

Eavesdropping in plants: delayed germination via biochemical recognition

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Running title: Eavesdropping in plants: delayed germination

1 **Summary**

2

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

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

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

22 **4. *Synthesis:*** Coupling ample theoretical support with empirical evidence here and elsewhere, an
23 ‘eavesdrop-and-wait’ competition avoidance strategy could be a common phenomenon. Our
24 findings suggest sympatric association may contribute to evolution of species-specific BR and that

25 seed traits are important in its development. The underlying mechanism may be simple
26 phytochemical-induced hormonal regulation. Factors preclude BR from being ubiquitous but
27 nonetheless, BR provides a potentially powerful mechanism by which the spatiotemporal diversity
28 of some communities is structured. Lastly, allelopathy may be erroneously invoked when
29 phytochemical-induced germination reduction occurs but a toxicity mechanism has not been
30 elucidated. In many cases, this fits more with the BRH than classic allelopathy.

31
32 **Key-words:** Allelopathy, autotoxicity, *Centaurea maculosa*, coevolution, historic interactions,
33 intraspecific recognition, novel weapons hypothesis, plant community structure, spatiotemporal
34 biodiversity, sympatric species recognition

35

36 **Introduction**

37

38 Seeds have sophisticated abilities to assess whether their contemporary environment is conducive
39 for establishment and rightly so, the timing of germination for many species is the most important
40 decision a plant faces and is expected to be under strong selection (Cohen 1967; Brown & Venable
41 1986; Venable & Brown 1988; Hierro *et al.* 2009). Myriad ecological factors that signify
42 establishment potential affect germination (Baskin & Baskin 1998) and seeds of some species use
43 conspecific and heterospecific phytochemicals as indicators of local competition magnitude
44 (Preston & Baldwin 1999; Dyer, Fenech & Rice 2000; Krock *et al.* 2002; Dyer 2004; Turkington *et*
45 *al.* 2005; Tielbörger & Prasse 2009; Orrock & Christopher 2010). Presence of species facilitating
46 establishment increases the germination of others (Lortie & Turkington 2002) and members of the
47 parasitic *Orobanche* and *Striga* (Orobanchaceae) will not germinate unless exposed to
48 phytochemicals of their obligate host (Bouwmeester 2003; Plakhine, Ziadna & Joel 2009).

49 Emerging work thus suggests some seeds use biological chemicals as adaptive signals to assess
50 presence of hosts, facilitators and competitors but the prevalence of this mechanism remains
51 unclear, including the role that species traits and historical interactions have in its occurrence.

52 A 90 year-old debate on the relative strengths of the individualistic, organismal and
53 integrated community concept has produced tremendous insight into the interplay between
54 stochastic processes and highly interdependent relationships on community structure (Clemens
55 1916; Gleason 1926; Lortie *et al.* 2004; Ricklefs 2008; Brooker *et al.* 2009). An important
56 emerging question is whether the outcome of species interactions depends on their history of
57 association and empirical evidence suggests strong sympatric association can affect local
58 community composition, species coexistence and ecosystem function (Lortie *et al.* 2004; Callaway
59 2007; Castillo, Verdu & Valiente-Banuet 2010; Inderjit *et al.* 2011; Verdu & Valiente-Banuet
60 2011). Importantly, prolonged plant interactions can drive adaptive responses that are specific to
61 particular taxa (Preston, Betts & Baldwin 2002; Hierro & Callaway 2003; Ehlers & Thompson
62 2004; Novoplansky 2009; Thorpe *et al.* 2011; Soliveres, Torices & Maestre 2012).

63 One such community-structuring force that invokes evolutionary and often coevolutionary
64 dynamics is allelopathy (Rabotnov 1982; Mallik & Pellissier 2000; Callaway & Ridenour 2004),
65 which is the release of phytotoxic chemicals by one plant that inhibits germination or growth of
66 other plants. It has traditionally been viewed as a form of interference competition, where
67 allelopathic chemicals (allelochemicals) released into the environment disrupt the metabolism of
68 recipient plants or their soil mutualists, and a mode by which some non-native plants become
69 successful invaders is through allelochemical release (Bais *et al.* 2003; Hierro & Callaway 2003;
70 Callaway & Ridenour 2004; Hierro, Maron & Callaway 2005; Callaway *et al.* 2008; Thorpe &
71 Callaway 2011). Because members of recently invaded communities have had no historic
72 association with these novel phytotoxins, and thus little time to evolve counter-defenses, their

73 inhibitory effects on native residents are often much stronger than in the communities in which the
74 toxins originally evolved. This “Novel Weapons Hypothesis” (NWH) has been demonstrated in
75 several different plant systems (Callaway & Aschehoug 2000; Prati & Bossdorf 2004; Inderjit *et al.*
76 2011; Svensson *et al.* 2013).

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

87 Plant taxa have unique exudate chemistries (Bais *et al.* 2004) and if a lineage has had
88 historically poor recruitment in the presence of another, there is potential for species-specific BR to
89 evolve. On the other hand, if amounts of commonly produced exudates (e.g., sugars, phenolics,
90 amino acids, anti-fungals; see Bais *et al.* 2004) reliably signify local competition magnitude,
91 similar to the dose-dependent responses in allelopathic systems (Perry *et al.* 2005; Inderjit *et al.*
92 2008), some seeds may simply cue in on this “phytogenic” background to gauge general
93 neighborhood qualities. In addition, because conspecifics frequently interact and share a more
94 similar niche than heterospecifics (Armas & Pugnaire 2011), intraspecific BR may be common.
95 Lastly, the well established positive relationship between seed size and establishment probability
96 (Jakobsson & Eriksson 2000; Moles & Westoby 2004, 2006) suggests that as seed reserves decline,

97 selection should intensify for predicting establishment potential based on phytochemical proxies of
98 contemporary neighborhood competitiveness. Small-seeded species also tend to have long-lived
99 soil seed banks (Moles & Westoby 2004, 2006) and thus there is greater probability in these taxa of
100 an establishment opportunity occurring from phytochemically delayed germination.

101 We simultaneously tested the non-mutually exclusive NWH and BRH by exposing six
102 allopatric and six sympatric species to the phytochemical leachates of all 12 test species, including
103 water and the reportedly allelopathic European *Centaurea maculosa* Lam. (*C. stoebe* L.; Bais *et al.*
104 2003 but see Blair *et al.* 2005; Stermitz, Hufbauer and Vivanco 2009) as respective negative and
105 positive controls. The NWH and different forms of the BRH make distinct predictions of
106 germination behavior following leachate exposure (Fig. 1). For each species, these include: 1)
107 germination is lower following leachate exposure of sympatric compared to allopatric species
108 (species-specific BRH), 2) intraspecific leachates reduce germination more than those of
109 heterospecifics, irrespective of region of origin (intraspecific BRH), 3) sympatric and allopatric
110 leachates reduce germination more than water (phytogenic BRH) and 4) leachates from allopatric
111 relative to sympatric species reduce germination (NWH). Seed mass of our test species spanned
112 over one order of magnitude from each region and we were thus also able to test the hypothesis that
113 occurrence of BR systems is more likely in smaller seeded species. We found support for
114 hypotheses 1-3 as well as BR responses in small-seeded species only, and discuss the implications
115 of BR to community structure and the ecological conditions under which a BR strategy is expected
116 to evolve.

117

118 **Materials and methods**

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120 **EXPERIMENTAL SET-UP**

121
122 In March 2007, we evaluated the seedling emergence potential of 20 and 13 perennial grass
123 and forb species respectively native to and sympatric in central North American (NA) and
124 Argentinean La Pampa (SA) grasslands by sowing them in flats containing sterilized, field-
125 collected Canfield silt-loam soil. Seeds of SA species were hand-collected in one La Pampa locale
126 and NA species were purchased from Earthskin Nursery (Mason City, IL 62664, USA), where
127 species of central Illinois ecotypes were collected from local prairie remnants and grown for
128 commercial value. From the pool of species that emerged at a high percentage (e.g., ~40% or
129 more), we chose six species from each region to evaluate their seedling emergence response to
130 phytochemicals from sympatric and allopatric species, *C. maculosa* and water. Because selective
131 pressure on using phytochemical cues to assess local competition intensity may intensify as seed
132 reserves decline, we also chose species such that a wide seed size range was represented from each
133 region (NA: 0.382-4.35 mg/seed, SA: 0.200-4.99 mg/seed; Table 1).

134 For phytochemical leachate sources, we grew monocultures of each NA and SA species as
135 well as *C. maculosa* in three separate 53 × 28 × 6 cm drained flats containing sterilized topsoil for
136 seven weeks in a greenhouse ($n = 13$ species). We wanted to maximize the potential for detecting a
137 BR response and thus used autoclave-sterilized soil in all experimental phases because soil
138 microbes can use phytochemicals as a carbon source, diminishing any phytochemical-induced
139 germination response (Kaur *et al.* 2009; Ehlers 2011). Flats of each species were watered through
140 the aboveground vegetation with deionized water (dH₂O) such that a total excess of 1.8 ± 0.1 L of
141 water was collected in water-tight flats. This leachate, which should contain water-soluble
142 phytochemicals exuded primarily from the roots, was then used to water seeds of the species grown
143 for phytochemical collection. In this way, all 12 test species were separately subjected to the
144 leachates of all other species, including a negative (dH₂O) and positive control (*C. maculosa*).

145 In July 2007, 33 seeds of each species were evenly sown on the soil surface of separate 9 ×
146 9 × 7 cm square pots and covered with 2-3 mm of soil. We then watered all pots (n = 504) with 25
147 ml of their respective leachate 2-3 times per week and recorded seedling emergence once per week
148 for five weeks. Because seeds often rely on several germination cues simultaneously (Preston &
149 Baldwin 1999) and light, if sufficiently strong, can override germination inhibitors (Baskin &
150 Baskin 1998), we installed shade cloth over all pots before the experiment. This approximated
151 photosynthetically active radiation (PAR) at the soil surface of an intact tallgrass prairie canopy
152 (Lane, Coffin & Lauenroth 2000; 0-5% ambient PAR) and reduced average ambient PAR
153 transmittance to 7.1%. Ambient PAR at 1200 hr and under shade cloth respectively averaged 1680
154 and 120 $\mu\text{mol}/\text{m}^2/\text{sec}$, as measured with an AccuPAR LP-80 ceptometer (Decagon Devices, Inc.).

155

156 EXPERIMENTAL DESIGN AND DATA ANALYSIS

157

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

169 as a fixed and species as a random factor, and tested whether the leachate of each species had
170 greater inhibitory effects on emergence of allopatric compared to sympatric species.

171 Because we foresaw potential for a weak BR signal and did not want to lose much power in
172 correcting for multiple ANOVA tests, we set experimentwise α at 0.10 and considered each of the
173 12 ANOVAs to be statistically significant at $P = 0.10/12 = 0.0083$. If significant leachate effects
174 on total seedling emergence occurred at this level, we used linear contrasts to test for treatment
175 differences at $\alpha = 0.05$ in all preplanned comparisons and Tukey-Kramer adjustments to maintain
176 an experimentwise error rate of 0.05 in post hoc tests. No heterogeneity of variance occurred
177 among treatments for emergence (Levine's HOV test: $P > 0.100$) but for dry weights per seedling,
178 log transformation of *Monarda fistulosa* L. was employed to yield treatment homogeneity of
179 variance ($P = 0.117$). Because phytochemical cues that signify a competitive environment can
180 accelerate germination (Dyer, Fenech & Rice 2000; Tielbörger & Prasse 2009; Orrock &
181 Christopher 2011), we also tested for NA and SA leachate effects on emergence rates for all species
182 using one-way ANOVAs. Here, we compared treatment means when $\geq 50\%$ of the final seedling
183 number for each species had emerged, which in all cases was after 1-2 weeks of leachate exposure.

184 To test for a relationship between seed size and BR response, we ran a Mann-Whitney U
185 test, assigning species ranked by seed mass into categories of whether BR was observed – this
186 could be a species-specific or phytogeneric BR response. We also ran this test using species
187 exhibiting species-specific BR only. Seeds of many species may frequently be exposed to
188 intraspecific phytochemicals and thus conspecific BR may be common. To assess this, we ran a
189 Wilcoxon signed-rank test using all species, assigning intraspecific leachate effects on seedling
190 emergence a value between 1-12 (i.e., from highest to lowest emergence percentage relative to
191 heterospecific leachates; Table 1) and testing whether this rank differed from the null hypothesis
192 median prediction of 6.5. All statistical tests were performed using SPSS (IBM®, version 18).

193 Support for “species-specific BR” does not necessarily imply that a species seeds respond
194 to *all* sympatric species but suggests they respond to some of them. Also, none of these hypotheses
195 are mutually exclusive and if several operate on a particular species, finding support for any one is
196 less likely. For example, the “intraspecific BRH” predicts that seeds respond strongest to
197 conspecific leachates but if some sympatric and allopatric species also reduce germination, it would
198 be more difficult to detect. By the same token, it would be difficult to detect species-specific BR
199 and the NWH if both simultaneously operate. As such, all of our tests are deemed conservative.
200 All hypotheses are based on the assumption that competitive interactions in a high resource
201 grassland environment are the norm and that species historically facilitating establishment of other
202 species is uncommon (but see Callaway & Walker 1997; Lortie & Turkington 2002; Bruno,
203 Stachowicz & Bertness 2003; Brooker *et al.* 2008; Gross *et al.* 2013).

204

205 **Results**

206

207 GENERAL LEACHATE EFFECTS AND PHYTOGENERIC BIOCHEMICAL 208 RECOGNITION

209 Leachate effects on total seedling emergence were significant for four species, with the
210 water treatment resulting in higher emergence relative to at least one of the phytochemical leachate
211 treatments ($F_{3,38} \geq 4.96$, $P \leq 0.005$). Emergence following water exposure was 44% to 131%
212 higher than NA, SA and *C. maculosa* leachate exposure for *M. fistulosa* and *Gaillardia*
213 *megapotamica* var. *scabiosoides* (Spreng.) Baker (Table 1, Tukey-adjusted HSD: $P \leq 0.023$), \geq
214 71% higher than NA and SA leachates for *Stipa eryostachia* Kunth (Table 1, $P \leq 0.006$) and 29%
215 higher than NA leachates for *Ratibida pinnata* (Vent.) Barnhart ($P = 0.027$). *Gaillardia*
216 *megapotamica* and *S. eryostachia* had higher emergence in the presence of water relative to

217 sympatric and allopatric leachates, the latter of which did not differ ($P \geq 0.711$). No NA nor SA
218 leachate reduced emergence of allopatric species more than those that were sympatric ($F_{1,408} \leq$
219 1.26 , $P \geq 0.262$, linear contrasts).

220

221 SPECIES-SPECIFIC BIOCHEMICAL RECOGNITION

222 Relative to allopatric leachates, exposure of sympatric leachates to *M. fistulosa* and *R.*
223 *pinnata* seeds respectively lowered their total seedling emergence by 27% and 13%, with a net
224 reduction of 10.3% in each case (Fig. 2, *M. fistulosa*: $F_{1,34} = 11.2$, $P = 0.002$; *R. pinnata*: $F_{1,34} =$
225 7.55 , $P = 0.010$; linear contrasts). Following the removal of intraspecific leachates from the
226 analyses, sympatric and allopatric leachate effects on emergence percentage remained virtually
227 unchanged and significant for both species, indicating that heterospecific sympatric phytochemicals
228 were driving the effects (*M. fistulosa*: $F_{1,31} = 9.05$, $P = 0.005$; *R. pinnata*: $F_{1,31} = 5.46$, $P = 0.026$).
229 Dry weight per seedling of *M. fistulosa* and *R. pinnata* did not differ among water and NA and SA
230 leachates ($F_{2,27} \leq 2.11$, $P \geq 0.142$). Seedling emergence rates differed between NA and SA
231 leachates for *M. fistulosa* only, with sympatric NA leachates significantly slowing emergence ($F_{1,34}$
232 $= 11.92$, $P = 0.002$).

233

234 INTRASPECIFIC BIOCHEMICAL RECOGNITION

235 For each species, intraspecific leachates were assigned a rank between 1 and 12 to designate
236 the order in which they affected seedling emergence relative to heterospecific leachates (Table 1).
237 Using a Wilcoxon signed-rank test, we tested whether the observed value for intraspecific leachate
238 effects differed from the null hypothesis expectation of 6.5 and found they reduced emergence
239 compared to heterospecific sympatric and allopatric leachates ($P = 0.020$, mean: 9.3 ± 2.0 (95%

240 CI)). Relative to the other 11 heterospecific leachates, intraspecific leachates resulted in the lowest
241 or second lowest emergence percentage for six of 12 species (Table 1).

242

243 SEED SIZE EFFECTS ON BIOCHEMICAL RECOGNITION

244 Small-seeded species were more likely than those with larger seeds to exhibit species-
245 specific or phytogeneric BR, with only the four smallest seeded species demonstrating BR
246 responses (Fig. 3, Mann-Whitney U: $P = 0.007$). The second and third smallest seeded species
247 were the only ones to exhibit species-specific BR and the effect of seed size on this response was
248 marginally significant (Mann-Whitney U: $P = 0.086$). Seed size was not a significant predictor of
249 emergence responses to intraspecific relative to interspecific leachates (Table 1, $r^2 = 0.041$, $P =$
250 0.527), suggesting development of intraspecific BR may not depend heavily on seed size.

251

252 *C. MACULOSA* EFFECTS ON SEEDLING EMERGENCE

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

258

259 Discussion

260

261 EVIDENCE FOR AND THE ADAPTIVE SIGNIFICANCE OF BIOCHEMICAL

262 RECOGNITION

263 The vast majority of seedlings perish for numerous reasons, many of which are stochastic,
264 but fairly reliable cues that signify degree of neighborhood competition exist for seeds and many of
265 these are chemical in nature (Bergelson & Perry 1989; Dyer, Fenech & Rice 2000; Preston, Betts &
266 Baldwin 2002; Dyer 2004; Tielbörger & Prasse 2009). We found two species had 13% and 27%
267 germination reductions following exposure to phytochemicals of sympatric relative to allopatric
268 species (Fig. 2). This opposes what is expected if the NWH was operating strongly and provides
269 evidence that some seeds have developed mechanisms to assess presence of particular taxa and
270 make germination decisions based on them (Preston & Baldwin 1999; Preston, Betts & Baldwin
271 2002). If correct, this chemically induced response suggests that historic interactions can
272 adaptively shape niche regeneration breadth and lends support to the proposition that some
273 communities are in part structured by sympatric evolutionary association (Clements 1916; Lortie *et*
274 *al.* 2004; Brooker *et al.* 2009). In addition, detecting presence of specific neighbors that confer
275 negative fitness consequences may increase establishment potential of all BR-possessing members,
276 thereby increasing community spatiotemporal diversity (also see Lortie *et al.* 2005; Turkington *et*
277 *al.* 2005). It is unlikely that osmotic potential differentials drove these effects (i.e., reduced
278 germination and growth from osmotic-induced low water uptake rates) because seedling growth did
279 not differ between allopatric and sympatric leachates and water (see Wardle, Nicholson and Ahmed
280 1992) and there is no reason to suspect allopatric leachates had inherently lower osmotic potentials.
281 Other systems that support species-specific BR include plants that inhibit germination of species
282 sharing a long history of sympatry but have no effect on those where past interactions are absent
283 (Preston & Baldwin 1999; Preston, Betts & Baldwin 2002; Renne *et al.* 2004).

284 Evolutionary theory with respect to allelopathy predicts that as encounters with toxins
285 increase in frequency, species should develop resistance to them (Rabotnov 1982). Given that
286 intraspecific and even sib-interactions frequently occur in many species (Cheplick 1992; Dyer

287 2004), it is difficult to imagine that strong resistance to within-species allelochemicals is not
288 commonplace. Moreover, conspecifics share a more similar niche than heterospecifics, their
289 interactions are generally stronger (Armas & Pugnaire 2011) and the BRH predicts chemical
290 recognition is most likely to develop here. We found intraspecific leachates significantly lowered
291 emergence compared to heterospecific leachates and interpret this as a convincing signature of
292 intraspecific BR (Table 1; also see Dyer, Fenech & Rice 2000; Dyer 2004; Turkington *et al.* 2005;
293 Orrock & Christopher 2010). If correct, this represents a potentially widespread and important
294 mechanism by which secondary metabolites may structure plant populations. Interestingly, seed
295 size did not affect emergence responses to intraspecific leachates, suggesting development of
296 conspecific recognition is independent of seed reserves, at least for small-seeded grassland species.
297 We add that unless a non-osmotic potential-based mechanism of toxicity has been established,
298 these oft interpreted ‘autotoxic’ effects (e.g., Alias *et al.* 2006) fit more with the intraspecific BRH
299 and in many cases, should be interpreted as such (see Perry *et al.* 2005 for a good autotoxicity
300 example).

301 Most plants compete for limited water, light and nutrient resources and thus it is possible
302 that concentrations of commonly produced exudates (e.g., sugars, phenolics, amino acids, anti-
303 fungals; see Bais *et al.* 2004) reliably indicate local competition magnitude. Relative to water, we
304 found that three species had emergence reductions following allopatric and sympatric leachate
305 exposure, suggesting some seeds cue in on a “phytogenic” background to gauge establishment
306 potential based on general neighborhood qualities. In communities characterized by rapid
307 compositional change, this non-specific eavesdropping may be particularly adaptive if quantities of
308 commonly produced phytochemicals are good proxies of contemporary competition. Our test of
309 the phytogenic BRH is potentially problematic because species-specific BR and the NWH may

310 have simultaneously operated, but in no case did allopatric leachates reduce germination more than
311 sympatric leachates so we consider this possibility highly unlikely.

312 All seeds face formidable challenges establishing under competitive conditions and the
313 severity of this increases as seed size and their concurrent reserves decline (Jakobsson & Eriksson
314 2000; Moles & Westoby 2004, 2006). Larger seeds also tend to have higher predation rates and
315 shorter lived soil seed banks (Moles & Westoby 2004, 2006) and thus the benefits of a
316 phytochemical-induced ‘sit-and-wait’ strategy likely decline as seed size increases (see below).
317 We found smaller seeded species were significantly more likely to exhibit BR (Fig. 3) and interpret
318 this as a manifestation of greater selective pressure on them to assess neighborhood competition
319 potential via BR. Kos and Poschlod (2008) found osmotic potential gradients did not differentially
320 affect germination of small- and large-seeded species from a phylogenetically diverse assemblage
321 and thus we feel osmotic differentials did not drive the observed responses. Interestingly, Dyer
322 (2004) found germination of small seeds of the dimorphic seeded *Aegilops triuncialis* L. was
323 inhibited when large-seeded siblings were in close proximity, but not vice versa, and suggested this
324 represented a greater fine-tuned assessment of the biotic environment by small seeds.

325

326 EVOLUTION OF BIOCHEMICAL RECOGNITION

327 For BR to evolve, the expected benefit:cost ratio of delayed germination *must* outweigh that
328 of ‘making the best of a bad situation’ under current conditions. Critically important factors
329 affecting its evolution include competition-dependent rates of pre-reproductive mortality as well as
330 reproductive output from successfully establishing individuals. If negative population growth
331 results from strong competition (e.g., high seedling mortality and low seed production from a few
332 successful individuals), phytochemical-induced germination delays would be profitable as long as
333 loss from soil seed bank decay is less than the fitness reductions incurred from emerging under

334 current conditions. Seed bank longevity is thus also important and the selective advantages of BR
335 become greater the longer seeds remain viable and the more limited establishment opportunities are
336 (Cohen 1967; Renne *et al.* 2004). We submit that selection on BR systems may even drive the
337 evolution of seed dormancy and not vice versa (also see Venable and Brown 1988).

338 Systems in which plants are most likely to develop some form of BR are those where: 1)
339 competition-induced seedling mortality is predictably high and reproductive output is low, 2) shade
340 intolerance is common, [including low phenotypic plasticity in low light levels](#) and 3) the frequency
341 of competitor-based establishment opportunities is generally shorter than the rate of soil seed bank
342 decay (Renne *et al.* 2004, e.g., ruderal communities, grasslands, savannas, chaparrals). BR is less
343 likely when soil seed bank longevity is low (e.g., forest canopy species) or contemporary
344 phytochemical cues, or lack thereof, are unreliable indicators of lifetime fitness (Cohen 1967). The
345 latter may occur in lineages that have historically been subjected to high post-emergence, density-
346 independent mortality (e.g., fire, late freezes, trampling) or a rapid change in neighborhood
347 competitive strength from stochastic disturbance (e.g., sporadic, intensely grazed systems).
348 Dominant community members are most likely to be chemically recognized because they are by
349 definition common, tend to exhibit temporal stability in biomass (Roscher *et al.* 2011) and may
350 represent a consistent competitive element. However, unless subordinate-dominant species
351 interactions are rare or consistently one-sided, species spanning a competitive hierarchy may not
352 differ in the incidence of species-specific BR because established subordinates can exert strong
353 size-asymmetric competition on establishing dominants (Schwinning & Weiner 1998).
354 [Intraspecific BR is also most likely to be developed in dominants, as simple probability dictates](#)
355 [their interactions with conspecifics are more frequent than intraspecific interactions among](#)
356 [uncommon subordinates with unclumped distributions.](#)

357

358 **Conclusions**

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

381

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383
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390

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548

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

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

Family	Species	mg/seed*	Species-specific BR†	Phytogeneric BR‡	Intraspecific leachate ranking¶
<u>North America (central Illinois ecotypes)</u>					
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
<u>Argentina (La Pampa province ecotypes)</u>					
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 euclastron</i> Steud.	4.55	ns	ns	7
Poaceae	<i>Stipa eryostachia</i> Kunth	0.200	ns	p = 0.004	11

553 * mg/seed is based on weighing 200 seeds and estimating individual seed mass. Dispersal
 554 structures were removed from all species before weighing.

555 † Species exhibiting greater germination reduction following exposure to leachates of sympatric relative to
556 allopatric species (supporting species-specific BR).

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

559 ¶ Intraspecific leachate rankings from 1-12 designate, from highest to lowest, the order in which
560 intraspecific leachates affected emergence relative to the 11 heterospecific leachates (Mann-Whitney U: P
561 = 0.007).

562

563 FIGURE LEGEND

564

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

567

568 **Fig. 2.** *Ratibida pinnata* and *Monarda fistulosa* respectively had 13% and 27% lower seedling
569 emergence when exposed to leachates of sympatric relative to allopatric species ($F_{1,34} \geq 7.55$, $P \leq$
570 0.010), supporting species-specific biochemical recognition. Removal of intraspecific leachates
571 from the analysis did not change these outcomes, indicating heterospecific sympatric
572 phytochemicals were driving the effects. Means \pm 1 SE are shown. Note that of our 12 test species,
573 seed mass of these species was respectively the third and second smallest.

574

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

579

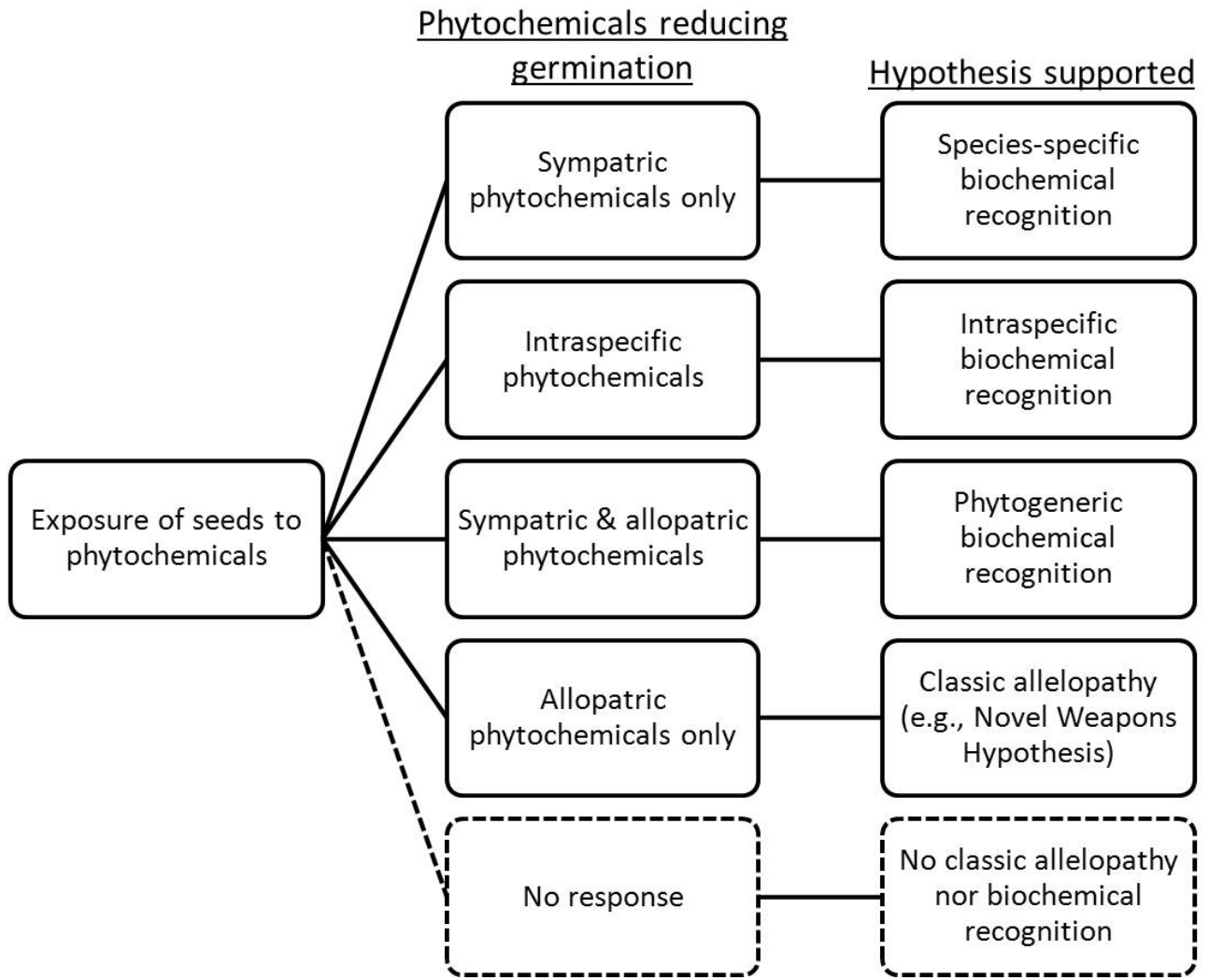


Figure 2

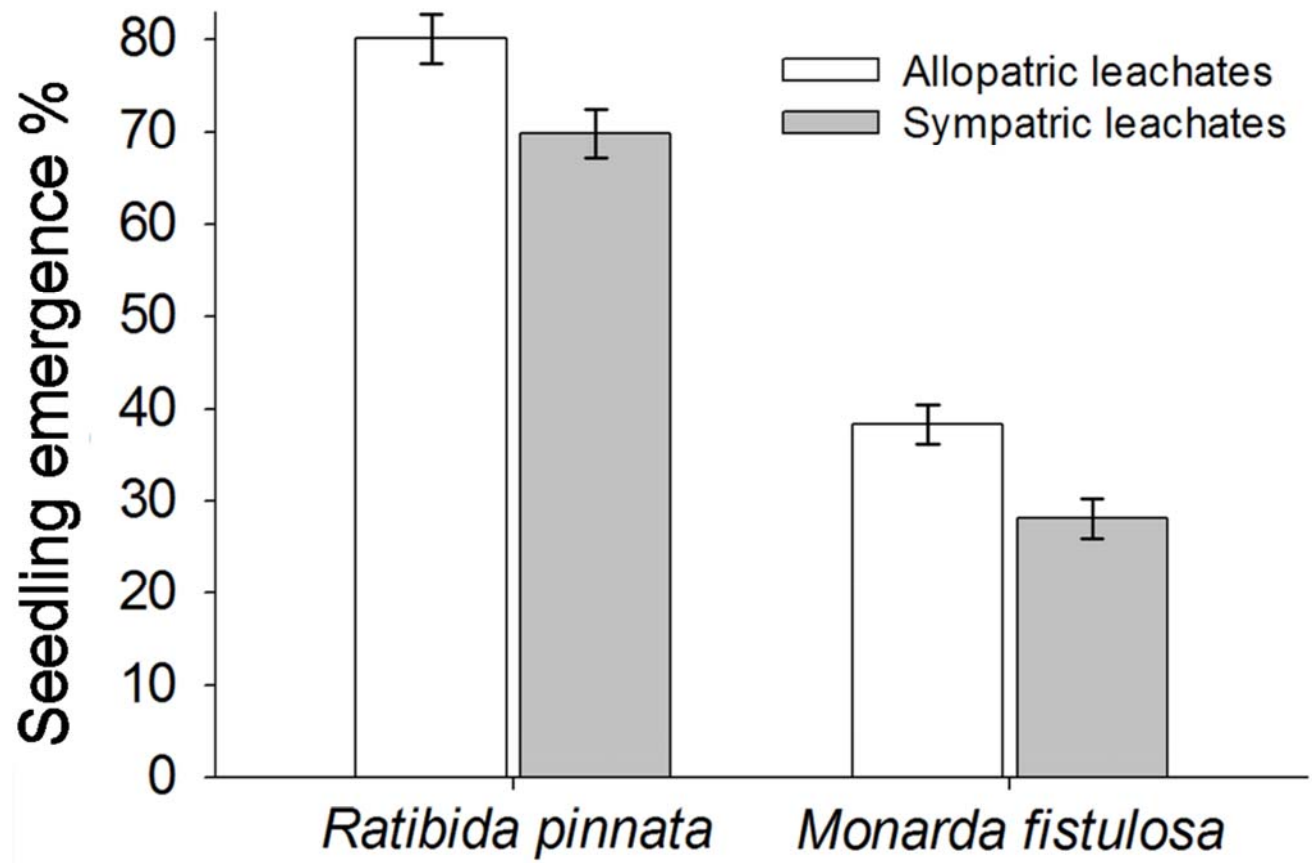


Figure 3

