



To what extent is fennel crop dependent on insect pollination?

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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*, Mill.) is an aromatic mass-flowering crop cultivated in the south of France for its essential oil that contains anethole. This compound is of food and economical interest because of its anise flavour, which 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 (fruit weight and amount of anethole per hectare) between insect pollinated flowers and bagged flowers. We also checked the ability of reproduction by self-pollination in this species, and the existence of a reproductive limitation linked to insect activity. We have shown that pollination without insects leads to reproductive failure in fennel. At the field level, insect pollination is fundamental to improve the fruit initiation rate, the fruit weight and the amount of anethole per hectare leading to an estimated 86 % dependence of fennel on insect pollination. Finally, we showed no reproductive limitation with the current state of insect activity. This study adds new evidence of the importance of insects in agroecosystems to sustain food production.

1. Introduction

Animal pollination enhances the sexual reproductive success of most wild and cultivated plants (Ollerton et al., 2011). While the importance of insects for crop yields is currently well-known for numerous systems (Bartomeus et al., 2014; Garibaldi et al., 2017; Klein et al., 2007; Williams, 2002), the question of the role of insects still arises for some major crops, such as aromatic ones. Agricultural production overall has been shown to be reduced by three to eight percent without insect pollination (Gallai et al., 2009). Crop dependence on insects is defined by the proportion of the decrease in yield when pollination occurs without insects (Klein et al., 2007; Martin et al., 2019). Insect pollination is irrelevant for reproduction in more than half of the leading global crops (for example, quinoa, lentils, pepper, or vine grape; Klein et al., 2007). And the yield of many others few depends on insects with a decrease in production varying between 0 % and 10 % (tomato, papaya; Klein et al., 2007), and 10–40 % (eggplant, strawberry), since some cultivated plants

are also capable of self-pollination or wind pollination (Culley et al., 2002). In contrast, some cultivated plants are highly dependent (40–90 %) on insects for their reproduction such as cucumber, avocado, almond, and peach, and others ones are almost entirely dependent (more than 90 %) such as melon, zucchini, and apple (Klein et al., 2007). In the current context, where various pressures are known to lead to a decline of insects, such as soil degradation, loss of habitats and resources, introduction of invasive species, and agricultural intensification (Wagner, 2020), it is important to understand the level of dependence of crop yields on insects.

Seed spices are very important crops in the world with a particular interest in the medical, pharmaceutical, chemical, food and agribusiness industries. The FAO estimated the anises, star anise, fennel and coriander production at almost 2 274,700 billion tones in 2020 in the main producing countries (especially numerous in the South of Asia) not including the countries with smaller production (as France). In Europe, fennel production represents more than 600,000 tons per year, produced

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in Italy, France, Spain, Germany, and the Netherlands. The fennel production in France accounts for a few thousand tons a year, mainly cultivated in the South of France (CTIFL¹), a part of the Mediterranean basin biodiversity Hot Spot (Médail and Quézel, 1999). Fennel (*Foeniculum vulgare* Mill.) is a generalist entomophilous plant (Chaudhary, 2006; Koul et al., 1996; Kumar and Singh, 2017a; Schurr et al., 2021) cultivated for its essential oil which contains an interesting organic compound with an anise flavor: the *trans*-anethole from the phenylpropene family. This compound is mostly produced by the fennel fruits (Koul et al., 1996; Salami et al., 2016). The fennel literature mentions that the plant species may be able to self-pollinate (Meena et al., 2015; Shojaiefar et al., 2021), even if protandry (i.e. the development of the male reproductive part of a flower earlier than the female reproductive part) is also shown (Meena et al., 2015; Németh et al., 1999). Studies on the production of fennel fruits and essential oil of the progenies from self-pollinated seeds compared to open pollinated seeds had already been done but results are inconsistent. It was shown that progenies from self-pollinated seeds produced more essential oil than from cross-pollination (Salami et al., 2016) but Shojaiefar et al. (2021) have rather shown a decrease in the fruit yield and the amount of essential oil in progenies from self-pollinated seeds. Thus, it appears to be essential to further explore the effect of the reproduction type on the crop production. To date, few authors have explored the direct influence (not on progeny) of insects on the fruit and seed set of fennel plants. Klein et al. (2007) classified fennel among highly insect dependent crops (40–90 %) for seed production; moreover, insect pollination has been shown to directly improve fennel seed set (Chaudhary, 2006; Kumar and Singh, 2017b; Meena et al., 2015) and had been linked to the production of essential oil (Schurr et al., 2021). For example, Chaudhary et al. (2006) found a decrease of 82 % of the seed weight per plant in pollination without insects compared to insect pollination. However, studies exploring the degree of dependence on insects for fennel reproduction are still scarce, especially field studies involving a high richness of insect. More investigations are then needed to better understand the reproductive fennel system within real-world crop fields. To sustainably support fennel production, it is important to understand and quantify the role of insect pollination in the reproduction of fennel with regard to the protandry of this species.

In this study, using bagged flowers in a large field-experiment, we analyzed the dependence of fennel on insect pollination by comparing fennel pollination with (observed fennel visitor) or without insects on (1) the fruit initiation rate and (2) the fruit weight per umbel. In addition to previous studies, we also focused on the fennel reproductive success and on the yield (at the field level) by comparing pollination treatments with or without insects on (3) the fruit weight per hectare, and (4) the amount of anethole per hectare immediately retrieved from fruits. Finally, we verified (a) whether fennel can be self-pollinated based on spontaneous and manual self-pollination, (b) whether fennel can reproduce without insect pollination by comparing open pollination and spontaneous self-pollination, and (c) whether fennel reproduction is limited by insect activity by comparing open pollination and controlled manual saturation by allopollen added under open-pollination conditions.

2. Materials and methods

This experimentation was conducted between June and September 2019.

2.1. Study area and plant species

The study area was in southern France (“Plateau de Valensole”,

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

2.2. Flower number estimation

For each of the 12 studied fields, we randomly selected three separate 1 square meters. In each square meter, we counted the number of plants ($N = 36$). We then calculated the mean number of plants per square meter and extrapolated the number of plants per hectare by multiplying this mean by 10,000. Per field, respectively ten fennel plants from the field blooming in July, but five fennel plants from the field blooming in August (this was intended to reduce the time spent in the field, as we noticed that five plants were enough for the estimation of flower number), were randomly selected ($N = 90$ plants). During the flowering peak, the number of bloomed umbels (randomly selected between primary and secondary umbels and between different sizes) was exhaustively counted on each selected plant and then averaged. We thus could estimate the number of umbels per hectare. On each plant, we selected three umbels for which we exhaustively counted the number of umbellets per umbel ($N = 270$ umbels). We then counted the number of flowers per umbellet on five umbellets for each selected umbel ($N = 1350$ umbellets) to obtain the mean number of flowers per umbellet. Finally, the mean number of flowers per hectare was estimated for each fennel type (July and August bloom).

2.3. Controlled pollination procedure and fruit set

To study the dependence of fennel on insects, we performed four controlled pollination treatments. Two pollination treatments with insects and two without insects (bagged flowers): (1) “open pollination” [OP] (control): the flower left free to be visited by insects (no bagging); (2) “open pollination + supplementation” [OPS]: no bagging and manual saturation by allopollen from umbels of other fennel plants (at least three meters away, in the same field); the pollen was added by rubbing the pollinated umbel with another umbel; (3) “spontaneous self-pollination” [SSP]: bagging of the umbel to avoid visits by insects and no manual pollen addition; and (4) “manual self-pollination” [MSP]: bagging of the umbel and manual self-pollination with pollen from another umbel from the same plant (i.e., geitonogamy); the pollen was added by rubbing the pollinated umbel with another umbel from the same plant.

From each of the 12 studied fennel fields, we randomly selected ten fennel plants to apply each of the four pollination treatments per plant. The plants were selected at least five meters from the field edge. On each plant, we selected four secondary umbels at similar stages of development (bagged or marked “stage 2”, when umbel deployed, with long umbellets ray, and green flower buds, and pollinated at the beginning of the flowering until the end “stages 3–6”, Appendix B) and similar sizes, that were identified by a colored wool thread, and we applied either OP,

¹ <https://memento.ctifl.fr/fiche/legumes/fenouil> (website visited on May 20, 2022)

Table 1

Number of flowers per umbellet, umbellets per umbel, umbels per plant and plant per m² for the two fennel bloom and the average between both.

Number	July bloom (mean ± SE)	August bloom (mean ± SE)	Average (mean ± SE)
Flowers per umbellet	21.52 ± 7.35	16.57 ± 6.83	19.87 ± 7.55
Umbellets per umbel	22.06 ± 5.62	13.43 ± 3.96	19.19 ± 6.54
Umbels per plant	6.07 ± 1.26	11.10 ± 5.81	7.74 ± 4.21
Plant per 1 m ² square	11.89 ± 3.79	11.13 ± 13.28	12.11 ± 4.90
Flowers per hectare	342,624,426 ± 1,972,575	274,926,539 ± 7,857,095	357,402,796 ± 10,185,981

OPS, MSP or SSP pollination treatment. All flowers of each selected umbel were pollinated. There were four treatments hosted on each plant. In total, 480 umbels were pollinated (120 per treatment, of which 60 were from the July bloom, and 60 were from the August bloom). Fennel has a flowering shift within its umbels from outside to inside. We suspected that the receptivity of flowers at the umbel scale extended over several days. We pollinated the fennel flowers from the start of the flowering of the umbel's outer flowers. We then repeated the treatments at least three times and continued if needed every day until the end of the flowering of the umbel's inner flowers. We pollinated each umbel at different times of the day, for example, umbels pollinated in the morning on day one were pollinated in the afternoon on day two and in the middle of the day on day three, etc. We rubbed the pollinated umbel with another umbel ensuring that the stamens of the pollinating umbel touch each flower of the pollinated umbel for a few seconds. For each umbel, we counted the number of umbellets. Using our estimation of the number of flowers per umbellet for each fennel type (July of August bloom; see details Table 1.), we then estimated the number of pollinated flowers for each pollination treatment. The pollinated umbels were then collected just before harvest by farmer: August 8–19 for the July bloom and September 6–17 for the August bloom. The rate of fruit initiation (%) was calculated for each pollinated umbel by dividing the number of initiated fruits by the estimated number of pollinated flowers per umbel. This fruit initiation rate was then analysed by treatments without separating rates by field or by individual. All fruits from each collected umbel were separated from the umbel remnants by hand and then they were weighed together for each umbel. The weight per fruit was then calculated by dividing this total weight of the fruits per pollinated umbel by the number of initiated fruits. We then estimated the fruit weight per hectare using our estimation of the number of umbels per hectare.

2.4. Phytochemical content

Because of time and financial limits, we chose a subsample of fruits from 240 of the total pollinated umbels (N = 60 per treatment). Phytochemicals (i.e. chemical compounds made by plants) from the fruits were obtained from the dried fruits (left in the open air then placed for 24 h in an oven at 40 °C). For each pollinated umbel, some fruits (the exact number was dependent on their mass) were selected to have a constant "extraction volume/test sample" ratio corresponding to 0.1 g of fruit. For extraction, after preliminary tests, and following method used in Rivoal et al. (2010), fruits were put for 30 min in cyclohexane solvent with ultrasound bath, then filtered immediately. The fruit extracts were then analyzed using a gas chromatograph (GC, 6890 N, Agilent Technologies®) coupled to a mass spectrometer detector (MSD 5973, Agilent Technologies®). The fruit extracts were then analyzed using a gas chromatograph (GC, 6890 N, Agilent Technologies®) coupled to a mass spectrometer detector (MSD 5973, Agilent Technologies®). We injected 2.5 µL of the extraction solution into a capillary chromatographic column (HP5-MS, 30 m length, 0.25 mm diameter, 0.25 µm film thickness,

Agilent Technologies®, split 90/10). The molecules were separated using a gradient temperature from 40 °C to 280 °C at a rate of 3 °C.min⁻¹ with a constant helium flow of 1.0 mL.min⁻¹. The accuracy and precision of the method were determined through replicate analyses (dodecane). Molecule identification in chromatograms was achieved using spectral libraries (NIST library), spectra from injected standards of high purity (> 99 %, Sigma Aldrich®, France), and by comparison of our experimental and retention indices calculated using a homologous series of C8–C20 *n*-alkanes. As standards, the monoterpenes compounds α-pinene, γ-terpinene, limonene, anethole and 1,8-cineole were quantified in mg / g of fruit. As we had the fruit weight, the number of fruits per umbel, and the number of umbels per hectare, we then could extrapolate the amount of anethole per hectare produced, in mg per hectare.

2.5. Statistical analysis

All the statistical analysis were conducted using the R software (version 4.0.2 2020–06–22; R Core Team, 2020). The NA values were removed before analyses.

To explain differences in fruit initiation rate per umbel (proportion; response variable) we used the pollination treatments and fennel types (July or August bloom) as predictor variables. We used a generalized linear mixed-effects model with binomial error distribution (glmer) including a random effect for the identity of the experimental field and plant, adding the argument "weight" in the "glmer" function that considers the total number of flowers per umbel using the package "lme4" (Bates et al., 2011). To explain differences in the fruit weight per umbel, the fruit weight per hectare and the amount of anethole from fruit per hectare (response variables), we used the pollination treatments and fennel types (July or August bloom) as predictor variables. We then used three separate linear mixed-effects models for each response variable including a random effect for the identity of the experimental field and plant (using the package "lme4" and "lmerTest" to obtain p-values; Kuznetsova et al., 2015). To explain differences in the fruit weight per umbel, we added the argument "weight" in the "glmer" function that considers the total number of fruits per umbel. To explain differences in the fruit weight per hectare and the amount of anethole per hectare, we added the argument "weight" in the "glmer" function that considers the estimation of the number of fruits per hectare. If the models did not fit well, and residuals had non-normal distribution, we used a "log+ 1" transformation (i.e., for the response variable of the amount of anethole per hectare). The significance of models was tested with a Wald test using the Anova function in the "car" package (Fox and Weisberg, 2019). We then realized post-hoc tests for each of response variable model to compare treatments using general linear hypotheses based on the "Tukey" test ("glht" function in the "multcomp" package; Hothorn et al., 2016).

3. Results

3.1. Flower number estimation

Using the mean of (1) flowers per umbellets, (2) umbellets per umbel, (3) umbel per plants and (4) plants per hectare, we estimated (a) the number of fennel flowers per umbel at 381 flowers, and (b) the number of flowers per hectare at almost 360 million (343 million for the July bloom, and 275 million for the August bloom). Details of estimations are available in the Table 1.

3.2. Fruit initiation rate

We found an initiation rate of fennel fruits of 35.65 ± 20.97 % (mean ± SD) for insect pollination treatments: 38.70 ± 19.63 % for the OP treatment and of 36.29 ± 21.95 % for the OPS treatment, compared to 4.98 ± 10.20 % for the treatments of pollination without insects: 6.06 ±

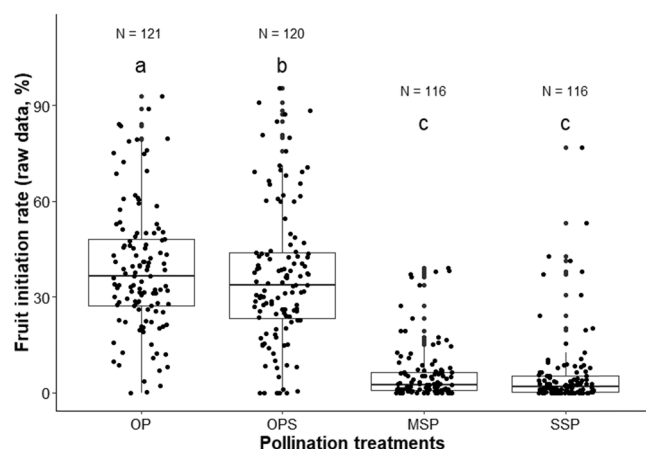


Fig. 1. Fennel fruit initiation rate (raw data, %) according to pollination treatments (OP = open pollination; OPS = open pollination + supplementation; MSP = manual self-pollination and SSP = spontaneous self-pollination). Black points represent each data. The vertical bars represent 1.5 times the interquartile range over the 25/75th percentile. Letter N represents the number of values after NA removal. Letters a, b and c represent the significance of the difference between treatments according to the general linear hypotheses from the generalized linear mixed-effects model with binomial error distribution (glmer) including a random effect for the identity of the experimental field and plant, and the total number of flowers per umbel in the argument “weight”.

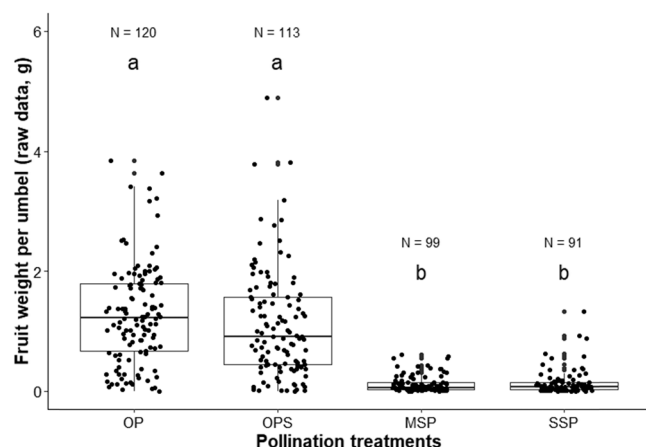


Fig. 2. Fennel fruit weight per umbel (raw data, g) according to pollination treatments (OP = open pollination; OPS = open pollination + supplementation; MSP = manual self-pollination and SSP = spontaneous self-pollination). Black points represent each data value. The vertical bars represent 1.5 times the interquartile range over the 25/75th percentile. Letter N represents the number of values after NA removal. Letters a and b represent the significance of the difference between treatment according to the general linear hypotheses from the linear mixed-effects model (lmer) including a random effect for the identity of the experimental field and plant, and the total number of fruits per umbel in the argument “weight”.

9.01 % (mean $\pm$ SD) for the MSP treatment, 5.79 $\pm$ 11.56 % for the SSP treatment. The initiation rate of the fennel fruits dropped by 86 % when pollination occurred without insects.

The generalized linear mixed-effects model explaining the differences in the fruit initiation rate according to pollination treatments and fennel types (July or August bloom) has shown no differences between fennel types (Chisquare = 0.09, df = 1, p-value = 0.76) but a difference between treatments (Chisquare = 25,313.20, df = 3, p-value < 0.001).

The general linear hypotheses showed a better fruit initiation rate for OP treatment than OPS (OPS - OP: Estimate = - 0.04 %; p-value = 0.03), MSP (MSP - OP: Estimate = - 2.51 %; p-value < 0.001), and SSP

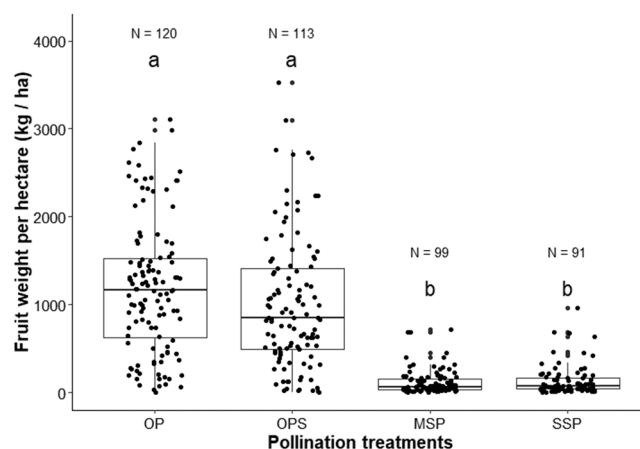


Fig. 3. Fennel fruit weight per hectare (raw data, kg / ha) according to pollination treatments (OP = open pollination; OPS = open pollination + supplementation; MSP = manual self-pollination and SSP = spontaneous self-pollination). Black points represent each data value. The vertical bars represent 1.5 times the interquartile range over the 25/75th percentile. Letter N represents the number of values after NA removal. Letters a and b represent the significance of the difference between treatment according to the general linear hypotheses from the linear mixed-effects model (lmer) including a random effect for the identity of the experimental field and plant, and the estimation of the number of fruits per hectare in the argument “weight”.

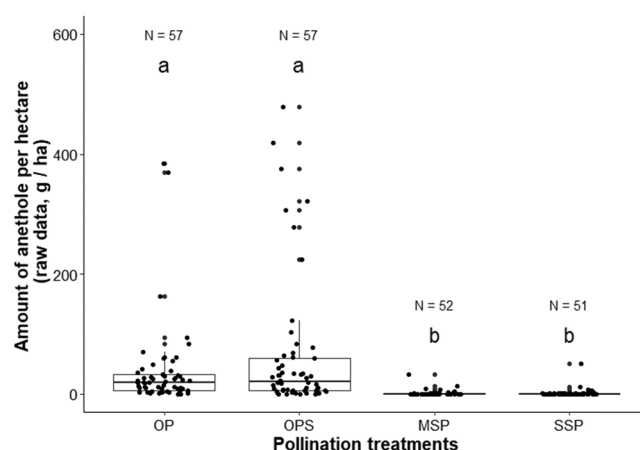


Fig. 4. Fennel fruit amount of anethole per hectare (raw data, g / ha) according to pollination treatments (OP = open pollination; OPS = open pollination + supplementation; MSP = manual self-pollination and SSP = spontaneous self-pollination). Black points represent each data value. The vertical bars represent 1.5 times the interquartile range over the 25/75th percentile. Letter N represents the number of values after NA removal. Letters a and b represent the significance of the difference between treatment according to the general linear hypotheses from the linear mixed-effects model (lmer) including a random effect for the identity of the experimental field and plant, and the estimation of the number of fruits per hectare in the argument “weight”.

treatments (SSP - OP: Estimate = - 2.50 %; p-value < 0.001; Fig. 1). They also showed a better fruit initiation rate for OPS treatment than MSP (MSP - OPS: Estimate = - 2.48 %; p-value < 0.001) and SSP treatments (SSP - OPS: Estimate = - 2.47 %; p-value < 0.001; Fig. 1). Finally, they showed no differences between the fruit initiation rate between SSP and MSP treatments (SSP - MSP: Estimate = 0.001 %; p-value = 0.99; Fig. 1). Details of post-hoc comparisons from the model general linear hypotheses are available in [Appendix C, Table C1](#).

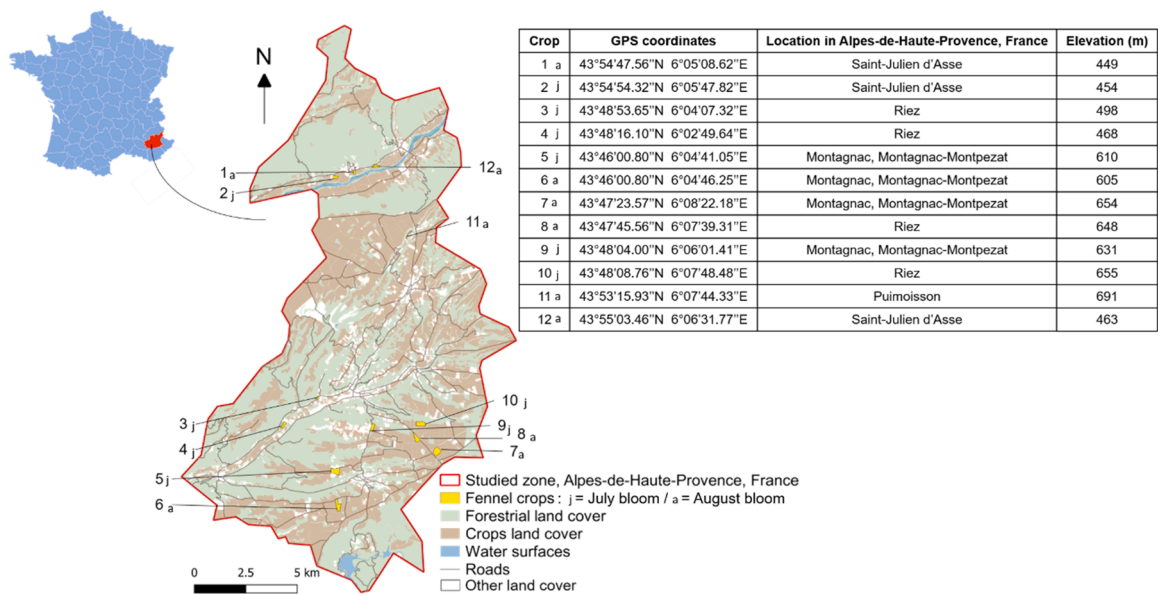


Fig. A1. The 12 studied fennel fields location and elevation in Alpes-de-Haute-Provence (France). The “a” and “j” letters correspond to the fennel types (July and August bloom): “j” means July bloom and “a” means August bloom.

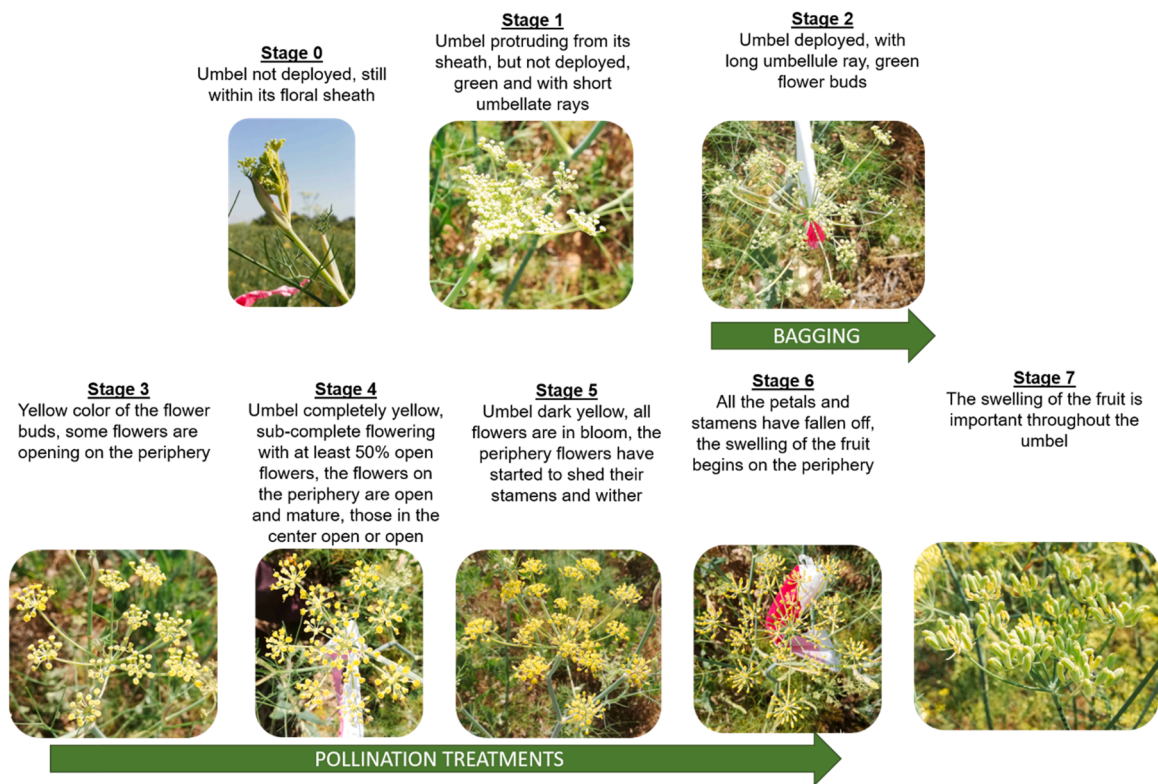


Fig. B1. Stages of the fennel umbels' development used to choose the moment of bagging, and of pollination treatments.

3.3. Fruit weight per umbel

We found fennel fruit weight per umbel of 1.25 ± 0.91 g (mean $\pm$ SD) for insect pollination treatments: 1.28 ± 0.83 g for the OP treatment and of 1.12 ± 0.90 g for the OPS treatment, compared to 0.14 ± 0.18 g for the treatments of pollination without insects: 0.15 ± 0.21 g for the MSP treatment, 0.13 ± 0.14 g for the SSP treatment.

The linear mixed-effects model explaining the differences in the fruit weight per umbel according to pollination treatments and fennel types

(July or August bloom) has shown differences between fennel types (Chisquare = 7.13, df = 1, p-value = 0.008) and between treatments (Chisquare = 112.16, df = 3, p-value < 0.001).

The fruit weight per umbel is better for the July bloom fennel crop than for the August bloom (Estimate = 0.65 g; SE = 0.24; df = 10.68; t-value = 2.67; p-value = 0.02). The general linear hypotheses showed a better fruit weight per umbel for OP treatment than MSP (MSP - OP: Estimate = - 1.34 g; p-value < 0.001) and SSP treatments (SSP - OP: Estimate = - 1.22 g; p-value < 0.001; Fig. 2). They also showed a better

Table C1

Difference in the fennel fruit initiation rate between treatments according to the general linear hypotheses from the generalized linear mixed-effects model with binomial error distribution (glmer) including a random effect for the identity of the experimental field and plant, and the total number of flowers per umbel in the argument “weight”.

Comparison	Estimate (%)	Standard error	z-value	p-value
OPS - OP	- 0.04	0.01	- 2.68	0.03
MSP - OP	- 2.51	0.02	- 113.61	< 0.001
SSP - OP	- 2.50	0.02	- 114.78	< 0.001
MSP - OPS	- 2.48	0.02	- 111.56	< 0.001
SSP - OPS	- 2.47	0.02	- 112.68	< 0.001
SSP - MSP	0.001	0.03	0.27	0.99

Table C2

Difference in the fennel fruit initiation rate between treatments according to the linear mixed-effects model (lmer) including a random effect for the identity of the experimental field and plant, and the total number of fruits per umbel in the argument “weight”.

Comparison	Estimate (g)	Standard error	z-value	p-value
OPS - OP	- 0.11	0.08	- 1.38	0.49
MSP - OP	- 1.34	0.16	- 8.17	< 0.001
SSP - OP	- 1.22	0.16	- 7.64	< 0.001
MSP - OPS	- 1.23	0.17	- 7.47	< 0.001
SSP - OPS	- 1.11	0.16	- 6.93	< 0.001
SSP - MSP	0.12	0.21	0.58	0.93

Table C3

Difference in the fennel fruit initiation rate between treatments according to the linear mixed-effects model (lmer) including a random effect for the identity of the experimental field and plant, and the estimation of the number of fruits per hectare in the argument “weight”.

Comparison	Estimate (kg/ha)	Standard error	z-value	p-value
OPS - OP	- 163.70	66.54	- 2.46	0.06
MSP - OP	- 1146.65	137.77	- 8.32	< 0.001
SSP - OP	- 1068.56	138.41	- 7.72	< 0.001
MSP - OPS	- 982.95	138.89	- 7.01	< 0.001
SSP - OPS	- 904.86	139.42	- 6.49	< 0.001
SSP - MSP	78.09	178.18	0.44	0.97

Table C4

Difference in the fennel fruit initiation rate between treatments according to the linear mixed-effects model (lmer) including a random effect for the identity of the experimental field and plant, and the estimation of the number of fruits per hectare in the argument “weight”.

Comparison	Estimate (g/ha)	Standard error	z-value	p-value
OPS - OP	0.22	0.15	1.54	0.39
MSP - OP	- 1.90	0.32	- 5.96	< 0.001
SSP - OP	- 2.33	0.32	- 7.22	< 0.001
MSP - OPS	- 2.12	0.32	- 7.91	< 0.001
SSP - OPS	- 2.56	0.32	- 7.91	< 0.001
SSP - MSP	- 0.44	0.42	- 1.03	0.72

fruit weight per umbel for OPS treatment than MSP (MSP - OPS: Estimate = - 1.23 g; p-value < 0.001) and SSP treatment MSP (SSP - OPS: Estimate = - 1.11 g; p-value < 0.001; Fig. 2). Finally, they showed no differences between the fruit weight per umbel between OPS and OP treatments (OPS - OP: Estimate = - 0.11 g; p-value = 0.49) neither between SSP and MSP treatments (SSP - MSP: Estimate = 0.12 g; p-value = 0.93; Fig. 2). Details of post-hoc comparisons from the model general linear hypotheses are available in [Appendix C, Table C2](#).

3.4. Fruit weight per hectare

We found fennel fruit weight per hectare of 1124.90 ± 775.91 kg / ha (mean $\pm$ SD) for insect pollination treatments: 1192.88 ± 750.13 kg / ha for the OP treatment and of 999.41 ± 744.24 kg / ha for the OPS treatment, compared to 124.14 ± 148.81 kg / ha for the treatments of pollination without insects: 129.83 ± 166.18 kg / ha for the MSP treatment, 117.79 ± 141.49 kg / ha for the SSP treatment.

The linear mixed-effects model explaining the differences in the fruit weight per hectare according to pollination treatments and fennel types (July or August bloom) has shown no differences between fennel types (Chisquare = 0.01, df = 1, p-value = 0.93) but a difference between treatments (Chisquare = 110.88, df = 3, p-value < 0.001).

The general linear hypotheses showed a better fruit weight per hectare for OP treatment than MSP (MSP - OP: Estimate = - 1146.65 kg / ha; p-value < 0.001) and SSP treatments (SSP - OP: Estimate = - 1068.56 kg / ha; p-value < 0.001; Fig. 3). They also showed a better fruit weight per hectare for OPS treatment than MSP (MSP - OPS: Estimate = - 982.95 kg / ha; p-value < 0.001) and SSP treatments (SSP - OPS: Estimate = - 904.86 kg / ha; p-value < 0.001; Fig. 3). Finally, they showed no differences between the fruit weight per hectare between OPS and OP treatment (OPS - OP: Estimate = - 163.70 kg / ha; p-value = 0.06) neither between SSP and MSP treatments (SSP - MSP: Estimate = 78.09 kg / ha; p-value = 0.97; Fig. 3). Details of post-hoc comparisons from the model general linear hypotheses are available in [Appendix C, Table C3](#).

3.5. Amount of anethole per hectare

We found fennel fruit amount of anethole per hectare of 51.55 ± 99.81 g / ha (mean $\pm$ SD) for insect pollination treatments: 37.64 ± 71.18 g / ha for the OP treatment and of 65.11 ± 112.03 g / ha for the OPS treatment, compared to 2.45 ± 8.39 g / ha for the treatments of pollination without insects: 2.05 ± 7.24 g / ha for the MSP treatment, 1.76 ± 4.99 g / g of fruit / ha for the SSP treatment.

The linear mixed-effects model explaining the differences in the amount of anethole from fruits per hectare according to pollination treatments and fennel types (July or August bloom) has shown no differences between fennel types (Chisquare = 0.56, df = 1, p-value = 0.46) but a difference between treatments (Chisquare = 95.64, df = 3, p-value < 0.001).

The general linear hypotheses showed a better amount of anethole from fruits per hectare for OP treatment than MSP (MSP - OP: Estimate = - 1.90 g / ha; p-value < 0.001) and SSP treatments (SSP - OP: Estimate = - 2.33 g / ha; p-value < 0.001; Fig. 4). They also showed a better amount of anethole from fruits per hectare for OPS treatment than MSP (MSP - OPS: Estimate = - 2.12 g / ha; p-value < 0.001) and SSP treatments (SSP - OPS: Estimate = - 2.56 g / ha; p-value < 0.001; Fig. 4). Finally, they showed no differences between the amount of anethole from fruits per hectare between OPS and OP treatment (OPS - OP: Estimate = 0.22 g / ha; p-value = 0.39) neither between SSP and MSP treatments (SSP - MSP: Estimate = - 0.44 g / ha; SE = 0.42; z-value = - 1.03; p-value = 0.72; Fig. 4). Details of post-hoc comparisons from the model general linear hypotheses are available in [Appendix C, Table C4](#).

4. Discussion

This study analyzed the degree of dependence of fennel on pollination by insects. This is one of the first studies linking the presence of insects to the quantity of anethole produced, a metabolite of high economic value. Our results suggest that most of the time, spontaneous and manual self-pollination lead to reproductive failure. Insect pollination improved the fruit initiation rate, and the fruit weight and the amount of anethole per hectare. Finally, our results seemed to show that no pollen limitation is occurring in our study system. This study adds new evidence of the importance of insects in agroecosystems to sustain food

production.

4.1. Fennel dependence on insect pollination

Our results confirmed those of previous studies showing a positive influence of insects pollination on crop yield (Garibaldi et al., 2016, 2013; Geslin et al., 2017; Woodcock et al., 2019), and especially for fennel seeds yield (Chaudhary, 2006; Kumar and Singh, 2017a; Meena et al., 2015). It has also been shown that this positive influence is found even in the plant progeny (Salami et al., 2016; Shojaeifar et al., 2021). There was no difference between spontaneous and manual self-pollination, both generally led to a low fructification rate suggesting that *F. vulgare* self-pollinates poorly. As manual self-pollination aimed to circumvent the protandry problem, the low success of this pollination method could be surprising and may be due to unsuitable manual pollination (flower damage, for example). For spontaneous self-pollination, it is probably due to the protandry which could act as a barrier more or less important to reproduction in self-pollination (Koul et al., 1996; Kumar and Singh, 2017a; Shojaeifar et al., 2021). This barrier could avoid the inbreeding depression found in this species (Shojaeifar et al., 2021). By contrast, insects, by visiting a lot of flowers at different stages, may work around the protandry of fennel flowers and optimize the reproduction. Moreover, insect pollinated flowers showed higher number of fruits and amount of anethole per hectare compared to bagged flowers. We can conclude that fennel is highly dependent on insects for reproduction. Many plant species rely heavily on insects for reproduction, both in wild species with implications for conservation (Schurr et al., 2019) and cultivated species with implications for global food sustainability (Gallai et al., 2009; Geslin et al., 2017, 2016; Klein et al., 2007). We confirmed that fennel dependence degree (86 % of reduction in the number of fruits without insects) is consistent with the 40–90 % noted by Klein et al. (2007) and 82 % shown by Chaudhary (2006). Nevertheless, there were still a small number of initiated fruits in self-pollination treatments, confirming that even if this species seems to be poorly spontaneously self-pollinate, it is nevertheless self-compatible (Koul et al., 1996). A similar lack of self-fertility (at the flower scale) but a successful geitonogamy linked to insect activity has been shown for others Apiaceae (the self-compatible coriander varieties) and fennel (Koul et al., 1989; Shojaeifar et al., 2021). Especially since, interestingly, the fruit weight was similar between open pollination and self-pollination, meaning that when a fruit is initiated, the quality of the fruit is consistent regardless of the mode of pollination. We can hypothesize that protandry in fennel could be more limiting at flower and umbel levels than at the plant level thanks to geitonogamy (Koul et al., 1996). This could also result from possible wind pollination (Klein et al., 2007) or from insects infiltrating in the bags without being noticed (thrips). Nevertheless, previous study which had circumvented the problem of insects infiltrating by using insecticide also showed some reproductive success in pollination without insects (Chaudhary, 2006). However, at the field level, we found a drop in the weight of the fruits per hectare in spontaneous and manual self-pollination compared to open pollination treatments. Some authors even explored the possibility that fennel could be selected for self-pollination, by developing productive pure line self-pollinated varieties, but with modified essential oil amount (Shojaeifar et al., 2021). In this way, a better understanding of plant dependence on insects could be useful for genetic research on improvement of crop varieties.

4.2. Reproduction limitation by insect activity

We showed a relatively low fructification rate in open pollination (< 40 %) compared to previous literature results (77 %; Kumar and Singh, 2017a). Falzari et al. (2005) studied the effect of the stand density of fennel on the pollen production and stigma receptivity and the subsequent influence on fennel yield. Results showed an increase of both pollen production and stigma receptivity and increase of the fennel yield

(Falzari et al., 2005). However, as the stand density in our field is relatively low (around 12 plants / m²), it can't entirely explain our low fruit set value. Other features seem to be able to influence the fruit set in open pollination. For example, we can't exclude a varietal effect or a lower efficiency of the pollinator cohort in our study area compared to other geographical areas. However, the initiation rate of the fruits was similar between open pollination and open pollination with allopollen supplementation. This is also the case of the amount of anethole produced per hectare. We expected an increase of these parameters with open pollination with allopollen supplementation. It seems that in our system, the fennel does not suffer from any pollen limitation. No pollen limitation means that, at this stage, even if insect pollination is still fundamental, it might be not necessary to increase insect abundance with the intention to improve production. Since the reproductive success of plants is linked to the amount of pollen received (Aizen and Harder, 2007), a greater abundance of insects could lead to pollen saturation of fennel flowers. This explanation would not be surprising given (1) the intense beekeeping activity in our study area, and (2) the great abundance and diversity of insects along fennel crops (Schurr et al., 2021) and within fennel flowers (Bharti et al., 2015; Chaudhary, 2006; Kumar and Rai, 2020; Meena et al., 2016, 2015; Ricciardelli D'Albore, 1986; Salami et al., 2016; Shilpa et al., 2014; Skaldina, 2020). However, not all insect species may be equal in their ability to carry a large amount of pollen or higher quality pollen, two other important factors in pollination (Aizen and Harder, 2007; Ollerton et al., 2011). Insect diversity could therefore be more important than insect abundance for the reproduction of fennel with regard to the complementarity hypothesis of functional diversity (Frund et al., 2013; Gagic et al., 2015; Schurr et al., 2021; Woodcock et al., 2019). Thus, our experimentation is a first step but it is essential to better understand the relative contributions of the different species of pollinators to crop pollination and yield depending on the degree of autogamy and reliance on insect pollination of the plant species (Klein et al., 2007; Schurr et al., 2021), foraging activity (visitors vs. pollinators; Ollerton, 2017), and the quantity and quality of pollen transported or deposited. It is also essential to analyze this relative contribution with regard to the landscape and local contexts, the production practices, and the context of climate change.

Finally, the fruit initiation rate appears to be higher for open pollination treatments without manual supplementation. As we used different donors, the possibility of inbreeding or incompatibility issues are excluded. We suspected no incompatibility issue as the fennel has been already shown self-compatible (Koul et al., 1996; Shojaeifar et al., 2021) but inbreeding depression was rather shown (Shojaeifar et al., 2021). We propose three explanations for this result. First, the supplementation technique used might not differentiate the quantity and quality or maturity of transported pollen, and either of the two factors could have a greater effect on fennel reproduction. But this explanation is closely dependent on the used number of different pollen donors' plants and flowers, pollen quality increasing with the increase in genetically different donors. Second, manual pollen saturation of flowers could lead to the obstruction of the not yet mature stigma, which would imply failure of fruit formation. Seed set is not necessarily maximised with the highest pollen deposition (Ornelas and Lara, 2009). Finally, hand manipulation could damage some parts of the flower, and if the style is damaged it can impact the fruit formation (Sáez et al., 2014a), or leading the plant to allocate its energy to repair the flower rather than to the fruit development.

4.3. Amount of anethole

Reproduction of fennel is important because the fruits are the part of the plant containing the greatest amount of essential oil (Hunault et al., 1989; Salami et al., 2016). The positive effect of insects on crops regarding quantity and quality of seeds and fruits, and by extension their economic value, has already been shown (Garibaldi et al., 2013; Woodcock et al., 2019). This has also been demonstrated for the amount

of essential oil and some metabolic contents of certain plants, such as mint and fennel (Nazem et al., 2019; Schurr et al., 2021). The literature has previously reported discrepancies about the effect of insects on fennel essential oil yield. Indeed, insect diversity had a positive influence on the amount of essential oil produced by fennel (Schurr et al., 2021). Nevertheless, other authors found that cross-pollination by insects had a negative effect on fennel progeny essential oil yield compared to progeny from self-pollinated seeds (Salami et al., 2016). The variability of results may be due to geographically contrasting insect communities, leading to variations in pollination efficiency. It could definitely also be due to varietal differences in fennel. This study showed that the amount of anethole from fennel fruit per hectare highly increased with insect pollination vs self-pollination. However, the anethole amount, beside the fact that it is dependent on the number of fruits, could be linked to other plant mechanisms. Indeed, some studies have shown that anethole could be toxic to certain insects, in particular pests (Alkan and Ertürk, 2020; Wang et al., 2021), without explaining the enzymatic mechanisms driving its synthesis. As such, over-exploitation of flowers by insects, potentially partially damaging flowers (Sáez et al., 2014b) could then lead to a defensive response resulting in increased anethole production. Nevertheless, even if the quality of production is increased, a too high density of insects could, in the long term, be negative for anethole production. It could be particularly the case in our study area where there is intense beekeeping (for honey purpose) that can lead to high honey bee density.

5. Conclusion

Here is one of the first studies comparing the fennel reproductive success and the subsequent yield in the amount of anethole between pollination with and without insects. We showed that the fennel crops, one of the main anise spice crops in the world, are highly dependent on insects for fruit formation, weight and anethole production. In agricultural landscapes, insects are increasingly threatened due to pesticide use and landscape uniformization (Brittain and Potts, 2011; Hendrickx et al., 2007; Kleijn et al., 2009; Vanbergen, 2021). It is important to consider the preservation of insects in agro-ecosystems together with the management of landscapes. This study proves once again that insects are essential to sustainably maintain the quality and diversity of global food production. In this sense, protecting insects, and biodiversity globally, also means enhancing livelihood and allowing food security which are the key challenges of our century (Garibaldi et al., 2016; Kremen and Merenlender, 2018).

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Declaration of competing interest

The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

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Appendix A

See Fig. A1.

Appendix B

See Fig. B1.

Appendix C. Details of post-hoc comparisons from the models general linear hypotheses

See Tables C1–C4.

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