Eavesdropping in plants: delayed germination via biochemical recognition

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Running title: Eavesdropping in plants: delayed germination
Summary

1. Allelopathy has traditionally been viewed as phytotoxic disruption of recipient plant metabolism and allelopathic effects are generally strongest on species lacking historic exposure to particular phytotoxins (Novel Weapons Hypothesis). However, mounting evidence suggests phytochemical-induced germination inhibition can be an adaptive response to competitive conditions, not the consequence of toxin exposure. That is, selective advantages can exist for seeds to chemically recognize potential competitor presence and defer germination until better establishment conditions occur. This Biochemical Recognition Hypothesis (BRH) contrasts the allelopathy paradigm by predicting greater germination inhibition following phytochemical exposure of sympatric compared to allopatric species.

2. In a greenhouse, we grew 12 species native to Argentinean and North American grasslands and tested whether phytochemical leachates from co-occurring species reduced seedling emergence more than those having no historic association.

3. Two species had 13% and 27% emergence reductions following leachate exposure of sympatric relative to allopatric species, supporting species-specific BR. Intraspecific leachates reduced emergence more than those from heterospecifics, suggesting within-species BR may be common. Only the four smallest seeded species exhibited heterospecific BR responses, suggesting selection for assessing local competition potential may intensify as seed reserves decline. Importantly, leachate origin did not affect seedling biomass nor accelerate germination, indicating a non-toxic biochemical effect on germination reduction but not growth.

4. Synthesis: Coupling ample theoretical support with empirical evidence here and elsewhere, an ‘eavesdrop-and-wait’ competition avoidance strategy could be a common phenomenon. Our findings suggest sympatric association may contribute to evolution of species-specific BR and that
seed traits are important in its development. The underlying mechanism may be simple phytochemical-induced hormonal regulation. Factors preclude BR from being ubiquitous but nonetheless, BR provides a potentially powerful mechanism by which the spatiotemporal diversity of some communities is structured. Lastly, allelopathy may be erroneously invoked when phytochemical-induced germination reduction occurs but a toxicity mechanism has not been elucidated. In many cases, this fits more with the BRH than classic allelopathy.

**Key-words:** Allelopathy, autotoxicity, *Centaurea maculosa*, coevolution, historic interactions, intraspecific recognition, novel weapons hypothesis, plant community structure, spatiotemporal biodiversity, sympatric species recognition

**Introduction**

Seeds have sophisticated abilities to assess whether their contemporary environment is conducive for establishment and rightly so, the timing of germination for many species is the most important decision a plant faces and is expected to be under strong selection (Cohen 1967; Brown & Venable 1986; Venable & Brown 1988; Hierro *et al.* 2009). Myriad ecological factors that signify establishment potential affect germination (Baskin & Baskin 1998) and seeds of some species use conspecific and heterospecific phytochemicals as indicators of local competition magnitude (Preston & Baldwin 1999; Dyer, Fenech & Rice 2000; Krock *et al.* 2002; Dyer 2004; Turkington *et al.* 2005; Tielbörger & Prasse 2009; Orrock & Christopher 2010). Presence of species facilitating establishment increases the germination of others (Lortie & Turkington 2002) and members of the parasitic *Orobanche* and *Striga* (Orobanchaceae) will not germinate unless exposed to phytochemicals of their obligate host (Bouwmeester 2003; Plakhine, Ziadna & Joel 2009).
Emerging work thus suggests some seeds use biological chemicals as adaptive signals to assess presence of hosts, facilitators and competitors but the prevalence of this mechanism remains unclear, including the role that species traits and historical interactions have in its occurrence.

A 90 year-old debate on the relative strengths of the individualistic, organismal and integrated community concept has produced tremendous insight into the interplay between stochastic processes and highly interdependent relationships on community structure (Clemens 1916; Gleason 1926; Lortie et al. 2004; Ricklefs 2008; Brooker et al. 2009). An important emerging question is whether the outcome of species interactions depends on their history of association and empirical evidence suggests strong sympatric association can affect local community composition, species coexistence and ecosystem function (Lortie et al. 2004; Callaway 2007; Castillo, Verdu & Valiente-Banuet 2010; Inderjit et al. 2011; Verdu & Valiente-Banuet 2011). Importantly, prolonged plant interactions can drive adaptive responses that are specific to particular taxa (Preston, Betts & Baldwin 2002; Hierro & Callaway 2003; Ehlers & Thompson 2004; Novoplansky 2009; Thorpe et al. 2011; Soliveres, Torices & Maestre 2012).

One such community-structuring force that invokes evolutionary and often coevolutionary dynamics is allelopathy (Rabotnov 1982; Mallik & Pellissier 2000; Callaway & Ridenour 2004), which is the release of phytotoxic chemicals by one plant that inhibits germination or growth of other plants. It has traditionally been viewed as a form of interference competition, where allelopathic chemicals (allelochemicals) released into the environment disrupt the metabolism of recipient plants or their soil mutualists, and a mode by which some non-native plants become successful invaders is through allelochemical release (Bais et al. 2003; Hierro & Callaway 2003; Callaway & Ridenour 2004; Hierro, Maron & Callaway 2005; Callaway et al. 2008; Thorpe & Callaway 2011). Because members of recently invaded communities have had no historic association with these novel phytotoxins, and thus little time to evolve counter-defenses, their
inhibitory effects on native residents are often much stronger than in the communities in which the toxins originally evolved. This “Novel Weapons Hypothesis” (NWH) has been demonstrated in several different plant systems (Callaway & Aschehoug 2000; Prati & Bossdorf 2004; Inderjit et al. 2011; Svensson et al. 2013).

Although this hypothesis enjoys empirical support, other modes of action may reduce germination following phytochemical exposure but where no direct chemical attack is occurring. Specifically, the “Biochemical Recognition Hypothesis” (BRH, sensu Renne et al. 2004) views some putative allelopathic responses as an adaptive reduction in germination through the recognition of other plant’s chemicals (Preston & Baldwin 1999; Preston, Betts & Baldwin 2002; Dyer 2004; Tielbörger & Prasse 2009). That is, selective advantages can exist for seeds to chemically recognize the presence of potential competitors and defer germination until better conditions for establishment occur – this “listen-and-wait” eavesdropping strategy becomes progressively more advantageous as the fitness reductions incurred from emerging under current conditions exceed loss from soil seed bank decay (Cohen 1967).

Plant taxa have unique exudate chemistries (Bais et al. 2004) and if a lineage has had historically poor recruitment in the presence of another, there is potential for species-specific BR to evolve. On the other hand, if amounts of commonly produced exudates (e.g., sugars, phenolics, amino acids, anti-fungals; see Bais et al. 2004) reliably signify local competition magnitude, similar to the dose-dependent responses in allelopathic systems (Perry et al. 2005; Inderjit et al. 2008), some seeds may simply cue in on this “phytogenic” background to gauge general neighborhood qualities. In addition, because conspecifics frequently interact and share a more similar niche than heterospecifics (Armas & Pugnaire 2011), intraspecific BR may be common. Lastly, the well established positive relationship between seed size and establishment probability (Jakobsson & Eriksson 2000; Moles & Westoby 2004, 2006) suggests that as seed reserves decline,
selection should intensify for predicting establishment potential based on phytochemical proxies of contemporary neighborhood competitiveness. Small-seeded species also tend to have long-lived soil seed banks (Moles & Westoby 2004, 2006) and thus there is greater probability in these taxa of an establishment opportunity occurring from phytochemically delayed germination.

We simultaneously tested the non-mutually exclusive NWH and BRH by exposing six allopatric and six sympatric species to the phytochemical leachates of all 12 test species, including water and the reportedly allelopathic European Centaurea maculosa Lam. (C. stoebe L.; Bais et al. 2003 but see Blair et al. 2005; Stermitz, Hufbauer and Vivanco 2009) as respective negative and positive controls. The NWH and different forms of the BRH make distinct predictions of germination behavior following leachate exposure (Fig. 1). For each species, these include: 1) germination is lower following leachate exposure of sympatric compared to allopatric species (species-specific BRH), 2) intraspecific leachates reduce germination more than those of heterospecifics, irrespective of region of origin (intraspecific BRH), 3) sympatric and allopatric leachates reduce germination more than water (phytogenic BRH) and 4) leachates from allopatric relative to sympatric species reduce germination (NWH). Seed mass of our test species spanned over one order of magnitude from each region and we were thus also able to test the hypothesis that occurrence of BR systems is more likely in smaller seeded species. We found support for hypotheses 1-3 as well as BR responses in small-seeded species only, and discuss the implications of BR to community structure and the ecological conditions under which a BR strategy is expected to evolve.

Materials and methods

EXPERIMENTAL SET-UP
In March 2007, we evaluated the seedling emergence potential of 20 and 13 perennial grass and forb species respectively native to and sympatric in central North American (NA) and Argentinean La Pampa (SA) grasslands by sowing them in flats containing sterilized, field-collected Canfield silt-loam soil. Seeds of SA species were hand-collected in one La Pampa locale and NA species were purchased from Earthskin Nursery (Mason City, IL 62664, USA), where species of central Illinois ecotypes were collected from local prairie remnants and grown for commercial value. From the pool of species that emerged at a high percentage (e.g., ~40% or more), we chose six species from each region to evaluate their seedling emergence response to phytochemicals from sympatric and allopatric species, *C. maculosa* and water. Because selective pressure on using phytochemical cues to assess local competition intensity may intensify as seed reserves decline, we also chose species such that a wide seed size range was represented from each region (NA: 0.382-4.35 mg/seed, SA: 0.200-4.99 mg/seed; Table 1).

For phytochemical leachate sources, we grew monocultures of each NA and SA species as well as *C. maculosa* in three separate 53 × 28 × 6 cm drained flats containing sterilized topsoil for seven weeks in a greenhouse (*n* = 13 species). We wanted to maximize the potential for detecting a BR response and thus used autoclave-sterilized soil in all experimental phases because soil microbes can use phytochemicals as a carbon source, diminishing any phytochemical-induced germination response (Kaur *et al*. 2009; Ehlers 2011). Flats of each species were watered through the aboveground vegetation with deionized water (dH$_2$O) such that a total excess of 1.8 ± 0.1 L of water was collected in water-tight flats. This leachate, which should contain water-soluble phytochemicals exuded primarily from the roots, was then used to water seeds of the species grown for phytochemical collection. In this way, all 12 test species were separately subjected to the leachates of all other species, including a negative (dH$_2$O) and positive control (*C. maculosa*).
In July 2007, 33 seeds of each species were evenly sown on the soil surface of separate 9 × 9 × 7 cm square pots and covered with 2-3 mm of soil. We then watered all pots (n = 504) with 25 ml of their respective leachate 2-3 times per week and recorded seedling emergence once per week for five weeks. Because seeds often rely on several germination cues simultaneously (Preston & Baldwin 1999) and light, if sufficiently strong, can override germination inhibitors (Baskin & Baskin 1998), we installed shade cloth over all pots before the experiment. This approximated photosynthetically active radiation (PAR) at the soil surface of an intact tallgrass prairie canopy (Lane, Coffin & Lauenroth 2000; 0-5% ambient PAR) and reduced average ambient PAR transmittance to 7.1%. Ambient PAR at 1200 hr and under shadecloth respectively averaged 1680 and 120 μmol/m²/sec, as measured with an AccuPAR LP-80 ceptometer (Decagon Devices, Inc.).

EXPERIMENTAL DESIGN AND DATA ANALYSIS

We analyzed our dataset using a one-way unbalanced analysis of variance (ANOVA), with four levels of the leachate treatment (i.e., NA, SA, *C. maculosa* and water). For each of our 12 test species, we considered seeded pots, which were randomly assigned to a leachate, as experimental units and had three replicates for *C. maculosa* and water and 18 replicates for each region (i.e., three leachate replicates of each of six species from NA and SA). For species with significant differences in total emergence between NA and SA leachates, we also removed the intraspecific leachate from the analysis and used a separate one-way ANOVA to explicitly test for heterospecific sympatric and allopatric leachate effects. To elucidate whether these responses were driven by non-toxic biochemicals, we harvested the seedlings of species exhibiting a BR response to sympatric species, and used a one-way ANOVA to test whether their dry weight per seedling differed between NA and SA leachates and water. We also used a two-way ANOVA, with region
as a fixed and species as a random factor, and tested whether the leachate of each species had greater inhibitory effects on emergence of allopatric compared to sympatric species.

Because we foresaw potential for a weak BR signal and did not want to lose much power in correcting for multiple ANOVA tests, we set experimentwise $\alpha$ at 0.10 and considered each of the 12 ANOVAs to be statistically significant at $P = 0.10/12 = 0.0083$. If significant leachate effects on total seedling emergence occurred at this level, we used linear contrasts to test for treatment differences at $\alpha = 0.05$ in all preplanned comparisons and Tukey-Kramer adjustments to maintain an experimentwise error rate of 0.05 in post hoc tests. No heterogeneity of variance occurred among treatments for emergence (Levine’s HOV test: $P > 0.100$) but for dry weights per seedling, log transformation of Monarda fistulosa L. was employed to yield treatment homogeneity of variance ($P = 0.117$). Because phytochemical cues that signify a competitive environment can accelerate germination (Dyer, Fenech & Rice 2000; Tielbörger & Prasse 2009; Orrock & Christopher 2011), we also tested for NA and SA leachate effects on emergence rates for all species using one-way ANOVAs. Here, we compared treatment means when $\geq 50\%$ of the final seedling number for each species had emerged, which in all cases was after 1-2 weeks of leachate exposure.

To test for a relationship between seed size and BR response, we ran a Mann-Whitney U test, assigning species ranked by seed mass into categories of whether BR was observed – this could be a species-specific or phytogeneric BR response. We also ran this test using species exhibiting species-specific BR only. Seeds of many species may frequently be exposed to intraspecific phytochemicals and thus conspecific BR may be common. To assess this, we ran a Wilcoxon signed-rank test using all species, assigning intraspecific leachate effects on seedling emergence a value between 1-12 (i.e., from highest to lowest emergence percentage relative to heterospecific leachates; Table 1) and testing whether this rank differed from the null hypothesis median prediction of 6.5. All statistical tests were performed using SPSS (IBM®, version 18).
Support for “species-specific BR” does not necessarily imply that a species seeds respond to all sympatric species but suggests they respond to some of them. Also, none of these hypotheses are mutually exclusive and if several operate on a particular species, finding support for any one is less likely. For example, the “intraspecific BRH” predicts that seeds respond strongest to conspecific leachates but if some sympatric and allopatric species also reduce germination, it would be more difficult to detect. By the same token, it would be difficult to detect species-specific BR and the NWH if both simultaneously operate. As such, all of our tests are deemed conservative.

All hypotheses are based on the assumption that competitive interactions in a high resource grassland environment are the norm and that species historically facilitating establishment of other species is uncommon (but see Callaway & Walker 1997; Lortie & Turkington 2002; Bruno, Stachowicz & Bertness 2003; Brooker et al. 2008; Gross et al. 2013).

Results

GENERAL LEACHATE EFFECTS AND PHYTOGENERIC BIOCHEMICAL RECOGNITION

Leachate effects on total seedling emergence were significant for four species, with the water treatment resulting in higher emergence relative to at least one of the phytochemical leachate treatments \( F_{3,38} \geq 4.96, P \leq 0.005 \). Emergence following water exposure was 44% to 131% higher than NA, SA and \( C. maculosa \) leachate exposure for \( M. fistulosa \) and \( G. megapotamica \) var. scabiosoides (Spreng.) Baker (Table 1, Tukey-adjusted HSD: \( P \leq 0.023 \)), \geq 71% higher than NA and SA leachates for \( S. eryostachia \) Kunth (Table 1, \( P \leq 0.006 \)) and 29% higher than NA leachates for \( R. pinnata \) (Vent.) Barnhart (\( P = 0.027 \)). \( G. megapotamica \) and \( S. eryostachia \) had higher emergence in the presence of water relative to
sympatric and allopatric leachates, the latter of which did not differ ($P \geq 0.711$). No NA nor SA leachate reduced emergence of allopatric species more than those that were sympatric ($F_{1,408} \leq 1.26$, $P \geq 0.262$, linear contrasts).

SPECIES-SPECIFIC BIOCHEMICAL RECOGNITION

Relative to allopatric leachates, exposure of sympatric leachates to *M. fistulosa* and *R. pinnata* seeds respectively lowered their total seedling emergence by 27% and 13%, with a net reduction of 10.3% in each case (Fig. 2, *M. fistulosa*: $F_{1,34} = 11.2$, $P = 0.002$; *R. pinnata*: $F_{1,34} = 7.55$, $P = 0.010$; linear contrasts). Following the removal of intraspecific leachates from the analyses, sympatric and allopatric leachate effects on emergence percentage remained virtually unchanged and significant for both species, indicating that heterospecific sympatric phytochemicals were driving the effects (*M. fistulosa*: $F_{1,31} = 9.05$, $P = 0.005$; *R. pinnata*: $F_{1,31} = 5.46$, $P = 0.026$).

Dry weight per seedling of *M. fistulosa* and *R. pinnata* did not differ among water and NA and SA leachates ($F_{2,27} \leq 2.11$, $P \geq 0.142$). Seedling emergence rates differed between NA and SA leachates for *M. fistulosa* only, with sympatric NA leachates significantly slowing emergence ($F_{1,34} = 11.92$, $P = 0.002$).

INTRASPECIFIC BIOCHEMICAL RECOGNITION

For each species, intraspecific leachates were assigned a rank between 1 and 12 to designate the order in which they affected seedling emergence relative to heterospecific leachates (Table 1). Using a Wilcoxon signed-rank test, we tested whether the observed value for intraspecific leachate effects differed from the null hypothesis expectation of 6.5 and found they reduced emergence compared to heterospecific sympatric and allopatric leachates ($P = 0.020$, mean: $9.3 \pm 2.0$ (95%...
CI)). Relative to the other 11 heterospecific leachates, intraspecific leachates resulted in the lowest or second lowest emergence percentage for six of 12 species (Table 1).

SEED SIZE EFFECTS ON BIOCHEMICAL RECOGNITION

Small-seeded species were more likely than those with larger seeds to exhibit species-specific or phytogeneric BR, with only the four smallest seeded species demonstrating BR responses (Fig. 3, Mann-Whitney U: $P = 0.007$). The second and third smallest seeded species were the only ones to exhibit species-specific BR and the effect of seed size on this response was marginally significant (Mann-Whitney U: $P = 0.086$). Seed size was not a significant predictor of emergence responses to intraspecific relative to interspecific leachates (Table 1, $r^2 = 0.041$, $P = 0.527$), suggesting development of intraspecific BR may not depend heavily on seed size.

C. MACULOSA EFFECTS ON SEEDLING EMERGENCE

*C. maculosa* leachate effects on total emergence did not differ between NA and SA leachates for any species except *S. eryostachia*, where this leachate increased emergence by $\geq 76\%$ relative to those from NA and SA ($F_{2,36} = 6.86$, Tukey-adjusted HSD: $P \leq 0.004$). Relative to water, emergence of *M. fistulosa* and *G. megapotamica* was reduced following exposure to *C. maculosa* leachates ($P \leq 0.027$).

Discussion

EVIDENCE FOR AND THE ADAPTIVE SIGNIFICANCE OF BIOCHEMICAL RECOGNITION
The vast majority of seedlings perish for numerous reasons, many of which are stochastic, but fairly reliable cues that signify degree of neighborhood competition exist for seeds and many of these are chemical in nature (Bergelson & Perry 1989; Dyer, Fenech & Rice 2000; Preston, Betts & Baldwin 2002; Dyer 2004; Tielbörger & Prasse 2009). We found two species had 13% and 27% germination reductions following exposure to phytochemicals of sympatric relative to allopatric species (Fig. 2). This opposes what is expected if the NWH was operating strongly and provides evidence that some seeds have developed mechanisms to assess presence of particular taxa and make germination decisions based on them (Preston & Baldwin 1999; Preston, Betts & Baldwin 2002). If correct, this chemically induced response suggests that historic interactions can adaptively shape niche regeneration breadth and lends support to the proposition that some communities are in part structured by sympatric evolutionary association (Clements 1916; Lortie et al. 2004; Brooker et al. 2009). In addition, detecting presence of specific neighbors that confer negative fitness consequences may increase establishment potential of all BR-possessing members, thereby increasing community spatiotemporal diversity (also see Lortie et al. 2005; Turkington et al. 2005). It is unlikely that osmotic potential differentials drove these effects (i.e., reduced germination and growth from osmotic-induced low water uptake rates) because seedling growth did not differ between allopatric and sympatric leachates and water (see Wardle, Nicholson and Ahmed 1992) and there is no reason to suspect allopatric leachates had inherently lower osmotic potentials. Other systems that support species-specific BR include plants that inhibit germination of species sharing a long history of sympatry but have no effect on those where past interactions are absent (Preston & Baldwin 1999; Preston, Betts & Baldwin 2002; Renne et al. 2004).

Evolutionary theory with respect to allelopathy predicts that as encounters with toxins increase in frequency, species should develop resistance to them (Rabotnov 1982). Given that intraspecific and even sib-interactions frequently occur in many species (Cheplick 1992; Dyer...
it is difficult to imagine that strong resistance to within-species allelochemicals is not commonplace. Moreover, conspecifics share a more similar niche than heterospecifics, their interactions are generally stronger (Armas & Pugnaire 2011) and the BRH predicts chemical recognition is most likely to develop here. We found intraspecific leachates significantly lowered emergence compared to heterospecific leachates and interpret this as a convincing signature of intraspecific BR (Table 1; also see Dyer, Fenech & Rice 2000; Dyer 2004; Turkington et al. 2005; Orrock & Christopher 2010). If correct, this represents a potentially widespread and important mechanism by which secondary metabolites may structure plant populations. Interestingly, seed size did not affect emergence responses to intraspecific leachates, suggesting development of conspecific recognition is independent of seed reserves, at least for small-seeded grassland species.

We add that unless a non-osmotic potential-based mechanism of toxicity has been established, these oft interpreted ‘autotoxic’ effects (e.g., Alias et al. 2006) fit more with the intraspecific BRH and in many cases, should be interpreted as such (see Perry et al. 2005 for a good autotoxicity example).

Most plants compete for limited water, light and nutrient resources and thus it is possible that concentrations of commonly produced exudates (e.g., sugars, phenolics, amino acids, anti-fungals; see Bais et al. 2004) reliably indicate local competition magnitude. Relative to water, we found that three species had emergence reductions following allopatric and sympatric leachate exposure, suggesting some seeds cue in on a “phytogenic” background to gauge establishment potential based on general neighborhood qualities. In communities characterized by rapid compositional change, this non-specific eavesdropping may be particularly adaptive if quantities of commonly produced phytochemicals are good proxies of contemporary competition. Our test of the phytogenic BRH is potentially problematic because species-specific BR and the NWH may
have simultaneously operated, but in no case did allopatric leachates reduce germination more than sympatric leachates so we consider this possibility highly unlikely.

All seeds face formidable challenges establishing under competitive conditions and the severity of this increases as seed size and their concurrent reserves decline (Jakobsson & Eriksson 2000; Moles & Westoby 2004, 2006). Larger seeds also tend to have higher predation rates and shorter lived soil seed banks (Moles & Westoby 2004, 2006) and thus the benefits of a phytochemical-induced ‘sit-and-wait’ strategy likely decline as seed size increases (see below). We found smaller seeded species were significantly more likely to exhibit BR (Fig. 3) and interpret this as a manifestation of greater selective pressure on them to assess neighborhood competition potential via BR. Kos and Poschlod (2008) found osmotic potential gradients did not differentially affect germination of small- and large-seeded species from a phylogenetically diverse assemblage and thus we feel osmotic differentials did not drive the observed responses. Interestingly, Dyer (2004) found germination of small seeds of the dimorphic seeded *Aegilops triuncialis* L. was inhibited when large-seeded siblings were in close proximity, but not vice versa, and suggested this represented a greater fine-tuned assessment of the biotic environment by small seeds.

**EVOLUTION OF BIOCHEMICAL RECOGNITION**

For BR to evolve, the expected benefit:cost ratio of delayed germination *must* outweigh that of ‘making the best of a bad situation’ under current conditions. Critically important factors affecting its evolution include competition-dependent rates of pre-reproductive mortality as well as reproductive output from successfully establishing individuals. If negative population growth results from strong competition (e.g., high seedling mortality and low seed production from a few successful individuals), phytochemical-induced germination delays would be profitable as long as loss from soil seed bank decay is less than the fitness reductions incurred from emerging under
current conditions. Seed bank longevity is thus also important and the selective advantages of BR become greater the longer seeds remain viable and the more limited establishment opportunities are (Cohen 1967; Renne et al. 2004). We submit that selection on BR systems may even drive the evolution of seed dormancy and not vice versa (also see Venable and Brown 1988).

Systems in which plants are most likely to develop some form of BR are those where: 1) competition-induced seedling mortality is predictably high and reproductive output is low, 2) shade intolerance is common, including low phenotypic plasticity in low light levels and 3) the frequency of competitor-based establishment opportunities is generally shorter than the rate of soil seed bank decay (Renne et al. 2004, e.g., ruderal communities, grasslands, savannas, chaparrals). BR is less likely when soil seed bank longevity is low (e.g., forest canopy species) or contemporary phytochemical cues, or lack thereof, are unreliable indicators of lifetime fitness (Cohen 1967). The latter may occur in lineages that have historically been subjected to high post-emergence, density-independent mortality (e.g., fire, late freezes, trampling) or a rapid change in neighborhood competitive strength from stochastic disturbance (e.g., sporadic, intensely grazed systems).

Dominant community members are most likely to be chemically recognized because they are by definition common, tend to exhibit temporal stability in biomass (Roscher et al. 2011) and may represent a consistent competitive element. However, unless subordinate-dominant species interactions are rare or consistently one-sided, species spanning a competitive hierarchy may not differ in the incidence of species-specific BR because established subordinates can exert strong size-asymmetric competition on establishing dominants (Schwinning & Weiner 1998).

Intraspecific BR is also most likely to be developed in dominants, as simple probability dictates their interactions with conspecifics are more frequent than intraspecific interactions among uncommon subordinates with unclumped distributions.
Conclusions

Our only criteria for choosing the 12 test species from a larger pool was that each had to germinate at high rates and a wide seed size range was represented. Given this novel, albeit ‘shotgun’ approach, it is perhaps surprising that two species exhibited species-specific BR, three exhibited phytogeneric BR and intraspecific BR emerged as common. Additionally, smaller seeded species were most likely to exhibit BR responses. Coupling multiple lines of theoretical support with empirical evidence here and elsewhere, it thus appears this ‘eavesdrop-and-wait’ competition avoidance strategy could be common in some systems. Several key factors preclude it from being ubiquitous but nonetheless, BR provides a potentially powerful mechanism by which some plant populations, and the spatial and temporal diversity of some communities, may be structured. Species-specific and phytogeneric BR was supported, implying that Clementsian and Gleasonian processes may operate simultaneously in the same plant community. We focused on phytochemical-induced germination delays as adaptive responses to competitive conditions but fully expect future examples of species-specific BR in systems where particular species periodically facilitate establishment and subsequent reproduction of beneficiaries (Callaway & Walker 1997; Bruno, Stachowicz & Bertness 2003; Brooker et al. 2008; Gross et al. 2013), and recognition of facilitators stimulates germination (Lortie & Turkington 2002; Bouwmeester 2003; Plakhine, Ziadna & Joel 2009). Given that adaptive strategies in germination behavior can form rapidly (Hierro et al. 2009), we expect ongoing refinement of BR systems as ecological factors alter the strength and even directionality of existing relationships, past interactions are lost and interactions with novel species form – these expected changes come from the enormous selective pressure on seeds to accurately evaluate biotic-based establishment potential and based on their assessment, make the critically important and irreversible decision to germinate.
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References


Cohen, D. (1967) Optimizing reproduction in a randomly varying environment when a correlation may exist between the conditions at the time a choice has to be made and the subsequent outcome. *Journal of Theoretical Biology, 16*, 1–14.


Supporting Information Additional Supporting Information may be found in the online version of this article: Appendix S1. (to be submitted upon acceptance.)

Table 1. Taxonomically diverse assemblage of 12 test species from North American and Argentinean grasslands.

<table>
<thead>
<tr>
<th>Family</th>
<th>Species</th>
<th>mg/seed*</th>
<th>Species-specific BR†</th>
<th>Phytogenic BR‡</th>
<th>Intraspecific leachate ranking¶</th>
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<td><strong>North America (central Illinois ecotypes)</strong></td>
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<td>Asteraceae</td>
<td>Heliopsis helianthoides (L.) Sweet</td>
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<td>ns</td>
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<td>p = 0.010</td>
<td>ns</td>
<td>11</td>
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<td>p = 0.002</td>
<td>p = 0.005</td>
<td>10.5</td>
</tr>
<tr>
<td>Poaceae</td>
<td>Andropogon gerardii Vitman.</td>
<td>2.13</td>
<td>ns</td>
<td>ns</td>
<td>7</td>
</tr>
<tr>
<td><strong>Argentina (La Pampa province ecotypes)</strong></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Asteraceae</td>
<td>Gaillardia megapotamica var. scabiosoides</td>
<td>1.47</td>
<td>ns</td>
<td>p = 0.001</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>(Spreng.) Baker</td>
<td></td>
<td></td>
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<tr>
<td>Asteraceae</td>
<td>Thelesperma megapotamicum (Spreng.) Kuntze</td>
<td>2.13</td>
<td>ns</td>
<td>ns</td>
<td>12</td>
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<tr>
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<td>4.76</td>
<td>ns</td>
<td>ns</td>
<td>10</td>
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<tr>
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<td>ns</td>
<td>ns</td>
<td>12</td>
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<tr>
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<td>ns</td>
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<tr>
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<td>Stipa eryostachia Kunth</td>
<td>0.200</td>
<td>ns</td>
<td>p = 0.004</td>
<td>11</td>
</tr>
</tbody>
</table>

* mg/seed is based on weighing 200 seeds and estimating individual seed mass. Dispersal structures were removed from all species before weighing.
† Species exhibiting greater germination reduction following exposure to leachates of sympatric relative to allopatric species (supporting species-specific BR).

‡ Species with greater germination reduction when exposed allopatric and sympatric leachates than to water (supporting phylogenetic BR).

¶ Intraspecific leachate rankings from 1-12 designate, from highest to lowest, the order in which intraspecific leachates affected emergence relative to the 11 heterospecific leachates (Mann-Whitney U: $P = 0.007$).
Fig. 1. Flowchart showing support for various hypotheses based on germination responses to different leachate sources (see text for details).

Fig. 2. *Ratibida pinnata* and *Monarda fistulosa* respectively had 13% and 27% lower seedling emergence when exposed to leachates of sympatric relative to allopatric species ($F_{1,34} \geq 7.55$, $P \leq 0.010$), supporting species-specific biochemical recognition. Removal of intraspecific leachates from the analysis did not change these outcomes, indicating heterospecific sympatric phytochemicals were driving the effects. Means ± 1 SE are shown. Note that of our 12 test species, seed mass of these species was respectively the third and second smallest.

Fig. 3. Seed mass effects on biochemical recognition (BR) responses. We observed an inverse relationship between seed mass and whether species-specific or phytogeneric BR responses occurred (Mann-Whitney U: $P = 0.007$). A 25-fold difference in seed mass (mg/seed) existed between the smallest and largest seeded species (Table 1).
Figure 1

**Phytochemicals reducing germination**

- Sympatric phytochemicals only
- Intraspecific phytochemicals
- Sympatric & allopatric phytochemicals
- Allopatric phytochemicals only
- No response

**Hypothesis supported**

- Species-specific biochemical recognition
- Intraspecific biochemical recognition
- Phytogeneric biochemical recognition
- Classic allelopathy (e.g., Novel Weapons Hypothesis)
- No classic allelopathy nor biochemical recognition
Figure 2

The graph shows the seedling emergence percentage for two species, *Ratibida pinnata* and *Monarda fistulosa*, under two conditions: Allopatric leachates (open bars) and Sympatric leachates (solid bars). The y-axis represents the seedling emergence percentage, ranging from 0 to 80. The x-axis lists the two species. The data indicates a higher seedling emergence for *Ratibida pinnata* under both conditions, with *Monarda fistulosa* showing a lower emergence under Allopatric leachates compared to Sympatric leachates.
Figure 3

The graph shows a scatter plot with the y-axis labeled "mg/seed" and the x-axis divided into two categories: BR response and No BR response. The plot displays data points indicating the mg/seed values for seeds with and without BR response.