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35 **Different strategies for breaking physical seed dormancy in field conditions in two**

36 **fruit morphs of *Vachellia caven* (Fabaceae)**

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47   **Running head:** Strategies for breaking PY in morphs of *V. caven*

48   **Keywords:** burial experiment, environmental conditions, Fabaceae, fruit morphology,

49   physical dormancy, seed coat structure, *Vachellia caven*.

52

Abstract

Differences in fruit morphology among or within species might indicate differences in other regenerative traits, such as seed dormancy and germination. In species with physical dormancy (PY), environmental conditions are suggested as responsible for dormancy break in field. Seeds of *Vachellia caven* have PY. This species exhibits two fruit morphs highly represented in Córdoba forests, Argentina: one is dehiscent and the other is indehiscent. In this study we performed a burial experiment with the aim to determine if the differences in *V. caven* fruit morphology were related to different patterns of PY break of their seeds in field conditions. We related these patterns to 1) environmental conditions that could influence the loss of PY, and 2) histological features of the lens zone. Seeds of both morphs exhibited dormancy break within 14 months of the start of the experiment, but with different patterns. The dehiscent morph showed an abrupt percentage of seeds that broke dormancy 14 months after the beginning of the experiment, probably after undergoing environmental changes similar to those suggested by the two-stage softening model. The indehiscent morph showed a gradual increase in seeds that broke dormancy, not clearly related to any of the environmental variables studied. No differences in seed coat structure of the lens zone were observed between morphs. The existence of both morphs could confer the species with higher possibilities of establishing and coping with environmental heterogeneity. Those characteristics contribute to the understanding of the success of this species in open and disturbed environments.

Keywords: burial experiment, environmental conditions, Fabaceae, fruit morphology, physical dormancy, seed coat structure, *Vachellia caven*.

Introduction

Seed dormancy mechanisms in plants have evolved to optimize the time of germination, avoiding germination in periods that are only ephemerally favourable and, therefore, increasing seedling survival (Fenner and Thompson, 2005; Baskin and Baskin, 2014; Willis *et al.,* 2014). Recently, it has also been suggested that physical dormancy (hereafter, PY) has evolved to allow seeds to escape seed predation, preventing the production and release of volatile compounds detectable by post-dispersal seed predators (Paulsen *et al.,* 2013). PY is caused by one or more layers of tightly packed palisade cells in the seed or fruit coat; such layers are impermeable to water and, once the seed or fruit coat becomes permeable, dormancy cannot be reversed (Baskin *et al.,* 2000; Baskin and Baskin, 2014). It has been suggested that seed hardness protects against microbial attack and extends seed longevity and persistence in the soil seed bank (Dalling *et al.,* 2011; Jarasuriya *et al.,* 2015). Therefore, PY has a great ecological importance (Allen and Meyer, 1998).

Hard seeds can be softened artificially by different mechanisms such as mechanical or acid scarification (Baskin and Baskin, 2014). However, the mechanisms involved in breaking physical dormancy under field conditions are scarcely known and seem to be highly variable among species (Van Assche *et al.,* 2003; Van Assche and Vandelook, 2006; Gama-Arachchige *et al.,* 2012; Jaganathan *et al.,* 2017). Seed softening might occur in response to fire (Moreira and Pausas, 2012; Jarasuriya *et al.,* 2015; Liyanage and Ooi, 2015; Cochrane, 2017), daily fluctuating temperatures (Van Assche *et al.,* 2003, Baskin and Baskin, 2014), moisture changes (Baskin and Baskin, 2014), animal ingestion of seeds or fruits (Venier *et al.,* 2012a; Jaganathan *et al.,* 2016), the action of soil microorganisms (Soriano *et al.,* 2014), or a set of environmental conditions acting during seed storage in the soil (Jaganathan *et al.,*

99   2017; Liu *et al*., 2017). Moreover, it has been suggested that differences in timing of PY

100 break in field conditions may be related to differences in the seed coat structure, especially to

101 epidermis thickness of the lens, a potential water-gap in the seed coat consisting of a point of

102 weakness of elongated palisade epidermal cells that eventually split apart, allowing water

103 entry to the seed in the final stage of seed softening (Baskin, 2003; Taylor, 2005; Venier *et*

104 *al*., 2012b; Jaganathan *et al.,* 2017). Water gaps open in response to an appropriate

105 environmental signal. Thus, understanding how timing of germination of seeds with PY is

106 controlled in in field conditions is necessary to determine the environmental conditions that

107 the water gap needs to open (Baskin, 2003).

108   Some species exhibit differences in fruit morphology; those different fruits may be

109 found in a single individual plant, a phenomenon known as heterocarpy or heteromorphism

110 (Lu *et al.,* 2010; 2015; Baskin *et al.,* 2014) or, although much less common, individuals

111 plants with a single morph, which may constitute different varieties of the same species

112 (Pometti *et al.,* 2010). The production of seeds/fruits of different morphologies could be an

113 adaptation of species to spatio-temporal variability of habitats (Imbert, 2002; Baskin and

114 Baskin, 2014; Lu *et al.,* 2010; 2015; Baskin *et al.,* 2014). The fruits or seeds within a species

115 may vary in size, color or morphology, as well as in dispersal, dormancy or germination

116 characteristics (Lu *et al.,* 2010; Baskin and Baskin, 2014; Baskin *et al.,* 2014; Zang *et al*.,

117 2016). Different trade-offs among species that differ in fruit features have been described

118 (Lu *et al.,* 2010; 2013; 2015; Baskin *et al.,* 2014). For example, diaspore dimorphism was

119 found to be associated with the diversification of the degree of dormancy and the spread of

120 offspring in time and space (Baskin *et al.,* 2014; Zang *et al.,* 2016 and reference therein).

121 Studies about the relationship of fruits with different morphology and their type of dormancy

122 were performed mostly in seeds with physiological dormancy (Baskin *et al.,* 2014; Zang *et*

123 *al.,* 2016), whereas studies on seeds with PY are scarce.

124   *Vachellia caven* (<u>Gillies</u> ex <u>Hook.</u> & <u>Arn.</u>) <u>Seigler</u> & <u>Ebinger</u> (ex *Acacia caven*

125 <u>Gillies</u> ex <u>Hook.</u> & <u>Arn.</u>; Seigler and Ebinger, 2005) is a tree native to Argentina. The

126 species has PY (Funes and Venier, 2006) and previous studies have shown that neither high

127 temperatures that simulate a fire nor cattle ingestion may break seed dormancy in this species

128 (Venier *et al.,* 2017; Venier *et al.,* 2012a). Previous works in some Fabaceae species reported

129 a seasonal pattern in dormancy loss, suggesting that fluctuating temperatures may be a key

130 factor to break dormancy and trigger germination (Van Assche *et al.,* 2003). *Vachellia caven*

131 exhibits five types of fruit morphs (Pometti *et al.,* 2010). Two of them are highly represented

132 in Córdoba forests, Argentina; they differ in their mechanisms for releasing the seeds, with

133 one being dehiscent and the other, indehiscent. Those morphs may be found in different

134 individuals (Pometti *et al.,* 2010). The presence of individuals with different fruit

135 morphologies has led some authors to consider the existence of two varieties of this species –

136 i.e., *V. caven var. caven* (indehiscent fruits) and *V. caven var. dehicents* (dehiscent fruits)

137 (Aronson, 1992; Pometti *et al.,* 2010). However, this species can also be considered

138 heteromorphic because its different fruit morphs can be found in a single individual

139 (Baranelli *et al.,* 1995).

140   The presence of the two fruit morphs (indehiscent and dehiscent) in *V. caven* may

141 have ecological significance. For example, seeds from the two fruit morphs might differ in

142 their PY breaking behaviour. Accordingly, we expect that the morph that exposes the seeds

143 earlier (dehiscent) would break seed dormancy as fast as possible in order to exploit the

144 favourable conditions to germinate as soon as they are dispersed and to reduce their exposure

145 to post-dispersal seed predators (Chambers and McMahon, 1994; Crawley, 2000).

146 Furthermore, those fruits that retain the seeds within the fruit for a longer time (indehiscent

147 fruit morph) could break seed dormancy later, since it would be favourable for them to keep

148 dormancy until the disperser has brought them to a new place or fruit dehiscence has

149 occurred. Therefore, we performed a burial experiment to explore the patterns of dormancy

150 break in field conditions in seeds with PY from two different fruit morphs of *V. caven.*

151 Moreover, we related these patterns to 1) environmental conditions that could influence the

152 loss of PY, and 2) histological features of the lens that could differ between seeds of both

153 fruit morphs, and could explain different mechanism of dormancy break in field conditions.

154

155 **Materials and methods**

156 *Study species*

157   *Vachellia caven* is one of the most widespread shrub or tree species of subtropical

158 South America; in Argentina it occurs mainly in lowland and mountain arid and semiarid

159 forests from central to northern regions (Aronson, 1992; Zuloaga and Morrone, 1999). This

160 species shows remarkable climatic tolerance and ecological adaptability and is able to

161 colonize areas degraded by anthropogenic activities, such as intense agriculture, grazing or

162 fire (Aronson, 1992; Cabido *et al.,* 1994). Most individuals of this species are usually shrubs

163 of 1-3 m in height, with some individuals attaining 8-10 m in height (Aronson, 1992).

164 Flowering occurs in August-September (Cialdella, 1984), and fruits ripen from January to

165 February and are dispersed from the tree from February-March to April. The fruits are

166 dispersed by cattle (observed in the indehiscent morph; Venier *et al.,* 2012a) and by medium-

167 size birds (Aronson, 1992), and seeds could be removed by ants (personal observation).

168 Without the intervention of dispersers, the indehiscent fruit morph would release the seeds

169 before the new reproductive season (personal observation). Seeds of *V. caven* have PY

170 conferred by lignified epidermal palisade-like cells and the presence of callose in the cuticle

171 that would make the seed coat impermeable to water (Venier *et al.,* 2012b). Previous studies

172 observed that this species may germinate at different temperatures, with optimal

173 temperatures being 25/15°C and 35/20° C (Funes and Venier, 2006).

174

175 *Study area*

176   The study area is located in the Chaco Serrano in Córdoba province, central

177 Argentina, at the southern end of the Gran Chaco. The vegetation is a mosaic of a seasonally

178 subtropical forest dominated by *Lithraea molleoides*, *Zantoxylum coco* and *Prosopis* spp.

179 (Giorgis *et al.,* 2011). The climate is monsoonal; mean annual temperature is 19 °C and

180 mean annual rainfall is 700 mm (which mainly falls in summer, between November and

181 March), with a long dry season in winter, from April to October (Capitanelli, 1979).

182

183 *Seed collection and initial characterization*

184   In January and February 2014 we collected fruits from the two fruit morphs

185 belonging to *V. caven*. In the study area a single individual may have either both fruit morphs

186 or only one morph. The fruits were collected from at least 20 different individuals from the

187 Chaco Serrano of Córdoba, Argentina. For each fruit morph we mixed the seeds from all the

188 individuals and performed a pool of seeds for each morph. From this pool, we randomly

189 chose 100 seeds of each morph and weighed each of them with a precision balance ($\pm$ 0.1

190 mg). In addition, in order to obtain the initial degree of PY in seeds of each morph before the

191   burial experiment, we conducted a germination experiment in the laboratory. Three replicates

192   (20 seeds each) of each of the two morphs were put to germinate for 30 days. After that

193   period, the seeds were scarified individually with sandpaper and placed again to germinate

194   for 30 days. Seeds were placed in 9-cm diameter Petri dishes on filter paper moistened with

195   distilled water and incubated in a germination chamber at 25 ± 1 °C (12/12 h daily

196   photoperiod, light density of about 30 $\mu mol/m^2/s$).

197

198   ***Burial experiment***

199   To study patterns of dormancy break in field conditions, in April 2014 we buried

200   fresh seeds of *V. caven* in 0.3-mm mesh nylon bags in the field (Van Staden *et al.,* 1994).

201   We closed the bags with sealing tape of different colors in order to distinguish the morphs

202   at the time of exhumation. The date for seed burial corresponds to the end of the primary

203   dispersal of both morphs in field. The burial area was located in La Bolsa (31° 44' 16.46''

204   S, 64°25' 31'' W), Córdoba, Argentina, in the Chaco Serrano region where the species

205   occurs naturally. The burial area was 2.5 x 5 m and the bags were buried at 5 cm deep in

206   the soil and protected against large seed removers with a wire cloth. In order to cover

207   potential local heterogeneity within the burial area, bags were randomly distributed but

208   ensuring that always, in each point of the burial area, bags of both morphs were presented.

209   Each nylon bag contained 100 fresh seeds. Every three months since the start of the

210   experiment, five nylon bags per morph were extracted from the soil. Thus, we buried 55

211   nylon bags per morph with a total of 5500 seeds of each morph. However, the experiment

212   ends when 25 nylon bags were extracted because by that time all seeds of both morphs

213 within the extracted bags had died or germinated, and therefore there were no dormant

214 seeds. The experiment lasted 14 months.

215   Environmental conditions were measured in the burial area. Daily maximum,

216 minimum and average temperatures were recorded using two sensors (Thermochron

217 iButton DS1921G) which obtained data every three hours during the whole experiment. In

218 addition, a rain gauge was placed next to the burial area and the amount of rain (mm) was

219 recorded after every rainy event.

220   After each exhumation, we spread the content of each bag and counted: a) the

221 number of clearly germinated seeds –i.e., seeds with easily distinguishable roots - (hereafter

222 emerged seedlings); b) the number of hard seeds; c) the number of dead seeds –i.e., seeds

223 that exhibited a high degree of fungal infection or that were rotted- and d) the number of

224 missing seeds -i.e., even though the bags were not damaged we detected the lack of some

225 seeds, probably due to a high seed decomposition or germination events long time before

226 bag extraction. The seeds that were classified as hard seeds (item b) were allowed to

227 germinate in Petri dishes (with a maximum of 25 seeds each) for 30 days, and those that

228 had not germinated after that period were scarified with a sandpaper and placed to

229 germinate for 30 days. This procedure was followed in order to reclassify the hard seeds

230 into the following categories: b1) germinable (seeds that germinated in the laboratory

231 without scarification); b2) dormant (hard seeds that germinated in the laboratory after

232 mechanical scarification); b3) dead (seeds that were detected as dead in the laboratory

233 experiment and seeds that died in the burial experiment (item c)). Finally, we obtained five

234 categories of seeds: 1) emerged (germinated under field conditions), 2) germinable

235   (germinated in the laboratory without scarification), 3) dormant (germinated in the

236   laboratory after scarification), 4) dead (rotted or showing fungal infection) and 5) missing

237   (Holmes and Moll, 1990).

238   In addition, in order to know if the buried seeds effectively need an environmental

239   signal for breaking dormancy, other seeds of both fruit morphs were maintained in the

240   laboratory under dark and dry conditions during the whole burial experiment. From this

241   laboratory pool, on each exhumation date, seeds of each morph (three Petri dishes per

242   morph, 15 seeds each) were also allowed to germinate in a germination chamber for 30

243   days (control experiment). After that period, seeds that had not germinated were scarified

244   and placed again in the germination chamber for 30 days. These seeds were classified as

245   germinable, dormant or dead. For all the experiments the germination chamber was set at

246     $25°C \pm 1\ °C$ (12/12 h daily photoperiod, light density of about $30\ \mu$ mol/m2/s). Seeds were

247   checked for germination twice a week and germinated seeds were recorded and removed.

248   The Petri dishes were moistened when necessary.

249 *Histological measurements of the seed coat*

250   In order to relate the patterns of dormancy break in field in seeds with PY to

251 histological features of the lens that could differ between morphs, the structure of the

252 epidermis in the lens zone was studied in seeds of both fruit morphs of *V. caven*. Five seeds

253 obtained from mature fruits of each morph were used for histological characterization of the

254 palisade cell layer in the lens zone. To soften the seeds, they were scarified with sandpaper at

255 the opposite side of the hilar zone and placed to imbibe water in Petri dishes on filter paper

256 moistened with distilled water. The seeds were cut into thick portions near the hilar zone

257 with a razor blade, fixed in 2.5 glutaraldehyde– 2% paraformaldehyde, and embedded in

258 Spurr low viscosity resin (embedding media kit ®. Data sheet # 217 Polysciences, INC).

259 Semi-thin sections (0.3-0.4 µm thick) were made using a diamond knife of an

260 ultramicrotome (Leica Ultracut R). The sections were stained with 1% Aniline Toluidine as a

261 general stain to distinguish lignified and pectic cell walls, and observed under a light

262 microscope (Nikon Eclipse – Ti). A digital photograph of each seed was taken to measure

263 the length of the epidermis using the imaging software Nis Elements-BR. Measurements

264 were made in radial lines through epidermis, from the cuticle to the parenchyma layer. In *V.*

265 *caven*, the lens zone is a depression in the seed coat and might correspond to a thin structure

266 of the epidermis that is different from the rest of the seed coat epidermis (Venier *et al.,*

267 2012b). Thus, in each seed, height of the epidermis was measured at three points: in the

268 center of the lens and to the left and right sides of the lens.

269

270 *Data analysis*

271   Differences in seed mass between seeds of both fruit morphs were evaluated using

272 General Linear Models with a gaussian error structure. Differences between fruit morphs and

273 dates (five exhumation dates) in seed proportion in each of the five classes of seeds were

274 analyzed with a Generalized Linear Model (GLMs) with binomial error structure with a logit

275 link function or quasi-binomial error structure when data were overdispersed. For missing

276 and dormant seeds, dates with zero seeds in those categories were not included in the

277 analysis. When differences among treatments were significant, "Di Rienzo, Guzmán and

278 Casanoves" (DCG) a posteriori test was performed for all the variables (Di Rienzo et al.,

279 2002). Histological measurements of seed coats were analyzed with General Linear Models

280   with a gaussian error structure correcting for the heteroscedasticity in the factor morph using

281   the function *varIdent*. All analyses were performed using the software InfoStat (Di Rienzo et

282   al., 2009) and its interface in R (R 2.15.0, R development core team 2012).

283   **Results**

284   *Initial seed characterization*

285   Seed mass was not statistically different between morphs ($\bar{x}$ for both morphs±SE $=0.1\pm1.3$ x

286   10^{-3} g; F=0.13; *P*=0.714). In the laboratory, before the burial experiment (April 2014), the

287   proportion of germinable seeds (without scarification) was low (less than 0.20) and did not

288   differ between fruit morphs (t=1.31; *P*=0.2390; Fig. 1); hence, both fruit morphs had a

289   similar degree of initial PY. Moreover, scarified seeds of both fruit morphs showed high

290   germination proportion, without significant differences between them (t= - 0.51; *P*=0.6258;

291   Fig. 1).

292   *Burial experiment*

293   Seeds of the two morphs of *V. caven* showed different temporal patterns of PY break

294   under field conditions (Fig. 2). For the indehiscent fruit morph, a gradual increase in the

295   number of emerged seedlings was observed during the experiment, whereas the dehiscent

296   fruit morph showed a remarkable increase of emerged seedlings at the end of the

297   experiment. The number of emerged seedlings was different between fruit morphs on most

298   of the exhumation dates, with a higher percentage of emerged seedlings for the indehiscent

299   morph on all the dates except in June 2015, when the pattern was reversed and a higher

300   germination percentage was observed in the dehiscent morph (Fig. 2 a, Table 1).

301   Temperature and precipitation decreased from the first autumn until the middle of the

302   winter was observed (Fig. 2 b). After that, an increase in temperature and precipitation was

303   recorded until the beginning of summer. During spring and early in summer maximum soil

304   temperatures were reached, and the highest thermal amplitude was recorded. The maximum

305   amount of precipitation occurred in February. No freezing event occurred throughout the

306   year.

307   The different proportions of non-emerged seedlings (germinable, dormant, dead and

308   missing seeds) from exhumed bags per fruit morph and date are summarized in Figure 3 a-

309   b. Significant differences were observed in the proportion of germinable and dormant seeds

310   between fruit morphs and among dates (Table 1). The highest germinable seeds values were

311   observed on the first, second and fourth exhumation dates in the indehiscent morph,

312   whereas very few germinable seeds were observed in the dehiscent fruit morph on all dates.

313   The highest proportions of dormant seeds were observed on the first three dates for the

314   dehiscent fruit morph, being lowest on the fourth date for both morphs; neither morph had

315   dormant seeds on the last date. Dead and missing seed proportions were not different

316   between fruit morphs and among exhumation dates (Table 1; Fig. 3a-b).

317   The pool of seeds that were mantained in the laboratory (control) showed no

318   differences in the proportion of germinable, dormant or dead seeds between fruit morphs or

319   dates (Fig. 3 c-d; Supplementary material Table S2). The proportion of dormant seeds was

320   high for both fruit morphs during the whole experiment in the laboratory.

321   *Histological measurements of seed coat*

14

322   The mean height of the epidermis in the center of the lens did not showed significant

323   differences between seeds from both fruit morphs ($\bar{x} \pm$ S.E $= 71.22 \pm 3.84$ μm; $\bar{x} \pm$ S.E $=$

324   61.73 ± 5.09 μm, for seeds of the indehiscent and dehiscent fruit morph respectively;

325   F=2.22, *P*=0.1749). Also, no differences were found for the length of the epidermis at the

326   other two measured points (Left of the lens: Indehiscent morph $\bar{x} \pm$ S.E $= 82.89 \pm 2.75$ μm.

327   Dehiscent morph $\bar{x} \pm$ S.E $= 81.28 \pm 7.76$ μm; F=0.03; *P*=0.8782. Right of the lens:

328   Indehiscent morph $\bar{x} \pm$ S.E $= 84.99 \pm 3.58$ μm. Dehiscent morph $\bar{x} \pm$ S.E $= 79.4 \pm 7.96$ μm;

329   F=0.41; *P*=0.5397).

330   **Discussion**

331   Species that show different fruit morphologies might also differ in other

332   regenerative traits, such as dormancy and germination (Lu *et al.,* 2010; 2013; 2015; Baskin

333   *et al.,* 2014; Zang *et al.,* 2016). In this study, we analyzed PY break in field conditions of

334   two different fruit morphs of *Vachellia caven*. Consistent differences in the temporal

335   patterns of dormancy break were observed between seeds of both fruit morphs. Contrary to

336   our predictions, the seeds of the indehiscent morph started germination earlier and showed

337   a gradual increase in the percentage of emerged seedlings throughout the experiment.

338   Furthermore, the dehiscent morph showed a marked seedling emergence at the end of the

339   experiment. All in all, we observed that in seeds of both fruit morphs different signals

340   might be involved in breaking PY (Van Assche and Vanderlook, 2006). Therefore, the

341   existence of both fruit morphs might confer the species with different strategies a) to cope

342   with environmental heterogeneity; b) to reduce attack by seed predators through staggered

343 seed germination (Dalling *et al.,* 2011) or by preventing the attack by rodents, which cannot

344 easily detect buried impermeable seeds (Paulsen *et al.,* 2013).

345   Most seeds of the dehiscent morph germinated abruptly by the end of the

346 experiment after being in the soil for one year, showing that even though their seeds are

347 released early from the fruit, they may persist dormant in the soil until germination.

348 However, before germination occurred we did not observe a single environmental signal,

349 such as frost; certainly, temperatures at 5 cm in soil depth during winter were 5°C only on

350 two or three dates. If those temperatures had boosted dormancy break, a high number of

351 seedlings would have emerged during spring or summer (2014-2015; Fig. 2 a-b). Therefore,

352 one possible explanation for the high percentage of emerged seedlings observed in the

353 dehiscent fruit morph in June 2015 is that seeds from this fruit morph requires a period of

354 high temperatures and great thermal amplitude –December 2014 to March 2015; Fig. 2b-

355 followed by a decrease in temperature and thermal amplitude –from April 2015- in order to

356 break seed dormancy. Taylor (2005) proposed a two-stage softening model for other

357 Fabaceae species. This model includes a first preconditioning stage produced by constant or

358 fluctuating temperatures, and is accelerated by increasing temperatures and humidity

359 typical of summer. The seeds remain impermeable during this step; then there is the second

360 stage of softening, which is achieved by fluctuating temperatures of summer or autumn.

361 Seeds that achieve softening in autumn generally need lower temperatures during the final

362 stage, as our dehiscent morph. A similar mechanism was more recently described by Gama-

363 Arachchige *et al.* (2012) through the stepwise PY-breaking behavior of *Geranium*

364 *carolinianum*. Also, a sensitivity cycling, of alternating temperatures or alterations of wet-

365 dry conditions to dormancy break in seeds with PY has been proposed in previous studies

366 (Van Assche *et al.,* 2003; Baskin, 2003; Van Assche and Vanderlook, 2006; Jayasuriya *et*

367 *al.,* 2009).

368 The indehiscent fruit morph exhibited a gradual increase in the percentage of

369 emerged seedlings since July 2014 (three months after the start of the experiment). This

370 pattern seems to be related to a gradual increase in thermal amplitudes, at least on the first

371 three exhumation dates (Fig. 2a-b). However, the environmental variables recorded during

372 our burial experiment do not clearly support why seed germination still increased when

373 thermal amplitude decreased. Although there is no strong evidence to support this

374 phenomenon, it has been suggested that dormancy break could be mediated by

375 microorganisms, particularly when a low germination percentage with no clear seasonal

376 pattern is observed, similar to our indehiscent morph (Van Assche *et al.,* 2003). The

377 presence of a permanent number of non-dormant seeds might be a strategy to explore novel

378 habitats, with seeds ready to germinate in the new environment (Willis *et al*., 2014). The

379 fact that this morph has a fruit as a dispersal unit, which is prepared for being dispersed

380 longer distances (Aronson, 1992), might be complemented with their capacity to reach

381 novel environments with a number of seeds able to establish.

382 It is striking that most of the seeds of both morphs germinated in the beginning of

383 winter –i.e., the highest percentage of emerged seedlings of the indehiscent morph and

384 almost all the emerged seedlings of the dehiscent morph- when precipitation start to

385 decrease until spring. However, a previous study observed that this species has a high

386 germination at 20/10 °C (Venier *et al.,* 2017) and a high survival of seedlings under water

387 stress (Venier *et al.,* 2013), suggesting that seeds could survive with low water availability

388 in the environment and remain in the seedling stage until the rainy season. This may be a

389   strategy to ensure their establishment, capitalizing the resources mobilized by the first rains

390   of spring, when much of the annual vegetation has still not established –i.e., establishing *V.*

391   *caven* seedlings in a moment of low competition.

392   The seed softening process in species with PY may take place over periods ranging

393   from a few weeks to many years (Taylor, 2005). The species *V. caven* as a whole showed

394   dormancy break of its seeds in 14 months. This period is brief compared to other species

395   with PY (Van Assche *et al.,* 2003; Cuneo *et al.,* 2010; Marchante *et al.,* 2010) but there are

396   species that showed similar amount of time for breaking PY (Holmes and Moll, 1990) or

397   even lower (Gama-Arachchige *et al.,* 2012). The high field germination of *V. caven*

398   suggests that, after a disturbance, this species might recolonize new areas from the seeds

399   available in the soil only in the year after the disturbance occurs. However, studies using

400   different burial depths and climatic conditions are necessary to have a broader view of the

401   regeneration behavior of this species.

402   The proportion of germinable seeds (i.e., seeds that germinated in the laboratory

403   without scarification) of both morphs was low on the five exhumation dates, suggesting

404   that most of the seeds that break dormancy in the soil obtained a sufficient amount of water

405   to germinate under field conditions. In other Fabaceae (*Delonix regia*) the number of

406   germinable seeds after being buried was higher in a dry year than in a humid year

407   (Jaganathan *et al.,* 2017). Therefore, considering that our experiment was performed in a

408   humid year, the amount of germinable seeds could increase in drier years (precipitation

409   from April 2014 to April 2015 in our experiment was 1033.5 mm, whereas the historical

410   precipitation for the study area is 725.5 mm (CIMA-CONICET-UBA)). In addition, during

411   the burial experiment the proportion of dormant seeds was higher in the dehiscent than in

412   the indehiscent fruit morph, with no dormant seeds being recorded on the last exhumation

413   date for either morph. When the dormant seeds were scarified and set to germinate, we

414   observed that most of the seeds were viable. Additionally, in both morphs, in most of the

415   bags exhumed, a proportion of the seeds were dead. Probably, some of these dead seeds had

416   germinated and decomposed after germination long before the end of the experiment, so we

417   were not able to discriminate if they had germinated or simply died due to the action of

418   microorganisms (Dalling *et al.,* 2011). All in all, the amount of dead seeds was not different

419   between morphs and did not modify the patterns observed in emerged seedlings. Finally,

420   control seeds that were maintained in the laboratory while the burial experiment was

421   underway did not show an increase in the proportion of germinable seeds, which also

422   suggests the need for an environmental signal for breaking PY.

423   The different patterns of PY loss shown by seeds of both fruit morphs of *V. caven*,

424   would not be related to differences in the histological structure of the lens, since the results

425   of the measurements in the lens area showed no significant differences between them.

426   Nevertheless, other aspects of the epidermis, such as lignin concentration and how tightly

427   packed the cells are in the palisade layer, are important in determining whether passage of

428   water into the seed is blocked (Kelly *et al.,* 1992; Baskin *et al.,* 2000), and should be

429   incorporated in future studies.

430   In summary, in this study we observed two different strategies for breaking PY in

431   seeds associated with two different fruit morphs (dehiscent and indehiscent) in *V. caven*.

432   The presence of these two different PY break seed behavior, even within the same plant

433   individual, could be considered a bet-hedging strategy and therefore could confer the

434   species with higher possibilities of establishing and coping with environmental

435 heterogeneity (Venable, 2007; Jaganathan, 2016). Those characteristics may contribute to

436 the understanding of the success of this species in open and disturbed environments where

437 *V. caven* mainly occurs (Zak and Cabido, 2002), showing that PY has ecological

438 significance for species in plant communities.

439

451 **Conflicts of interest**

452 None

453

454 **Supplementary materials**

455 **Table S1.** Mean and standard error for the proportion of seeds of the different categories in

456 relation to morphs and exhumation dates.

457   **Table S2.** Statistics and *p* values for the control experiment separated by type of seeds

458   (germinable, dormant, dead).

459

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610

Figure references

Figure 1. Seed germination proportion of scarified (grey) and non-scarified (white) seeds of the dehiscent and indehiscent morph of *Vachellia caven,* in the laboratory before the burial experiment (April 2014).

Figure 2. Proportion of emerged seedlings from two morphs of *Vachellia caven.* Seeds were buried under field conditions and exhumed on five dates. (a) Germination proportion of seeds from indehiscent (white) and dehiscent (grey) morphs (mean ± S.E.). Different letters over each bar indicate significant differences in the post-hoc test for the interaction term (morph x date). (b) Temperatures and precipitation during the burial experiment. Lines represent absolute month maximun (dotted), minimum (dashed) and mean (continuous) temperatures (°C); barrs represent total monthly precipitation (mm).

Figure 3. Mean ± S.E. of the cumulative proportion of dormant (white), germinable (grey), dead (light grey) and missing (black) seeds, inside exhumed bags (a-b) and control seeds (maintained under laboratory conditions) (c-d), of the two morphs of *Vachellia caven* on five dates.

628 **Table 1.** Statistics (t values) and *P* values for the proportion of seeds under different

629 conditions in relation to morphs (dehiscent and indehiscent), exhumation date (five dates)

630 and their interaction. Statistics were obtained from Generalized Linear Models (see Table

631 S1 of the Supplementary material for means and standard error of each seed category on

632 each date). Significant p values (*P*≤0.05) are indicated in bold.

	Seed condition after exhumation									
	Emerged		Germinable		Dormant		Dead		Missing	
	t	*P*	t	*P*	t	*P*	t	*P*	t	*P*
Morph	6.776	**<0.0001**	4.090	**0.0027**	4.075	**0.0002**	1.461	0.151	1.380	0.188
Date	5.943	**<0.0001**	3.665	**0.0006**	4.495	**0.003**	1.017	0.314	2.099	0.053
Morph*Date	0.325	**<0.0001**	2.112	**0.0401**	0.288	0.109	0.892	0.3771	1.155	0.266

633