



Do seeds sense each other? Testing for density-dependent germination in desert perennial plants

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The timing of seedling emergence may strongly affect fitness in competitive environments. Therefore, selection should favour mechanisms that allow sensing neighbours prior to emergence. We tested whether or not germination is affected by density and identity of neighbouring seeds or seedlings of desert perennial plants. Based on theory, we predicted that germination fractions are independent of neighbouring seeds, that germination is accelerated in dense interspecific neighbourhoods, and neighbour effects are caused by seedlings, not by seeds. We examined germination fraction and timing of four naturally coexisting sandy desert perennial species in low versus high seed densities in both intra- and interspecific neighbourhoods, and with and without removal of newly emerged seedlings. Neighbours accelerated germination independent of density and this pattern was apparently caused by the presence of early emerging seedlings. Germination fractions were lower in high seed densities even when neighbours did not germinate, indicating that seeds were able to sense each other prior to emergence. Early germination may be adaptive because fast emerging seedlings may gain a competitive edge over slow emerging ones. However, since seeds that did not germinate died, delayed germination may only be advantageous for mother plants when sib competition is intense. Another key finding was a competitive hierarchy with late successional species germinating faster and inhibiting germination of pioneer species. This indicates that successional processes may be directed as early as during germination.

Competition is a major force determining structure and function of natural plant communities. Density-dependence affects plants at many different life history stages and previous studies suggested that importance and intensity of competition may shift predictably through the life cycle of a plant (Goldberg et al. 2001, Mitri 2006, Schiffrers and Tielbörger 2006). However, most experiments on plant–plant interactions have looked at survival and growth as the major response variables and few studies have investigated the degree to which competition may be detected already at the very beginning of a plant's life. This is surprising since it is known that the time at which a plant germinates relative to its neighbours has large consequences for lifetime fitness (reviewed by Verdú and Traveset 2005). For example, a head start of as little as a few hours may result in the so-called priority effect, i.e. competitive dominance and increased growth and fecundity (Fowler 1984, Weiner 1985, Verdú and Traveset 2005). Therefore, there should be strong selection for mechanisms that allow seeds to evaluate neighbour conditions prior to emergence and to plastically respond to them.

Theoretical models have shown that a major possibility to spread extinction risk in unpredictably varying environments is to postpone germination to more favourable years (Cohen 1966, Venable and Lawlor 1980, Valleriani 2005).

Furthermore, theory suggests that fitness is maximised when germination is triggered by an environmental cue that is indicative for the favourability of the upcoming season (Cohen 1967, Venable and Lawlor 1980, Tielbörger and Valleriani 2005). Therefore, plants have developed a number of mechanisms allowing them to assess the best time for germination. In unpredictable environments such as deserts, a main cue for germination is the moisture surrounding the seed. However, environmental quality includes also the biotic environment, and germination behaviour of seeds may indeed depend on the density of neighbouring seeds or seedlings (Miller et al. 1994, Lortie and Turkington 2002a, 2002b, Grundy et al. 2003, Dyer 2004, Kluth and Bruelheide 2005, Li et al. 2005, Turkington et al. 2005). When reviewing these and earlier studies, one must distinguish between two types of plastic germination response to neighbours. The first possibility is to stay dormant for at least one year and delay germination to another season (Lortie and Turkington 2002b, Kluth and Bruelheide 2005). Alternatively, plants may vary the timing of germination within a season, e.g. exhibit accelerated seedling emergence in competitive neighbourhoods (Dyer et al. 2000). The relative selective advantage of either of the two strategies depends critically on the life history of the studied organisms. For example, perennials are less likely

than coexisting annuals to delay germination to later seasons because seed longevity and adult longevity are two alternative mechanisms for surviving extended unfavourable periods (Rees 1994, 1996, Tuljapurkar and Wiener 2000). Accelerating germination within a season is not only advantageous but includes a risk of mortality associated with premature emergence. Therefore, perennial plants, which may balance the risk of seedling mortality by adult longevity, should benefit more than annuals from early seedling emergence (Dyer et al. 2000, Verdú and Traveset 2005), while annuals should be more likely than perennials to postpone germination to following seasons.

Despite the fundamental differences between perennials and annuals, the vast majority of previous studies on density-dependent germination has focused on annuals (Kluth and Bruelheide 2005, Li et al. 2005, Turkington et al. 2005). Very few studies have looked at the effects of sowing density on timing of emergence (Bergelson and Perry 1989, Dyer et al. 2000, Turkington et al. 2005) and the only study done with perennials (Dyer et al. 2000) did not use naturally co-occurring species. Finally, previous studies of density-dependent germination have speculated but not tested whether the effect is mainly due to the presence of seeds or newly emerging seedlings (summarised in Turkington et al. 2005). Clearly, the existence of a general mechanism allowing seeds to communicate prior to emergence would be highly intriguing. However, common sense dictates that physiologically active seedlings are more likely than relatively inert seeds to alter the physical or chemical conditions in their surrounding and thus affect their neighbours. Unfortunately, the differences between seed and seedling effects on germination have not been explicitly tested before.

Here, we studied density-dependent germination for four coexisting desert perennial plant species of a sand dune ecosystem in intra- and interspecific neighbourhoods, and we evaluated whether seeds may sense each other prior to emergence. We tested the following hypotheses:

1. Neighbours accelerate germination of perennial plants but do not affect germination fractions. This hypothesis is based on the above considerations of low selection pressure on seed dormancy in perennials.
2. Germination is accelerated in interspecific but not in intraspecific neighbourhood. This hypothesis is in accordance with the only study that has explicitly separated between these two types of neighbours (Dyer et al. 2000).
3. Neighbour effects on seed germination are caused by seedlings, i.e. seeds do not sense each other. Since no study has compared seed and seedling effects, we base this hypothesis on common sense.

Material and methods

Study system

The study was conducted with perennial plant species growing at the Nizzana research site in the northwestern Negev desert of Israel. The site is located at the Egyptian–Israeli border, approximately 60 km southwest of Be’er

Sheva and is characterised by linear west–east trending sand dunes. The rainy season and, accordingly, the growing season of plants extends from October to May. Average annual rainfall is 90 mm with high inter-annual variation (Berkowicz et al. 1995). Seeds of four native perennial species were collected for the study: *Stipagrostis scoparia* (Poaceae), *Moltkiopsis ciliata* (Boraginaceae), *Artemisia monosperma* (Asteraceae) and *Cornulaca monacantha* (Chenopodiaceae). In the following, only genera names are used for the four species. The species were selected because they are among the most abundant species in the study area, they dominate distinct successional stages across the dunes and they are thus representative for different habitat types. *Stipagrostis* is a pioneer species with a high potential for vegetative reproduction that colonises shifting sands (Danin 1996, Tielbörger 1997). In the research site, it dominates the mobile dune tops where it forms phytogenic hillocks of up to 10 m in diameter. The adjacent semi-stabilised slopes represent a consecutive stage and are dominated by the dwarf shrub *Moltkiopsis*. *Cornulaca* is an abundant shrub of up to 3 m in diameter that grows throughout the area but forms monospecific stands in stable areas with hard microbiotic crusts. *Artemisia* dominates fully stabilised areas along the dune bases (Tielbörger 1997). All species may reproduce vegetatively and from seeds but vegetative propagation is particularly common for *Stipagrostis* and *Moltkiopsis* whose roots and rhizomes are frequently covered and uncovered in shifting sand. A likely successional sequence has been postulated to start in mobile sands with *Stipagrostis*, which is gradually replaced by *Moltkiopsis*, while *Artemisia* and *Cornulaca* characterise stabilised areas of the species-rich interdunes (Danin 1996, Tielbörger 1997). These areas exhibit a relatively low turnover in species and plant cover and are regarded as the most advanced successional stage (Malkinson and Kadmon 2003).

Germination experiments

Seed collection, storage, design of germination experiments and viability tests followed a standard protocol that has been successfully applied for annuals and perennials from the study area (Tielbörger and Valleriani 2005). Fresh seeds were collected during the time of seed set of each species. Seeds of *Artemisia* and *Cornulaca* ripen in autumn and were collected in December of the first study season, while seeds of *Stipagrostis* and *Moltkiopsis* were collected in the following spring (April–May). Seeds were taken from at least 50 different randomly selected individuals per species growing in an area of approximately 2 km². After seed collection, seeds of all individuals of one species were mixed thoroughly, put into several small cotton bags and placed in the field for overwintering under natural conditions. It should be noted that fresh seeds of the study species are not dormant and can germinate immediately after dispersal (Tielbörger unpubl.), and that overwintering was done only for mimicking natural conditions rather than for breaking summer dormancy. Short before the onset of the following rainy season (September), the seeds were retrieved from the field. Eight replicates of the following seed mixtures were produced: 10 seeds of each species plus 10

seeds of one of four possible neighbour species (i.e. same species or one of the three other species each), and 10 seeds of each species plus 30 seeds of a neighbour species. In order to ensure independence of samples, eight separate mixtures were used for each target species in the low density-treatment. Thus, the design was full factorial with neighbour density (10 vs 30), target species (four species) and neighbour species (four species) as three independent factors. Suitable sowing densities were selected after determining natural densities of seeds in the soil. To that end, soil samples (20 × 20 cm, 5 cm depth) were collected before the onset of the rainy season and soil seed numbers counted. Thirty samples were collected randomly at each of three catenary positions: dune top (dominated by *Stipagrostis*), dune slope (*Moltkiopsis*), interdune areas (*Cornulaca*, *Artemisia*, and other species) and in two microhabitats—the areas under shrubs and open areas between shrubs. This differentiation was made because we know from previous studies (Tielbörger and Kadmon 1997) that secondary seed dispersal by wind causes accumulation of seeds beneath shrubs. The findings confirmed that sowing densities were within the range of naturally occurring soil seed densities and median densities under shrubs were similar to (interdune: 19.05) or lower (slope: 14.3; top: 2.0) than in our low density treatment (Fig. 1). It should be noted that the soil seed bank comprised of few species on the dune top and slope (because of dominance of few species), while seed bank samples from the interdune included a diverse mixture of species.

The batches of seeds were sown in a regular manner into petri dishes of 10 cm diameter and 1.5 cm depth that were filled with sand. In intraspecific treatments, ten target seeds were selected randomly and marked with toothpicks. All seeds were then covered with a thin layer of sand and

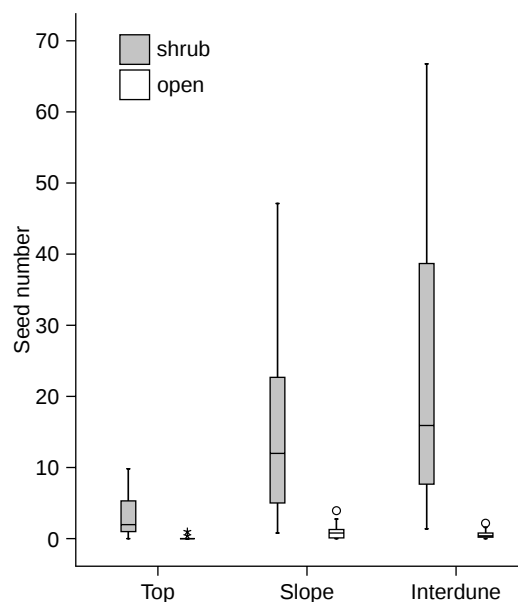


Figure 1. Median number of seeds in the soil in three catenary positions (dune top, slope, interdune) and two microhabitats (under shrubs, open areas). Numbers were calculated for the area of a petri dish (78.5 cm²); boxes include quartiles, whiskers 75% percentiles. Outliers (10%) were deleted for graphical presentation.

watered regularly, because previous trials have indicated that this procedure allows maximum germination despite the limited amount of substrate. Furthermore, sowing into shallow petri dishes allowed detection of germination at the earliest possible stage – a prerequisite for our experiment. Because seeds were lifted up by the emerging radicle, germination could be detected very early and most seedlings could be carefully removed before emergence of cotyledons. Germination is completed with the breaking of the endosperm by the radicle (Finch-Savage and Leubner-Metzger 2006), and we therefore use the notion 'germination' throughout the manuscript. It should be noted, however, that some individuals of the small-seeded species *Stipagrostis* and *Artemisia* were detected only after the emergence of the cotyledons.

The petri dishes were arranged randomly in an aerated greenhouse in Jerusalem, where they experienced day lengths and temperatures similar to those in the field. Irrigation started simultaneously with the onset of rain (end of October). Excessive amounts of water were provided and percolation of the water through holes in the bottom of the dishes was allowed. Therefore, germination was unlikely to be limited by water or salts. New germination events (radicle emergence or early cotyledon emergence) were recorded daily. After approximately six weeks of watering, no further germination was observed and the samples were allowed to dry out after carefully removing the seedlings. Previous trials with several annual and perennial plant species, including our four focal species, have shown that seeds of perennials die after experiencing a wet season (greenhouse or field), while annual seeds remain dormant for several consecutive seasons (Prasse and Tielbörger unpubl., Tielbörger and Valleriani 2005). To ensure that this applied also for our current experiment, we performed viability tests on the non-germinating seeds. Petri dishes with remaining seeds were stored in a common garden for overwintering (i.e. drought and natural summer temperatures and day lengths) and watered again in the greenhouse during the following season. After four weeks of irrigation, intact seeds were poked with a needle to observe whether they have fleshy embryos (method adopted from Pake and Venable 1996). This method was chosen since tetrazolium tests are not feasible for species with very small seeds (e.g. *Artemisia*), they yield spurious results when the seeds are covered with fungi or bacteria, and gibberelic acid does not induce germination of potentially dormant seeds in many of the coexisting species.

A parallel experiment was done to evaluate whether germination is primarily affected by neighbouring seeds or by seedlings. We established eight replicates of monospecific samples with 40 seeds each, where seedlings were removed immediately after radicle emergence and we compared germination with the high-density trials where seedlings were not removed. The dishes used for these experiments were placed randomly among the dishes from the density experiment and they were checked every 6–8 h to ensure removal of seedlings at the earliest possible stage.

Germination fractions were estimated for each petri dish by dividing the number of target seedlings by the number of target seeds supplemented. In intraspecific mixtures, ten seeds were selected randomly as targets and marked with

toothpicks. Note that estimates based on the selected targets did not differ from estimates based on all seeds.

Time to germination in days was calculated for each sample as follows:

$$\text{time to germination} = \frac{\sum (i \times n_i)}{\sum n_i}$$

with i being the number of days since first watering and n_i being the number of germinating seeds (radicle emergence) at day i .

The effect of neighbour species and neighbour density on germination was tested with generalized linear models for *Artemisia* and *Cornulaca*. Germination fraction and days to germination were the dependent variables in the models, and neighbour species and neighbour density the fixed factors. A binomial distribution with logit link function was used for the germination fraction data, normal distribution with logit link function was used for time to germination. Post hoc tests reducing false discovery rate (Benjamini and Hochberg 1995) were performed for each target species separately, to test for differences in germination between densities and neighbour species. Due to extremely low germination in *Stipagrostis* and *Moltkiopsis*, GLM could not reliably be applied for these two species (many dishes with zero germination, many missing values for determining time to germination). Instead, we performed Fisher's exact tests to evaluate differences between neighbour species in the number of dishes with at least one germinating seed.

The data of the removal experiments was less problematic (i.e. germination was observed in all treatments) and for comparing the effect of neighbours with and without removal, separate GLM were constructed. Germination

fraction and time to germination were the dependent variables in the models, and target species and treatment (removal of seedlings vs no removal) fixed factors using similar distributions and link functions as above. Post hoc tests correcting for false discovery rate (Benjamini and Hochberg 1995) were performed for comparing germination fractions and timing between species and between treatments within species if the GLM yielded significant interactions.

Results

Neighbour effect on germination fraction

Germination fractions were strongly species-specific ranging from less than 2% (*Stipagrostis*) and 3% (*Moltkiopsis*) to relatively high germination fractions for *Cornulaca* (35%) and *Artemisia* (60%, Fig. 2). The effect of neighbour species on germination fraction differed between target species: *Stipagrostis* did not emerge in interspecific neighbourhoods and germination of *Moltkiopsis* was inhibited by the presence of *Cornulaca* and *Artemisia*. This suppression of germination of *Stipagrostis* and *Moltkiopsis* by later successional species was highly significant ($p < 0.01$) in Fisher's exact tests. Specifically, *Stipagrostis* germinated in 6 out of 16 cases (8 seedlings altogether) when sown with itself, and in none out of 48 cases when sown in interspecific mixtures. *Moltkiopsis* germinated in 5 out of 16 cases in intraspecific mixture, in 8 cases when sown with *Stipagrostis*, in 3 cases with *Cornulaca* and in none of the 16 cases with *Artemisia* as neighbour (Fig. 2). It must be noted that the suppression

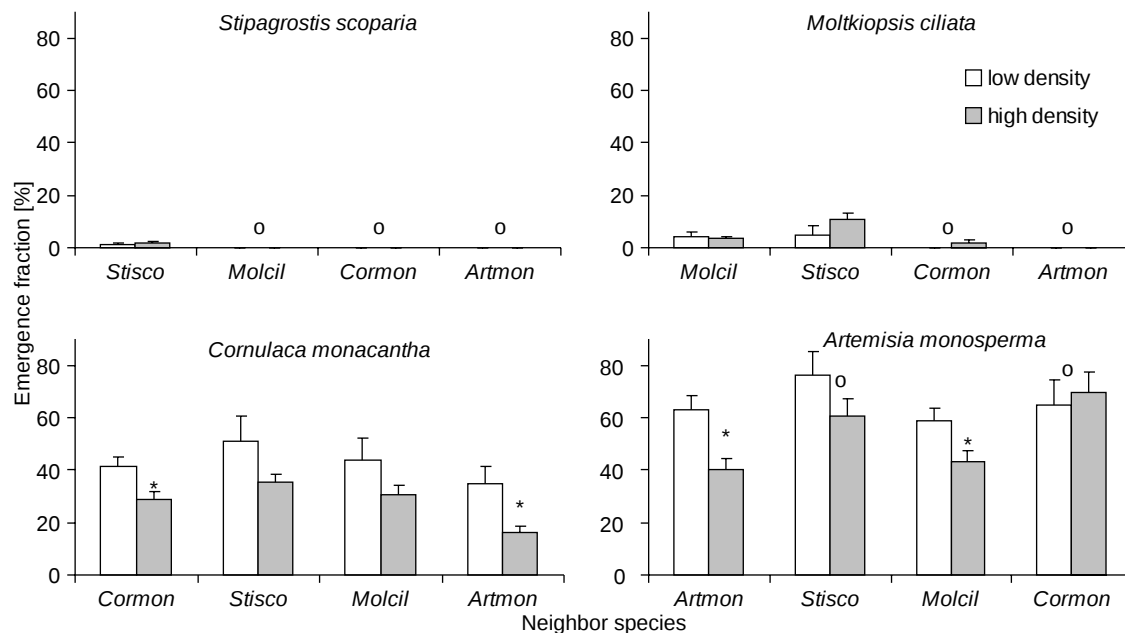


Figure 2. Mean (+SE) percent germination for seeds of four perennial plant species sown in two densities and with different neighbour species. Asterisks show significant differences between densities, circles indicate significant differences between interspecific and intraspecific neighbourhoods (false discovery rate post hoc tests). For *Stipagrostis* and *Moltkiopsis*, circles indicate significant differences to intraspecific treatment in the number of dishes with germination (Fisher's exact tests). Seedling numbers in dishes with germination (from left to right) were 2 and 6 (*Stipagrostis*), and 7, 11, 4, 17, and 3 (*Moltkiopsis*). Abbreviations: Stisco- *Stipagrostis scoparia*; Molcil- *Moltkiopsis ciliata*; Cormon- *Cornulaca monacantha*; Artmon- *Artemisia monosperma*.

Table 1. Results (Wald χ^2) of generalized linear models constructed to test for the effect of neighbour species (neighbour), and neighbour density on germination fractions (Germ) and time to germination (Time) for *Cornulaca* and *Artemisia*. Note that due to low germination fractions and missing cells, no reliable models could be generated for *Stipagrostis* and *Moltkiopsis*, and data for these species were analysed with nonparametric tests.

Source of variation (DF)	<i>Cornulaca</i>		<i>Artemisia</i>	
	Germ	Time	Germ	Time
Neighbour (3)	16.76***	58.87***	39.64***	103.94***
Density (1)	24.74***	20.04**	24.41***	3.35
Neighbour $\times$ Density (3)	1.75	7.92***	10.70*	4.00

of germination by later successional species was also observed in samples where *Stipagrostis* and *Moltkiopsis* served as neighbours and not as targets.

Neighbour effects were species-specific in all species and treatments, including the intraspecific treatment, and the presence of *Artemisia* had the largest negative effect on germination fraction. With a single exception (*Moltkiopsis* with *Stipagrostis*), germination fraction was consistently lower in high-density treatments (Table 1, Fig. 2). This effect was statistically significant for *Cornulaca* and *Artemisia* in intraspecific neighbourhood, for *Cornulaca* with *Artemisia* as neighbour, and for *Artemisia* with *Moltkiopsis* (Fig. 2).

Neighbour effect on time to germination

Time to germination differed largely between target species and neighbour species. Intraspecific mixtures tended to have a shorter time to germination than interspecific mixtures for *Cornulaca* and *Artemisia*, but this difference was significant in only one case (*Artemisia* with *Moltkiopsis*). Species differed largely in germination speed, with *Artemisia* being

the fastest to emerge in all conditions (Fig. 3). Density effects were significant for *Cornulaca* (faster germination in low density treatments), but a significant density $\times$ neighbour interaction (Table 1) indicated that this trend was statistically significant in only one case (*Artemisia* with *Cornulaca*, Fig. 3).

Effect of seedling removal on germination

Similar to the seed density manipulations, germination fractions differed significantly between species (Table 2). *Artemisia* exhibited the highest, *Cornulaca* intermediate, and the other two species relatively low germination fractions (Fig. 4). There was no significant effect of seedling removal or its interaction with species. However, timing of germination differed consistently between species and removal treatments (Table 2). Specifically, germination was significantly accelerated in the presence of seedlings for all study species (Fig. 4). Post hoc tests revealed that *Artemisia* was the fastest species to emerge, while *Stipagrostis* had the longest time to germination (Fig. 4).

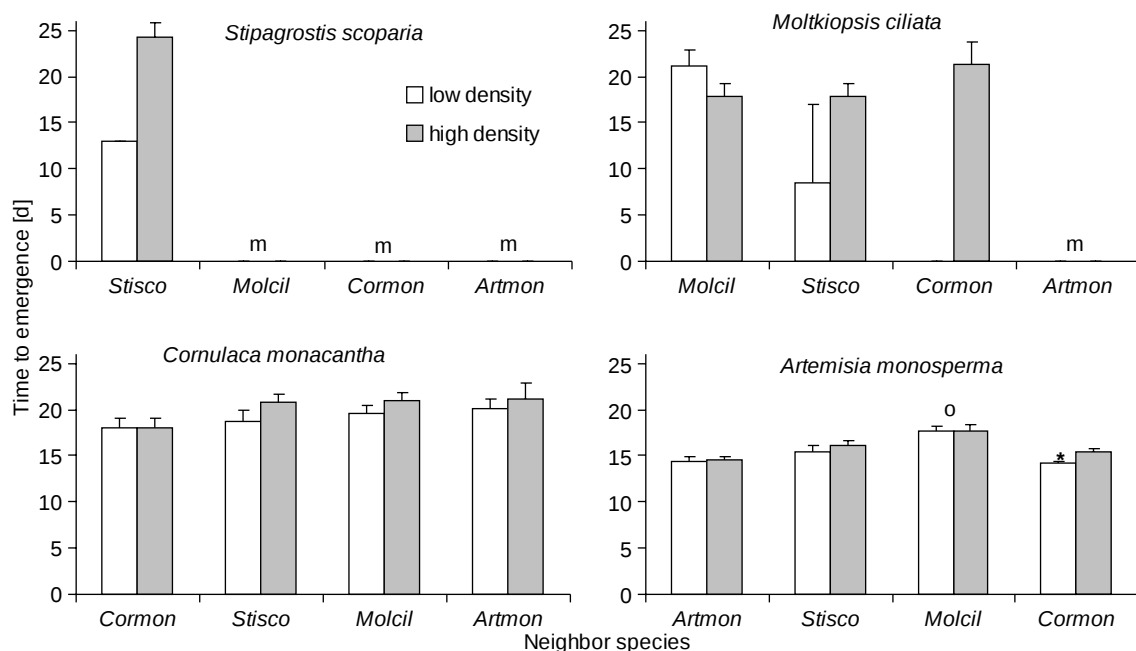


Figure 3. Mean (+SE) time to germination in days for four perennial plant species (Fig. 2) with seeds of different neighbour species sown in two densities. The asterisk indicates a significant difference between densities, the circle indicates a significant difference between inter- and intraspecific neighbourhood (false discovery rate post hoc tests). m indicates missing values (no germination).

Table 2. Results (Wald χ^2) of generalized linear models constructed to test for the effect of seedling removal vs no removal and target species on germination fractions and time to germination.

Source of variation (DF)	Dependent variable	
	Germination fraction	Time to germination
Species (3)	256.97***	44.20***
Removal (1)	0.07	29.16***
Species $\times$ Removal (3)	5.50	0.99

***p < 0.001

None of the seeds that failed to germinate in the main experiment germinated in the following season. Subsequent poking of the nongerminating seeds confirmed that they were dead (i.e. empty or damaged embryo) and not dormant.

Discussion

Our overall results indicate that neighbouring seeds may significantly affect both the fraction of germinating seeds as well as the time to germination, and that there was a consistent competitive hierarchy. In the following, we discuss our findings with respect to our initial hypotheses.

Effect of neighbours on germination timing

The removal experiments partly supported our hypothesis in that the presence of intraspecific neighbouring seedlings led to accelerated germination in all four species. Interestingly, there was no effect of (intraspecific) sowing density on germination speed indicating that merely the presence, not the density of neighbours speeds up germination. Fast

germination is an adaptation to pre-empt space in highly competitive environments, and many previous studies have demonstrated that even a small head start can translate into a considerable advantage for early-germinating seeds (Ross and Harper 1972, Fowler 1984, Weiner 1985, Turkington et al. 2005, Verdú and Traveset 2005). This priority effect should be particularly important in arid environments, where seedlings have to grow fast in a very short season to survive the summer drought. At the study site, recruitment of perennial plants, including our focal species, is indeed an extremely rare event with average survival fraction of naturally germinating seedlings to the third season after germination ranging from zero to 1.6% (Prasse and Tielbörger unpubl.). This highlights the high importance for early germination in the studied system.

Previous studies on neighbour effects on the timing of germination are relatively rare and only one study has explicitly compared intra- vs interspecific neighbourhoods (Dyer et al. 2000). Because in that study, emergence of a perennial bunchgrass native to California was accelerated in the presence of two non-native annual grasses, we hypothesised that the priority effect would be more pronounced in interspecific neighbourhoods. Surprisingly, we did not find clear differences between neighbour species in their ability to modify germination timing of targets. However, Dyer et al. (2000) investigated plant species that do not co-occur in nature. Our findings therefore suggest that in natural plant communities, selection pressure for a priority effect may be independent of the identity of the neighbour species and is similarly large for all component species of a plant community.

Effect of neighbours on germination fraction

Interestingly, there were clearly negative effects of both density and identity of neighbouring seeds on germination fractions. Namely, germination of *Stipagrostis* and *Moltkiopsis* was almost entirely suppressed in the presence of the two other species, and *Artemisia* and *Cornulaca* showed generally lower germination fractions when sown in high intra- and interspecific densities. Even when treating the findings for *Stipagrostis* and *Moltkiopsis* with caution, this indicates that there was negative density-dependent germination in both inter- and intraspecific neighbourhoods. The advantage of this behaviour is obvious for those individuals or species that suppressed others (i.e. *Artemisia* and *Cornulaca*). However, because non-germinating seeds die, selection for the evolution of density dependent germination is difficult to understand.

Negative density-dependence in germination has been demonstrated in several previous studies, but all have focused on plants that were capable of postponing germination to the following season (Palmbad 1968, Ross and Harper 1972, Linhart 1976, Bergelson and Perry 1986, Murray 1998, Goldberg et al. 2001, Lortie and Turkington 2002a, Grundy et al. 2003, Kluth and Bruehlheide 2005, Li et al. 2005, Turkington et al. 2005). For these plants, there is an advantage of not germinating under competitive conditions in the first year, because they may exploit more favourable conditions in later years. For perennials without a permanent seed bank there is no such

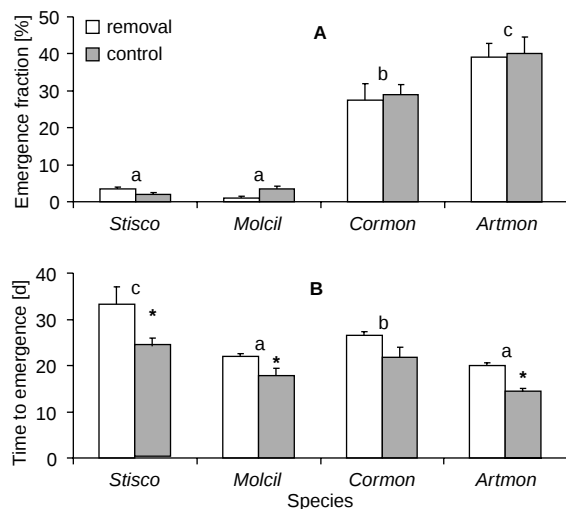


Figure 4. Mean (+SE) percentage of emerging seeds (A) and mean (+SE) time to germination in days (B) for four perennial plant species (Fig. 2) sown in high densities (40 seeds). Emerging seedlings were either left intact (control) or removed immediately after germination (removal). Asterisks indicate significant differences between the treatments, different letters indicate differences among species (false discovery rate post hoc tests).

advantage and selection should favour immediate germination of all seeds. Indeed, the few previous tests for density-dependent germination in perennial plants did not show any effect (Howard and Goldberg 2001, Orth et al. 2003). We suggest that the pattern detected in our study may be understood in the context of a parent–offspring conflict about optimal germination time. Theoretically, offspring fitness in unpredictably varying environments is maximised under immediate germination, while it pays for the mother plant to delay germination (Ellner 1986, Nilsson et al. 1994). These models were developed for annuals with seed dormancy, but Nilsson et al. (1994) demonstrated that maintaining a high fraction of nongerminating seeds may be advantageous for the mother plant even when seed survival is very low. This pattern emerges when maternal fitness loss due to sibling competition is larger than the fitness gain from a few more vigorous seedlings. Another advantage for the mother plant evolves when even non-germinating seeds inhibit germination of other species, such as observed for *Moltkiopsis* and *Stipagrostis* as neighbours. In that case the likelihood for conspecifics (i.e. most likely offspring from the same mother) to emerge in the same place in the following years is increased. This may lead to selection for a mechanism allowing sacrificing a fraction of the offspring for the benefit of the germinating fraction. Practically, such a mechanism could evolve when seed germination is maternally controlled such as by chemical or physical properties of the seed coat (Baskin and Baskin 2004). This is interesting because it would indicate that neighbours could induce secondary physical dormancy (sensu Baskin and Baskin 2004), because other dormancy types are mostly controlled by the embryo (Finch-Savage and Leubner-Metzger 2006).

Do seeds sense each other?

Previous studies that detected density-dependent germination have speculated about whether this effect may be due to the presence of seeds or fully emerged seedlings (Waite and Hutchings 1978, Lortie and Turkington 2002a, 2002b, Turkington et al. 2005). However, there have been virtually no experiments to separate between these two possibilities. Our results from the removal experiments suggest that we need to distinguish between the effects of neighbours on germination timing and on the fraction of emerging seeds.

Time to germination differed markedly between removal and control treatments for all species with germination accelerated in the presence of seedlings. This indicates that time to germination was affected by the presence of seedlings, rather than seeds. Though previous studies have not explicitly separated seed and seedling effects, there is evidence for effects of seedlings on germination of seeds and these include both facilitative (Bergelson and Perry 1989, Lortie and Turkington 2002b) and competitive effects (Juhren et al. 1956, Ross and Harper 1972, Inouye 1980, Shaw and Antonovics 1986). Similar to our study, Bergelson and Perry (1989) found accelerated emergence in the presence of previously planted seeds. Though we planted all seeds simultaneously, a closer look at the relationship between germination timing and competitive effect may indicate whether it is seeds or seedlings that affect

germination of neighbours. In our study, the competitive hierarchy was related to the timing of germination, i.e. the fastest species (*Artemisia*) was both the one exhibiting the highest germination fractions as well as the one with the largest potential to suppress germination of other species. At the same time, the apparently competitively inferior *Stipagrostis* had the longest time to germination. However, the relationship between time to germination and competitive hierarchy was not consistent, because *Moltkiopsis*, which was suppressed by *Artemisia* and *Cornulaca*, germinated at similar rates as *Artemisia*.

In contrast to neighbour effects on timing of germination, there were reduced germination fractions under high seed densities, and this negative effect could be observed even when the neighbours did not germinate (*Stipagrostis* and *Moltkiopsis*). Furthermore, the removal experiments showed that germination fractions were not higher without neighbours. In combination these findings are highly suggestive for the existence of a mechanism allowing seeds to sense each other even prior to emergence. Though previous studies have never explicitly differentiated between effects of seeds and seedlings, there is evidence that leachate from intact seeds (Qadir and Abbasi 1971, Picman and Picman 1984, Bergelson and Perry 1986, Murray 1998) or maternal surface hormones and sibling inhibition (Dyer 2004, Kucera et al. 2005) may control emergence of other seeds. Such allelopathic effects should be highly species-specific, suggesting that they may have played a role in our study. *Artemisia monosperma* had the largest negative effects, and this species belongs to a genus for which allelopathic substances have been found before (Friedman et al. 1977, Ferreira and Janick 2004). However, almost all other species had some effect on one or more other species and *Cornulaca* and *Artemisia* exhibited intraspecific effects. It is therefore more likely that there is some ubiquitous, non-allelopathic mechanism allowing seeds to sense each other. Detailed studies about the regulation of seed dormancy were mostly done with annuals (reviewed by Finch-Savage and Leubner-Metzger 2006), and we must be careful to transfer available results to plants without primary dormancy such as our perennial species. Yet, studies about enforced dormancy in *Nicotiana attenuata* suggest that methyl jasmonate, abscisic acid (ABA) and certain terpenes may be rather ubiquitous substances inhibiting seed germination (Krock et al. 2002, Preston et al. 2004). Though ABA does not inhibit breaking of the seed coat, it may inhibit radicle emergence (Finch-Savage and Leubner-Metzger 2006) and it may thus have played a role in our study. However, the above substances were found only in intact neighbouring plants or in plant litter and seeds or seedlings had no inhibitory effect (Preston et al. 2004). Because previous studies have been quite speculative about the nature of a mechanism of density-dependent germination, it would be highly interesting for future studies to investigate the physiological basis of communication between seeds in the soil.

Competitive hierarchy and successional sequence

Neighbour effects in our study were highly species-specific with a clear competitive hierarchy, ranging from *Stipagrostis*, over *Moltkiopsis* and *Cornulaca* to the competitively

superior *Artemisia*. This hierarchy is striking because it correlates with the proposed successional sequence at the research site (Tielbörger 1997). Namely, species with low germination fractions in interspecific neighbourhoods were those from the pioneer stages (*Stipagrostis* and *Moltkiopsis*) indicating that they could be displaced by other species in the course of succession as soil seed densities increase. Given their overall low seed viability, there must be some other trait allowing these two species to dominate the pioneer stages, which is most likely their high potential for vegetative reproduction in mobile sand (Danin 1996, Tielbörger 1997). In addition, parallel experiments have shown that seeds of *Stipagrostis* sown in stabilised sand in the field (late successional stages) do not germinate while they exhibit detectable germination on mobile dune crests, where late successional species had mostly low germination (Prasse and Tielbörger unpubl.). Our current findings indicate that the pattern observed in the field may be partly explained by high seed densities in the stable interdune areas. We therefore propose that early germination is primarily a means of pre-empting space in late successional stages, i.e. under highly competitive conditions (Fowler 1984, Weiner 1985, Verdú and Traveset 2005). To the best of our knowledge, this is the first evidence suggesting that the direction of successional processes could be affected by density and identity of seeds in the soil. Therefore, a major challenge for future research is to evaluate the importance of density-dependent germination for spatio-temporal dynamics of natural plant communities under field conditions.

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