</td>
<td>$\chi^2_{(df)}$</td>
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<td>800.58</td>
<td>332.72 (3,168)</td>
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<td>Density (D)</td>
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<td>762.09</td>
<td>309.73 (1,168)</td>
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<td>762.09</td>
<td>24.25 (1,168)</td>
</tr>
<tr>
<td>$S \times D \times Ls$</td>
<td>198.11 (6,168)</td>
<td>0.03</td>
<td></td>
</tr>
</tbody>
</table>

Fig. 4 The germination fraction (means ± SE) of *Ambrosia artemisiifolia* seeds in experimentally manipulated competitive environments for the low- (A) and high-density treatments (B). The solid line indicates the germination fraction under control situation (without neighbour). Asterisks show significant pairwise contrasts between control vs. each competitive neighbourhood: ‘***’ $P < 0.001$, ‘*’ $P < 0.05$. Capital letters indicate significant differences at $P < 0.05$ as obtained by the Tukey’s HSD test for the density × life stage interaction, therefore differences between the two different density treatments, i.e. the two graphs.

NS). High-density treatments resulted in lower seedling biomass at the end of the experiment (Table 2). Both the low- and high-density adult neighbourhood decreased the biomass of *A. artemisiifolia* seedlings according to the density × life stage interactions (Fig. 6). The highest biomass of *A. artemisiifolia* seedlings was gained in the neighbourhood of *D. carota* compared with the other species (Tukey’s post hoc test; *A. artemisiifolia* vs. *D. carota*: diff = −0.004, $P < 0.001$; *A. artemisiifolia* vs. *E. tetragonum*: NS; *A. artemisiifolia* vs. *E. annua*: NS; *D. carota* vs. *E. tetragonum*: diff = 0.003, $P < 0.001$; *D. carota* vs. *E. annua*: diff = 0.002, $P < 0.001$; *E. tetragonum* vs. *E. annua*: NS). The species × life stage interaction revealed that the biomass of *A. artemisiifolia* seedlings was significantly reduced in case they were grown together with conspecific seedlings (Tukey’s post hoc test; *A. artemisiifolia* vs. *D. carota*: diff = −8.10, $P = 0.001$; *A. artemisiifolia* vs. *E. annua*: diff = −6.11, $P = 0.02$; *A. artemisiifolia* vs. *E. tetragonum*: diff = 6.58, $P = 0.02$), and even more when they developed in the neighbourhood of each one of the adult plant species tested. The presence of intraspecific competitive neighbourhood of seeds at high density also modified the final seedling biomass compared with the control, competition-free set-up (Fig. 6).

In the no competitive environment situation, the germinated seeds represented 82.5 ± 14.88% (mean ± SD), dead seeds 13.75 ± 9.91%, while only a small fraction of seeds remained alive but dormant in the soil (3.75 ± 5.82%).

**Discussion**

**Highly flexible germination time in Ambrosia artemisiifolia seeds**

Miller (1987) found that the cost associated with a 3-day delay in emergence is a 97% reduction in mean biomass in *A. artemisiifolia* under natural/field conditions in the presence of competitors. Therefore, evolution should favour the ability to react to a future competitive environment and avoid it by accelerated germination. In the experiment reported here, we found significantly accelerated emergence of *A. artemisiifolia* seeds within several experimental competitive environments. Both density and identity of competitors at all life stages had significantly different effects on
the timing of germination of *A. artemisiifolia* seeds. Surprisingly, the presence of *E. annua* at seedling and even at seed stage provoked a more rapid germination compared with the other tested competitor species and the control situation. Testing the effect of seeds as competitive neighbours has been neglected until the present, when some recent studies have made the distinction between the effect of seeds and seedlings on the germination of species and found that seeds can influence the germination parameters of target and competitor species reciprocally (e.g. Tielbörger & Prasse, 2009). We assume that the accelerated germination found in our case was tightly linked with the germination pattern of competitors, as *E. annua* had the most rapid and most vigorous germination among the tested competitors (Figs 1 and 2). By germinating simultaneously or with a slight delay compared with its competitors, *A. artemisiifolia* can capitalise resources and occupy ‘safe sites’, because it grows very fast, and it is known to have a suppressive effect on the seedlings of other species and found that seeds can influence the germination parameters of target and competitor species reciprocally (e.g. Tielbörger & Prasse, 2009). We assume that the accelerated germination found in our case was tightly linked with the germination pattern of competitors, as *E. annua* had the most rapid and most vigorous germination among the tested competitors (Figs 1 and 2). By germinating simultaneously or with a slight delay compared with its competitors, *A. artemisiifolia* can capitalise resources and occupy ‘safe sites’, because it grows very fast, and it is known to have a suppressive effect on the seedlings of other species (Miller & Werner, 1987). Therefore, in the presence of other seeds or seedlings *A. artemisiifolia* adopts the competitive confrontation strategy (Novoplansky, 2009).

In contrast, the presence of particular plant species at the adult stage and mostly at high density delayed the emergence of *A. artemisiifolia*. We suppose that the delayed germination can be linked to the induction of secondary dormancy in *A. artemisiifolia* seeds. The shading effects and/or the water uptake by neighbouring adult plants could cause the postponed germination of certain seeds. The same factors and/or the increased soil temperature later in the season may have induced secondary dormancy in the remaining seeds (Baskin & Baskin, 1980). The fraction of dormant seeds was the highest in the neighbourhood of adult *E. tetragonum* individuals at high density, in support of this assumption, where we detected the highest germination delay of more than 12 days compared with the control.

**Plastic environmental cueing rather than bet-hedging germination in Ambrosia artemisiifolia seeds**

Environmental cueing may induce germination under favourable conditions, but those conditions may not persist long enough for survival and reproduction. Therefore, temporally unpredictable environments will select towards two alternative, but not exclusive strategies: (i) preserving a fraction of dormant seeds under presently favourable conditions (bet-hedging strategy) or (ii) developing the ability to correctly respond to environmental cues or signals predicting future environmental conditions (Petrů & Tielbörger, 2008). Bet-hedging dormancy, as a complement to germination cueing, is likely to be the most consistently favourable germination strategy (Donohue et al., 2010).

Based on our results, the majority of viable seeds germinated in adequate conditions without competition and only a small fraction of the recovered seeds were
dormant after the germination period (3.8 ± 5.8%). Similarly, a study on nine invasive *A. artemisiifolia* populations also found low levels of dormant seeds (2.8 ± 3.14%) in the upper 5 cm of the soil (Fumanal et al., 2008). Although these dormancy fractions are very low compared with, for example, those found in desert annuals, where it was definitely proved that dormancy is a bet-hedging trait (Venable, 2007), they can form a considerable seed reserve considering the reproductive output of *A. artemisiifolia* (Fumanal et al., 2007). These fractions of seeds can contribute to the build-up of a persistent seed bank across years that assure long-term persistence in unpredictable environments, such as old fields. We are aware that our results are not precise expressions of bet-hedging germination, but they can be considered for further investigations.

Our results demonstrate the ability of *A. artemisiifolia* seeds to distinguish between the severity of competitive environment into which they will emerge and to respond to it not only by adjusting their time of emergence, but also by the fraction germinating. The ratio of germinating seeds was highly dependent on the neighbours’ life stage and density, as high-density adult neighbours hindered seed germination and induced secondary dormancy compared with seeds or seedlings. Non-dormant seeds of *A. artemisiifolia* enter secondary dormancy when they are kept in darkness at temperatures favourable for germination or at naturally increasing soil temperatures in spring (Baskin & Baskin, 1980). The light requirement for germination in the relatively big seeded *A. artemisiifolia* (Fumanal et al., 2007) can function as a mechanism for detecting open soil surfaces in closed vegetation (Kotorov & Leps, 1999). This prevents *A. artemisiifolia* seeds from recruiting in conditions where individuals may have low chances of survival because of competition from established neighbouring plants (competitive avoidance strategy, Novoplansky, 2009).

**Reliable environmental perception in Ambrosia artemisiifolia seeds**

We focused on biomass as a measure of performance in seedlings, because it directly quantified seedling vigour and it is usually strongly related to other measures of seedling performance (Orrock & Christopher, 2010). Data on seedling biomass confirmed the ability of *A. artemisiifolia* seeds to react to environmental cues and predict the future competitive environment in the case of heterospecific, but not in case of conspecific neighbours.

Seedling biomass was affected by adult neighbours, but not by seeds or seedlings in interspecific competitive environments. The presence of certain established heterospecific neighbours caused both delayed germination of *A. artemisiifolia* seeds and restrained subsequent seedling development. The competitive pressure exerted by the two superior competitors (*E. tetragonum* and *E. annuus*) can be explained by their larger relative stature and early growth in comparison with the other two competitors (*A. artemisiifolia* and *D. carota*). *Erigon anmus* is a winter annual of North American origin, where it co-occurs with *A. artemisiifolia* in annual dominated fields (Raynal & Bazzaz, 1975). Earlier studies have reported that *A. artemisiifolia* is suppressed by *E. annuus* under natural conditions, and the same was shown in pairwise competition experiments in their native range (Raynal & Bazzaz, 1975). The biennial *D. carota* proved to be an ideal neighbour for germinating seeds and also for developing seedlings. This interaction can be labelled neutral, as a positive interaction (facilitation) would assume significantly increased values compared with competition-free control conditions, which was not the case.

As *A. artemisiifolia* produces a high number of seeds and it does not have a specific dispersal strategy, a con-
siderable amount of seeds falls in the immediate vicinity of the mother plants. Therefore, intraspecific competition is expected to be high due to the dense seed bank and the large number of emerging seedlings under natural conditions (Fumanal et al., 2008). It seems that A. artemisiifolia seeds are adapted to germinate at high conspecific density, because they showed the highest germination fraction in monocultures. However, the biomass of seedlings was significantly reduced in the presence of conspecific seedlings and even next to seed neighbours in high densities compared with the control. In this respect, our study supports the niche theory of co-existence and adds to a large body of evidence that intraspecific competition is greater than interspecific competition, because conspecific individuals share similar resource requirements (MacArthur & Levins, 1967). Based on our results, it seems that intraspecific competition is the dominant force influencing population size and performance of A. artemisiifolia in bare ground without major competitive forces.

Conclusions and insights for Ambrosia artemisiifolia invasion

Plastic responses to environmental cueing in A. artemisiifolia seeds is a mechanism for occupying ephemeral bare ground surfaces. The ‘selection’ of suitable habitats for establishment without possible competitive neighbours is achieved by reacting to the density, identity and the life stage of competitors. Ambrosia artemisiifolia showed reduced and delayed germination in the presence of adult competitors, which indicated that resident plants can affect this exotic invader through competition. Adult neighbouring species can act upon germination, seedling recruitment and growth, as well.

Some concerns can be raised about the general validity of our results, because our experiment had no repetition in time and was restricted to certain environmental conditions. Therefore, further studies should be made involving different soil types, various abiotic conditions and further competitor species. Nevertheless, our results indicate that the ability to react to likely future competitive environments can be added to the list of traits (high pollen and seed production, high phenotypic plasticity, generalist character) that might make A. artemisiifolia one of the most successful invasive species of disturbed habitats in Europe.

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