1   Impact of invasive bees on plant-pollinator interactions and reproductive success of plant

2   species in mixed *Nothofagus antarctica* forests

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16   **Abstract**

17   Invasive social bees can alter plant-pollinator interactions with detrimental effects on both

18   partners. However, most studies have focused on one invasive bee species, while the

19   interactions among two or more species remain poorly understood. Also, many study sites had

20   a history of invasive bees, being hard to find sites with historical low abundances. In

21   Patagonia, *Bombus ruderatus* (F.) invasion begun in 1993 and *B. terrestris* (L.) in 2006.

22   Though honey bees (*Apis mellifera, L.*) introduction started in 1859, their density is still low

23   in some parts. By experimentally increasing honey bee densities, we evaluated the effect of

honey bees and bumblebees floral visitation on native pollinators' floral visitation, pollen

deposition, and reproductive success of three plant species in mixed *Nothofagus antarctica*

forests of northern Patagonia: *Oxalis valdiviensis*, *Mutisia spinosa,* and *Cirsium vulgare*. Our

results show that exotic bees became the main floral visitors. No negative association was

found between invasive bees and native pollinators' visitation rates, but there was evidence of

potential competition between honey bees and bumblebees. Floral neighborhood diversity

played an important role in pollinators' behavior. Conspecific pollen deposition was high for

all species, while deposition of heterospecific pollen was very high in *M. spinosa* and *C.*

vulgare. Not as expected, honey bees visitation rate had a negative effect on heterospecific

pollen deposition in *C. vulgare*. For *O. valdiviensis*, exotic visitation rates increased

conspecific pollen deposition, which was positively related to reproductive success. Although

exotic bees became main floral visitors, their contribution to reproductive success was only

clear for one species.

Keywords: honey bees, bumblebees, exotic pollinators, pollination

Introduction

Invasive bee species can alter native plant-pollinator interactions, with effects on both

partners. As they can reach high numbers, exotic bees can compete with native pollinators for

floral resources and nesting sites (Goulson 2003, Mallinger 2017, Agüero *et al* 2018). As a

consequence, native pollinator fitness and population dynamics can be compromised

(Thomson 2004, Paini & Roberts 2005). Moreover, the displacement of native pollinators

might affect plant species partners thus the entire communities of both plants and pollinators

are subject to modifications (Aizen *et al* 2008). Yet most studies have focused on the impact

48   of one single invasive species on pollination patterns while the interaction between two or

49   more invasive species remains unexplored.

50   Invasive bees can affect pollination outcomes in both direct and indirect ways. Directly, they

51   can trigger pervasive negative effects on pollen flow if they behave as pollen thieves or if they

52   promote selfing (Mendes do Carmo *et al* 2004, Traveset & Richardson 2006, Dohzono *et al*

53   2008). Because some invasive bees (e.g. honey bees and bumblebees) are generalist species

54   (i.e., forage on a wide range of plant species) they are associated with an increase in

55   heterospecific pollen deposition (Traveset & Richardson 2006, Marrero *et al* 2016).

56   Indirectly, they can affect pollination by modifying the behavior of more efficient pollinators

57   or by reducing pollen availability (Dohzono *et al* 2008). These modifications to pollen

58   quantity/quality balance are expected to negatively affect plant reproductive success

59   (Dohzono *et al* 2008, Magrach *et al* 2017, Valido *et al* 2019). It is thus necessary to assess the

60   impact of invaders on plant-pollinator interaction patterns and plant reproductive success to

61   better understand and forecast the response of plant communities to the introduction of novel

62   invasive pollinator species.

63   Worldwide, honey bees (*Apis mellifera* L.) and bumblebees (*Bombus* spp.) are recognized as

64   some of the most important invasive pollinator species (Stout & Morales 2009). Honey bees

65   have been introduced for honey production and crop pollination all over the world except

66   Antarctica (Moritz *et al* 2005, Abrol 2012). The spread of some bumblebee species is more

67   recent and is related to crop pollination (Stout & Morales 2009). In the last decades, two

68   species of bumblebees began to invade Argentinean Patagonia, after being introduced into

69   Chile. It is thought that *Bombus ruderatus* (F.) invasion begun in 1993 and *Bombus terrestris*

70   (L.) in 2006 (Roig Alsina & Aizen 1996, Torretta *et al* 2006, Morales *et al* 2013). *Apis*

71   *mellifera* introduction started in 1859 (Pérez Rosales 1859, Sanguinetti & Singer 2014).

72   Although numerous studies show that these social bees can disrupt plant-pollinator

73   interactions with negative effects on both partners (Morales & Aizen 2006, Aizen *et al* 2008,

74   Santos *et al* 2012, Magrach *et al* 2017, Valido *et al* 2019), no one has assessed the combined

75   effect of the two groups of pollinators (i.e. honey bees and bumblebees) on plant-pollinator

76   interaction and reproductive success of plant biota in Argentina's Patagonia.

77   Mixed *Nothofagus antarctica* forests of northern Patagonia represent one of the main forestal

78   community of the Argentine Patagonia. This community is one of the most diverse

79   ecosystems in the region and is characterized by many plant species that rely on animal-

80   mediated pollination (Aizen & Ezcurra 1998, Speziale *et al* 2010). The relative recent

81   invasion of *Bombus terrestris* and *B. ruderatus* to mixed *Nothofagus antarctica* forests of

82   northern Patagonia and the low density of honey bees in some sites provide a good scenario

83   for experimental sites that are relatively new to the presence of invasive bees. This also allows

84   us to study their potential novel effects when they coexist. Therefore, by experimentally

85   increasing honey bee density in a locality where invasive bumblebees (*B. ruderatus* and *B.*

86   *terrestris*) are common flower visitors we studied the effect of both groups of pollinators on

87   plant-pollinator interaction in three different plant species of the mixed *Nothofagus antarctica*

88   forests. To do this, we evaluated four different aspects: i) behavioral response of pollinators to

89   the floral neighborhood, ii) the relationship between native and invasive pollinators visitation

90   rates (i.e. potential competition), iii) changes in pollen deposition patterns and iv) changes in

91   reproductive success of the three plant species. First, we expect that an increase in floral

92   neighborhood diversity will lead to an increase in the visitation rate of invasive bees on focal

93   plant individuals. Second, that an increase in invasive bee visits relates negatively to native

94   pollinators visits. Third, we expect that the increase in exotic pollinators visitation rates will

95   increase both conspecific and heterospecific pollen deposition. Finally, we hypothesize that

96   this increase in pollen deposition not necessarily will lead to an increase in reproductive

97   success because of a decrease in pollen quality.

98

99   **Materials and methods**

100   I.   Study Site

101   The study was performed at the proximity of the locality El Foyel, Río Negro, Argentina (S

102   41°38'48.44''; W 71°29'59.06''). This ecosystem represents a forest/steppe ecotone,

103   dominated by *Nothofagus antarctica* (Forst.) Oerst., accompanied with other woody species

104   such as *Lomatia hirsuta* (Lam.) Diels ex J.F. Macbr., *Schinus patagonicus* (Phil.) I. M. Johnst.

105   ex Cabrera and *Diostea juncea* (Gillies ex Hook.) Miers (Gyenge *et al* 2009). The climate is

106   characterized by a mean annual temperature of 9.7 ℃ (mean range 3.1 ℃-16.7 ℃) and an

107   average annual rainfall of 920 mm, with a probability of frost throughout the year (Reque *et*

108   *al* 2007).

109   II.   Studied Species

110   *Oxalis valdiviensis* Barnéoud (Oxalidaceae) is a perennial herb, 5-25 cm tall (Zuloaga &

111   Belgrano 2017) with cymose inflorescences bearing 9-31 flowers. Flowers are yellow and up

112   to 25 mm in diameter (Fig. 1A) and the fruit is a capsule. It is an endemic species to

113   Argentinean and Chilean Andes.

114   *Mutisia spinosa* Ruiz & Pav. (Asteraceae) is a semi-woody climber plant (Zuloaga &

115   Belgrano 2017). Flowers are disposed of in solitary heterogamy capitula. Ray flowers (8-10)

116   with ligulate light pink corollas (Fig. 1B). The tube is ca. 15 mm long and the ligule is elliptic,

117   ca. 30 × 8 mm. Numerous disk flowers with tubular yellow corollas, tube ca. 20 mm long.

118   The fruit is a glabrous achene 15 mm long. It is an endemic species to southern Argentina and

119   Chile.

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120 *Cirsium vulgare* (Savi) Ten. (Asteraceae) is an annual or biennial herb that growths 0.5-2 m

121 tall (Zuloaga & Belgrano 2017). The inflorescence consists of big capitula (mean = 15.7 mm,

122 Fenner *et al* 2002) with purple bisexual flowers (Fig. 1C). The fruit is an achene 3.5-4.8 mm

123 long, slightly asymmetrical. Although native to Europe, West, Central and South Asia, North

124 Africa and the Azores Islands; it is naturalized in most part of Argentina.

125 All three plant species whose blooming period overlap and have entomophilous pollination

126 (Coulin personal observations, Madjidian *et al* 2008, Morales & Aizen 2002). Particularly,

127 previous studies in this region demonstrated that for *M. spinosa* main pollinator used to be the

128 native giant bumblebee *Bombus dahlbomii* (Guer.) whose population is declining currently

129 (Madjidian *et al* 2008).

130   III.   Experimental design

131 In the study site, exotic bumblebees (*B. terrestris* and *B. ruderatus*) density (0.0097

132 individuals/m^2) is higer than honey bee density (0.000065 individuals/m^2) (Coulin *et al* 2019).

133 In order to increase honey bee abundance in relation to exotic bumblebees, we brought 10

134 beehives to the site on January 12, 2017, and taken care of during the experiment in the site.

135 During the flowering season, we selected 16 individuals for each plant species. Plant

136 individuals were separated more than 2 m from each other and marked with a code number.

137 All individuals were located no more than 1 km from the hives so that they were included in

138 the normal honey bee foraging range, which is 2-3 km (Abrol 2012). In each analyzed

139 individuals, we marked closed buds to identify which flowers were only exposed to an

140 increase in honey bee abundance for pollen deposition and reproductive success analysis.

141   IV.   Visitation rates and floral neighborhood characterization

142 We measured visitation rates on all plant individuals from January 26, 2017 to February 20,

143 2017. To estimate visitation rates on each focal plant, we registered the total number of floral

144   visitors during a specific period of time on a known (previously counted) number of flowers.

145   It is worth to note that only those floral visitors that touched the reproductive parts of the

146   flower were taken into account as a legitimate visit. Census started in the morning (ca. 10:00

147   hs) up to afternoon (ca. 16:30 hs). To avoid variation due to differences in the time of the day,

148   we changed the order of individual census in every repetition. Mean total visit time for each

149   plant individual is ca. 54 minutes.

150   We also characterized the flowering neighborhood in a circular plot (1 m of radius) around

151   each studied individual. We registered in each plot the number of flowering individuals for all

152   plant species and the number of open flowers.

153   V.   Pollen deposition

154   We collected at least 10-20 flowers for each individual in post-anthesis from the previously

155   marked buds and store them in 70 % alcohol. In the lab, we randomly selected three flowers

156   from each individual from each species. First, we separated the stigmatic area of each flower

157   and macerated for 24 hours in NaOH: water 1:10 (w:v). Then, we added a drop of melted

158   glycerol-gelatine containing safranin on a slide, place one stigma and squashed it carefully

159   with a coverslip (Zarlavsky 2014). The safranin is used to stain the pollen grains and allowed

160   better identification. Later, using a microscope, we counted the number of conspecific and

161   heterospecific pollen grains on the stigma. For the identification, we analyzed the pollen

162   morphology of each studied species by applying the same method to pollen grains directed

163   collected from the anthers. For further information, we also consulted the corresponding

164   bibliography.

165   VI.   Reproductive success

166   In each individual, we also marked at least three buds or pre-anthesis capitula that were

167   exposed to an increase in honey bee abundance. When the fruits of each plant individual were

168 matured, we collected them (capsules of *Oxalis* were removed pre-dehiscence). Each

169 capitulum of both Asteraceae species was collected and restored in paper bags, while *O.*

170 *valdiviensis* fruits were stored in 70 % alcohol for later analysis.

171 For each *O. valdiviensis* fruit, we counted the number of well-formed seeds (viable) in each

172 locule. Seeds that were disintegrated or too small with a different color were considered as

173 atrophied. We also counted the number of ovules per locule which we observed varied

174 between two and three among individuals. Therefore, we estimated the correct value for each

175 individual by counting the number of ovules per locule in three flowers per individual.

176 Differences in ovule production have also been observed in other *Oxalis* species: *O. alpina*

177 (Weller 1981); *O. magnifica* (Guth & Weller 1986), *O. corniculata* (Abid 2010). For

178 Asteraceae species, we distinguished for each capitulum the number of well-formed achenes

179 (healthy) from those that were hollow or shrunken. Achenes that are hollow or shrunken may

180 indicate that they are infertile (Michaux 1989).

181 In the case of *O. valdiviensis,* we could not analyze 5 of the 16 individuals for reproductive

182 success. For *M. spinosa*, for three individuals we could only analyze two capitulum, for two

183 one capitulum and for one individual we did not have data. Those individuals for which we

184 did not have data were not included in the model. Finally, for 6 individuals of *C.vulgare* we

185 analyzed two capitulum and for one, only one capitulum.

186   VII.   Data analysis

187 For describing floral neighborhood diversity we calculated Simpson´s diversity (1-D)

188 (Simpson 1949, Lande 1996). We grouped the floral visitors into three different categories:

189 honey bees (*A. mellifera*), exotic bumblebees (*B. terrestris* and *B. ruderatus*) and native

190 pollinators. Native pollinators include all other species visiting the flowers.

191   We calculated the visitation rate (measured as individuals/min*flower or capitulum) per

192   pollinator category and both the number of conspecific and heterospecific pollen grains on

193   stigma for each plant individual for each plant species. For *C. vulgare* and *M. spinosa,* we

194   calculated the reproductive success as the number of healthy achenes divided by the total

195   number of achenes. For *O. valdiviensis*, we calculated the reproductive success as the number

196   of viable seeds divided by the total number of ovules.

197   We used structural equation modeling (SEM) to fit a path analysis to test our multiple

198   hypotheses. SEM allows testing hypothesis related to causal relationships even in complex

199   models (Mitchell 1992). We used the "SEM" function in the R package Lavaan (Rosseel

200   2012) for fitting all of our structural equations. Path analysis assumes normality so we

201   transformed the variables to improve the normality of distribution: square root transformation

202   for honey bees, bumblebees and native pollinators visitation rate, conspecific pollen grains

203   and heterospecific pollen grains values and arcsin square root transformation for Simpson

204   diversity index and for reproductive success values. We evaluated models to fit with a chi-

205   squared test and Comparative Fit Index (CFI). We incorporated CFI because is less sensitive

206   to sample size (Ainur *et al* 2017).

207   We constructed an initial theoretical model for the three species (Fig. 4, Appendix). Our

208   model proposed causal links for our six variables of interest: Simpson´s diversity (1-D),

209   honey bees visitation rate, native pollinators visitation rate, bumblebees (*B. terrestris* and *B.*

210   *ruderatus*) visitation rate , conspecific pollen grains (mean number of conspecific pollen

211   grains deposited on stigma), heterospecific pollen grains (mean number of heterospecific

212   pollen grains deposited on stigma) and reproductive success (number of viable seeds/total

213   number of ovules for *O. valdiviensis* or healthy achenes/ total number of achenes for *M.*

214   *spinosa* and *C. vulgare*) (Fig 4, Appendix).

215   Initially, the Chi-square value > 0.05 and CFI << 0.95, which indicates that goodness of fit of

216   the models is not good (Hu & Bentler 1999). In order to improve the models, we included

217   paths suggested by the analysis of the modification indices (MIs) (Mitchell 1992, Rosseel

218   2012). We only included parameters that have a biological interest. After this, the goodness of

219   fit of each model improved considerably (chi-square value > 0.05 and CFI ≥ 0.95). This is

220   why the final structural equations differ from our initial theoretical model for the Asteraceae

221   species. Additionally, we also analyzed the correlation among the transformed variables by

222   using Pearson´s r ('record' function of the 'Hmisc' R-package) (Table 2, Appendix).

223

224   **Results**

225   For all studied species, data fitted properly our proposed model after incorporating influential

226   relationships based on modifications indices: *O. valdiviensis*: *P*-value (Chi-square) = 0.319;

227   CFI = 0.93; *M. spinosa*: *P*-value (Chi-square) = 0.466; CFI = 1.00; *C. vulgare*: *P*-value (Chi-

228   square) = 0.361 ; CFI > 0.97. In general, there is a good correlation between path analysis

229   results and Pearson correlation values, although the level of significance may differ for some

230   relations between variables (Table 2, Appendix)

231   I.   Pollinator categories visitation rate and floral neighborhood diversity

232   Mean visitation rates were higher for honey bees and native pollinators in *O. valdiviensis* and

233   for bumblebees follow by honey bees in both *M. spinosa* and *C. vulgare* (Fig 2). Path analysis

234   shows that floral neighborhood had more frequently a significate positive effect on at least a

235   pollinator category visitation rate for all plant species. In this sense, plant neighborhood

236   diversity had a significant positive effect on native pollinators in *O. valdiviensis,* a significant

237   positive effect on honey bees in *M. spinosa* and on honey bees in *C. vulgare* (Fig. 3).

238   Contrary, it had a negative effect on bumblebees visitation rate on *C. vulgare*.

239   II.   Potential competition with native pollinators

240 We found no significant negative effect of exotic pollinators, honey bees and bumblebees, on

241 native visitation rate in any plant species (Fig. 3).Instead, we found a significant positive

242 relation between exotic bumblebees and native pollinators visitation rate in *M. spinosa* (Fig

243 3). We also found a significant positive relation between honey bee and bumblebees visitation

244 rate in *C. vulgare*. We did not observe that any encounter between invasive bees and native

245 floral visitors disrupted the foraging behavior of the last ones.

246   III.   Pollen deposition

247 Mean conspecific pollen grains deposited on stigma was one order of magnitude higher for *O.*

248 *valdiviensis* (339.2 ± 55.12) than *M. spinosa* (92.11 ± 11.15) and *C. vulgare* (30.96 ± 4.56)

249 (Table 1). Contrarily, mean heterospecific pollen grains deposited on stigma were highest for

250 *C. vulgare* (35.25 ± 7.20), followed by *M. spinosa* (26.38 ± 4.54) and *O. valdiviensis* (11.53 ±

251 4.18) (Table 1). For *O. valdiviensis*, we found a significant effect of honey bees and native

252 pollinators visitation rate on mean conspecific pollen deposition and a marginal significant

253 relation between bumblebees and conspecific pollen grains (Fig. 3). Moreover, native

254 pollinators visitation rate had an also positive effect on heterospecific pollen deposition in *O.*

255 *valdiviensis* (Fig. 3). Bumblebees visitation rate had a marginally significant positive effect on

256 heterospecific pollen grains in *C. vulgare*. While, contrarily to our hypothesis, we found that

257 honey bees visitation rate had a significant negative effect on heterospecific pollen deposition

258 in *C. vulgare* (Fig. 3). We found no effect between pollinators categories and pollen

259 deposition in *M. spinosa* (Fig. 3)

260

261   IV.   Reproductive success

262 Mean reproductive success was extremely high for *C. vulgare* (0.92 ± 0.03), while *O.*

263 *valdiviensis* and *M. spinosa* had similar values (0.63 ± 0.05 and 0.64 ± 0.03, respectively)

264 (Table 1). Based on our path analysis, we found a marginal significant relation between

265 conspecific pollen deposition and reproductive success of *O. valdiviensis* (Fig 3). As expected

266 we found a negative but marginal significant relation between heterospecific pollen

267 deposition and reproductive success also for *O. valdiviensis* (Fig 3).

268

269 **Discussion**

270 Our study shows that invasive bees became main floral visitors for the three studied plant

271 species, with the diversity of floral neighborhood playing a considerable role in their

272 behavior. Honey bees and native visitors were the main pollinators of *O. valdiviensis*

273 contributing to conspecific pollen deposition, which marginally related to an increase in

274 reproductive success. *Cirsium vulgare*, an exotic species mainly pollinated by exotic bees,

275 had a high reproductive success. *Mutisia spinosa*, a native species whose local main pollinator

276 is in decline and for which we did not record any visit (e.i., *Bombus dahlbomii*), had

277 surprisingly an intermediate reproductive success. Invasive bumblebees may be contributing,

278 at least partially, to the pollination of this species.

279 Flower neighborhood diversity was an important component shaping the foraging behavior of

280 pollinators (i.e. visitation rate), especially for honey bees (Fig 3). We found a predominantly

281 positive effect of flower diversity on pollinator visitation rates, a pattern that has been

282 highlighted in several studies (Sih & Baltus 1987, Molina-Montenegro *et al* 2008, Muñoz &

283 Cavieres 2008). Mechanisms such as the combined attraction by convergent floral syndromes

284 or plants providing complementary resources can increase generalist pollinator visits

285 (Ghazoul 2006). Solitary bees with small foraging ranges can also tend to nest in areas with

high floral diversity and resource availability (Bruckman & Campbell 2014). However, competition rather than facilitation can also occur between plants, the result depending on factors such as the composition and density of floral neighborhoods (Ghazoul 2006, Morales & Traveset 2009, Waters *et al* 2014). In our study, *Cirsium vulgare* co-flowering around *M. spinosa* may be more attractive for bumblebees. This exotic thistle was the most common species present in *M. spinosa* floral neighborhood and bumblebees have innate preferences for violet and blue colors (Giurfa *et al* 1995).

Contrary to our hypothesis, we did not found a negative relation between invasive bees and native pollinators visitation rate that could suggest a potential competition or displacement. Instead, we found a positive relation in *M. spinosa*. A positive relation between invasive bees and native pollinators has already been observed (Mallinger *et al* 2017). However, the absence of potential competition cannot be ruled out. The positive relationship can be by the preference of both pollinators for the same plant individual or by competition between them. The second hypothesis is based on the idea that if the competition is reducing the resource availability per flower, animals could be visiting more flowers for collecting the quantity they require (Maloof & Inouye 2000). For *O. valdiviensis* and *C. vulgare*, the absence of a negative interaction may be because the actual density of exotic bees is not sufficient to reduce resource availability.

The hypothesis related to resource reduction could also explain the positive relation between honey bee and bumblebee visitation rates in *C. vulgare*. These generalist bees have a high preference for this exotic plant species (Morales & Aizen 2006). If visits by honey bees and bumblebees reduce the amount of nectar and/or pollen per capitulum, both can increase the number of visits to obtain the quantity they require. The fact that we observed honey bees and bumblebees fighting for foraging in capitulum also supports this. Interspecific scent marks could also explain this behavior: bumblebees and honey bees tend to avoid or depart quickly

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311   from flowers that were previously visited by an individual of the different species (Stout &

312   Goulson 2001).

313   As expected, we found that honey bee and bumblebee visitation rates related positively to

314   conspecific pollen deposition but only in *O. valdiviensis*. Honey bees can be efficient

315   pollinators outside their native range because they are generalists, and their pollination

316   effectiveness can be similar to that of native pollinators (Hung *et al* 2018). Even if they are

317   less efficient, their high abundance may compensate it (Agüero *et al* 2018). In the studied

318   region, exotic bumblebees have demonstrated to be efficient pollinators, for example, of some

319   orchids species (Sanguinetti & Singer 2014). Even though we did not found the same pattern

320   for *M. spinosa* and *C. vulgare,* the mean number of conspecific pollen grains was very high as

321   Asteraceae flowers only have one ovule per flower. This shows that these species could not be

322   experiencing pollen limitation, at least at the quantity level (Aizen & Harder 2007). This also

323   suggests that the lack of a clear increase in conspecific pollen deposition with an increase in

324   pollinator visitation rates could be due to pollen saturation (Aizen & Harder 2007).

325   Contrarily to our hypothesis, we did not find a relevant positive effect of invasive bees

326   visitation rates on heterospecific pollen deposition. Only bumblebees visitation rate had a

327   marginally positive effect in *C. vulgare*. Interestingly, honey bee visitation rate had a

328   negative relation. Although honey bees and bumblebees are both generalist species, they are

329   both also characterized by their flower constancy (Goulson 2010, Grüter & Ratnieks 2011).

330   Flower constancy is relatively higher for honey bees (Goulson 2010) and this characteristic

331   may reduce the chances of deposition of heterospecific pollen on stigmas. However, it is

332   important to note that *C. vulgare*, which was mainly visited by both categories of invasive

333   bees, had the highest proportion of heterospecific pollen deposition. Native pollinators

334   visitation rate related positively to heterospecific pollen deposition in *O. valdiviensis*,

335 probably because different species with different behavior and morphology were grouped in
336 this category.

337 An increase in exotic bees visitation rates may be beneficial for the reproductive success of *O.*
338 *valdiviensis*. All the pollinator categories contributed to conspecific pollen deposition and
339 there was a significant effect of conspecific and heterospecific pollen deposition on
340 reproductive success. Despite the high conspecific pollen deposition, pollen quality limitation
341 can be important cause *O. valdiviensis* presents tristyly as a crossbreeding system: three
342 different morphotypes differ in the length of the style and the length of the two whorls of the
343 stamen (Fyfe 1950). Effective pollination occurs when pollen comes from individuals with
344 different morphotypes. Honey bees tend to forage several flowers of the same inflorescence or
345 individual before moving to another, which could reduce the quality of the pollen (i.e.,
346 increasing geitonogamy) (Paton 1997). Another interesting aspect is that heterospecific pollen
347 grains, although mean grains were high, were related to reproductive success. Heterospecific
348 pollen can reduce reproductive output by different mechanisms such as allelopathic inhibition
349 of conspecific pollen (Morales & Traveset 2008). Those detrimental effects can even be
350 independent of conspecific pollen load size (Arceo-Gómez & Ashman 2011).

351 We observed no effect of conspecific pollen deposition on the reproductive success of *M.*
352 *spinosa*. Their primary pollinator is *Bombus dahlbomii*, whose population is declining, and it
353 has been suggested that invasive bumblebees may not be able to replace it (Madjidian *et al*
354 2008). In our study mean reproductive success was 64 %, even though exotic bumblebees
355 were the main pollinators. The number of deposited conspecific pollen grains on stigma was
356 very high, suggesting that other factors are limiting its reproductive success such as pollen
357 quality or nutrient limitation (De Jong & Klinkhamer 1989, Niesenbaum 1993, Morales &
358 Traveset 2008).

359 *Cirsium vulgare*, an exotic species mainly visited by both exotic bees, had an extremely high

360 reproductive success showing that this plant species does not suffer from a limitation in pollen

361 quantity or quality. Positive interaction between invasive bees and exotic plant species has

362 already been demonstrated (Goulson 2003, Morales *et al* 2009, Traveset & Richardson 2014).

363 Even though we did not found a positive relation between exotic bees and conspecific pollen

364 deposition, this data may suggest that both honey bees and bumblebees are contributing

365 positively to the reproductive success of *C. vulgare*.

366 Based on our data, we can conclude that invasive bees do not compromise the reproductive

367 success of the studied plant species. It is important to bear in mind that our work is based on

368 variation in the visitation rates at the individual level during one growing season and native

369 pollinators were analyzed as "one group". However, it is an important contribution to

370 understanding the processes associated with the potential impact of invasive bees in the area

371 and serves as a guide for future research.

372

381

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530

531 **Figures and tables**

532 **Fig 1.** Flowers and inflorescences of the three studied species (a) *Oxalis valdiviensis*, (b)

533 *Mutisia spinosa* and (c) *Cirsium vulgare* and one of the invasive bumblebee species: *Bombus*

534 *terrestris*.

535 **Fig 2.** Barplot showing visitation rates of each defined pollinators categories (bumblebees,

536 honey bees and native pollinators) for the three studied plant species (*Oxalis valdiviensis*,

537 *Mutisia spinosa,* and *Cirsium vulgare*). Error lines indicate standard errors (SE). For each

538 plant species, different letters indicate values that differ significantly (first ANOVA,

539 then Tukey's test: P-value < 0.05).

540 **Fig 3.** Path analysis showing the most important causal relationships between the variables of

541 interest for each of the studied plant species (a) *Oxalis valdiviensis*, (b) *Mutisia spinosa* and

542   (c) *Cirsium vulgare*.Variables: Simpson´s diversity (1-D), honey bees v. r. (honey bees

543   visitation rate), native pollinators v. r. (mean native pollinators visitation rate), bumblebees v.

544   r. (mean *Bombus terrestris* and *B. ruderatus* visitation rate), conspecific pollen grains,

545   heterospecific pollen grains and reproductive success (number of viable seeds/ total number

546   of ovules for *O. valdiviensis* or healthy achenes/ total number of achenes for *M. spinosa* and

547   *C. vulgare*). The models show the magnitude of the standardized coefficients of each path

548   (arrows width) and their significance (**: *P*-value < 0.05) or marginal significance (*: *P*-

549   value 0.05-0.1). Full lines represent positive effects and dotted lines negative effects.

550

551   **Table 1.** The mean and standard error (SE) of the number of conspecific and heterospecific

552   pollen grains deposited on stigma and the reproductive success for the three plant species

553   (*Oxalis valdiviensis*, *Mutisia spinosa* and *Cirsium vulgare*). For each column, different letters

554   indicate values that differ significantly (first ANOVA, then Tukey's test: *P*-value < 0.05).

555

556   **Figures and tables on Appendix**

557   **Fig 4.** Theoretical path diagram incorporating all the hypothetical causal relationships

558   between the variables of interest for the three studied plant species. Variables: Simpson´s

559   diversity (1-D), honey bees v. r. (honey bees visitation rate), native pollinators v. r. (mean

560   native pollinators visitation rate), bumblebees v. r. (mean *Bombus terrestris* and *B. ruderatus*

561   visitation rate), conspecific pollen grains, heterospecific pollen grains and reproductive

562   success (number of viable seeds/ total number of ovules for *O. valdiviensis* or healthy/ total

563   number of achenes for *M. spinosa* and *C. vulgare*). Full lines represent positive effects and

564   dotted lines negative effects.

565

566   **Table 2.** Pearson correlation coefficients for all transformed variables of interest for each of

567   the three plant species (a) *Oxalis valdiviensis*, (b) *Mutisia spinosa* and (c) *Cirsium vulgare*.

568   Asterisks indicate significant differences (**: *P*-value < 0.05) or marginal significant

569   differences (*: *P*-value 0.05-0.1).

	Simpson´s diversity	Honey bees visitation rate	Native pollinators visitation rate	Bumblebees visitation rate	Conspecific pollen grains	Heterospecific pollen grains
(a) Oxalis valdiviensis						
Honey bees visitation rate	0.01					
Native pollinators visitation rate	0.57*	-0.11				
Bumblebees visitation rate	-0.05	-0.15	0.30			
Conspecific pollen grains	0.14	0.66**	0.38	0.32		
Heterospecific pollen grains	0.70**	0.27	0.55*	0.26	0.49	
Reproductive success	-0.30	-0.07	0.07	0.30	0.27	-0.25
(b) Mutisia spinosa						
Honey bees visitation rate	0.58**					
Native pollinators visitation rate	-0.05	-0.25				
Bumblebees visitation rate	-0.50*	-0.40	0.55**			
Conspecific pollen grains	0.36	0.02	-0.16	-0.27		
Heterospecific pollen grains	0.13	-0.15	-0.06	-0.08	0.46*	
Reproductive success	0.01	0.13	0.22	0.04	-0.29	-0.39
(c) Cirsium vulgare						
Honey bees visitation rate	0.65**					
Native pollinators visitation rate	0.33	0.31				
Bumblebees visitation rate	-0.09	0.55**	-0.04			
Conspecific pollen grains	0.06	0.27	-0.01	0.18		
Heterospecific pollen grains	-0.68**	-0.44*	-0.21	0.10	-0.15	
Reproductive success	-0.45*	-0.30	-0.05	-0.06	-0.01	0.22

table

Plant species	Conspecific pollen grains	Heterospecific pollen grains	Reproductive success
Oxalis valdiviensis	339.21 (± 55.12) a	11.53 (± 4.18) b	0.63 (± 0.05) b
Mutisia spinosa	92.11 (± 11.15) b	26.38 (± 4.54) ab	0.64 (± 0.03) b
Cirsium vulgare	30.96 (± 4.56) b	35.25 (± 7.20) a	0.92 (± 0.03) a

| *Oxalis valdiviensis* | 339.21 (± 55.12) a | 11.53 (± 4.18) b | 0.63 (± 0.05) b |
| *Mutisia spinosa* | 92.11 (± 11.15) b | 26.38 (± 4.54) ab | 0.64 (± 0.03) b |

a) Oxalis valdiviensis

b) Mutisia spinosa

c) Cirsium vulgare