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## RESEARCH PAPER

## Bees improve oil quality of oilseed rape

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## ABSTRACT

Global demand for vegetable oil is projected to expand to 28 Mt by 2028. Oilseed rape (OSR) is the predominant oilseed crop in Europe. Its nutritional value is determined by seed oil content and fatty acids (FAs) composition. Insect pollination increases seed oil content, however, its benefit on FAs composition remains unknown. In this study, we address this gap by investigating the variation of OSR seed oil content and FAs composition along a gradient of bee abundance in farmed OSR fields. We used a pollinator exclusion experiment to disentangle the contribution of pollination processes, i.e. wind, self and insect pollination. We show that bees improved OSR nutritional value by increasing amount of poly-unsaturated FAs and decreasing the amount saturated and trans-saturated FAs. In addition, oil content was higher in seeds with than without access to pollinators. Our study provides for the first time, to the best of our knowledge, evidence that insect pollination enhances the quality value of OSR oil by affecting FAs composition. Such effect implies potential negative consequences with any further decline of pollinator abundance. More investigations are also needed to better understand the mechanisms by which insect pollination affects FAs composition, and the way to enhance such mechanisms.

## Introduction

Balancing biodiversity conservation with food security and the preservation of a broader set of ecosystem services, in a context of global change, is among the greatest contemporary challenges (Crist et al., 2017). Crop pollination by animals is a clear example of nature's contribution to people within productive ecosystems (Millennium Ecosystem Assessment, 2005): 70 % of crops worldwide depend to some extent on pollinators for their yield (Klein et al., 2007). In a context of increasing food demand, pollination service may thus enhance food security. However, pollen limitation was shown in several crops (Aizen & Harder, 2009; Martin et al., 2019), which may thus jeopardize this aim. In addition to improving crop yields, pollinators can improve crop quality, as found for apple (*Malus Domestica*) or strawberry (*Fragaria x ananassa*, Bartomeus et al., 2014; Garratt et al., 2014; Gazzea et al., 2023; Klatt et al., 2014). However, insect pollination benefits in terms of crop quality are not universal. For example, Bartomeus et al. (2014) did

not find any effect of insect pollination on the nitrogen content of bean seeds, and Garratt et al. (2014) revealed that insect-pollination can either reduce or improve apple quality. This latter pattern was related to two mechanisms: a modification of physiological trade-off, i.e., apple trees pollinated by insects showed higher fruit number per tree but a lower mineral concentration per apple (Garratt et al., 2014), or an improved pollination rate resulting from outcross pollen (Stein et al., 2017).

Oilseed rape (OSR, *Brassica napus*) is the main oil crop in the European union (FAOSTAT, 2014). Oilseed crops are used for human food, livestock, and bioenergy production (Jahreis & Schäfer, 2011). OSR seeds are rich in oil (42–48 %; Bommarco et al., 2012; Rathke et al., 2006) and represent one-third of the amount of oil consumed in Europe (data for 2013, www.oilworld.biz). Several studies showed that oilseed rape yield benefits from insect pollination with increases of up to 35 % (Bartomeus et al., 2014; Bommarco et al., 2012; Perrot et al., 2018). The dependence of OSR on insect pollination however varies among

Data availability statement Data are available on request by email to the authors.

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cultivars (Hudewenz et al., 2014) and OSR type (hybrid versus open-pollinated cultivar, Marini et al., 2015). Although few studies have investigated how insect pollination affects OSR quality (Bartomeus et al., 2014; Bommarco et al., 2012; Marini et al., 2015; Oz et al., 2008), results obtained so far show that oil content per seed increases by 1.28 to 6 % when comparing seeds from flowers with and without access to pollinators. Quality of oil crops was first characterized by high oil content, but also by low percentage of trans-saturated and saturated fatty acids (Anderson et al., 2010; de Souza et al., 2015), high percentage of unsaturated fatty acid, the latter being composed of monounsaturated and polyunsaturated fatty acids (Carrillo et al., 2012; Ruxton et al., 2004), and a low ratio of omega-6 to omega-3 fatty acids (lower than 4; Simopoulos, 2002, here after called " $\omega 6/\omega 3$ "). Indeed, trans-fatty and saturated fatty acid diet were associated with human diseases as cardio-vascular disease or type-2 diabetes, while diets that preferentially rely on unsaturated fatty acids, especially polyunsaturated fatty acids with ratio  $\omega 6/\omega 3$  lower than 4 have a protective effect as anti-inflammatory effects or prevent cardiovascular diseases (see Dhaka et al. 2011, Lenighan et al. 2019, Simopoulos 2002). Because oil content and/or fatty acids composition are improved in fruits resulting from out-crossing pollination (Brittain et al., 2014; Kämper et al., 2021), the potential role of insect-pollination on OSR oil seed quality is questioned. So far, however, to our knowledge, the relationships between insect pollination and fatty acids composition or ratio  $\omega 6/\omega 3$  fatty acids in OSR seeds have never been investigated.

In this study, we examined OSR seed oil content and fatty acids composition in 72 farmers' fields from 2013 to 2016. Fields were selected along a gradient of bee abundance (Perrot et al., 2018). We further combined this natural gradient of bee abundance with a pollinator-exclusion experiment where flowers from the same plant had selectively access to self-pollination, wind, small, or large insect pollination. This exclusion experiment allowed exploring which pollen vector (wind, insect, self) would best improve crop quality but also to disentangle the effect of pollinators on crop quality through a modification of physiological trade-off or a preferential investment in out-fertilized seeds. We therefore assessed both the effect of insect pollination and bee abundance on seed oil content (Bommarco et al., 2012) and fatty acids composition. We predicted that bee abundance would increase oil content as well as improve fatty acids composition, i.e. higher unsaturated fatty acids and lower trans and saturated fatty acids in seeds from flowers with pollinator access as observed in previous studies in other crops (Brittain et al., 2014; Negussie et al., 2015).

## Materials and methods

### Study site, experimental fields and landscape context

The study was conducted between 2013 and 2016 in the LTSER "Zone Atelier Plaine & Val-de Sèvre" (ZA-PVS, 450 km<sup>2</sup>), a study site located in the south of Deux-Sèvres district, central western France (Bretagnolle et al., 2018). In the same study site, previous studies had shown a positive effect of bee abundance (i.e. honeybee and wild bees mainly *Lasioglossum* sp) on oilseed rape yield (Catarino et al., 2019; Perrot et al., 2018, 2022). Only winter OSR is cultivated in the LTSER, representing usually 8 %–10 % of the agricultural surface. Experiments were conducted directly in farm fields, without requesting any modifications to the practices from the farmers. All selected fields were cultivated using conventional agriculture, but with various nitrogen amendments and pesticide loads. Fields were chosen within 1 km<sup>2</sup> square landscapes, the latter being selected to represent density

gradients of three environmental features: semi-natural habitats (hedgerows and forest fragments), meadows, and organically farmed fields (see Sirami et al. 2019). All these landscape features are known to influence strongly pollinator abundance (Kennedy et al., 2013). Within the selected squares, an OSR field was chosen if present (usually, there was only one OSR field). On average, OSR fields were at 361.84 m (65.43 m to 1147.25 m) distant to the nearest OSR neighbour. Field size ranged from 0.99 to 23.08 ha (mean 6.37 ha). Field soil type belonged to four classes: three classes of highly calcareous soils, with a depth of 20 cm ( $n = 37$ ), 30 cm ( $n = 22$ ) or 40 cm ( $n = 7$ ), and one class with red silt over limestone ( $n = 6$ ), according to the IGCS soil map (<https://www.geoportail.gouv.fr/>). All OSR crops were under conventional farming.

### Study design

In total, 72 OSR fields were used for this study (7, 21, 25, 19 in 2013, 2014, 2015 and 2016, respectively). Information on practices (yield, plant cultivar and fertiliser) were obtained by farmers' survey at the end of each cropping season (see Perrot et al. 2018). In each field, three individual OSR plants were selected at two different positions: one position at the edge of the field (between 0 and 5 m from field border) and one 20 m in the core field, i.e. a total of six plants per field were available (Perrot et al., 2018). On each individual plant, three (2013), two (2014) and four (2015–16) secondary branches were selected on which we implemented pollination exclusion treatments. For each individual plant, branches were selected so as to be at the same flowering stage, and close (adjacent) to each other. The different experimental treatments allowed for self-pollination (SF), wind-pollination (W), and small-bodied (SP) or large-bodied (LP) insect pollinators. One of the branches was used as a control ( $N = 373$  branches in total), i.e. all its flowers were accessible to all vectors (insects, wind and self-pollination, i.e. "SF+W+SP+LP", here after called "open treatment"). A second branch was caged with a small mesh bag (mesh size=0.6 mm,  $N = 248$  branches), in which flowers could only be pollinated by self- or wind pollination ("SF+W"). In 2013, 2015 and 2016, a third branch was caged with a large mesh (mesh size=3 mm,  $N = 226$  branches), thus allowing self-, wind and small-insect pollination ("SF+W+SP"). Finally, in 2015 and 2016 a fourth treatment was added, using an osmolux bag (Pantek, France,  $N = 154$  branches), allowing only gas exchange and thus excluding all types but self-pollination ("SF", call after "self-treatment"). In 2013 only, each treatment was replicated within each plant (i.e. two open, large and little mesh branches per plant), otherwise no replication was done. For all treatments, bags were installed before the onset of flowering. Plants were visited weekly to adjust bags, i.e. bags were lifted upwards to cover new or future flowers leaving outside flowers that were faded. Bags were removed after the last flower had faded. All manipulations were made gently to avoid effect on seed development (Jacobs et al., 2009). We collected the branches five days before harvest by separating experimental (caged) and open branches from the rest of the plant and stored them in individual paper bags.

### Seed weight, oil extraction, identification and calculation of fatty acid

Once branches were brought back to the laboratory, all bags were left 48 h in a heat chamber at 60 °C. Then for each treatment branch, three seeds were selected randomly from all seeds of the branch, individually weighed, and grouped together with a grinder mixed with 7 ml of iso-propanol:hexane, then oil content was evaporated at 40–50 °C during one night following Warren et al. (1988). Oil extracted was weighed and % of lipid was expressed as the ratio of oil weight out of dry weight of the

three seeds. Oil was then stored at  $-20\text{ }^{\circ}\text{C}$  with chloroform until FA analysis.

FA analysis was performed during 2016/17 winter. FA composition was determined in 65 (due to budget limitations) of the 72 fields (7–21–25–12 for 2013–16) by mixing, for each field, oil samples per treatment to reduce the economic cost of the analyses. Gas chromatography coupled to flame ionization detection (GC-FID) was used to determine FA composition. FAs were transmethylated for GC-FID following the Morrison & Smith procedure (Morrison & Smith, 1964) on 3 mg of oil. The FA composition was then determined on a Hewlett Packard Model 5890 chromatograph (Palo Alto, CA, USA) using a CPSIL-88 column (100 m  $\times$  0.25 mm i.d. film thickness 0.2  $\mu\text{m}$ , Varian, Les Ulis France) equipped with a flame ionisation detector. Hydrogen was used as a carrier gas (inlet pressure, 210 kPa). FAs were identified by comparison with commercial synthetic standards (Sigma-Aldrich, France). The data were processed using the EZChrom Elit software (Agilent Technologies, Massy, France). FA relative percentage was calculated based on the peak area of a FA to the total peak area of all FAs.

### Pollinator sampling

Bee abundance was sampled during the OSR flowering period, using two different and complementary methods, colored pan-traps and transect, since wild bee abundance was found to be better estimated by pan-traps and honeybees by transect (Perrot et al., 2018, see also Westphal et al. 2008). Twelve (2013–15) or three pan-traps (2016) filled with water and organic soap were put into OSR fields during 4 days at two different positions in 2013–2015 (field and core field) and only in core field in 2016 (Perrot et al., 2018), being of three colors: white, blue and yellow (see Westphal et al. 2008). Transect method also varied slightly among years: systematic sweep netting was performed in 2013–2014, while visual counting and sweep netting capture was performed in 2015–2016. Both sweep net and visual counting were performed along a transect at the edge of the field and another one at the field centre (50 m). In both, the transect length was 50 m. For sweep netting, the observer swept along all transects. In visual count, the transect always lasted 10 min (but when a bee was caught, the time necessary to identify or place the bee in a tube was not counted). Durations were measured by a hand chronometer. For sweep netting, all pollinators caught by the net were kept for later identification. For visual counting, pollinators were visually identified if possible, and only unidentified bees were kept for later identification. All pollinators caught were then identified to species at the laboratory. For pan-traps, bee abundance was estimated by a nested procedure, starting with mean count per bowl colour and position in the field (core vs. edge), then averaging per position in the field, and finally per field (Perrot et al., 2018). In this study, we focused on *Lasioglossum* spp. (the main wild bee genus, accounting for 55 % of all captures) and honeybees, as they were found to be the main contributors to OSR yield in this study site (Perrot et al., 2018). From these two abundances, we derived a metric called “bee abundance” which was the sum of z-scores for sweep net and pan trap counts in 2013 and 2014, and for visual counts and pan traps in 2015 and 2016. (see Catarino et al. 2019).

### Statistical analyses

First, we examined the effect of insect pollination on oil content. We used a linear mixed model (LMM) with %lipid as dependent variable. We included, as explanatory variables, the treatment (four levels) to identify which pollination vector (wind, insect, self) would improve %lipid, as well as bee abundance and its interaction with treatment. Because fertilizer intensity, oilseed rape variety ID and temperature are known to

modify oil content, they were added to the model as co-variables as well as year and soil type categorized in calcareous or red soils. We also included the interaction between bee abundance and variety ID as a co-variable because the effect of insect pollination can depend on variety ID (Hudewenz et al., 2014). Fertilizer intensity was the sum of nitrogen (applied on average: 175.1 kg/ha, range: 89.6–323.9), phosphorus (54.7 kg/ha, 0–156.6) and potassium (29.9 kg/ha, 0–270) centered and scaled before summing up (see Perrot et al. 2022). OSR fields were sown with two main varieties DK Exstorm (17 fields) and DK Expertise (17 fields) both representing 23.6 % of the fields and the remaining fields (52.7 %) were sown with 18 different varieties ( $2.11 \pm 0.17$  SD fields per variety ID). Variety IDs were thus grouped into three categories: DK Exstorm, DK Expertise and “others varieties”. Robustness analyses were conducted by replacing ‘variety ID’ by ‘varietal category’, i.e. hybrid (88.9 % of fields) and open-cultivar (11.1 %) but this did not modify any results (see Appendix A). Because temperature was shown to modify FA composition and lipid content (Baux et al., 2013; Rathke et al., 2006), we included average minimal temperature to second half of oil accumulation period in OSR seeds (i.e. in our study from the 1st May to the 15th June) following (Baux et al., 2013). Plant ID, nested in field ID were included as random factor in the model. A Tukey post-hoc test was then performed to evaluate differences between treatment modalities or between variety ID/ varietal categories. To ensure that insect pollination affected %lipid by modifying lipid weight rather than seed weight, two supplementary LMMs were realised with lipid weight or seed weight as dependent variable and the same explanatory, co- and random variables as for %lipid LMM.

Then we explored the effect of insect pollination on FA composition by investigating the variation in the proportion of saturated, mono-unsaturated (including trans-saturated FAs) and poly-unsaturated FAs (hereafter FA groups), the w6/w3 ratio as well as the proportion of each FAs (hereafter FA profile). As previously, we built LMMs, for FA groups, FA profile and the w6/w3 ratio. Because FAs that composed FA groups and FA profile were strongly correlated, we conducted two principal component analyses (PCA) to reduce dimensionality for FA group and FA profile. For FA group, a first PCA was realised with the three group of FAs, i.e. saturated, mono-unsaturated (including trans-saturated FAs) and poly-unsaturated. For FA profile, a second PCA was realised with the 18 different FAs. The two first components of each PCA were extracted and used as dependent variables in LMMs (i.e. four models, two for FA groups and two for FA profiles). For each PCA, FA groups and profile were centered and then log-ratio transformed (i.e., taking the log of the ratio between observed frequencies and their geometric mean), as advised by Aitchison (1986) for compositional data. The same explanatory variables and co-variables used in the %lipid LMM were used in these latter models. Field ID was included as random factor in these models as FA composition was grouped by treatment for FA extraction method.

All analyses were performed using the software R (R Core team, 2020). For each LMMs model, we used a multi-model inferences analysis (i.e. model averaging) that consists in retaining all models with AICc values with a difference lower than 2.0 compared to the best model, and then a parameter estimate is obtained by averaging over all models in the top model following the so-called “zero method” method i.e. a parameter estimate of zero is substituted into those models where the given parameter is absent (Burnham & Anderson, 2002; Grueber et al., 2011). We also checked for collinearity between explanatory variables using variance inflation factor (VIF) on residuals of the complete model (without interaction). We found no collinearity (all VIF < 1.22). We also checked for spatial autocorrelation, which was detected for the LMM including the second PCA axis (Moran  $I = -0.14$ ,  $p\text{-val} = 0.034$ ). We thus accounted for this spatial structure by adding an exponential spatial

autocorrelation structure in this model. Finally, residuals of all LMM models were visually checked, and the residuals dispersion was tested with the DHARMA package (Hartig, 2018). No under- or overdispersion of residuals was found (all  $p$ -values > 0.088). In all LMMs, bee abundance was  $\log(x + \text{abs}(\text{min}) + 1)$  transformed to meet the model assumptions, i.e. “min” is the minimum value to take account of a value less than zero because of previous centred transformation. We used the “glmmTMB” package for LMMs, “MuMin” for model averaging (Barton & Barton, 2018), “car” for VIF analyses (Firth et al., 2009), “emmeans”

package (Lenth et al., 2019) for Tukey post hoc test and “composition” for centred log ratio transformation of FA groups and profile.

### Results

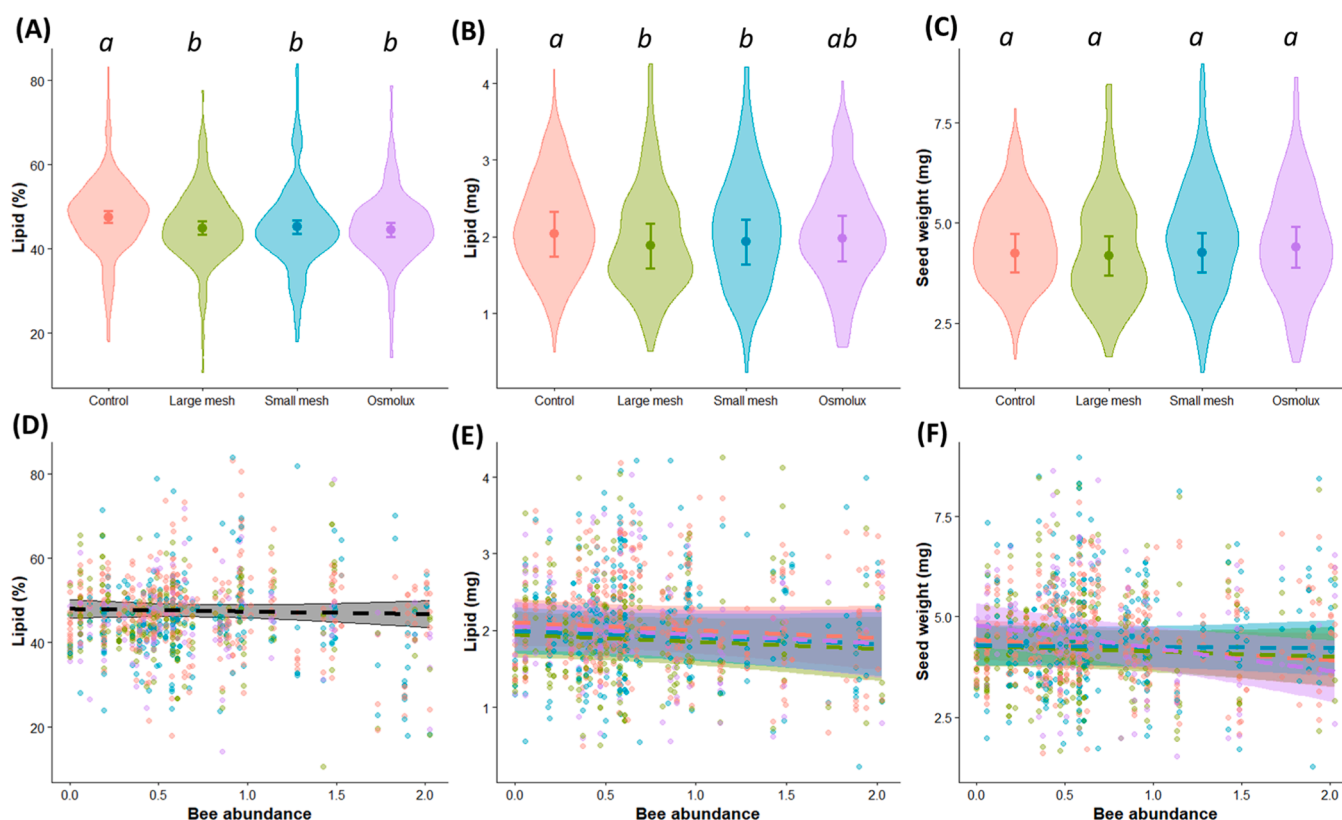
#### Effect of insect pollination on oilseed rape oil content

Over the four years, open OSR seeds contained on average 2.1 mg lipids (confidence interval: 1.7–2.6 mg) which represented 48.0 %

**Table 1**

Output of linear mixed models explaining OSR oil content by bee abundance, pollination-exclusion treatments and their interaction as well as by soil type, fertilizer intensity use, temperature, variety ID with this interaction with bee abundance. Estimated coefficient (“Est”), standard errors of this coefficients (Std), z-value and p-value are given for each explanatory variable for the average models. Significant results are bolded. “-” indicates explanatory variable was not kept in model selection.

	Lipid (%)				Lipid (mg)				Seed weight (mg)			
	Est	Std	z-val	p-val	Est	Std	z-val	p-val	Est	Std	z-val	p-val
Large mesh (treatment)	-2.6	<b>0.6</b>	<b>4.6</b>	<b>&lt;0.001</b>	-0.2	<b>0.0</b>	<b>3.4</b>	<b>0.001</b>	-0.2	0.1	1.3	0.188
Small mesh (treatment)	-2.4	<b>0.6</b>	<b>4.3</b>	<b>&lt;0.001</b>	-0.1	<b>0.1</b>	<b>2.1</b>	<b>0.035</b>	-0.1	0.1	1.2	0.235
Self (treatment)	-3.0	<b>0.7</b>	<b>4.5</b>	<b>&lt;0.001</b>	-0.1	0.1	0.9	0.342	<b>0.4</b>	<b>0.1</b>	<b>2.6</b>	<b>0.009</b>
Bee abundance	-0.8	1.3	0.6	0.536	-0.1	0.1	1.0	0.313	-0.2	0.2	1.6	0.106
Bee x Large mesh	-	-	-	-	0.0	0.0	0.2	0.828	0.1	0.2	0.8	0.429
Bee x Small mesh	-	-	-	-	0.0	0.0	0.3	0.749	0.2	0.1	1.7	0.099
Bee x Self	-	-	-	-	0.0	0.0	0.2	0.822	-0.3	0.2	1.8	0.074
Fertilizer	0.0	0.1	0.1	0.944	0.0	0.0	0.3	0.728	0.0	0.0	0.6	0.558
Temperature	18.8	12.6	1.5	0.137	0.0	0.2	0.1	0.899	-0.4	0.8	0.5	0.624
Red soil (soil type)	-0.5	1.6	0.3	0.733	0.0	0.0	0.1	0.941	-	-	-	-
Open cultivar (varietal)	-0.5	<b>2.0</b>	<b>0.3</b>	0.798	-	-	-	-	-	-	-	-
Bee x Open cultivar	0.7	<b>2.7</b>	<b>0.3</b>	0.792	-	-	-	-	-	-	-	-
2014 (year)	-	-	-	-	0.3	0.2	1.6	0.109	<b>0.6</b>	<b>0.3</b>	<b>2.2</b>	<b>0.030</b>
2015 (year)	-	-	-	-	0.3	0.2	1.9	0.057	<b>0.8</b>	<b>0.3</b>	<b>3.1</b>	<b>0.002</b>
2016 (year)	-	-	-	-	-0.2	0.2	1.5	0.143	-0.7	<b>0.3</b>	<b>2.6</b>	<b>0.010</b>



**Fig. 1.** Effect of (A–C) treatments and (D–G) bee abundance on (first column)%lipid, (second column) lipid weight and (third column) seed weight. Dots in (A–C) represent mean for each treatment and bars, confidence interval of prediction as predicted by linear mixed models. Open treatment is in red, large mesh in green, small mesh in blue and self in purple. Violin graphs represent distribution of each variable per treatment. For linear regression plots (D,E), when interactions of bee abundance and treatments are not significant, black lines represent relations over the four treatments and when this interaction is significant, one line was drawn per treatment as predicted by linear mixed models. Dashed lines represent non-significant relations ( $p$ -val < 0.10) and solid one, significant relation.

(43.9–52.2 %) of seed weight. The lipid in seeds in open treatment was significantly higher than in the other treatments (Table 1, Fig. 1A). The increase was 2.5 % (confidence interval: 2.5–2.8 %) compared to seeds in the large mesh treatment (“SF+W+SP” pollination), 2.4 % (2.3–2.6 %) in small mesh (“SF+W” pollination) and 3 % (2.8–3.3 %) in self (“W” pollination) treatments. Lipid weight was also higher in open seeds compared to seeds in the pollination-exclusion treatments (large mesh: between 0.16 mg (0.15–0.16 mg), small mesh: 0.11 mg (0.10–0.11 mg) and self: 0.07 mg (0.05–0.08 mg); Table 1, Fig. 1B). Open seed weight varied between 1.62 and 7.9 mg (average: 4.5 mg) but did not differ between treatments (Fig. 1.C). No differences were observed in seed weight between open and small mesh treatments (Table 1, Fig. 1C). Overall therefore, access to large pollinators (the open situation) significantly increased lipid and to a lesser extent, lipid weight, at the expense of seed weight.

Although access to pollinators increased lipid, we found no relationship between lipid and bee abundance (Table 1, Fig. 1D). Similarly, no significant relationship was found between lipid weight and bee abundance (Table 1, Fig. 1E). By contrast, in the self-treatment, seed weight significantly decreased with bee abundance (Table 1, Fig. 1F). Temperature was positively correlated to lipid but neither fertilization intensity, variety ID or varietal type significantly affected lipid, lipid or seed weight. These variables also did not influence the effect of bee abundance on lipid, lipid or seed weight either (Table 1, Appendix A).

*Effect of insect pollination on oilseed rape fatty acid composition*

OSR seeds were composed of ~8 % saturated FAs (hereafter SFA) and ~90 % of unsaturated FAs (hereafter UFA) among which ~64 % monounsaturated FAs (hereafter MUFA) and ~26.0 % polyunsaturated FAs (hereafter PUFA, Table 3). The main MUFA was the oleic acid (C18:1), which represented around 57 % of the FAs (Table 3). Trans-oleic acid, a trans-saturated FA (TFA) belonging to MUFA, was found in a very low amount, i.e. ~1.8 % (Table 3). Linoleic acid was the most abundant PUFA (18.5 %) and the main omega-6 FA (Table 3); the other omega-6 FA, eicosadienoic acid, represented 0.10 % of the FAs. Alpha-linolenic acid was the second most abundant PUFA (8.5 %) and the only omega-3 FA. The w6/w3 ratio was around 2.3 (Table 3).

Results from LMMs on PCA axes showed that the pollinator-exclusion treatments affected OSR seed FAs composition (Table 2, Fig. 2). Open seeds had higher MUFA (especially oleic acid) and lower SFA (especially stearic acid) and PUFA than seeds in the pollinator-exclusion treatments except for self-treatment which showed higher PUFA than open seed (see coloured points in Fig. 2 and Table 2). Finally, open seeds had a lower ω6/ω3 ratio than seeds in other treatments (Tables 2, 3).

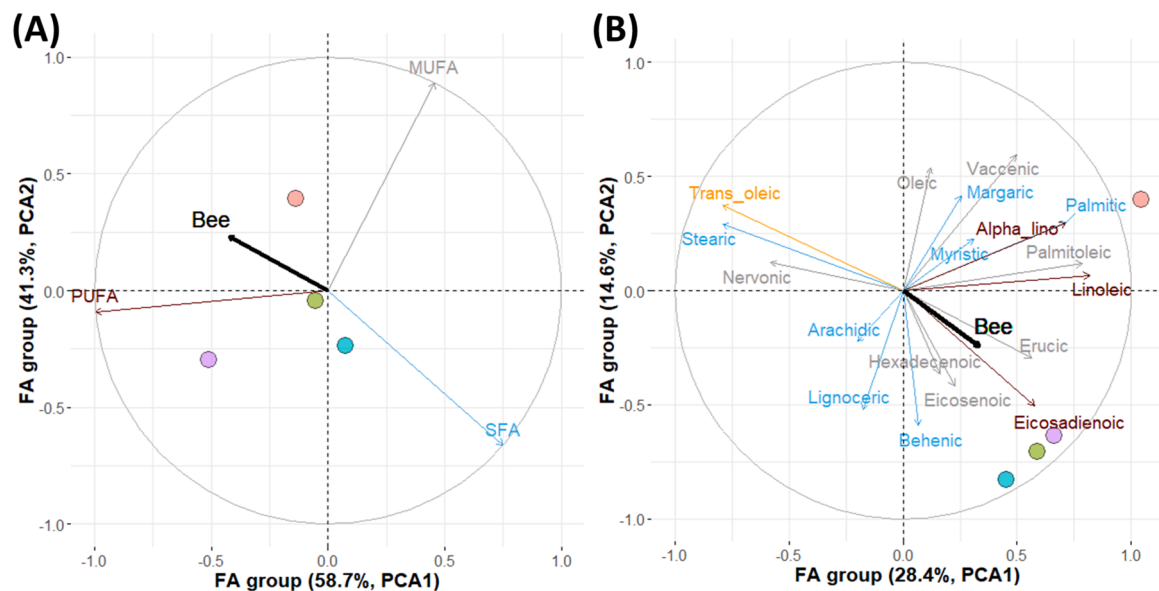
Regarding FA composition according to bee abundance confirms these results for MUFA and SFA that were respectively positively and negatively correlated to bee abundance (Table 2, Fig. 2). Bee abundance was positively correlated to PUFA (Table 2, Fig. 2), and to the amount of alpha-linolenic (the main ω3) and linoleic (the main ω6) acids (Table 2, Fig. 2B), resulting in an absence of effect of bee abundance on the ω6/ω3 ratio. We also found that bee abundance was negatively correlated to SFA (Table 2, Fig. 2B). Interestingly, there was no interaction between bee abundance and treatment effect in FA composition (Table 2). Here again, neither fertilization intensity, temperature, variety ID nor varietal type modified ω6/ω3 ratio, FA group or profile (Table 2, Appendix A), hence having no significant effect on OSR seed oil quality.

**Discussion**

In this study, performed in 72 farmed OSR fields from 2013 to 2016, we found that insect pollination plays a key role in OSR quality. Oil seeds are associated with potential human health benefits when they contain high amounts of poly-unsaturated FAs and low amounts of saturated and trans-saturated FAs, as well as being a source of omega-3 fatty acids, especially the alpha-linolenic acid. We found that seeds from flowers

**Table 2** Output of linear mixed models explaining OSR fatty acid composition, by bee abundance, pollination-exclusion treatments and their interaction as well as by soil type, fertilizer intensity use, temperature, variety ID with this interaction with bee abundance. Estimated coefficient (“Est”), standard errors of this coefficients (Std), z-value and p-value are given for each explanatory variable for the average models. For FA group (PCA2), exponential spatial autocorrelation structure was used to take into account spatial dependencies in the data (see Methods). Significant results are bolded. “-” indicates explanatory variable was not kept in model selection.

	FA group (PCA1)				FA group (PCA2)				FA profile (PCA1)				FA profile (PCA2)				ω6 on ω3			
	Est	Std	z-val	p-val	Est	Std	z-val	p-val	Est	Std	z-val	p-val	Est	Std	z-val	p-val	Est	Std	z-val	p-val
Large mesh (treatment)	0.1	0.1	0.6	0.538	-0.4	0.1	3.3	0.001	-0.5	0.2	2.4	0.018	-1.1	0.2	5.4	<0.001	0.1	0.0	2.6	0.009
Small mesh (treatment)	0.2	0.1	1.8	0.068	-0.6	0.1	5.5	<0.001	-0.6	0.2	3.5	<0.001	-1.2	0.2	6.9	<0.001	0.1	0.0	3.4	0.001
Self (treatment)	-0.4	0.1	2.6	0.010	-0.7	0.1	4.9	<0.001	-0.4	0.2	1.9	0.062	-1.0	0.2	4.7	<0.001	0.0	0.0	0.0	0.989
Bee abundance	-0.4	0.2	2.3	0.023	0.1	0.2	0.4	0.707	0.3	0.3	1.1	0.257	-0.2	0.3	0.8	0.409	0.1	0.1	1.2	0.223
Bee x Large mesh	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Bee x Small mesh	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Bee x Self	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Fertilizer	-	-	-	-	-	-	-	-	0.0	0.0	0.2	0.842	0.0	0.0	0.5	0.632	0.0	0.0	0.2	0.874
Temperature	0.2	0.8	0.3	0.788	-1.4	1.9	0.7	0.478	-	-	-	-	-	-	-	-	0.0	0.2	0.2	0.848
Red soil (soil type)	0.2	0.3	0.5	0.601	-0.1	0.3	0.4	0.702	-0.1	0.2	0.3	0.775	-	-	-	-	0.0	0.0	0.1	0.886
Dk exstorn (variety)	-	-	-	-	-	-	-	-	0.1	0.3	0.4	0.705	0.4	0.4	1.0	0.306	-	-	-	-
Others (variety)	-	-	-	-	-	-	-	-	0.1	0.2	0.4	0.716	0.6	0.5	1.3	0.198	-	-	-	-
Bee x Dk exstorn	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Bee x Others	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2014 (year)	0.7	0.4	1.8	0.069	-	-	-	-	-1.8	0.5	3.5	<0.001	2.7	0.5	5.2	<0.001	-0.1	0.1	1.0	0.296
2015 (year)	0.9	0.3	2.6	0.009	-	-	-	-	-3.5	0.5	7.5	<0.001	2.1	0.5	4.3	<0.001	-0.1	0.1	0.9	0.385
2016 (year)	-1.4	0.4	4.0	<0.001	-	-	-	-	0.9	0.5	1.8	0.068	1.2	0.5	2.3	0.024	-0.1	0.1	0.7	0.502



**Fig. 2.** Principal component analyses and effect of treatments or bee abundance on (A) fatty acid group and (B) fatty acid profile. Coloured arrows represent FA groups or FAs correlations among the two first principal components. Smaller angles of the arrows indicate stronger correlations between FA groups or FAs correlation. PUFAs are in brown, MUFAs in grey and SFA in light blue. For FA profile, we separate trans-oleic from MUFAs which is represent in orange. Dots in (1-2) represent means for each treatment as predicted by linear mixed models on the two first principal components. The open treatment is in red, large mesh in green, small mesh in blue and self in purple. Black arrows represent correlations over the four treatments of bee abundance on the two first principal components as predicted by linear mixed models.

**Table 3**

Fatty acids composition of oilseed rape seeds collected in the 65 fields on plants with or without access to insect-pollination. Results are expressed as% of total FAs and are the mean value  $\pm$  standard error for the four treatments in the exclusion experiment. Mean, minimum and maximum values of FAs groups and main FAs from <sup>1</sup>Adamska et al. (2004), <sup>2</sup>Jahreis and Schäfer (2011), <sup>3</sup>Liersch et al. (2013), <sup>4</sup>Orsavova et al. (2015) are also given for comparison.

	This study				Litterature
	Open	Large mesh	Small mesh	Self	
Unsaturated FAs	90.5 ( $\pm 1.6$ )	90.3 ( $\pm 1.7$ )	89.7 ( $\pm 1.9$ )	90.1 ( $\pm 1.6$ )	93.1 (92.46–93.7) <sup>2,4</sup>
Polyunsaturated FAs	26.9 ( $\pm 2.5$ )	27.7 ( $\pm 3.2$ )	27.1 ( $\pm 2.8$ )	28.9 ( $\pm 3.8$ )	28.53 (20.9–32) <sup>2,4</sup>
Linoleic acid (C18:2)	18.5 ( $\pm 1.8$ )	19.3 ( $\pm 2.4$ )	18.8 ( $\pm 2$ )	19.9 ( $\pm 2.8$ )	20.54 (19.6–20.94) <sup>1,2,3,4</sup>
Alpha-linoleic acid (C18:3)	8.3 ( $\pm 0.8$ )	8.3 ( $\pm 1.1$ )	8.2 ( $\pm 1$ )	8.9 ( $\pm 1.2$ )	7.96 (1.2–10.67) <sup>1,2,3,4</sup>
Monounsaturated FAs	65.3 ( $\pm 2.3$ )	64.3 ( $\pm 3.1$ )	64.5 ( $\pm 2.7$ )	63 ( $\pm 3.9$ )	66 (60.9–71.1) <sup>2,4</sup>
Oleic acid (C18:1)	58.3 ( $\pm 2.4$ )	57.3 ( $\pm 3.3$ )	57.2 ( $\pm 2.7$ )	56 ( $\pm 3.6$ )	62.27 (60.9–63.3) <sup>1,3,4</sup>
Trans-saturated FAs	1.8 ( $\pm 1$ )	1.6 ( $\pm 0.9$ )	1.9 ( $\pm 1$ )	1.9 ( $\pm 0.9$ )	0.14 <sup>4</sup>
Saturated FAs	7.7 ( $\pm 0.8$ )	8.1 ( $\pm 0.9$ )	8.3 ( $\pm 1.1$ )	8 ( $\pm 0.9$ )	6.15 (6.2–6.3) <sup>2,4</sup>
Palmitic acid (C16:0)	4.5 ( $\pm 0.4$ )	4.7 ( $\pm 0.4$ )	4.8 ( $\pm 0.5$ )	4.7 ( $\pm 0.5$ )	4.65 (4.48–4.86) <sup>1,3,4</sup>
Stearic acid (C18:0)	2.2 ( $\pm 0.7$ )	2.2 ( $\pm 0.8$ )	2.3 ( $\pm 0.8$ )	2.3 ( $\pm 0.8$ )	1.64 (1.6–1.7) <sup>1,3,4</sup>
w6- w3 ratio	2.2 ( $\pm 0.2$ )	2.4 ( $\pm 0.3$ )	2.3 ( $\pm 0.2$ )	2.3 ( $\pm 0.2$ )	2 <sup>2</sup>

with access to pollinators (open treatment) had higher oil contents and mono-unsaturated FA but lower amounts of saturated and trans-saturated acids. Moreover, bee abundance was positively correlated to the amount of poly-unsaturated FAs and negatively to the amount of trans-saturated and saturated FAs, indicating that the abundance of pollinating bees further improved oil quality. Therefore, our results suggest that both the presence and the abundance of bees improve the nutritional value of OSR oil, and constitute the first evidence to the best of our knowledge, for such a positive role.

Seeds from flowers with access to pollinators (open treatment) showed a higher oil content (+2.4 %), a higher amount of mono-unsaturated FAs (+0.8 %) and a lower amount of saturated FAs (–0.5 %). Higher oil contents (of similar magnitude) were also found in insect-pollinated seeds compared to non-insect pollinated seeds (Bartomeus et al., 2014; Bommarco et al., 2012; Marini et al., 2015). More bees resulted in a higher amount of poly-unsaturated FAs, and a lower amount of saturated FAs especially stearic acid and trans-saturated FAs.

Our results are in agreement with previous studies which showed that insect-pollinated seeds had a higher amount of mono-unsaturated FAs and a lower amount of mono-unsaturated FAs than in self-pollinated seeds in almonds (Brittain et al., 2014) or in *Jatropha curcas* L. (Negusie et al., 2015). The effect of insect-pollination on the FA composition of oilseed rape seeds that we have detected in this study shows that bees improve seed quality, in addition to their positive effect on yields. Therefore, bee abundance enhances OSR production for farmers (yield), but may also improve seed quality, which might have some impact on human health (by modifying FAs composition). The causal effect of pollinators on seed quality remains, however, poorly understood. A potential mechanism explaining the positive effect of pollinators on oil content is that pollinators were shown to reduce flowering times and speed up seed maturation, which reduces green seed and increases oil content (Adamidis et al., 2019; Bommarco et al., 2012; Fairhurst et al., 2022; Oz et al., 2008). For FA composition, positive bee effects were detected independently of experimental treatment, thus of the

pollination process, which suggests that the bee effect results from plant resource allocation rather than pollination per se. From a carbon perspective, poly-unsaturated FAs cost more to produce and yield less energy when oxidized, than monounsaturated or saturated FAs (Linder, 2000). However, seeds rich in unsaturated FAs may have a competitive advantage by being able to germinate earlier and grow more rapidly at low temperatures (Linder, 2000). Consequently, an OSR plant may have an advantage to produce seeds with high amounts of poly-unsaturated FAs and low amounts of saturated FA, but this composition can be limited by the energy required to produce poly-unsaturated FA. Bee presence was also shown to reduce OSR blooming period, root biomass, number of pods or plant biomass (Adamidis et al., 2019; Fairhurst et al., 2022; Perrot et al., 2018). Consequently, plants pollinated by bees may invest less in producing flowers, roots and biomass, allowing higher allocation to the production of unsaturated FAs. However, since pollination-exclusion treatment and bee abundance showed different effects on FA composition, we cannot reject the possibility of a reduced seed formation due to the mesh in exclusion treatments, because the proportion of oleic acid or oil content increases with seed formation while linoleic decreases (Vera et al., 2007). Moreover, we did not find any correlation between bee abundance and oil content. Only a few exogenous pollen is necessary to increase OSR seed weight, or number of seeds per pods (Lankinen et al., 2018). This may explain why we detected a significant effect of insect pollination on oil content in the exclusion experiment, but not of the bee abundance gradient.

In our study, FAs composition showed lower oleic acid but higher saturated and trans-saturated FA, and similar poly-unsaturated percentage compared to those available from literature (Table 3). FAs composition did not differ between variety ID or varietal type, as opposed to the results found by Liersch et al. (2013), who found differences between cultivars for most FAs. For example, a higher amount of linoleic acid was found in the Kasub variety (20.19 %) than in the Lisek variety (18.6 %). Finally, a large part of the variance in FAs composition and oil content was explained by inter-annual variation (Tables 1 and 2). This could be explained by annual variation in water availability (Champolivier & Merrien, 1996) or in temperature (Baux et al., 2013), both known to modify OSR quality (Rathke et al., 2006). However, we found no strong effect of temperature on FA composition in our study. We cannot exclude that, additionally, these variations are related to inter-annual variations in honeybee and *Lassioglossum* abundances. These two pollinators may have different roles in crop pollination because of different foraging behavior. More investigations are therefore needed to understand the mechanisms underlying the effects of fertilisers, year and variations in bee abundance on oil quality. In addition, experiments at higher biological scale, i.e. in our case at plant scale need to be conducted to confirm our results observed at branch scale, as OSR plants can adapt their seed production strategy when insect pollination is scarce (Adamidis et al., 2019; Fairhurst et al., 2022).

## Conclusion

Our study confirms that pollinators impact not only yield but also quality of production, and that the latter should not be underestimated. Indeed such beneficial effect of bees on quality has economic benefits (Bommarco et al., 2012). According to the latter, each supplementary% unity in oil content increases oilseed rape sell price by 1.5 %. In a previous study in our site, we found that absence of bee pollination resulted in OSR yield of only 2.55 t/ha, while in the presence of abundant bees, yield reached up to 0.84 t/ha (Perrot et al., 2022). With a sell price of 373.7 €·t<sup>-1</sup> (reference price Euronext May 2015) and for an average field size of 6.7 ha, the total gain for farmers would be 6384.7 € per field (Perrot et al., 2022). Similarly, bee pollination, as found in the present study, would increase sell price by 3.75 % (thus reaching 387.7€ per ha) because of improved oil content of 2.4 % (2.3–2.6 %). At the entire field scale, bees will increase farmers' gain by 2421.5 € (361.4 €/ha), resulting mainly from yield increase (+313.9 €/ha, i.e. 86.8 % of the

increase) rather than yield quality (+47.5 €/ha). Hence the gain increase for the farmer resulting from quality improved by bees is approximately 13 % additional gain. Improving pollination service in such agroecosystems may therefore represent an economic added value that may balance biodiversity conservation in agricultural landscapes, since the latter requires ecological infrastructures at the expense of cropped areas (Montoya et al., 2019). Incentive monetary values could be proposed by agricultural cooperative to farmers, for promoting agricultural practices or landscape infrastructures that improve bee diversity and abundance, which in return may improve farmer's income.

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## CRediT authorship contribution statement

**Thomas Perrot:** Formal analysis, Writing – original draft. **Vincent Bretagnolle:** Visualization, Writing – review & editing. **Niyazi Acar:** Formal analysis, Writing – review & editing. **Valérie Febvret:** Formal analysis, Writing – review & editing. **Annick Matejcek:** Formal analysis, Writing – review & editing. **Stéphane Grégoire:** Visualization, Formal analysis, Writing – review & editing. **Sabrina Gaba:** Writing – original draft, Writing – review & editing.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## Supplementary materials

Supplementary material associated with this article can be found, in the online version, at [doi:10.1016/j.baae.2024.02.002](https://doi.org/10.1016/j.baae.2024.02.002).

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