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1 **Allelopathy by the invasive garden lupine inhibits the germination of native herbs**

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18

19 **Abstract**

20 Allelopathy is the stimulatory or inhibitory effect of one plant on another plant by release of phytochemicals. It  
21 can affect the competitive interactions between plants, but the strength of the effect may depend on the tissue of  
22 the donor plant and the phylogenetic relatedness of the interacting species. Here, we examined the allelopathic  
23 effects of the invasive garden lupine *Lupinus polyphyllus* (Fabaceae) on six native herbs from three families  
24 (Asteraceae, Caryophyllaceae, Fabaceae). We exposed the test species to aqueous leachates prepared from root  
25 or shoot tissue of *L. polyphyllus* and measured germination rate and germination time. Both leachates inhibited  
26 germination in all species (13–84%), but shoot leachate was more allelopathic than root leachate in case of  
27 Fabaceae and Caryophyllaceae. Plants from closely related Fabaceae were least affected by *L. polyphyllus*  
28 leachates in terms of germination rate, but leachates delayed their germination. These results demonstrate that  
29 the invasive *L. polyphyllus* can inhibit the germination of co-occurring native species and suggest that  
30 allelopathy likely contributes to its invasion success. Our results have implications for management of invasive  
31 *L. polyphyllus* populations, emphasising the importance of the removal of mown plant material to diminish  
32 inhibitory allelopathic effects on local plants.

33

34 **Keywords:** allelopathy, leachate, competition, invasive species, novel weapons-hypothesis, germination,  
35 inhibition

36 **Introduction**

37 Invasive plant species often alter the structure of their recipient community through competition (Levine et al.,  
38 2003). Such negative effects on neighboring plants can be mediated by allelopathy if the inhibitory effects are  
39 caused by the release of phytochemicals to the environment (Hierro & Callaway, 2003; Rice, 1984). Indeed,  
40 allelopathy is common among invasive plants (Kalisz et al., 2021) and the novel weapons hypothesis suggests  
41 that it is one of the main mechanisms explaining their invasion success (Callaway & Ridenour, 2004). The  
42 hypothesis postulates that allelopathic effects are particularly strong in the case of invasive plants because native  
43 species may lack tolerance to the allelopathic compounds of the invader owing to the short history of species  
44 coexistence.

45           The strength of allelopathic effects may depend on the evolutionary history of the competing species  
46 (Zhang et al., 2021). More closely related plant species are likely to be phytochemically more similar than  
47 distantly related ones due to the phylogenetic conservation of phytochemical composition in plants (Grutters et  
48 al., 2017; Wink, 2003). Plant species that do not share the compound profile may be more vulnerable to the  
49 allelopathic effects than those with similar profiles. In support of this prediction, the strength of the allelopathic  
50 inhibition increased with increasing phylogenetic distance of the studied species (Zhang et al., 2021). In addition  
51 to interspecific variation, plant tissues within an individual may differ in their allelopathic potential owing to  
52 differences in where allelopathic compounds are biosynthesized, stored, and how they are transported (Wink,  
53 1987). Accordingly, a meta-analysis revealed that leachates prepared from belowground parts of plants had  
54 weaker allelopathic effects than those prepared from aboveground parts (Zhang et al., 2021). Understanding the  
55 causes of among-species variation in responses to allelopathy can help predict effects of invasive species on  
56 native plant communities.

57           The garden lupine *Lupinus polyphyllus* (Lindl., Fabaceae) has become one of the most harmful  
58 invasive species in Europe over the course of the last century (Rumlerová et al., 2016). The species is a strong  
59 competitor that negatively affects plant community diversity by increasing the proportion of competitive species  
60 (Hansen et al., 2021) and reducing species richness (Ramula & Pihlaja, 2012). Litter leachates and leaf litter of  
61 *L. polyphyllus* inhibited the germination, root growth and seedling emergence of native European plant species

62 (Loydi et al., 2015). Whether roots also hold comparable allelopathic potential and whether the strength of the  
63 allelopathic effects are associated with species relatedness are unknown. To address these questions, we studied  
64 the allelopathic effects of *L. polyphyllus* on the germination of six native herbs from three different families  
65 (Asteraceae, Caryophyllaceae, Fabaceae) found to co-occur in the invasive range. We used aqueous leachates of  
66 roots and shoots to explore whether allelopathic compounds (if any) are released into the environment passively  
67 i.e. by rain water. We predicted that both leachates would reduce the germination success of the natives under  
68 laboratory conditions, but that shoot leachate would have stronger negative effects than root leachate. We also  
69 predicted that the allelopathic effects would be weaker on the more closely related species of Fabaceae compared  
70 to those from the other two plant families.

71

## 72 **Materials and methods**

### 73 *Study species*

74 Garden lupine *Lupinus polyphyllus* (Fabaceae) is a short-lived perennial herb native to western North America. It  
75 was introduced to Europe and other parts of the world as an ornamental plant in the 1800's, and escaped from  
76 gardens by the end of the century (Fremstad, 2010). The species is considered invasive in parts of Europe, New  
77 Zealand, Chile, and southern Australia (Fremstad, 2010; Meier et al., 2013). In Finland, it commonly occurs in  
78 road verges, wastelands, and forest understories (Fremstad, 2010), where it reduces the local plant diversity and  
79 insect abundance (Ramula & Pihlaja, 2012; Ramula & Sorvari, 2017). The leaves of *L. polyphyllus* from  
80 introduced populations contain high concentrations of alkaloids (Kalske et al., 2022), of which lupanine,  
81 sparteine and tigloyloxolupanine are known to have allelopathic potential (Muzquiz et al., 1994; Wink, 1983).

82 To explore the allelopathic effects of *L. polyphyllus* on co-occurring herbaceous native species,  
83 we used six common species as targets, two from each of three families. The species were sneezewort yarrow  
84 (*Achillea ptarmica* L., Asteraceae), dandelion (*Taraxacum* spp. F. H. Wigg., Asteraceae), clammy campion  
85 (*Viscaria vulgaris* Röhl., Caryophyllaceae), red campion (*Silene dioica* L. Clairv., Caryophyllaceae), white  
86 clover (*Trifolium repens* L., Fabaceae), and crimson clover (*Trifolium incarnatum* L., Fabaceae). All other  
87 species commonly co-occur with *L. polyphyllus* in Finland (S. Ramula, personal observation), except *T.*

88 *incarnatum* that is not native to the country. However, *T. incarnatum* is common in central Europe where it may  
89 compete with *L. polyphyllus*. We collected the seeds ourselves locally or bought them from national seed  
90 suppliers.

91

92 *Experimental setup*

93 We collected plant material for leachate preparation from one population of *L. polyphyllus* in Kaarina, Finland in  
94 early June 2021, c. 30 individuals pooled together. Roots and shoots were cleaned, separated and dried at 65 °C  
95 for 48h. After drying, we soaked coarsely ground dry plant material (either root or shoot) in deionized water  
96 (100 g dry material/1 L water) for 72h to prepare the leachate. Plant material was separated from water by cotton  
97 cloth. The ratio of dry plant material to water is comparable to the natural amount of litter to rain water in  
98 European semi-natural grasslands and, hence, results in realistic concentration of allelopathic compounds  
99 (Hovstad and Ohlson 2008; Loydi et al. 2015). In addition, by using only water we ensured our treatment is  
100 comparable to leaching of allelopathic compounds from plants by rain and gives a realistic estimation of the  
101 allelopathic potential of *L. polyphyllus*.

102 To explore the allelopathic effects of root and shoot leachates on the six co-occurring species, we  
103 germinated all species separately on petri dishes and exposed their seeds to either root leachate, shoot leachate or  
104 tap water (control). We did not control for differences in osmotic potential. We had 30 seeds per petri dish, and  
105 20 replicates in each of the three treatments per species, resulting in a total of 360 petri dishes. Bottoms of the  
106 petri dishes were covered with two layers of a paper towel to retain moisture. On the first day of the germination  
107 experiment, we added 10 mL of liquid to each petri dish and sealed it with parafilm. We kept the petri dishes in  
108 room temperature, in natural indirect light and arranged them randomly. We recorded the number of seeds  
109 germinated on each dish, and rearranged the dishes once every three days until 21 days had passed since sowing  
110 and maximum germination had been reached. We used germination rate (on days 3, 9, 15 and 21) and number of  
111 days to maximum germination in the analyses.

112

113 *Statistical analyses*

114 We analysed the data in R (R version 3.6.1; R Core Team 2020). We conducted repeated measures generalized  
115 linear mixed models (lme4::glmer; Bates et al. 2015) for germination rate (number of seeds germinated out of 30  
116 in each petri dish) with leachate treatment (control, root, shoot), plant family (Asteraceae, Caryophyllaceae,  
117 Fabaceae) and time (3, 9, 15 or 21 days since sowing) and all possible interactions among them as fixed  
118 categorical explanatory variables. Petri dish number was included as a random factor for the repeated measures  
119 structure. We specified a binomial distribution and logit link function, and obtained significance of the fixed  
120 variables from Wald's chi-square test. Our data met the model assumptions as the dispersion factor was close to  
121 one (dispersion = 0.995,  $P = 0.824$ ; DHARMA::testDispersion; Hartig 2022). To allow comparisons of the effect  
122 of allelopathy across plant families with different germination rates, we calculated effect sizes of the root and  
123 shoot leachates on germination rate on day 21. We used natural logarithmic response ratios with the following  
124 formula  $\ln RR = \ln(\bar{X}_T / \bar{X}_C)$  where  $\bar{X}_T$  is the mean of the leachate treatment (root or shoot) and  $\bar{X}_C$  the mean of the  
125 control within each family (SingleCaseES::LRRd; Hedges *et al.* 1999). We calculated 95 % confidence intervals  
126 for  $\ln RR$  to compare the effect of the leachate treatments between plant families. Negative values of  $\ln RR$   
127 indicate inhibition of germination in the leachate treatments compared to control. Finally, for the time in days to  
128 maximum germination, we conducted a linear model (stats::lm) with leachate treatment, plant family and their  
129 interaction as fixed explanatory variables. We inspected the residuals of the model to ensure they met the  
130 assumptions of normality and homogeneity of variances. We determined the significance of fixed variables using  
131  $F$ -tests (stats::anova).

132

### 133 **Results**

134 Both root and shoot leachates inhibited germination across the observation period in all three plant families, with  
135 the strength of the inhibition depending on the plant family (a significant three-way interaction; Table 1; Figure  
136 1). In Asteraceae, seeds exposed to root and shoot leachates had 35% and 47% lower germination rates than  
137 those in control treatment, respectively, but germination rate did not differ between the two leachate treatments  
138 (Figure 1). Root and shoot leachates inhibited germination compared to control also in Caryophyllaceae (32%  
139 and 84%, respectively), but shoot leachate was over four times as effective in preventing germination than root

140 leachate (Table 2, Figure 1). Fabaceae had the highest germination rate of all plant families, while similar to  
141 Caryophyllaceae, shoot leachate inhibited germination more than root leachate (root 13 %, shoot 40%, Figure 1).  
142 Overall, inhibition of germination was weaker in Fabaceae than in Asteraceae (roots) and Caryophyllaceae  
143 (shoots) based on comparison of the effect sizes and 95 % confidence intervals (Table 2).

144 Plant families were differently affected by the leachates also in terms of germination time (treatment  
145 × family; Table 1). Plants in Fabaceae reached their maximum germination first out of all plant families (Figure  
146 2), and were the only ones where leachate treatment delayed germination. Seeds exposed to root and shoot  
147 leachates germinated on average two and three days later than seeds in control treatment, respectively (Figure 2).  
148 In Asteraceae and Caryophyllaceae, germination time did not differ between treatments (Figure 2).

149

## 150 **Discussion**

151 We found strong allelopathic effects of *L. polyphyllus* on the germination of co-occurring native herbs from  
152 three different plant families. Leachate from shoots inhibited germination more than leachate from roots in plants  
153 from Caryophyllaceae and Fabaceae, whereas in plants from Asteraceae the effect of the two leachate types was  
154 similar in magnitude. Relatedness of the target species to *L. polyphyllus* affected the strength of allelopathy as  
155 the inhibitory effects on germination rate were strongest in the two more distantly related families, Asteraceae  
156 and Caryophyllaceae. Plants in Fabaceae that are closely related to *L. polyphyllus* were least affected by  
157 allelopathy in terms of germination rate. However, they were the only ones where allelopathy affected  
158 germination time, with shoot leachate delaying germination. This is the first study that reports differences in  
159 allelopathic potential between tissue types of *L. polyphyllus* and links them to phylogenetic relatedness.

160 The main unique group of phytochemicals in the genus *Lupinus* that are known to cause allelopathic  
161 effects are quinolozidine alkaloids (Wink, 1983, 2019). Although alkaloids in general are not highly soluble in  
162 neutral water used in our leachate preparation, some lupine alkaloids are an exception (Rumble et al., 2018) and  
163 they are known to be excreted into water from seeds (Wink, 1983). Furthermore, the detection of quinolizidine  
164 alkaloids (lupanine, sparteine, hydroxylupanine) from *L. angustifolius* and *L. albus* in soil pore water coincided  
165 with rainfall (Hama & Strobel, 2020), suggesting that leaching by rain is an important release pathway for



166 allelopathic compounds in lupines. Moreover, the presence of activated carbon increased the biomass of the  
167 native perennial herb *Anthriscus sylvestris* (Apiaceae) compared to control treatment when grown in competition  
168 with *L. polyphyllus*, indicating that phytochemicals contribute to the inhibitory allelopathic effects of this  
169 invasive plant (Lyytinen & Lindström, 2019). In addition to phytochemicals, osmotic potential of the leachate  
170 can explain some of the inhibitory allelopathic effects (Wardle et al., 1992) and has previously been shown to  
171 contribute to allelopathic effects of *L. polyphyllus* on the germination of co-existing native species (Loydi et al.,  
172 2015). While we can neither rule out the effect of osmotic potential nor determine the compounds of the  
173 leachates that caused the observed allelopathy, we know quinolizidine alkaloids are present in leaves of *L.*  
174 *polyphyllus* from invasive populations in high concentrations (Kalske et al., 2022) and they could have  
175 contributed to the inhibitory allelopathic effects. Although we only consider one population in this study, the  
176 concentrations and composition of quinolizidine alkaloids are similar across populations of the species in  
177 Finland (Kalske et al., 2022), suggesting that these results may be more broadly applicable.

178 Leachates prepared from shoots had stronger negative allelopathic effects than leachates prepared  
179 from roots in two out of the three plant families (Caryophyllaceae and Fabaceae). The allelopathic potential of  
180 litter leachate of *L. polyphyllus* in a previous study (Loydi et al., 2015) is similar in magnitude to our findings,  
181 but the allelopathic potential of roots of *L. polyphyllus* was previously unknown. Generally, leachates prepared  
182 from underground plant parts have weaker negative allelopathic effects than those prepared from aboveground  
183 parts (Zhang et al., 2021), which fits well with our findings. The synthesis of quinolizidine alkaloids occurs only  
184 in the leaves, and although they are present in all tissues of *L. polyphyllus*, quinolizidine alkaloid concentrations  
185 are higher in stem tissues than in roots (Wink & Hartmann, 1981). This uneven distribution of potentially  
186 allelopathic compounds in the plant could explain the differences in the strength of allelopathic inhibition  
187 between the two tissue types.

188 Relatedness of the species appears to affect the strength of the allelopathic effects. Germination rate  
189 was strongly inhibited by *L. polyphyllus* in plants in the families Asteraceae and Caryophyllaceae, and especially  
190 so by shoot leachate in Caryophyllaceae (lnRR -1.84). The plants in Fabaceae that are in the same family as  
191 *Lupinus* were least sensitive to the leachate treatments in terms of germination rate, but they were the only ones

192 for which shoot leachate increased germination time. These results are partly in accordance with those of Zhang  
193 et al. (2021) who demonstrated weaker negative allelopathic effects on closely related species than on more  
194 distantly related species. Phylogenetic conservatism of phytochemical composition likely explains this pattern,  
195 although species of *Trifolium* (used here) do not produce quinolizidine alkaloids as do species of *Lupinus* (Wink,  
196 2003). This phytochemical difference can be the reason for the observed delay in germination. Furthermore, it is  
197 possible that the mechanism of allelopathy changes with species relatedness, having weaker effects on  
198 germination rate but stronger effects on germination time in more closely related species.

199 Overall, the inhibitory allelopathy of *L. polyphyllus* is likely to contribute to some of the effects this  
200 invasive species has on the plant communities. The invasion of *L. polyphyllus* homogenizes plant community  
201 composition, increases the proportion of competitive species (Hansen et al., 2021) and reduces overall species  
202 richness (Ramula & Pihlaja, 2012). The species and families that are more sensitive to inhibitory allelopathy of  
203 *L. polyphyllus* are likely the ones to decline in its presence, such as more distantly related species. As such, our  
204 results are in line with the novel weapons hypothesis that proposes allelopathy as one of the main mechanisms  
205 explaining plant invasions (Callaway & Ridenour, 2004). Nevertheless, allelopathic potential needs to be  
206 compared between native and introduced populations of the species to confirm the novel weapons hypothesis. A  
207 more thorough understanding of the consequences of allelopathy by *L. polyphyllus* for plant communities would  
208 require studying the effects on plant growth in addition to germination. This may be particularly important for  
209 plant species with vegetative reproduction.

210 Importantly, the observed strong allelopathic effects of shoots have implications for management of  
211 invasive *L. polyphyllus*. Mowing or grazing of the aboveground biomass is a common and recommended  
212 practice of management for invasions by this species (e.g. Fremstad 2010). Even if mowing as such can be  
213 effective in reducing population growth of *L. polyphyllus* (Ramula, 2020), the speed of recovery of the native  
214 community may depend on how mowed plant material is handled. Because mowed shoots of the invader may  
215 cause strong allelopathic effects if left at the site, it may be important to collect and dispose the litter after  
216 mowing to promote re-establishment of the native flora. Positive aspect of our results is that as allelopathy by

217 roots is weaker than that by shoots, many of the negative allelopathic effects of *L. polyphyllus* can be alleviated  
218 by removal of aboveground parts only.

219

220 **Competing interests:** The authors declare there are no competing interests.

221 **Author contribution statement:** EM and SR designed the study and conducted the experiment, AK and EM  
222 analyzed the data, AK wrote the first version of the manuscript, EM and SR commented on the manuscript.

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224 **Data availability statement:** Data will be deposited to Dryad upon acceptance of the manuscript.

225

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304

305 **Table 1.** Results from mixed models analysing the effects of *Lupinus polyphyllus* root and shoot leachates on the  
 306 germination rates and germination times of native herbs from three families.

	Germination rate			Germination time		
	$X^2$	Df	<i>P</i>	<i>F</i>	Df	<i>P</i>
Treatment (control, root, shoot)	342.31	2	< 0.001	1.67	2	0.190
Family (3 levels)	418.67	2	< 0.001	318.02	2	< 0.001
Time (4 levels)	1213.23	3	< 0.001	.	.	.
Treatment × Family	58.81	4	< 0.001	4.37	4	0.002
Treatment × Time	218.04	6	< 0.001	.	.	.
Time × Family	635.66	6	< 0.001	.	.	.
Treatment × Family × Time	81.66	12	< 0.001	.	.	.
Residual				.	345	

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310 **Table 2.** Natural logarithm response ratios and 95 % confidence intervals (lnRR, 95 % CI) for the effect of root  
 311 and shoot leachates of *Lupinus polyphyllus* on the germination rate of native herbs from three families.  
 312 Germination was measured 21 days since sowing and negative values indicate inhibition of germination.

<b>Plant family</b>	<b>Leachate</b>	<b>lnRR</b>	<b>95 % CI</b>	
Asteraceae	Root	-0.44	-0.68	-0.20
	Shoot	-0.64	-0.88	-0.40
Caryophyllaceae	Root	-0.38	-0.59	-0.17
	Shoot	-1.84	-2.08	-1.60
Fabaceae	Root	-0.14	-0.19	-0.08
	Shoot	-0.50	-0.60	-0.40

313

314 **Figure 1.** The effects of *Lupinus polyphyllus* root and shoot leachates on the germination rate (estimated  
315 marginal means  $\pm$  SE) of native herbs from three families. Points that do not share the same letter differ at a  
316 given time point within a panel ( $P < 0.05$ , Tukey's test). Y-axis is the same for all panels.

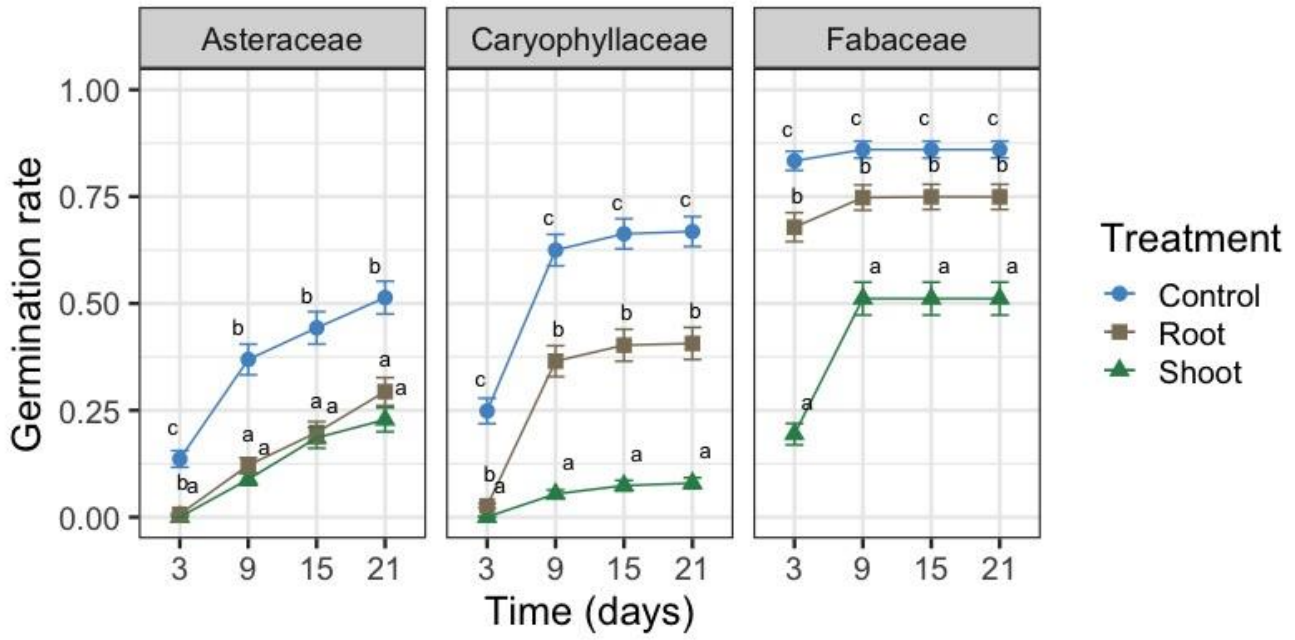
317

318 **Figure 2.** The effects of *Lupinus polyphyllus* root and shoot leachates on the germination time (estimated  
319 marginal means  $\pm$  SE) of native herbs from three families. Bars that do not share the same letter differ within  
320 family ( $P < 0.05$ , Tukey's test), ns = not significant. Ast = Asteraceae, Car = Caryophyllaceae, Fab = Fabaceae.

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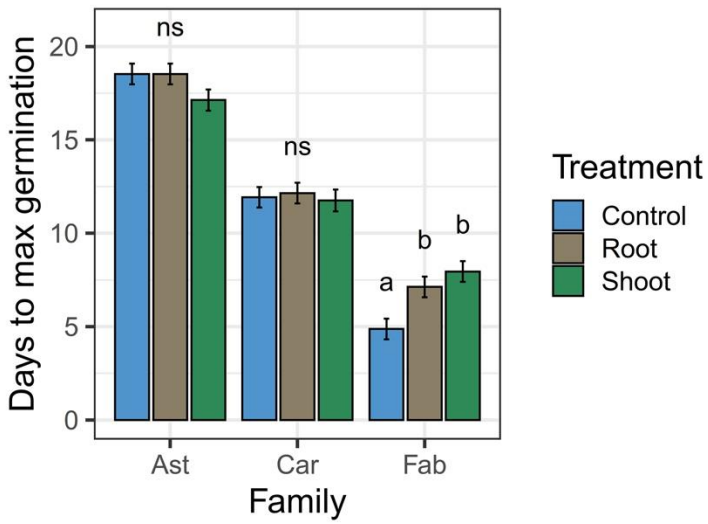
322 **Figure 1.**



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325 **Figure 2.**



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