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Is fennel crop dependent on insect pollination?

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Highlights

- Fennel (*Foeniculum vulgare*) is of food and economical particular interest
- Crop production were compared between insect pollinated flowers and bagged flowers
- The fennel fruit set dropped by 92% when reproduction occurred without insect
- The fennel fruit weight and their yield in *trans*-anethole increased with insect pollination
- Insects are essential to sustain aromatic fennel production

15

Abstract

The importance of insects for global food crop production is starting to be well-known but the level of dependence on insects is less referenced, especially for some aromatic crops. Fennel (*Foeniculum vulgare*) is an aromatic mass-flowering crop cultivated in the southern of France for its essential oil that contains *trans*-anethole of food and economical particular interest since it is used to elaborate anise drinks. Here, we have characterized the dependence of fennel on insect pollination. We used four controlled pollination treatments to compare the reproductive success (fruit initiation rate, fruit weight), and the fennel yield (weight of the fruits and amount of *trans*-anethole at the crop level) between insect pollinated flowers and bagged flowers. We also checked the ability of reproduction by autogamy in this species, and the existence of a reproductive limitation linked to insect activity. We have shown that pollination without insect

27 leads to reproductive failure in fennel which is not an autogamous plant species. At the crop
28 level, insect pollination is fundamental to improve the fruit initiation rate, the weight of the
29 fruits and the amount of *trans*-anethole leading to an 92% dependence of fennel on insect
30 pollination. Finally, we showed no reproductive limitation with the current state of insect
31 activity. We are thus providing interesting clues to managers and producers to support the
32 production of fennel crops, showing once again that insects are essential to sustain global food
33 production.

34

35 **Keywords**

36 Fennel crops; Insect diversity and abundance; Fruit set; Fennel yield; *Trans*-anethole

37

38 **1. Introduction**

39 Animal pollination enhances the sexual reproductive success of most wild and cultivated
40 plants (Ollerton et al., 2011). While the importance of insects for crop yields, at least to some
41 extent, is currently well-known (Bartomeus et al., 2014; Garibaldi et al., 2017; Klein et al.,
42 2007; Williams, 2002), the question of the role of insects still arises for some major crops, such
43 as aromatic crops. Due to this dependence, agricultural production overall has been shown to
44 be reduced by three to eight percent without insect pollination (Gallai et al., 2009). Crop
45 dependence on insects is defined by the proportion of the decrease in yield when pollination
46 occurs without insects (Klein et al., 2007; Martin et al., 2019). Insect pollination is irrelevant
47 for reproduction in more than half of the leading global crops (for example, quinoa, lentils,
48 pepper, or vine grape; Klein et al. 2007). In contrast, some cultivated plants are highly
49 dependent (40-90%) on insects for their reproduction such as cucumber, avocado, almond, and
50 peach, and others ones are almost entirely dependent (more than 90%) such as melons,

51 zucchinis, and apples (Klein et al., 2007). In contrast, the yield of many others depends less on
52 insects with a decrease in production varying between 10-40% (eggplant, strawberry) and 0-
53 10% (tomato, papaya ; Klein et al. 2007), since some cultivated plants are also capable of self-
54 pollination or wind pollination (Culley et al., 2002). In the current context, where various
55 pressures are known to lead to a decline of insects, such as soil degradation, loss of habitats and
56 resources, the introduction of invasive species, and agricultural intensification (Sánchez-Bayo
57 and Wyckhuys, 2019), it is important to understand the level of dependence of crop yields on
58 insects.

59 Fennel (*Foeniculum vulgare*) is a generalist entomophilous plant (Schurr et al., 2021)
60 of particular economic interest that is cultivated in the southern of France. Fennel essential oil
61 (EO) contains an interesting organic compound with an anise flavor, *trans*-anethole, from the
62 phenylpropene family. This compound is mostly produced by the fruits of fennel (Salami et al.,
63 2016). To date, few authors have explored the importance of insects for the fruit set of fennel
64 crops (Chaudhary, 2006; Meena et al., 2015; Schurr et al., 2021). We also suspected that the
65 production of phytometabolite compounds was directly influenced by insect activity. Indeed,
66 self-pollination compared to cross- or open-pollination positively influences the production of
67 fennel essential oil and some of its secondary metabolites (Salami et al., 2016). The fennel
68 literature mentions that the plant species may be able to self-pollinate (Meena et al., 2015), but
69 protandry is also presumed (Meena et al., 2015; Németh et al., 1999). Furthermore, insect
70 pollination has been shown to improve fennel seed set (Meena et al., 2015; Salami et al., 2016)
71 and the production of essential oil (Schurr et al., 2021), but the question of the degree of
72 dependence of fennel reproduction on insects has not been explored. To sustainably support
73 fennel production, it is important to understand and quantify the role of insect pollination in the
74 reproduction of fennel with regard to the supposed protandry of this species.

75 In this study, we analyzed the dependence of fennel on insect pollination by comparing
76 fennel pollination with or without insects for (1) fruit initiation rate, (2) the weight of fruits and
77 (3) the fruit weight per hectare, and (4) the amount of *trans*-anethol retrieved from fruits per
78 hectare. Finally, we verified (a) whether fennel can be autogamous based on spontaneous
79 autogamy and controlled manual autogamy, (b) whether fennel can reproduce without insect
80 pollination by comparing free pollination and spontaneous autogamy, and (c) whether fennel
81 reproduction is limited by insect activity by comparing free pollination and controlled manual
82 saturation by allopollen added under open-pollination conditions.

83

84 **2. Materials and methods**

85 2.1. Study area and plant species

86 The fennel (*Foeniculum vulgare*, Apiaceae) populations used in this study were located
87 in southern France (“Plateau de Valensole”, Alpes-de-Haute-Provence; Appendix A). In this
88 area, fennel is cultivated for local anise spirit production. This species originally came from the
89 Mediterranean basin but has been naturalized in many regions around the world, and fennel is
90 now cultivated practically worldwide (Europe, Asia and North America; Badgujar et al. 2014).
91 This species can achieve 2.5 m in height and be annual, biennial or perennial (Piccaglia and
92 Marotti, 2001). Fennel has numerous small yellow flowers organized in umbellets forming
93 large-flat inflorescences named umbels. Each fertilized fennel flower may produce an oblong
94 to ovoid fruit, which is called a diachene. A diachene is composed of two achenes which each
95 can hold one seed. The studied variety is “*Jupiter*”, developed by the Pernod-Ricard France®
96 company. This variety is sown two times a year, resulting in two summer blooms. The first
97 bloom occurs at the end of June, and it is harvested in early August (the plants being sown in
98 the previous year). The second bloom occurs at the end of July until harvest in mid-September

99 (the plants being sown in March of the current year). Twelve crops were selected for our study
100 (Appendix A), including six from the first bloom and six from the second bloom.

101

102 2.2. Flower number estimation

103 For each of the 12 studied crops (six from the July bloom, six from the August bloom),
104 we counted the number of plants per m², three times, exhaustively (N = 36). We then estimated
105 the number of plants per hectare. Per crop, respectively 10 fennel plants from the crops
106 blooming in July, and 5 fennel plants from the crops blooming in August, were randomly
107 selected. The number of bloomed umbels was exhaustively counted on each selected plant (N
108 = 95 plants). We thus could estimate the number of umbels per hectare. On each plant, we
109 selected three umbels for which we exhaustively counted the number of umbellets per umbel
110 (N = 270 umbels). We then counted the number of flowers per umbellet on five umbellets for
111 each selected umbel (N = 1 350 umbellets) to obtain the mean number of flowers per umbellet.
112 Finally, the mean number of flowers per hectare was estimated.

113

114 2.3. Controlled pollination procedure and fruit set

115 To study the dependence of fennel on insects, we performed four controlled pollination
116 treatments. Two pollination treatments with insects and two without insects (bagged flowers) :
117 (1) “open pollination” [OP] (control): the flower left free to be visited by insects (no bagging);
118 (2) “open pollination + supplementation” [OPS]: no bagging and manual saturation by
119 allopollen from umbels of other fennel plants (at least three meters away, in the same crop); the
120 pollen was added by rubbing the treated umbel with another umbel; (3) “spontaneous
121 autogamy” [SA]: bagging of the umbel to avoid visits by insects and no manual pollen addition;
122 and (4) “manual autogamy” [MA]: bagging of the umbel and manual self-pollination with

123 pollen from another umbel from the same plant (*i.e.*, geitonogamy); the pollen was added by
124 rubbing the treated umbel with another umbel from the same plant.

125 From each of the 12 studied fennel crops, we randomly selected ten fennel plants to
126 apply each of the four pollination treatments. The plants were selected from crops at least five
127 meters from the field edge. On each plant, we selected four umbels at similar stages of
128 development (Appendix B) that were identified by a colored wool thread, and we applied either
129 OP, OPS, MA or SA pollination treatment. All flowers of each selected umbel were treated.
130 There were four treatments hosted on each plant. In total, 480 umbels were treated (120 per
131 treatment, of which 60 were from the July bloom, and 60 were from the August bloom). We
132 treated the fennel flowers from the start of the flowering of umbel outer flowers. We then
133 repeated the treatments at least three times and continued if needed every day until the end of
134 the flowering of the inner flowers. Using our estimation of the number of flowers per umbellet,
135 we estimated the number of pollinated flowers for each pollination treatment. The treated
136 umbels were then collected just before harvest: August 8-19 for the July bloom and September
137 6-17 for the August bloom. The rate of fruit initiation (%) was calculated by dividing the
138 number of initiated fruits by the estimated number of treated flowers per umbel. All fruits from
139 each collected umbel were separated from the umbel remnants by hand and then they were
140 weighed together for each umbel. The weight per fruit was then calculated by dividing this total
141 weight of the fruits per treated umbel by the number of initiated fruits. We then estimated the
142 fruit weight per hectare using our estimation of the number of umbels per hectare.

143

144 2.4. Phytometabolites content

145 Phytometabolites from the fruits of a subsample of 220 of the total treated umbels (N=51
146 for the SA treatment, N=52 for the MA treatment, N=57 for the OP treatment and N=58 for the
147 OPS treatment) were obtained from the dried fruits placed in an ultrasound bath for a few

148 minutes before extraction with cyclohexane solvent. For each treated umbel, some fruits (the
149 exact number was dependent on their mass) were selected to have a constant “*extraction*
150 *volume/test sample*” ratio corresponding to 0.1 g of fruit. The fruit extracts were then analyzed
151 using a gas chromatograph (GC, 6890N, Agilent Technologies®) coupled to a mass
152 spectrometer detector (MSD 5973, Agilent Technologies ®). We injected 2.5 µL of the
153 extraction solution into a capillary chromatographic column (HP5-MS, 30 m length, 0.25 mm
154 diameter, 0.25 µm film thickness, Agilent Technologies®). The molecules were separated using
155 a gradient temperature from 40°C to 280°C at a rate of 3°C min⁻¹ with a constant helium flow
156 of 1.0 mL min⁻¹. The accuracy and precision of the method were determined through replicate
157 analyses (dodecane). Molecule identification in chromatograms was achieved using spectral
158 libraries (NIST library), spectra from injected standards of high purity (>99%, Sigma Aldrich®,
159 France), and by comparison of our experimental and retention indices calculated using a
160 homologous series of C8–C20 *n*-alkanes. As standards, the monoterpenes α -pinene, γ -
161 terpinene, limonene, *trans*-anethole and 1,8-cineole were chosen to quantify the final
162 concentrations of the measured terpenoids.

163

164 2.5. Statistical analysis

165 To explain the fruit initiation rate, the fruit weight, the fruit weight per hectare, and the
166 amount of *trans*-anethol per hectare, based on the pollination treatments, we used linear mixed-
167 effects models with “log+1” transformations, including a random effect for the identity of the
168 experimental crops (lmer; R software version 4.0.2 2020-06-22 R Core Team 2020). The
169 significance of these models was tested with an anova test.

170 We then conducted *a priori* tests (contrast analysis) between (1) the SA and MA
171 treatments to determine if fennel can be autogamous, (2) the OP and SA treatment to test the

172 dependence of fennel reproduction on insect pollination, and (3) the OP and OPS treatments for
173 testing for pollen limitations due to a lack of insect activity. We tested the difference between
174 these treatments for fruit initiation rate, the weight of the fruit and the fruit weight per hectare,
175 and the amount of *trans*-anethol from the fruit per hectare using the “emmeans” package (R
176 software version 4.0.2 2020-06-22 (R Core Team, 2020).

177

178 **3. Results**

179 3.1. Flower number estimation

180 We found a mean of 19.87 ± 7.55 flowers per umbellet (21.52 ± 7.35 for the July bloom
181 and 16.57 ± 6.83 for the August bloom). We calculated a mean of 19.19 ± 6.54 umbellets per
182 umbel (22.06 ± 5.62 in July and 13.43 ± 3.96 in August) and a mean of 7.74 ± 4.21 umbels per
183 plant (6.07 ± 1.26 in July and 11.10 ± 5.81 in August). Finally, we estimated approximately
184 $121,100 \pm 49,000$ plants per hectare ($118,900 \pm 37,900$ in July and $111,300 \pm 50,000$ in August).
185 We then estimated $357,402,796 \pm 10,185,981$ fennel flowers per hectare ($342,624,426 \pm$
186 $1,972,575$ for the July bloom and $274,926,539 \pm 7,857,095$ for the August bloom).

187

188 3.2. Fruit initiation rate

189 We found an initiation rate of fennel fruits of 37.65 ± 20.71 for insect pollination
190 treatment compared to 5.97 ± 10.41 for the treatment of pollination without insect ($38.70 \pm$
191 19.63 for the OP treatment, 36.60 ± 21.78 for the OPS treatment, 6.41 ± 9.57 for the MA
192 treatment, and 5.53 ± 11.22 for the SA treatment). The initiation rate of the fennel fruits dropped
193 by 92% when pollination occurred without insect. The fennel fruit initiation rate for the MA
194 and SA treatments was close to 0%.

195 The linear mixed-effects model explaining the variation of the fruit initiation rate
196 according to pollination treatments has shown a difference between treatments (F value =
197 247.640, p-value < 0.001). The contrast analysis showed no difference between the SA and MA
198 treatments (E= 0.139, p = 0.243). However, there was a significantly higher fruit initiation rate
199 for the OP treatment than for the SA treatment (E= 2.463, p < 0.001; Fig. 1). Finally, there was
200 a significant difference in the fruit initiation rate between the OP and OPS treatments (E= 0.260,
201 p = 0.027).

202

203 Fig. 1. Fennel fruit initiation rate (%) with a log+1 transformation for open pollination (OP; red dots) and for
204 spontaneous autogamy (SA; blue dots) - E= 1.291, p < 0.001. The vertical bars represent the 95% confidence
205 interval.

206

207 3.4. Fruit weight

208 The linear mixed-effects model explaining the weight of the fruits according to
209 pollination treatments has shown a difference between treatments (F value = 5.945, p-value <
210 0.001). The contrast analysis showed no difference in the weight of the fruits between the SA
211 and MA treatments (E = - 0.001, p = 0.133) or between the OP and SA treatments (E = 0.001,
212 p = 0.121). Finally, there was a significant difference in the fruit initiation rate between the OP
213 and OPS treatments (E = 0.001, p < 0.001).

214

215 3.5. The weight of the fruit per hectare

216 The linear mixed-effects model explaining the variation of the fruit initiation rate
217 according to pollination treatments has shown a difference between treatments (F value =

218 119.380, p -value < 0.001). The contrast analysis showed no difference between the SA and MA
219 treatments for the weight of the fruits per hectare ($E = 0.058$, $p = 0.781$). We found that the
220 weight of the fruits per hectare was significantly greater for the OP treatment than the SA
221 treatment ($E = 2.918$, $p < 0.001$; Fig. 2). Finally, there was a significant difference in the fruit
222 initiation rate between the OP and OPS treatments ($E = 0.481$, $p = 0.012$).

223

224 Fig. 2. Difference in the weight of the fennel fruits per hectare (kg/ha) with a log+1 transformation between the
225 open pollination (OP; red dots) and the spontaneous autogamy (SA; blue dots) - $E = 2.918$, $p < 0.001$

226

227 3.6. Amount of anethole per hectare

228 The linear mixed-effects model explaining the variation of the fruit initiation rate
229 according to pollination treatments has shown a difference between treatments (F value =
230 20.837, p -value < 0.001). The contrast analysis showed no difference between the SA and MA
231 treatments for the amount of anethole per hectare ($E = - 0.043$, $p = 0.866$). We found that the
232 amount of anethole per hectare was significantly greater for the OP treatment than the SA
233 treatment ($E = 1.443$, $p < 0.001$; Fig. 3). There was no difference between the OP and OPS
234 treatments in the amount of anethole per hectare ($E = 0.173$, $p = 0.474$).

235

236 Fig. 3. Difference between open pollination (OP; red dots) and spontaneous autogamy (SA; blue dots) for the
237 weight of the fruits per hectare (kg/ha) - $E = 1.443$, $p < 0.001$. The vertical bars represent the 95% confidence
238 interval.

239

240 4- Discussion

241 This study analyzed the degree of dependence of fennel on pollination by insects. This
242 is the first study linking the presence of insects to fruit set and to a metabolite of high economic
243 value: *trans*-anethole. Our results suggest that most of the time, spontaneous and manual
244 autogamy lead to reproductive failure. From a yield point of view, insect pollination is
245 fundamental to improve the number and the weight of fruits and the amount of *trans*-anethole
246 per hectare. Finally, these results showed reproductive limitations when there is a lack of insect
247 activity in fennel crops. Our results provide some clues to support and sustain the production
248 of fennel crops, and we believe that these results will be of interest to fennel managers and
249 producers.

250

251 4.1. Fennel dependence on insect pollination

252 Our results confirmed those of previous studies showing a positive influence of insects
253 on crop yield (Garibaldi et al., 2016, 2013; Geslin et al., 2017; Woodcock et al., 2019), and
254 especially for fennel seeds yield (Salami et al., 2016). There was no difference between
255 spontaneous and manual autogamy. Both of which generally led to reproductive failure
256 suggesting that *F. vulgare* self-pollinates poorly. This implies that fennel protandry could be a
257 barrier to reproduction success in spontaneous as manual autogamy. By contrast, insects by
258 visiting a lot of flowers at different stages thus may counter the problems linked to the protandry
259 of fennel flowers and optimize the reproduction. Moreover, insect pollinated flowers showed
260 better number of fruits and amount of anethole per hectare compared to bagged flowers. We
261 can conclude that fennel is highly dependent on insects for reproduction. Many plant species
262 rely heavily on insects for reproduction, both in wild species with implications for conservation
263 (Schurr et al., 2019) and cultivated species with implications for global food sustainability
264 (Gallai et al., 2009; Geslin et al., 2017, 2016; Klein et al., 2007). Klein et al. (2007) classified
265 fennel, based on the literature, among highly insect dependent crops (40-90%) for seed

266 production We have shown that dependence degree could be superior (92% of reduction in the
267 number of fruits without insect). Nevertheless, there were still a small number of initiated fruits
268 in self-pollination treatments. We can propose the hypothesis that protandry in fennel could be
269 more or less expressed at different levels in a plant (umbellets, umbels, plants). This could also
270 result from minimal but possible wind pollination (Klein et al., 2007) or from insects infiltrating
271 in the bags without being noticed (thrips). Especially since, interestingly, the weight of the fruits
272 was similar between open pollination and self-pollination, meaning that when a fruit is initiated,
273 the quality of the fruit is consistent regardless of the mode of pollination. However, at the crop
274 level, we found a drop in the weight of the fruits per hectare in spontaneous and manual
275 autogamy.

276

277 4.2. Reproduction limitation by insect activity

278 The initiation rate of the fruits was similar between open pollination and open
279 pollination with allopollen supplementation. This is also the case of the amount of anethole
280 produced per hectare. We expected an increase of these parameters with open pollination with
281 allopollen supplementation. It seems that in our system, the fennel does not suffer any pollen
282 limitation. Since the reproductive success of plants is linked to the amount of pollen received
283 (Aizen and Harder, 2007), a greater abundance of insects could lead to pollen saturation of
284 fennel flowers. This explanation would not be surprising given the great abundance and
285 diversity of insects along fennel crops (Schurr et al. 2021) and within fennel flowers (Bharti et
286 al., 2015; Chaudhary, 2006; Kumar and Rai, 2020; Meena et al., 2016, 2015; Ricciardelli
287 D'Albore, 1986; Salami et al., 2016; Shilpa et al., 2014; Skaldina, 2020). However, not all
288 insect species may be equal in their ability to carry a large amount of pollen or higher quality
289 pollen, two other important factors in pollination (Aizen and Harder, 2007; Ollerton et al.,
290 2011). Insect diversity could therefore be more important than insect abundance for the

291 reproduction of fennel with regard to the complementarity hypothesis of functional diversity
292 (Frund et al., 2013; Gagic et al., 2015; Schurr et al., 2021; Woodcock et al., 2019). Nevertheless,
293 the fruit weight, and the weight of the fruit per hectare appears to be higher for open pollination
294 treatments without manual supplementation. As we used different donors, the possibility of
295 inbreeding or incompatibility issues are excluded. We propose three explanations for this result.
296 First, the supplementation technique used might not differentiate the quantity and quality or
297 maturity of pollen transported, and either of the two factors could have a greater effect on fennel
298 reproduction. But this explanation is closely dependent on the used number of different pollen
299 donors' plants and flowers, pollen quality increasing with the increase in genetically different
300 donors. Second, manual pollen saturation of flowers could lead to the obstruction of the not yet
301 mature stigma, which would imply failure of fruit formation for one of the two available seeds.
302 Seed set is not necessary maximised with the highest pollen deposition (Ornelas and Lara,
303 2009). Finally, hand manipulation could damage some parts of the flower, and if the style is
304 damaged it can impact the fruit formation (Sáez et al., 2014), or leading the plant to allocate its
305 energy to repair the flowers rather than to the fruit development.

306

307 4.3. Amount of *trans*-anethole

308 Reproduction of fennel is important because the fruits are the part of the plant containing
309 the greatest amount of essential oil (Salami et al., 2016). Fennel is cultivated for its essential oil
310 that contains *trans*-anethole, which is used to elaborate anise drinks. The positive effect of
311 insects on crops regarding quantity and quality of seeds and fruits and by extension their
312 economic value, has already been shown (Garibaldi et al., 2013; Woodcock et al., 2019). This
313 has also been demonstrated for the amount of essential oil and some metabolic contents of
314 certain plants, such as mint and fennel (Nazem et al., 2019; Schurr et al., 2021). The literature
315 has previously reported discrepancies about the effect of insects on fennel essential oil yield.

316 Indeed, insect diversity had positive influence on the amount of essential oil produced by fennel
317 (Schurr et al., 2021). Nevertheless, other authors found that cross-pollination by insects had a
318 negative effect on fennel essential oil yield compared to self-pollination (Salami et al., 2016).
319 The variability of results may be due to geographically contrasting insect communities, leading
320 to variations in pollination efficiency. It could definitively also be due to varietal differences in
321 fennel. This study showed that the amount of *trans*-anethole from fennel fruit per hectare highly
322 increased with insect pollination vs self-pollination. Insects are thus essential to fennel crop
323 production.

324

325 **5. Conclusion**

326 Here is the first study compared the fennel reproductive success and the subsequent
327 yield in the amount of *trans*-anethole between bagged and unbagged fennel flowers. We showed
328 that the fennel crops are highly dependent on insects for fruit formation, weight and anethole
329 production. In agricultural landscapes, insects are more and more threatened due to pesticide
330 use and landscape uniformization (Brittain and Potts, 2011; Hendrickx et al., 2007; Kleijn et
331 al., 2009; Vanbergen, 2021). The preservation of insects can no longer be ignored in agro-
332 ecosystems and should be considered in landscape managements. This study proves once again
333 that insects are essential for us to sustainably maintain the quality and diversity of global food
334 production. In this sense, protecting insect, and biodiversity globally also means enhancing
335 livelihood and allowing food security which are the key challenges of our century (Garibaldi et
336 al., 2016; Kremen and Merenlender, 2018).

337

338 **Declaration of competing interest**

339 The authors declare no conflict of interest. The funders had no role in the design of the study;
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356

357 **Appendices**

358 Appendix A: The 12 studied fennel crops location and elevation in Alpes-de-Haute-Provence
359 (France).

360

361 Appendix B: Stages of the fennel umbels' development used to choose the moment of bagging,
362 and of pollination treatments.

363

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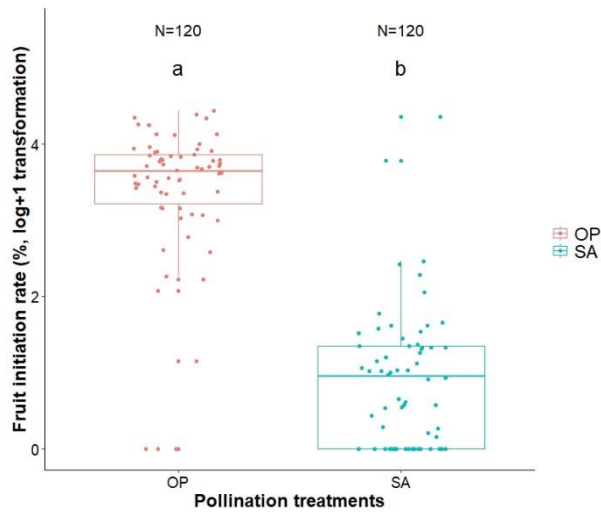
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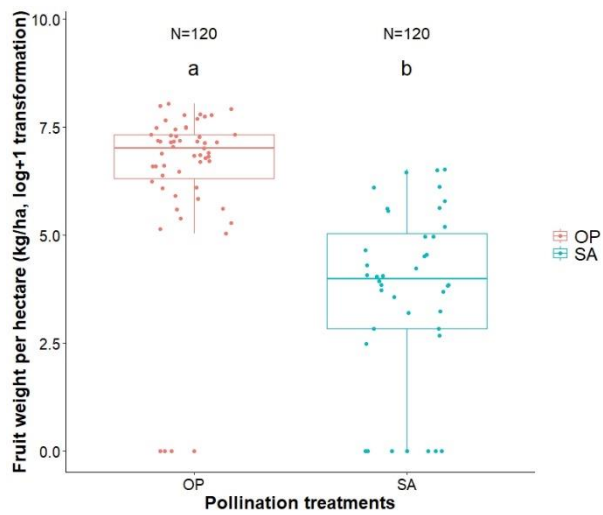
507 **Figures**



508

509 Fig. 1. Fennel fruit initiation rate (%) with a log+1 transformation for open pollination (OP; red dots) and for
510 spontaneous autogamy (SA; blue dots) - $E= 1.291$, $p < 0.001$. The vertical bars represent the 95% confidence
511 interval.

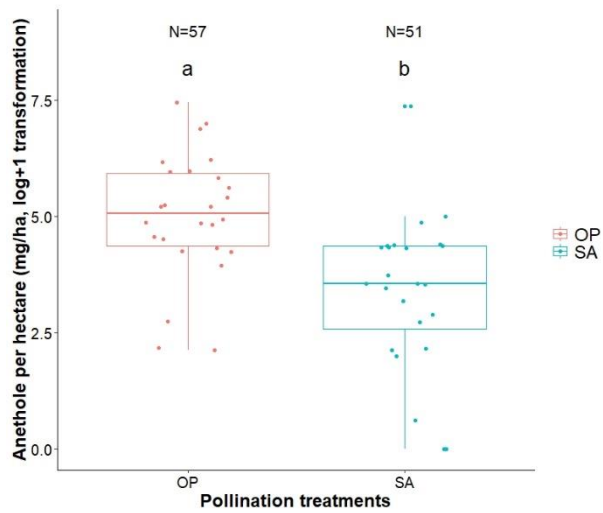
512



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514 Fig. 2. Difference in the weight of the fennel fruits per hectare (kg/ha) with a log+1 transformation between the
 515 open pollination (OP; red dots) and the spontaneous autogamy (SA; blue dots) - $E = 2.918$, $p < 0.001$

516



517

518 Fig. 3. Difference between open pollination (OP; red dots) and spontaneous autogamy (SA; blue dots) for the

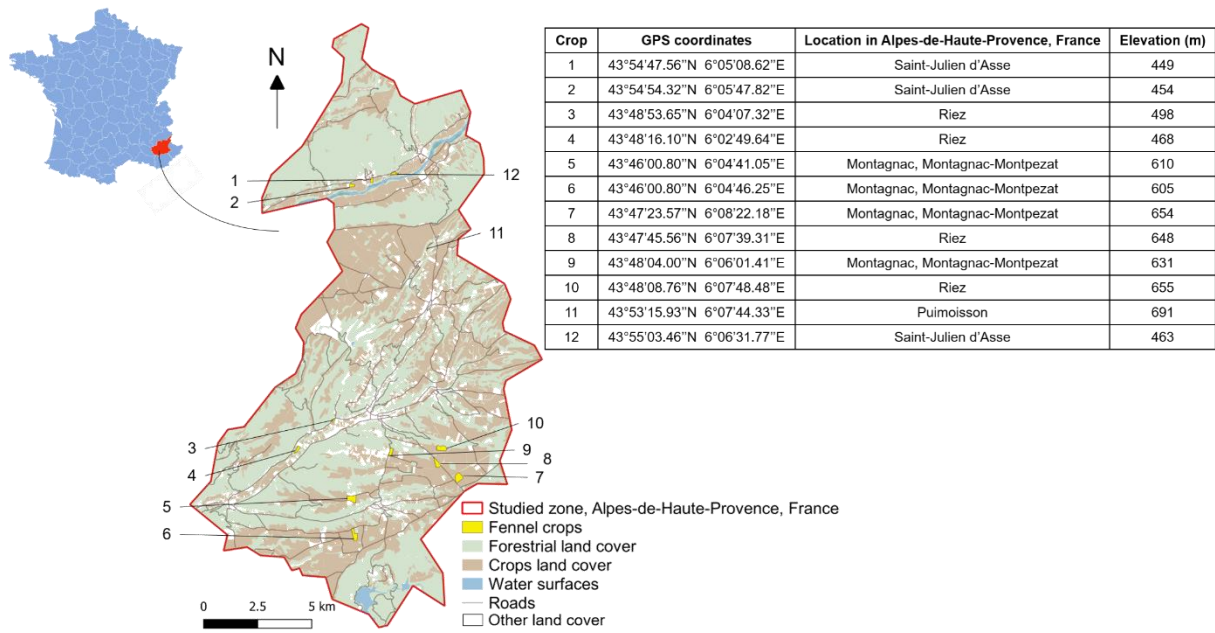
519 weight of the fruits per hectare (kg/ha) - $E = 1.443$, $p < 0.001$. The vertical bars represent the 95% confidence

520 interval.

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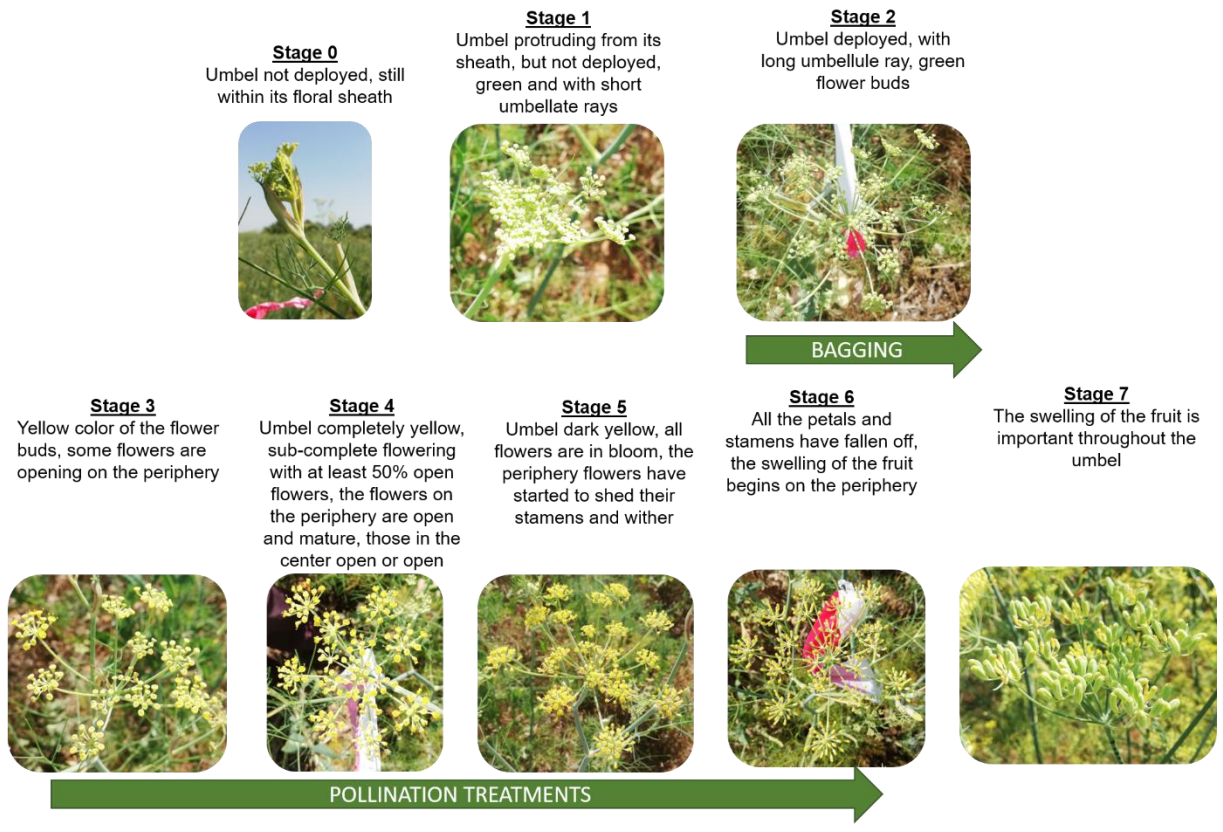
523 **Appendices**



524

525 Appendix A: The 12 studied fennel crops location and elevation in Alpes-de-Haute-Provence
 526 (France).

527



528

529 Appendix B: Stages of the fennel umbels' development used to choose the moment of bagging,
 530 and of pollination treatments.