

1 **Plant species variation in bottom-up effects across three trophic levels: A test of traits**  
2 **and mechanisms**

3

4 Running title: **Plant trait-mediated indirect effects**

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27 **ABSTRACT**

28 1. An increasing number of studies have addressed the mechanisms by which plant inter-  
29 specific variation influence interactions at higher trophic levels, but little is known about the  
30 underlying plant traits driving these dynamics.

31 2. Here we investigated the effects of host plant species on herbivore-parasitoid interactions  
32 and the underlying traits driving such effects. For this, we measured the abundance of seed-  
33 eating bruchids and their parasitoids across seven sympatric populations of the bean species  
34 *Phaseolus coccineus* and *P. vulgaris* in Central Mexico. To investigate the mechanisms  
35 underlying differences between bean species in bruchid-parasitoid interactions, we carried out  
36 two laboratory experiments to test whether bruchid and parasitoid performance differed  
37 between plant species. We also measured seed size and phenolic compounds to investigate if  
38 seed traits mediate bruchid-parasitoid interactions by influencing herbivore susceptibility or  
39 resistance to parasitoids.

40 3. Field surveys revealed that the rate of parasitoid recruitment to bruchids was significantly  
41 higher on *P. vulgaris* than on *P. coccineus*. Subsequent laboratory bioassays indicated that  
42 bruchids developed more slowly and exhibited lower fitness on *P. vulgaris* seeds than on *P.*  
43 *coccineus* seeds. Accordingly, we found that bean species differed in seed size, with *P.*  
44 *vulgaris* having smaller (less nutritious) seeds, which explains why bruchid development was  
45 slower on this plant species.

46 4. These results provide a mechanism for why bruchids exhibited higher parasitism rates on  
47 seeds of *P. vulgaris* in the field which could be due to Slow-Growth/High-Mortality effects, a  
48 smaller physical refuge provided by the seed, or both factors. The roles of these mechanisms  
49 remain inconclusive without further study.

50

51 **Keywords:** *bruchids, parasitoids, Phaseolus, phenolic compounds, seed size, trait-mediated*  
52 *indirect effects*

53

## 54 **INTRODUCTION**

55 The effects of plant phenotypic variation on consumers can be of environmental and/or  
56 genetic origin, and within the latter category effects may occur due to variation within (i.e.  
57 among genotypes) or among species. The effects of plant intra- and inter-specific variation on  
58 higher trophic levels have been documented most extensively within the context of plant-  
59 herbivore interactions, where plant genotype or species differences in defences and nutritional  
60 quality influence herbivore behaviour, abundance, and diversity (e.g. Karban, 1992; Hare,  
61 2002; Helms et al., 2004; Mooney & Singer, 2012). In addition, studies have also documented  
62 effects of plant intra- and inter-specific variation on higher trophic levels and entire food  
63 webs, with genotypes or species varying in community structure of associated arthropod  
64 faunas (Helms et al., 2004; Johnson & Agrawal, 2005; Whitham et al., 2006; Harvey et al.,  
65 2011; Mooney & Singer, 2012) and in the strength of consumptive interactions (Mooney &  
66 Agrawal, 2008; Abdala-Roberts et al., 2012).

67 Plant genetic or species effects on higher trophic levels may be direct or indirect  
68 (Hare, 2002). Within the latter category, there are two mechanisms by which these bottom-up  
69 effects are transmitted indirectly to higher trophic levels (e.g. herbivores and their enemies).  
70 First, plant trait variation can influence herbivore abundance, which in turn influences  
71 herbivore enemy abundance (“density-mediated indirect effect”; Mooney & Singer, 2012).  
72 Under this scenario, there are no changes in per capita interaction rates (i.e. strength or  
73 function of interactions; Wootton, 1994). Alternatively, plant genetic effects may indirectly  
74 influence herbivore enemies by altering herbivore traits (“trait-mediated indirect effect”;  
75 Mooney & Singer, 2012), leading to changes in the function of herbivore-enemy interactions

76 (interaction modification, sensu Wootton, 1994). For example, the Slow-Growth/High  
77 Mortality hypothesis poses that herbivore development on a poor quality host plant (species  
78 or genotype) will be slower than on a high quality host, exposing the herbivore to heightened  
79 predation risk and thus greater attack rates by natural enemies (Benrey & Denno, 1997;  
80 Moran & Hamilton, 1980; Williams, 1999). Alternatively, herbivores feeding on plants with  
81 high levels of chemical defences might sequester these chemicals which then serve as defence  
82 against their natural enemies, thus weakening the strength of natural enemy top-down effects  
83 (Moran & Thompson, 2001; Singer et al., 2014). In addition, there are a number of other  
84 mechanisms by which intra- or inter-specific variation in plant traits may modify herbivore-  
85 enemy interactions (reviewed by Hare 2002; Mooney and Singer 2012). Some of the most  
86 common ones are differences in the strength of plant volatile emissions (i.e. host location cue  
87 used by herbivore enemies) (Turlings et al., 1990; Dicke & van Loon, 2000), as well as  
88 variation in the degree to which host plants provide physical refuge for herbivores (e.g. Price  
89 1988; Andow, 1991), all of which may independently or in combination drive plant trait-  
90 mediated effects on associated interactions.

91         While there is a growing number of studies evaluating the effects of plant intra- and  
92 inter-specific variation on higher trophic levels (reviewed by Hare, 2002; Mooney & Singer,  
93 2012; Chen et al., 2015), comparatively fewer have examined the mechanisms underlying  
94 such effects (Abdala-Roberts & Mooney, 2013). In particular, separating density-mediated  
95 effects from interaction modifications is a key distinction in order to predict ecological and  
96 evolutionary consequences of plant phenotypic variation on higher trophic levels (Abrams,  
97 1982; Mooney & Singer, 2012). For instance, changes in the strength of species interactions  
98 (i.e. interaction modifications), rather than density-mediated effects, represent a primary  
99 source of evolutionary change due to alteration of selection on species traits (Miller & Travis,  
100 1996; Inouye & Stinchcombe, 2001; Abdala-Roberts & Mooney, 2015). A recent example

101 within the context of plant intra-specific variation documented that milkweed (*Asclepias*  
102 *syriaca*) genotype modified ant-aphid interactions, influencing the number of ants attracted  
103 per aphid (Mooney & Agrawal, 2008). In addition, another recent study focusing on plant  
104 species-level effects by Singer et al. (2012) reported density-dependent recruitment of  
105 insectivorous birds to caterpillars across eight tree species, with bird predation rates  
106 increasing with host tree quality, and where high quality host trees had higher caterpillar  
107 densities. Finally, although an increasing number of studies have broadly documented the  
108 mechanisms by which plant intra- and inter-specific are transmitted to higher trophic levels,  
109 the underlying plant traits driving such effects are not always addressed (Mooney & Singer,  
110 2012).

111 Here we investigated the effects of host plant species on consumptive interactions at  
112 higher trophic levels (herbivores and their enemies), as well as the underlying mechanisms  
113 driving such effects. To this end, we carried out a field study where we measured herbivory  
114 by seed-eating beetles (hereafter “bruchids”) and attack by their parasitoids across seven  
115 sympatric populations of two bean species of the genus *Phaseolus* (*P. coccineus* and *P.*  
116 *vulgaris*, Fabaceae) in Central Mexico. To evaluate the traits that mediate plant species’ effect  
117 on bruchids and parasitoids performance, we next carried out two laboratory experiments and  
118 measured seed traits related to plant resistance, including seed size, and seed chemical  
119 defences (i.e. phenolic compounds). By addressing these above, our work builds towards a  
120 more complete understanding of the mechanisms and underlying traits by which plant inter-  
121 specific variation cascades-up to influence higher trophic levels.

122

## 123 **MATERIAL AND METHODS**

### 124 **Study system**

125 We studied two bean species of the genus *Phaseolus* (Fabales: Fabaceae) in Central Mexico:  
126 *P. coccineus* and *P. vulgaris* (Fig. SM1). The center of origin of the genus is within Mexico,  
127 where the greatest diversity of wild and domesticated beans is found (Delgado-Salinas et al.,  
128 2006). *Phaseolus vulgaris* is naturally distributed at elevations ranging from 1300 to 1900 m,  
129 in tropical deciduous forests. Its flowers are white, pink, or purple, and give way to pods that  
130 are 8–20 cm long, pods are green, yellow, black, or purple and usually contain 4–6  
131 multicolored seeds (Gentry, 1969; Freytag & Debouck, 2002). The flowers of *P. coccineus*  
132 are red and they give way to dark green pods which 9-13 cm long, pods usually contain 3-5  
133 seeds which are black, white, cream or brown (Freytag & Debouck, 2002). *Phaseolus*  
134 *coccineus* is naturally distributed at higher elevations (>1400 m) in pine-oak forests, but  
135 populations of the two species exhibit extensive overlap, especially in Central Mexico  
136 (Freytag & Debouck, 2002). *Phaseolus coccineus* is largely allogamous and perennial, while  
137 *P. vulgaris* is autogamous and annual, nevertheless the reproductive phenology of both  
138 species largely overlap, fruiting from December to April (Gentry, 1969; Freytag & Debouck,  
139 2002). Both species are among the five within the genus that been domesticated (Delgado-  
140 Salinas et al., 2006). *Phaseolus coccineus* is cultivated mostly at the center of its wild form's  
141 distribution, and is mostly consumed locally, although its production has spread to South  
142 America and Europe (Delgado-Salinas, 1988). *Phaseolus vulgaris* is widely cultivated and is  
143 nowadays considered one of the most important legumes worldwide for direct human  
144 consumption (Broughton et al., 2003).

145 In Central Mexico, seeds of both species exhibit high rates of attack by three species  
146 of bruchid beetles (Coleoptera: Bruchidae), namely: *Acanthoscelides obtectus*, *A. obvelatus*,  
147 and *Zabrotes subfasciatus* (Fig. SM1) (Alvarez et al., 2005; Zaugg et al., 2013). These species  
148 are specialized on the genus *Phaseolus* and co-occur on the same host plants. *Acanthoscelides*  
149 species are distributed worldwide, and although the two species of this genus studied here

150 exhibit very few morphological differences, *A. obvelatus* is univoltine whereas *A. obtectus* is  
151 multivoltine (Alvarez et al., 2005). Both species lay their eggs on seeds within dry pods in the  
152 field or directly on the stored (harvested) beans. *Zabrotes subfasciatus* is multivoltine and  
153 restricted to Mexico and Central America (Zaugg et al., 2013) where it is also an important  
154 pest of stored beans as well as several other crop species. Females enter the mature pods  
155 through a hole in the pod and glue their eggs onto the seed coat (Campan & Benrey, 2006).  
156 Larvae feed inside the seeds and after four larval instars they pupate and emerge as adults  
157 (Campan & Benrey, 2006). More than one larva (3-4) of *Acanthoscelides* sp. and *Z.*  
158 *subfasciatus* can be typically found per pod (X. Moreira, personal observation).

159 In Central Mexico, the most common natural enemies of these bruchids are three  
160 parasitic wasps of the genus *Horismenus* (*H. missouriensis*, *H. butcheri*, and *H. depressus*;  
161 Hymenoptera: Eulophidae) and *Stenocorse bruchivora* (Hymenoptera: Braconidae) (Fig.  
162 SM1) (Hansson et al., 2004; Bonet, 2008; Zaugg et al., 2013). These solitary ectoparasitoids  
163 wasps are specialized on bean-feeding bruchid beetles (Aebi et al., 2008; Zaugg et al., 2013),  
164 and attack third and fourth instar larvae (Campan & Benrey, 2004). Other parasitoid wasps  
165 also found at our study sites are *Dinarmus basalis*, *Eupelmus* sp., *Chryseida* sp.,  
166 *Microdontomerus* sp., but these are usually present in much lower abundances.

167

### 168 **Field experiment: Effects of host plant species on seed herbivores, parasitoids and their** 169 **interactions**

170 Seeds of both *P. vulgaris* and *P. coccineus* were collected at seven sympatric populations in  
171 Central Mexico from December 2007 to March 2008 (Table SM1). The populations were  
172 located at roadsides, rural habitats, and close to cultivated crops at elevations ranging from  
173 1,791 to 2,039 m (Table SM1). At the end of the growing season (March), once pods were  
174 dry, at each site we collected all the mature bean pods present (approximately 50–100 per

175 plant) from three to a maximum of 80 randomly selected individuals depending on the  
176 population size. In total we sampled pods from 221 individuals, 70 were *P. coccineus* and 151  
177 were *P. vulgaris*.

178         Once collected, pods from each population were shelled; seeds were placed in  
179 ventilated plastic containers, and incubated under controlled conditions (26 °C day  
180 temperature, 14 hrs daylight, 70% R.h.). Each container was inspected daily for emerging  
181 bruchids and parasitoids. After emergence, all bruchids and parasitoids were recorded and  
182 identified to species or family level. Emerging insects were collected and individually stored  
183 in vials with 70% ethanol.

184         The effects of host plant species (*P. coccineus* vs. *P. vulgaris*) on bruchid and  
185 parasitoid abundance (mean number of individuals per plant) were analysed using linear  
186 mixed models (PROC MIXED in SAS 9.2, SAS Institute, Cary, NC) (Littell et al., 2006). The  
187 models included the effect of plant species treated as a fixed factor, and the effect of plant  
188 population treated as random because populations were randomly chosen and representative  
189 of sympatric populations in Central Mexico. To account for differences in the number of  
190 seeds sampled per plant (which would likely influence bruchid recruitment), we included the  
191 number of seeds per plant as a covariate in the bruchid abundance model. As bruchid  
192 abundance influences parasitoid recruitment, we included the number of bruchids as a  
193 covariate in the parasitoid abundance model. Normality was achieved by log-transforming  
194 original variables. We provide least square means  $\pm$  S. E. as descriptive statistics.

195         To investigate whether host plant species influenced bruchid-parasitoid interactions,  
196 we departed from the above model of parasitoid abundance and used a linear mixed model  
197 (PROC MIXED in SAS 9.2) that tested for the effects of plant species (fixed), number of  
198 bruchids, and their interaction on parasitoid abundance. As above, we included the effect of  
199 population treated as random. The interaction term tests for a difference between bean species

200 in the slope of the relationship between bruchid and parasitoid abundance which is indicative  
201 of trait-mediated interactions (Abdala-Roberts et al., 2012; Moreira et al., 2012), i.e. changes  
202 in the per capita effects of parasitoids on bruchids. Because we cannot match parasitoid and  
203 bruchid species (there were different species of both insects in the same seeds), all analyses  
204 were based upon pooled abundances across bruchid and parasitoid species.

205

### 206 **Laboratory experiments: Effects of host plant species on bruchid and parasitoid fitness** 207 **correlates**

208 To test for host plant species effects on bruchid and parasitoid fitness correlates, we reared  
209 insects on undamaged seeds of each bean species collected from four randomly chosen  
210 populations (Cuernavaca, San Jose de los Laureles, Malinalco and Tepoztlan, Table SM1).

211 For the bruchid performance experiment, we used *Z. subfasciatus* (multivoltine species  
212 easily reared in the laboratory) as the study model (Campan & Benrey, 2004). In February of  
213 2011, we collected several adult individuals of this bruchid species from our wild bean  
214 populations and reared them on cultivated seeds of red kidney beans in climate chambers (27  
215 °C and 70% Relative humidity) at the University of Neuchâtel (Switzerland). Since 2012 we  
216 have been maintaining a population of this species, and every year we add new individuals  
217 collected from the field to add genetic variability. We filled eight plastic containers (height:  
218 15 cm, diameter: 11 cm), with 50 undamaged seeds (one container per population) of both  
219 bean species (two bean species × four populations = eight containers). We then added 50 male  
220 and 50 female freshly emerged adult bruchids to each container. After three days of mating  
221 and subsequent oviposition on beans of each focal species, we randomly selected 25 bruchid-  
222 infested seeds per population per species and used a paintbrush to remove all but three newly  
223 laid eggs from the surface of each seed. Newly laid eggs (one day old) are milky white, and  
224 become transparent before the larvae hatch. We then placed five bruchid-infested seeds per

225 population in small plastic containers (height: 2 cm, diameter: 2.5 cm) (two bean species ×  
226 four populations × five containers = 40 containers) in the climate chamber (27 °C and 70%  
227 R.h.) and inspected them daily for newly emerged bruchids. On average, adult bruchids  
228 emerged 28 days later. Immediately after emergence, each individual was deep-frozen at -80  
229 °C and oven-dried for 48 h at 65 °C to constant weight. We recorded (i) the dry weight of each  
230 individual as a proxy of body size, (ii) developmental time of each individual (measured as  
231 the number of days until emergence), (iii) percentage of males (measured as the number of  
232 males divided by the total number of emerged bruchids) and (iv) survival (measured as the  
233 number of emerged bruchids divided by the number of eggs on the coat, which always three)  
234 (Campan & Benrey, 2004; Zaugg et al., 2013). For a closely related bruchid species  
235 (*Callosobruchus maculatus*, Coleoptera: Bruchidae), it has been shown that female eggs are  
236 allocated to high-quality hosts and male eggs to low-quality hosts so a higher percentage of  
237 emerging males is directly related to lower bruchid fitness (Spitzen & van Huis, 2005).

238 For the parasitoid performance experiment we used *S. bruchivora* as the study model  
239 (Campan & Benrey, 2004). We collected parasitoids from bruchid-infested seeds of wild bean  
240 populations in Mexico and reared them on red kidney bruchid-infested beans in climate  
241 chambers (27 °C and 70% R.h.) at the University of Neuchâtel. We carried out this  
242 experiment at the same time as the bruchid performance experiment. As above, we selected  
243 bruchid-infested seeds and removed all but three eggs from the coat of each seed. After seed  
244 selection, we filled small plastic containers (height: 2 cm, diameter: 2.5 cm) with five of these  
245 seeds (six containers per species and population, two bean species × four populations × six  
246 replicates = 48 containers) and placed these containers in a climate chamber (27 °C and 70%  
247 R.h.). Once bruchid larvae had reached the third or fourth instar inside the seed and can be  
248 parasitized (ca. 17 days after oviposition), we placed two inexperienced wasps (one male and  
249 one female) inside each plastic container (Campan & Benrey, 2004). We allowed the females

250 to parasitize for a period of three days (females lay one egg on each bruchid larva). During  
251 this time, we provided a drop of honey to enhance oogenesis (Schmale et al., 2001). Adult  
252 parasitoids emerged approximately 17 days later. Immediately after emergence, each  
253 parasitoid specimen was deep-frozen at -28 °C and oven-dried for 48 hrs at 65 °C to constant  
254 weight. We recorded (i) the dry weight and tibia length of each individual (using an ocular  
255 micrometer) as proxies of body size, (ii) percentage of males (measured as the number of  
256 males divided by the total number of emerged parasitoids), and (iii) success of parasitism  
257 (measured as the number of emerged parasitoids divided by [number of emerged bruchids +  
258 number of parasitoids]) (Campan & Benrey, 2004; Zaugg et al., 2013). We did not measure  
259 parasitoid developmental time because it was not possible to record the exact day when  
260 bruchid larvae were parasitized. As would be expected, our laboratory experiment was not  
261 free of limitations relative to field conditions (e.g. parasitoids were only given three days to  
262 parasitize their hosts, were confined in a small space with their hosts and were lacking some  
263 important host-plant cues), making a direct link between field and lab results more difficult.  
264 However, success of parasitism was similar and consistent with those observed in a previous  
265 field study with the same plant, bruchid and parasitoid species and in the same plant  
266 populations (Zaugg et al., 2013), and levels of bruchid parasitism in the lab fell within the  
267 range of parasitism rates observed in the field (Zaugg et al., 2013). These findings provide  
268 strong indication that some important aspects of the biology of bruchid-parasitoid interactions  
269 were preserved under laboratory conditions.

270         The effects of host plant species (*P. coccineus* vs. *P. vulgaris*) on bruchid (weight and  
271 developmental time) and parasitoid (weight and tibia length) fitness correlates were analysed  
272 using linear mixed models (PROC MIXED in SAS) (Littell et al., 2006). The main effect of  
273 host plant species was treated as a fixed factor whereas the effects of plant population and  
274 container (to account for repeated measures recorded on the same experimental unit) were

275 treated as random factors. Because female body size is larger for both bruchids and parasitoid,  
276 we separately analysed the weight, tibia length, and developmental time for males and  
277 females. For survival and male percentage of bruchids and success and male percentage of  
278 parasitoids (one value per container) we used linear mixed models (PROC MIXED in SAS)  
279 (Littell et al., 2006) with the host plant species as a fixed factor and plant population as a  
280 random factor. Normality was achieved by log-transforming original variables, except for  
281 tibia length of female parasitoids which was analysed with a generalized linear mixed model  
282 in PROC GLIMMIX using a Poisson distribution (log link function), as these data were non-  
283 normal after transformation. We use least square means  $\pm$  S. E. as descriptive statistics.

284

#### 285 **Seed traits: Plant species differences in seed size and chemical defences**

286 We measured the size of 54 randomly chosen seeds per plant species per population (same as  
287 above used for the bruchid and parasitoid performance bioassays; Table SM1). In total, we  
288 measured 432 seeds corresponding to 54 seeds  $\times$  four bean populations  $\times$  two bean species.

289 Using the same four populations, we also quantified the concentration of phenolic  
290 compounds in seeds of five randomly selected individuals per population, per species (we  
291 used a pool of 10 seeds per individual for phenolic extractions). In total, we quantified  
292 phenolic compounds from seeds of 40 individuals corresponding to four bean populations  $\times$   
293 two bean species  $\times$  five individuals. Phenolic compounds are widely recognized as herbivore  
294 feeding deterrents across many plant taxa (Salminen & Karonen, 2011; Mithöfer & Boland,  
295 2012). Extraction of phenolic compounds was carried out by using 300 mg of plant tissue  
296 with aqueous methanol (1:1 vol:vol) in an ultrasonic bath for 15 min, followed by  
297 centrifugation and subsequent dilution of the methanolic extract (Moreira et al., 2014).  
298 Samples were analysed by HPLC using a Grace C18 reversed phase column (3 $\mu$ m, 150 $\times$ 4.6  
299 mm, Grace Davison Discovery Science, Columbia, MD, USA) and an YL9100 instrument

300 (YL Instrument Co., the Republic of Korea), with diode array detection. The 15  $\mu$ l injection  
301 was eluted at a constant flow of 0.7 ml/min with a gradient of acetonitrile and 0.25%  
302 phosphoric acid in water as follows: from 80% to 50% water in 5 minutes, then from 50% to  
303 30% in five minutes, and kept at 30% for 7 minutes, and a final step from 30% to 5% in 4  
304 minutes, followed by 5 minutes of equilibration time. Peaks were detected by a diode array  
305 detector at 270 nm (for hydrolysable tannins), 320 nm (for ferulic acid derivatives), 370 nm  
306 (for flavonoids), and 500 nm (for anthocyanins). Absorbance spectra were recorded from 200  
307 to 900 nm. Peaks showing a characteristic absorption band of phenolics (Mabry et al., 1970)  
308 were recorded. Concentrations were calculated a standard curve that related peak areas to  
309 known ferulic acid, quercetin (for phenolics), and cyaniding (for anthocyanins) concentrations  
310 using 270 nm absorbance.

311 To test for bean species differences in seed size and phenolic compounds, we used  
312 linear mixed models (PROC MIXED in SAS) (Littell et al., 2006) testing for an effect of host  
313 plant species (fixed) and including plant population as a random factor. We report least square  
314 means  $\pm$  S. E. as descriptive statistics.

315

## 316 **RESULTS**

### 317 Field study: Effects of host plant species on seed herbivores, parasitoids and their interactions

318 The overall field sampling yielded a total of 143,203 seeds across all sympatric wild bean  
319 populations (28,820 in *P. coccineus* and 114,383 in *P. vulgaris*). From these, we recorded  
320 41,415 emerging arthropods, of which 32,411 were bruchid beetles and 9,004 were parasitic  
321 wasps.

322 Total bruchid abundance varied greatly among populations (within bean species). In  
323 particular, the mean number of bruchids per plant ranged from  $21.60 \pm 88.24$  to  $235.13 \pm$   
324  $49.33$  in *P. coccineus* (11-fold variation), and from  $73.11 \pm 65.77$  to  $323.67 \pm 113.93$  in *P.*

325 *vulgaris* (four-fold variation). Despite such extensive variation, host plant species did not  
326 have a significant effect on total bruchid abundance (Fig. 1a). Likewise, parasitoid abundance  
327 also varied extensively among populations, within bean species. In particular, the mean  
328 number of parasitoids per plant ranged from  $2.00 \pm 39.24$  to  $38.17 \pm 32.04$  in *P. coccineus*  
329 (19-fold variation) and from  $11.25 \pm 39.24$  to  $235.00 \pm 45.31$  in *P. vulgaris* (21-fold  
330 variation). In this case, we found a significant effect of plant species on total parasitoid  
331 abundance, with *P. vulgaris* seeds having 2.2-fold more parasitoids than *P. coccineus* seeds  
332 (Fig. 1b). In addition, the mechanistic model for parasitoid abundance revealed a significant  
333 plant species  $\times$  bruchid abundance interaction, i.e. bruchid-parasitoid interactions differed  
334 between bean species. This interaction occurred because the rate of parasitoid recruitment per  
335 bruchid was 2.1-fold greater for *P. vulgaris* than for *P. coccineus* ( $F_{1,210} = 10.85$ ,  $P = 0.001$ ;  
336 Fig. 2), indicating that trait differences between bean species mediated the strength of  
337 parasitoid-bruchid interactions.

338

339 Laboratory experiments: Effects of host plant species on bruchid and parasitoid fitness  
340 correlates

341 Overall, bruchids reared on *P. coccineus* seeds performed better than those reared on *P.*  
342 *vulgaris* seeds (Fig. 3). Firstly, the percentage of emerged bruchids per egg on *P. coccineus*  
343 seeds was 59% higher than in *P. vulgaris* seeds (Fig. 3a). Secondly, the dry weight of male  
344 and female bruchids on *P. coccineus* seeds was 21% and 11% higher, respectively, than on *P.*  
345 *vulgaris* seeds (Fig. 3d, 3f). Finally, developmental time of female bruchids on *P. coccineus*  
346 seeds was significantly shorter (by one day) than on *P. vulgaris* seeds (Fig. 3e). Plant species  
347 did not have a significant effect on the percentage of males that emerged from the seeds (Fig.  
348 3b).

349           There was some evidence (albeit weaker relative to bruchids) that host plant species  
350 influenced parasitoid performance. Specifically, the tibia length of male parasitoids (proxy of  
351 body size) reared on bruchid-infested *P. coccineus* seeds was 13% greater compared with  
352 males reared on seeds of *P. vulgaris* (Fig. 4d). However, plant species had no significant  
353 effects on success of parasitism (Fig. 4a), percentage of parasitoid males (Fig. 4b), male and  
354 female weight (Fig. 4c, 4e), or female tibia length (Fig. 4f).

355

### 356 Seed traits: Effects of host plant species on seed size and chemical defences

357 We found significant differences in seed size between plant species, with *P. coccineus* seeds  
358 being 40% larger than *P. vulgaris* seeds (Fig. 5a). We also found significant differences for  
359 total seed phenolics among plant species (Fig. 5b), with the concentration of total phenolics in  
360 *P. coccineus* seeds being 26% higher than in seeds of *P. vulgaris* (Fig. 5b). Analyses by  
361 phenolic compound class indicated that the concentration of flavonoids in seeds of *P.*  
362 *coccineus* was 38% higher than in seeds of *P. vulgaris* ( $337.2 \pm 35.7$  vs.  $243.7 \pm 35.4 \mu\text{g g}^{-1}$ ,  
363  $F_{1,34} = 11.09$ ,  $P = 0.002$ ). We found no significant differences between species for ferrulic  
364 acid derivatives (i.e. lignins) ( $59.0 \pm 6.9 \mu\text{g g}^{-1}$  in *P. coccineus* vs.  $50.8 \pm 6.9 \mu\text{g g}^{-1}$  in *P.*  
365 *vulgaris*;  $F_{1,34} = 0.86$ ,  $P = 0.361$ ) or anthocyanins ( $258.1 \pm 15.5 \mu\text{g g}^{-1}$  in *P. coccineus* vs.  
366  $225.8 \pm 15.5 \mu\text{g g}^{-1}$  in *P. vulgaris*;  $F_{1,35} = 2.16$ ,  $P = 0.151$ ), suggesting that flavonoids drove  
367 the overall difference in concentration of phenolics between species.

368

## 369 **DISCUSSION**

370 The mechanisms by which plant bottom-up effects influence higher trophic levels have been  
371 increasingly documented over last decade (e.g. Helms et al., 2004; Johnson & Agrawal, 2005;  
372 Mooney & Agrawal, 2008; Mooney & Singer, 2012; Singer et al., 2012; Petry et al., 2013). A  
373 common pathway for plant phenotypic variation to influence higher trophic levels is through

374 density-mediated effects where plant traits influence higher trophic levels through linear food  
375 chains (e.g. sequential changes in abundance across trophic levels) but consumer interactions  
376 are not modified (Mooney & Singer, 2012). However, there is also evidence that plant species  
377 or genotypes mediate consumer interactions indirectly via changes in consumer traits. For  
378 example, plant genetic types (e.g. species or genotypes) can indirectly influence natural  
379 enemies by affecting herbivore quality, susceptibility, and host location with this in turn  
380 driving changes in the strength of natural enemy top-down effects on herbivores (Turlings et  
381 al., 1990; Werner & Peacor, 2003; Helms et al., 2004; Mooney & Agrawal, 2008; Gols et al.,  
382 2009; Mooney & Singer, 2012; Singer et al., 2012). For example, Mooney & Agrawal (2008)  
383 showed that genotypes of the common milkweed indirectly influenced aphid-ant interactions  
384 by changing the quality and quantity of the aphids' honeydew. In this study, we provide  
385 similar evidence for effects of plant-species level variation on herbivore-enemy interactions  
386 (i.e. plant trait-mediated indirect effects) and further address the plant traits that are likely  
387 mediating such dynamics. In particular, our results show how phenotypic differences between  
388 *P. vulgaris* and *P. coccineus* resulted in changes in the function of bruchid-parasitoid  
389 interactions. We found that the rate of parasitoid recruitment to bruchids was higher on *P.*  
390 *vulgaris* seeds than on *P. coccineus* seeds. In addition, laboratory experiments showed that  
391 bruchids exhibited lower fitness (i.e. lower survival, reduced growth, and longer  
392 developmental times, albeit for females only in the latter case) on *P. vulgaris* seeds than on *P.*  
393 *coccineus* seeds. Overall, these results provide support for the Slow-Growth/High-Mortality  
394 hypothesis, which predicts that herbivore development on low-quality host plants should be  
395 slower, increasing the risk of herbivore mortality from natural enemies (Moran & Hamilton,  
396 1980; Clancy & Price, 1987; Benrey & Denno, 1997).

397         Although an increasing number of studies have identified the mechanistic pathways  
398 underlying plant bottom-up effects on consumers, in most cases there is no information on the

399 specific plant traits driving these dynamics (Hare, 2002). Our results provide strong evidence  
400 of seed traits potentially mediating plant inter-specific variation for bruchid-parasitoid  
401 interactions. Specifically, we found that *P. vulgaris* had smaller seeds than *P. coccineus*,  
402 which likely resulted in lower resource availability (and/or quality). Accordingly, previous  
403 studies have shown that seed predators and frugivores prefer larger seeds and fruits,  
404 presumably because they offer greater resource availability and/or quality (Janzen, 1971;  
405 Gómez & Zamora, 1994; Herrera, 2000). Potentially as a result of this difference, bruchid  
406 developmental time was longer on *P. vulgaris* seeds than on *P. coccineus* seeds with this in  
407 turn leading, at least in part, to stronger top-down effects by parasitoids on the former bean  
408 species (Fig. 2). In agreement with these findings, previous work in this system has shown  
409 that *Z. subfasciatus* beetles developed more slowly and produced smaller offspring when  
410 feeding on smaller and less nutritious seeds of wild *P. coccineus* and *P. vulgaris* populations  
411 compared with their cultivated counterparts which have larger and more nutritious seeds  
412 (Benrey et al., 1998; Campan & Benrey, 2006). Additionally, previous study has found that  
413 larger seeds of a closely related *Phaseolus* species (*P. lunatus*) contained higher protein  
414 contents than smaller ones (Hernández-Cumplido et al., unpublished data).

415         Despite evidence presented here in support of the Slow-Growth/High-Mortality  
416 hypothesis, we recognize that other unmeasured mechanisms (e.g. emission of plant induced  
417 volatiles; Turlings et al., 1990) might be also driving herbivore-parasitoid interactions in our  
418 study system. In particular, we speculate that parasitoids could have preferred to oviposit on  
419 *P. vulgaris* seeds which are smaller than *P. coccineus* seeds, because of easier access of the  
420 parasitoid ovipositors to the bruchid larvae inside (Sivinsky et al., 1997; Wang et al., 2009;  
421 Chen et al., 2015). Likewise, other seed traits such as coat thickness cannot be discarded as  
422 predictors of parasitoid attack. Based on this, our findings support the argument that seed  
423 traits, in particular size, are likely important in mediating variation in bruchid-parasitoid

424 interactions among species of *Phaseolus*. Finally, it is important to note that differences in  
425 bruchid parasitism between bean species might be partly due to changes in bruchid species  
426 composition (Schmitz et al., 2004; Mooney & Singer, 2012). Therefore, further work is  
427 necessary to improve our understanding on differences between bruchid species in their  
428 susceptibility to parasitoids, as well as if the parasitoid species studied are equivalent with  
429 respect to host use.

430         Interestingly, despite exhibiting greater size, and thus presumably higher resource  
431 availability, *P. coccineus* seeds also contained higher concentrations of phenolic compounds  
432 (especially flavonoids) than *P. vulgaris* seeds. Although higher concentrations of chemical  
433 defences are typically associated with reduced seed quality and would thus lead to lower  
434 bruchid performance on *P. coccineus*, there are at least two likely reasons why this was not  
435 the case. Firstly, bruchids feeding on *P. coccineus* seeds could have sequestered secondary  
436 metabolites, a phenomenon that is frequently exhibited by specialist insect herbivores (Opitz  
437 & Müller, 2009; Boeckler et al., 2011; Mason et al., 2014), and the sequestered compounds  
438 are in turn used by the herbivore as chemical defences against natural enemies (Boeckler et  
439 al., 2011). However, parasitoid fitness did not vary between bean species for any of the  
440 measured traits and for male tibia length the mean value was even greater for *P. coccineus*  
441 (suggesting higher fitness on this plant species), which argues that this mechanism was not at  
442 work. Alternatively, studies have shown that specialist insect herbivores can tolerate and even  
443 benefit from the consumption of highly defended plant tissues, through mechanisms of  
444 detoxification (Bernays, 1998; Mason et al., 2014). Under this scenario, parasitoid fitness  
445 would not be expected to vary between plant species, as observed in this study. In support of  
446 this interpretation, previous work has shown that *Z. subfasciatus* is able to tolerate high  
447 concentrations of cyanogenic compounds present in *P. lunatus* (lima bean) without exhibiting  
448 detectable reductions in fitness correlates (Shlichta et al., 2014).

449           In conclusion, we show that bottom-up effects of host plant species on higher trophic  
450 levels are driven by trait-mediated indirect effects. In particular, differences in seed quality  
451 between plant species presumably modified herbivore susceptibility and thus the strength of  
452 natural enemy top-down effects. We argue that further studies are needed to separate density-  
453 mediated from interaction modification effects as well as determine the underlying traits by  
454 which intra- and inter-specific variation influence herbivore-enemy interactions. In doing so,  
455 we will build a more robust body of theory explaining how plant intra- and inter-specific  
456 variation shapes associated consumer communities. Additionally, we suggest that future work  
457 should investigate the presence of opposing patterns of selection on traits that provide a direct  
458 fitness benefit to the plant but may also decrease fitness under a multi-trophic setting (Herrera  
459 et al., 2002; Agren et al., 2013). In our system, larger seeds will directly contribute to greater  
460 offspring survival and overall fitness but at the same time may also suffer from greater  
461 herbivore attack and weaker herbivore suppression by natural enemies (due to faster herbivore  
462 development).

463

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472

#### 473 **AUTHOR CONTRIBUTIONS**

474 XM formulated the idea of the manuscript. XM and BB designed the experiments. XM, SGK,  
475 JHC performed the experiments. SR analyzed phenolic compounds. XM analyzed the data.  
476 XM and LAR wrote the manuscript; and other authors provided editorial advice.

477

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666 **FIGURE LEGENDS**

667

668 **Figure 1.** Effects of host plant species (*Phaseolus coccineus* vs. *P. vulgaris*) on the mean  
669 number of (a) bruchid beetles and (b) bruchid parasitoids per plant across seven sympatric  
670 populations of these bean species located in Central Mexico. Bars are least square means  $\pm$   
671 s.e.m. (N = 70 for *P. coccineus* and N = 151 for *P. vulgaris*). F-values, degrees of freedom  
672 and associated significance levels (*P*) are shown.

673

674 **Figure 2.** Relationship between the mean number of parasitoids and bruchids per plant  
675 associated with (a) *Phaseolus coccineus* and (b) *P. vulgaris* across seven sympatric  
676 populations of both species located in Central Mexico. Each point represents an individual  
677 plant (N = 70 for *P. coccineus* and N = 151 for *P. vulgaris*). F-values, P-values, degrees of  
678 freedom, and predicted relationships are based upon simple linear regressions performed  
679 separately for each bean species. The slope of the relationship differed between host plant  
680 species ( $F_{1,210} = 10.85$ ,  $P = 0.001$ ), with the per capita rate of parasitoid recruitment being  
681 significantly greater (2.1-fold) for *P. vulgaris*.

682

683 **Figure 3.** Effects of host plant species (*Phaseolus coccineus* vs. *P. vulgaris*) on (a) survival of  
684 *Zabrotes subfasciatus* bruchids (percentage of emerged bruchids per number of glued eggs on  
685 the seed coat), (b) percentage of bruchid males (number of males divided by total  
686 individuals), (c) (e) developmental time (days until adult emergence) of male and female  
687 bruchids and (d, f) dry weight (in mg) of male and female bruchids in a laboratory  
688 experiment. Seeds were selected from four sympatric populations of both species located in  
689 Central Mexico. Bars are least square means  $\pm$  s.e.m. (N = 20). F-values, degrees of freedom

690 and associated significance levels ( $P$ ) are shown. Different letters indicate significant ( $P <$   
691 0.05) differences between host plant species.

692

693 **Figure 4.** Effects of host plant species (*Phaseolus coccineus* vs. *P. vulgaris*) on (a) success of  
694 parasitism of the parasitoid *Stenocorse bruchivora* (percentage of emerged parasitoids per  
695 number of glued bruchid eggs on the seed coat), (b) percentage of parasitoid males (number  
696 of males divided by total individuals), (c) (e) dry weight (in mg) of male and female  
697 parasitoids and (d, f) tibia length (ocular units) of male and female parasitoids in a laboratory  
698 experiment. Seeds were selected from four sympatric populations of both species located in  
699 Central Mexico. Bars are least square means  $\pm$  s.e.m. ( $N = 24$ ). F-values, degrees of freedom  
700 and associated significance levels ( $P$ ) are shown. Different letters indicate significant ( $P <$   
701 0.05) differences between host plant species.

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703 **Figure 5.** Effects of host plant species (*Phaseolus coccineus* vs. *P. vulgaris*) on (a) size (in  
704 cm) and (b) total phenolics (in  $\mu\text{g g}^{-1}$  d.w.) of seeds of each species. Seeds were selected from  
705 four sympatric populations of both species located in Central Mexico. Bars are least square  
706 means  $\pm$  s.e.m. ( $N = 216$  for seed size and  $N = 20$  for total phenolics). F-values, degrees of  
707 freedom and associated significance levels ( $P$ ) are shown.

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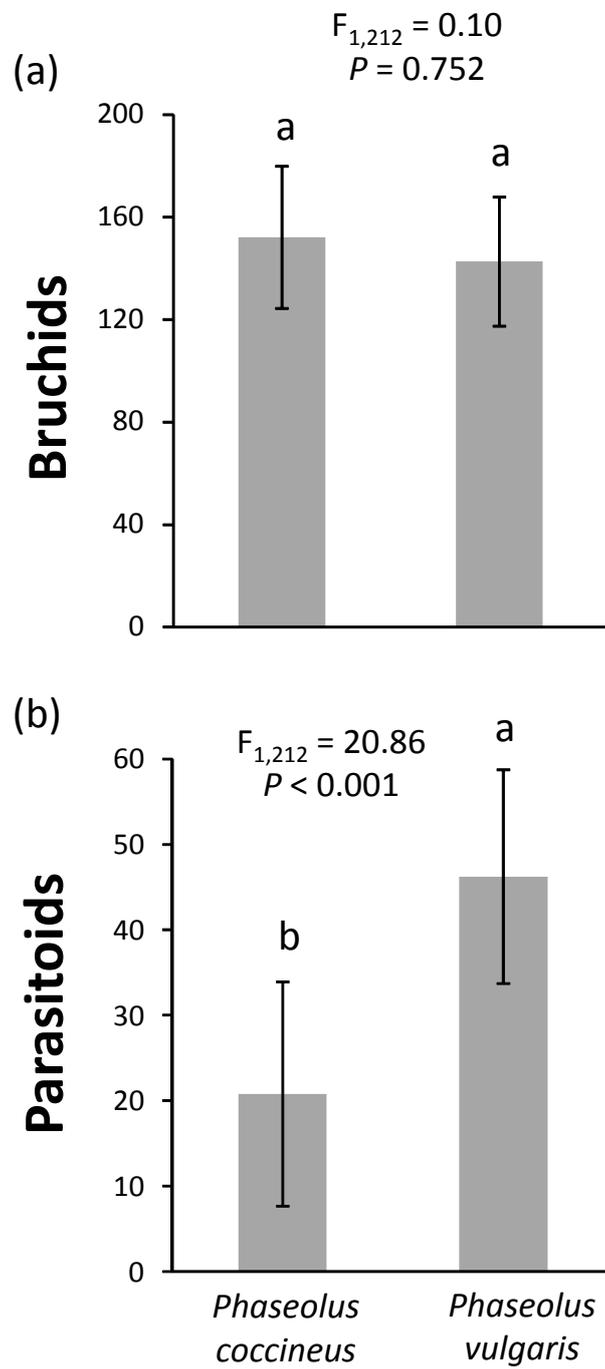
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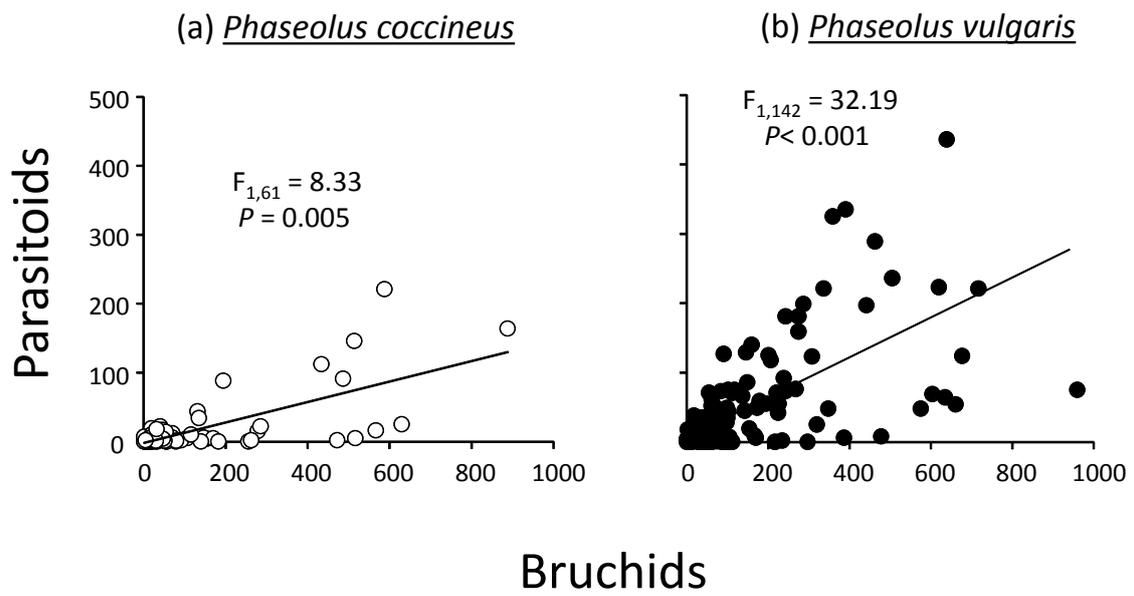
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719 **Figure 1.** Moreira et al

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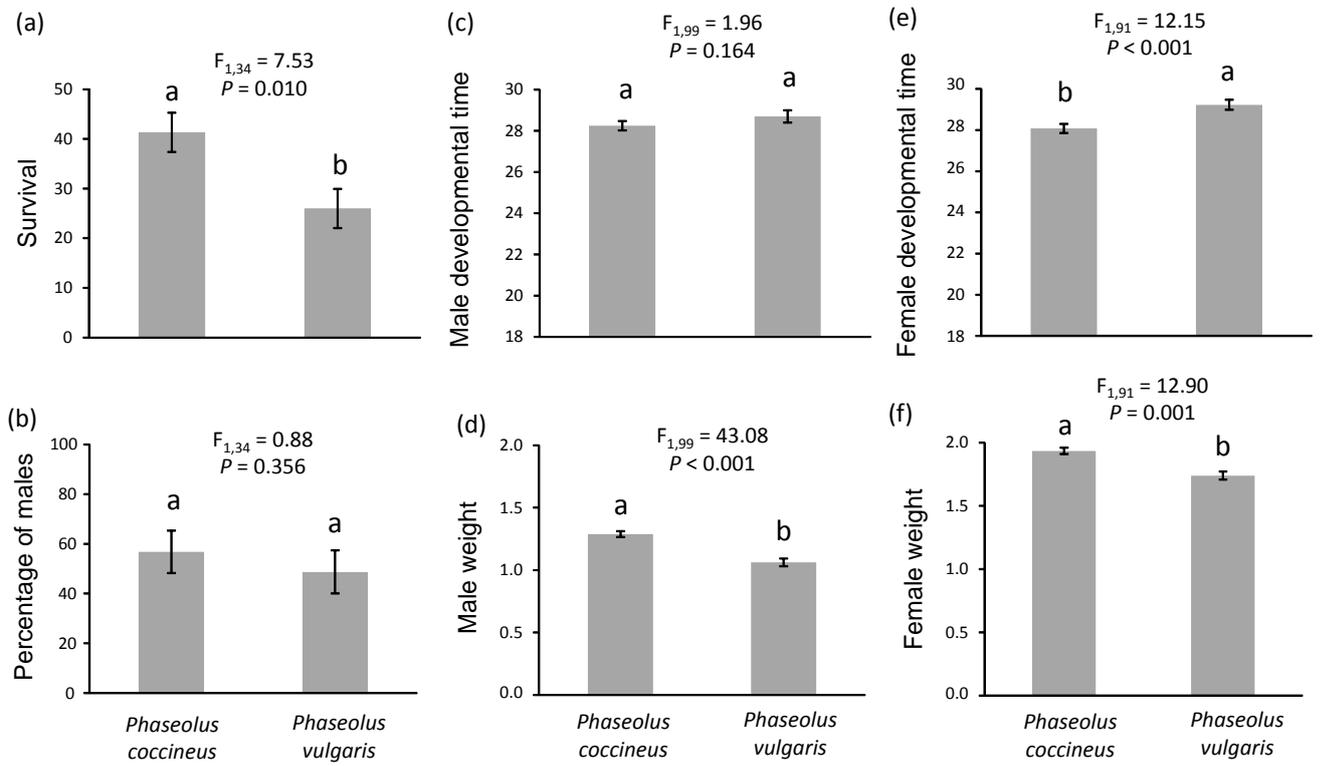
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**Figure 2.** Moreira et al

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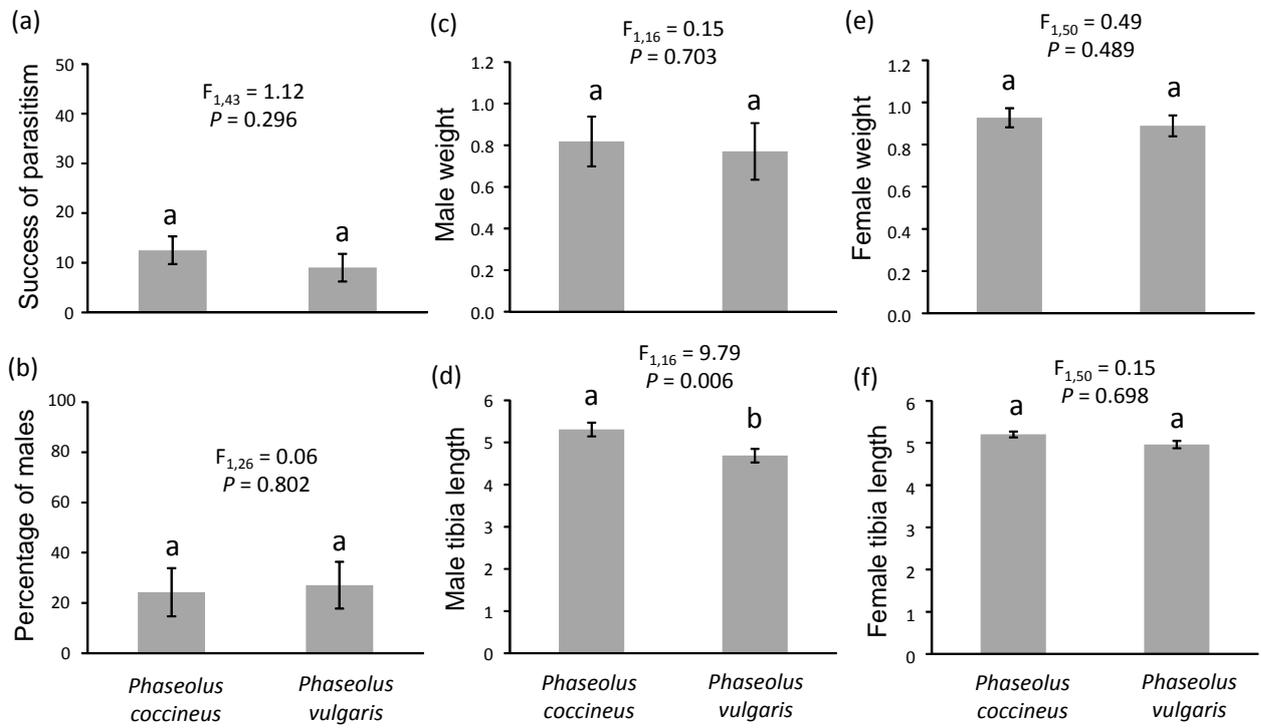
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**Figure 3.** Moreira et al

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760 **Figure 4.** Moreira et al

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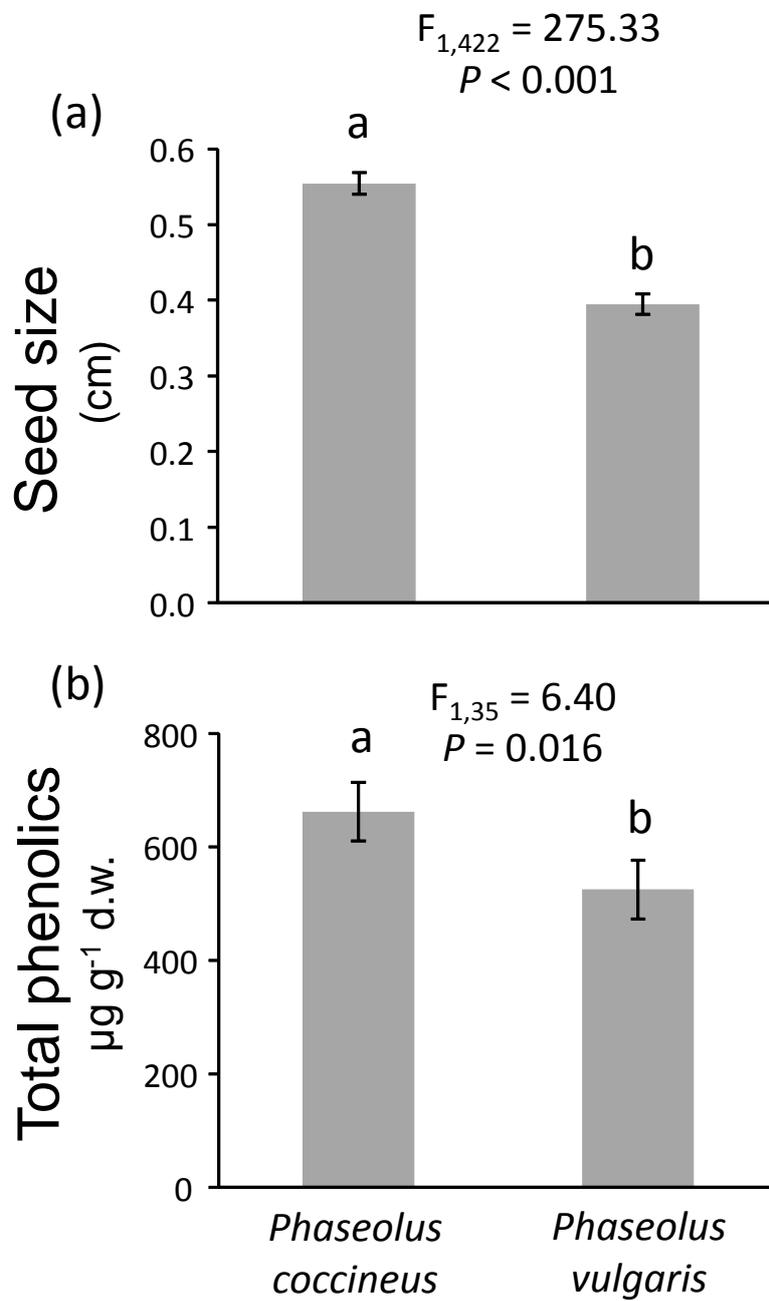
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774 **Figure 5.** Moreira et al

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