

# Eavesdropping in plants: delayed germination via biochemical recognition

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## Summary

1. Allelopathy has traditionally been viewed as a 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 glasshouse, 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 that 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 affecting these germination decisions may be simple phytochemical-induced hormonal regulation. Factors preclude BR from being ubiquitous but nonetheless, BR provides a potentially powerful mechanism by which some plant populations and the spatiotemporal diversity of some communities are 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*, competition avoidance strategy, historic interactions, intraspecific recognition, novel weapons hypothesis, plant population and community dynamics, 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

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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; Bidartondo & Read 2008), and members of the parasitic *Orobanchae* and *Striga* (Orobanchaceae) will not even germinate unless exposed to phytochemicals of their obligate host (Bouwmeester *et al.* 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 historic 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 (Clements 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).

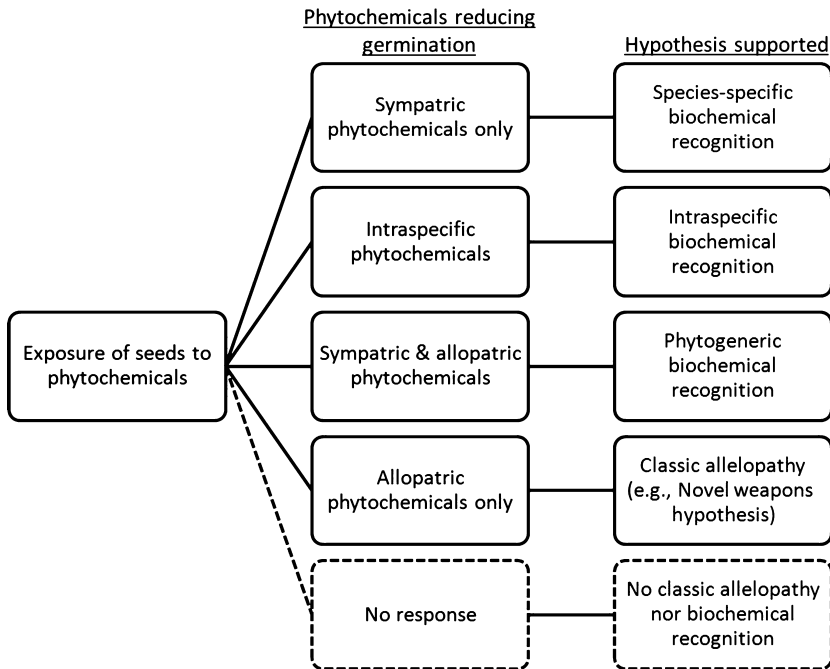
One such community-structuring force that invokes evolutionary and co-evolutionary dynamics is allelopathy (Rabotnov 1982; Mallik & Pellissier 2000; Callaway & Ridenour 2004), which is the release of phytotoxic chemicals by one plant that inhibits germination or growth of other plants. It has traditionally 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-defences, 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 'eavesdrop-and-wait' competition avoidance 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, antifungals; 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.* 2011), some seeds may simply cue in on the strength of this 'phytogenic' background to gauge general neighbourhood qualities and make germination decisions based on it. 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 neighbourhood 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 & Vivanco 2009) as respective negative and positive controls. The NWH and different forms of the BRH make distinct predictions of germination behaviour following leachate exposure (Fig. 1). For each species, these include the following: (i) germination is lower following leachate exposure of sympatric compared to allopatric species (species-specific BRH), (ii) intraspecific leachates reduce germination more than those of heterospecifics, irrespective of region of origin (intraspecific BRH), (iii) sympatric and allopatric leachates reduce germination more than



**Fig. 1.** Flowchart showing support for various hypotheses based on germination responses to different phytochemical sources (see text for details).

water (phytogeneric BRH) and (iv) 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 heterospecific BR responses in small-seeded species only, and discuss the implications of BR to community structure and the ecological conditions under which BR strategies are 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 common species from each region (NA: Johnson & Anderson 1986; Martin, Moloney & Wilsey 2005; SA: Cano, Fernández & Montes 1980; Rúgolo de Agrasar, Steibel & Troiani 2005) 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 magnitude 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<sup>-1</sup>, SA: 0.200–4.99 mg seed<sup>-1</sup>; 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 7 weeks in a glasshouse ( $n = 13$  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 above-ground vegetation with deionized water (dH<sub>2</sub>O) such that a total excess of  $1.8 \pm 0.1$  L of water was collected in watertight flats. This leachate, which should contain water-soluble phytochemicals exuded from roots and shoots, 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<sub>2</sub>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 5 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 h and that under shade cloth, respectively, averaged 1680 and 120  $\mu\text{mol m}^{-2} \text{sec}^{-1}$ , as measured with an AccuPAR LP-80 ceptometer (Decagon Devices, Inc., Pullman, Washington, USA).

### EXPERIMENTAL DESIGN AND DATA ANALYSIS

We analysed our data set 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,

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

Family	Species	mg seed <sup>-1</sup> *	Species-specific BR <sup>†</sup>	Phylogenetic BR <sup>‡</sup>	Intraspecific leachate ranking <sup>§</sup>
North America (Central Illinois ecotypes)					
Asteraceae	<i>Heliopsis helianthoides</i> (L.) Sweet	4.35	ns	ns	11
Asteraceae	<i>Parthenium integrifolium</i> L.	4.00	ns	ns	2
Asteraceae	<i>Ratibida pinnata</i> (Vent.) Barnhart	1.01	$P = 0.010$	ns	11
Fabaceae	<i>Dalea purpurea</i> Vent.	1.56	ns	ns	12
Lamiaceae	<i>Monarda fistulosa</i> L.	0.382	$P = 0.002$	$P = 0.005$	10.5
Poaceae	<i>Andropogon gerardii</i> Vitman.	2.13	ns	ns	7
Argentina (La Pampa province ecotypes)					
Asteraceae	<i>Gaillardia megapotamica</i> var. <i>scabiosoides</i> (Spreng.) Baker	1.47	ns	$P = 0.001$	6
Asteraceae	<i>Thelesperma megapotamicum</i> (Spreng.) Kuntze	2.13	ns	ns	12
Poaceae	<i>Aristida niederleinii</i> Mez	4.76	ns	ns	10
Poaceae	<i>Bromus brevis</i> Nees ex Steud.	4.99	ns	ns	12
Poaceae	<i>Hordeum euclaston</i> Steud.	4.55	ns	ns	7
Poaceae	<i>Stipa eryostachia</i> Kunth	0.200	ns	$P = 0.004$	11

\*mg seed<sup>-1</sup> is based on weighing 200 seeds and estimating individual seed mass. Dispersal structures were removed from all species before weighing.

<sup>†</sup>Species exhibiting greater germination reduction following exposure to leachates of sympatric relative to allopatric species (supporting species-specific BR).

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

<sup>§</sup>Intraspecific leachate rankings from 1 to 12 designate, from highest to lowest, the order in which intraspecific leachates affected emergence relative to the 11 heterospecific leachates (Mann–Whitney  $U$ :  $P = 0.007$ ).

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 pre-planned 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 2010), we also tested for NA and SA leachate effects on emergence rates for all species using one-way ANOVA. 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 phylogenetic 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 and 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, Chicago, Illinois, USA).

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 PHYTOGENETIC BIOCHEMICAL RECOGNITION

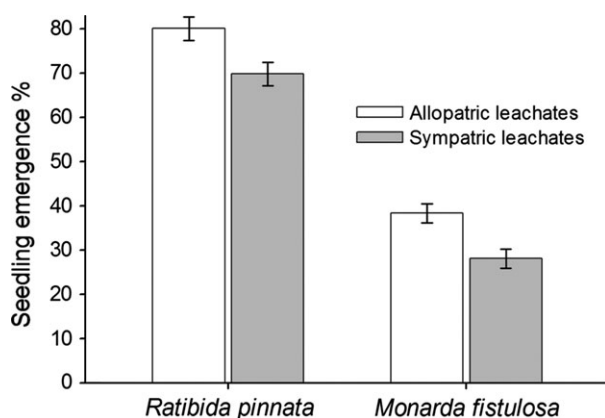
Leachate effects on total seedling emergence were significant for four species, with the water treatment resulting in higher



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 from 44% to 131% higher than NA, SA and *C. maculosa* leachate exposure for *M. fistulosa* and *Gaillardia megapotamica* var. *scabiosoides* (Spreng.) Baker (Table 1, Tukey-adjusted HSD:  $P \leq 0.023$ ),  $\geq 71\%$  higher than NA and SA leachates for *Stipa eryostachia* Kunth (Table 1,  $P \leq 0.006$ ) and 29% higher than NA leachates for *Ratibida pinnata* (Vent.) Barnhart ( $P = 0.027$ ). *Gaillardia 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 between 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 ( $F_{1,34} = 11.92$ ,  $P = 0.002$ ).



**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  $\pm 1$  SE are shown. Note that of our 12 test species, seed mass of these species was, respectively, the third and second smallest.

#### INTRASPECIFIC BIOCHEMICAL RECOGNITION

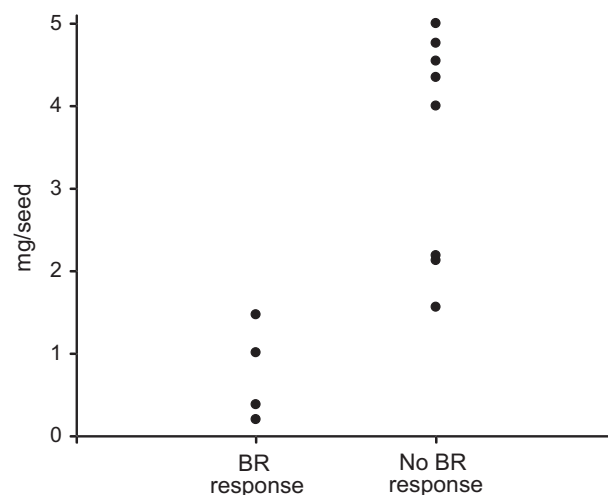
For each species, intraspecific leachates were assigned a rank between 1 and 12 to designate the order in which they affected seedling emergence relative to heterospecific leachates (Table 1). Using a Wilcoxon signed-rank test, we tested whether the observed value for intraspecific leachate effects differed 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

The effect of *C. maculosa* leachate 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



**Fig. 3.** Seed mass effects on heterospecific 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 ( $\text{mg seed}^{-1}$ ) existed between the smallest and largest seeded species (Table 1).

( $F_{2,36} = 6.86$ , Tukey-adjusted HSD:  $P \leq 0.004$ ). Relative to water, emergence of *M. fistulosa* and *G. megapota mica* 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 neighbourhood 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 the presence of specific neighbours 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 & 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 2004), 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 postulates that chemical recognition is most likely to develop where competition is predictably high. We found intraspecific leachates significantly lowered emergence compared to heterospecific leachates and interpret this as a convincing

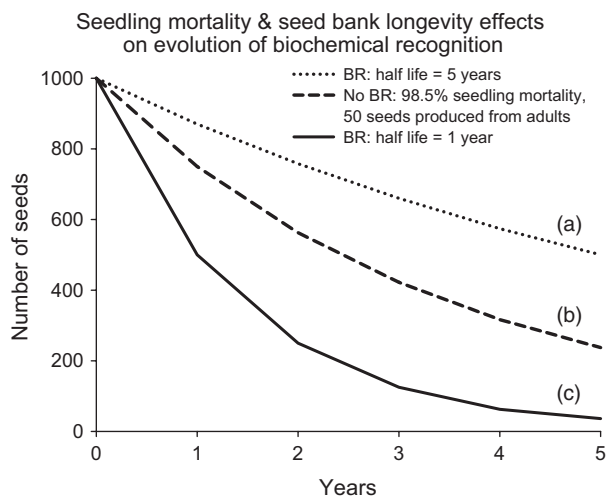
signature of intraspecific BR (Table 1; also see Dyer, Fenech & Rice 2000; Dyer 2004; Turkington *et al.* 2005; Orrock & Christopher 2010). If correct, this represents a potentially widespread and important mechanism by which secondary metabolites may structure plant populations. Interestingly, seed 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. Alías *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, antifungals; 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 'phytogenetic' background to gauge establishment potential based on general neighbourhood 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 phytogenetic 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 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 biochemically assess neighbourhood competition potential. Kos & Poschod (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* equal if not outweigh that of 'making the



**Fig. 4.** Under competitive conditions, the number of seeds in the soil seed bank for three genotypes (or species) that differ in their possession of biochemical recognition (BR) and soil seed bank decay rates (for simplicity, annuals are modelled). In (a) and (c), the half-lives of the soil seed bank are 1 and 5 years, respectively, and no seeds germinate because their BR systems signify poor establishment potential. In (b), the 'No BR strategy', all seeds germinate, seedlings undergo 98.5% mortality and successful individuals produce 50 seeds. Through time, there are proportionately more BR-possessing seeds available for establishment if the competitive conditions are relaxed, but only if they have a modestly long-lived soil seed bank.

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 (Fig. 4). Seed bank longevity is thus also important and the selective advantages of BR become greater the longer the 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 & Brown 1988). Ultimate empirical proof of its adaptive significance would require measuring fitness differentials between BR-possessing and non-possessing seeds (at the genotype or species level) under temporally varying competitive conditions (also see Cohen 1967).

Systems in which plants are most likely to develop some form of BR are those where: (i) competition-induced seedling mortality is predictably high, and subsequent reproductive output is low, (ii) shade intolerance is common, including low phenotypic plasticity in low light levels (i.e. BR is less likely if species with highly plastic, guerilla growth strategies enable individuals to occasionally find light gaps and reproduce) and (iii) 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; also see Fig. 4), shade tolerance is common and contemporary phytochemical cues, or lack thereof, are unreliable indicators of lifetime fitness (Cohen 1967; Donaldson-Matasci, Bergstrom & Lachmann 2013). The latter may occur in lineages that have historically been subjected to high post-emergence, density-independent mortality (e.g. fire, late freezes, drought, trampling) or a rapid change in neighbourhood 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, species spanning a competitive hierarchy may not appreciably 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 develop in dominants, as simple probability dictates their interactions with conspecifics are more frequent than intraspecific interactions among uncommon subordinates with unclumped distributions. Because related taxa release similar root exudates (Bais *et al.* 2004; e.g. isoflavonoid antimicrobials in the Fabaceae) and generally share a similar niche (Cadotte 2013), we suspect relatedness may contribute to phylogenetic-specific BR, even among historically allopatric taxa. Lastly, we view BR not as an 'all-or-nothing' phenomenon but as a system with varying degrees of development and sensitivity among lineages. Indeed, biochemicals are but one of many cues that can signify establishment potential (e.g. light quality and quantity, smoke, diurnal temperature fluctuations, soil nitrogen, CO<sub>2</sub> and moisture content; Baskin & Baskin 1998), and depending on their historic reliability in predicting realized fitness for a seed, other cues may either override or act in concert to strengthen these biotic signals (e.g. high vs. low light levels with a biochemical signal present).

## 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 phytochemical BR and intraspecific BR emerged as common. Additionally, smaller seeded species were most likely to exhibit heterospecific 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 evolutionary mechanism by which some plant populations, and the spatial and temporal diversity of some communities, may be structured.

Species-specific and phytogeneric BR were 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; Callaway 2007; Brooker *et al.* 2008; Gross *et al.* 2013), and recognition of facilitators stimulates germination (Lortie & Turkington 2002; Bouwmeester *et al.* 2003; Bidartondo & Read 2008; Plakhine, Ziadna & Joel 2009). Given that adaptive strategies in germination behaviour 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 predicted 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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