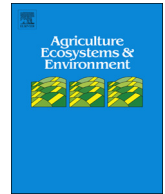




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## Bees increase oilseed rape yield under real field conditions

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## ABSTRACT

Oilseed rape (OSR, *Brassica napus* L.) is a common crop found in many European agricultural landscapes. It is pollinated by a wide variety of insects, but the reported contribution of pollinators to yield varies widely between studies (from 0 to 50%). Moreover, such a contribution has seldom been estimated at the field scale in real farming conditions. We analysed OSR yields in response to insect pollination; over four years, at two different scales: farm fields and individual plants. We used both empirical and experimental approaches along a gradient of pollinator diversity and abundance. The empirical approach was based on farm surveys (151 fields) while the experimental approach used various pollination exclusion methods (570 plants in 101 fields) to estimate the relative contributions of insect, wind, and self-pollination. The OSR yields were positively correlated to total bee abundance and bee genera diversity, through improved fruiting success and plant seed mass (after adjusting for plant biomass). Hoverfly diversity and abundance, and bumblebee abundance did not have any effect. The main OSR pollinators in our study were honeybees (*Apis mellifera*) and wild bees (*Lasioglossum* spp.). Yields were increased, on average, by up to 37.5% (27.7% – 47.5%) at field scale when bee genera diversity increased from a single genus to more than 10 genera (pan-trap data). Insect pollination contributed about 30% of plant yield. Self-pollination and wind pollination accounted for the remaining 70%, with self-pollination being the major contributor. Our study demonstrates that pollinator diversity and abundance, at least at very high levels, have a major effect on OSR yields. This suggests that establishing a monetary value for pollination services in OSR farming systems could be used to balance the cost of managing semi-natural habitats or meadows to maintain bees and other pollinators.

## 1. Introduction

In most angiosperms, pollen transfer depends on animals (Ollerton et al., 2011), and this holds true for both wild and domesticated plant species, of which 70% are pollinator dependent (Klein et al., 2007). The economic value of pollination service has been estimated at 10% (€149 billion) of yearly global world agricultural production (Gallai et al., 2009), being particularly important for the yield of many small farms (Garibaldi et al., 2016). The dependence of crop yields on insect pollination, however, varies widely between crops, from independent to obligate (Klein et al., 2007). Pollinators not only increase yields by increasing seed set, but they may also enhance crop quality (Bartomeus et al., 2014), and stabilise food production either in time (Garibaldi et al., 2011) or space (Deguines et al., 2014). However, despite the global importance of pollinators for food production, pollination is

rarely taken into account in the development of farming systems or practices (Breeze et al., 2014), partly because it is difficult to disentangle pollination by insects from other factors that affect yield (Marini et al., 2015). Additionally, there may be an order of magnitude variation in the effect of insect pollination on yields within a particular crop (Gallai et al., 2009). This variability is explained by the spatial variation of pollinator communities, leading to a spatial variation of pollination potential and pollen limitation (Gómez et al., 2010), reducing agricultural production (Wilcock and Neiland, 2002).

Oilseed rape (OSR, *Brassica napus* L.) is the fourth largest oil crop in terms of production in the world and the most common in the European Union (FAOSTAT, 2014). OSR is not only pollinated by insects but also by wind and self-pollination (Becker et al., 1992; Mesquida and Renard, 1982). Wind pollination is the transfer of pollen from one plant to another by passive wind transport, while self-pollination is the direct

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passage of pollen between the male and female parts of the same flower or between two flowers on the same plant. There is huge uncertainty in estimates of the relative importance of insect pollination for OSR yields, with reported values ranging from negligible (Sammegard et al., 2016) to 50% (Araneda Durán et al., 2010) with a range of values in between (Bartomeus et al., 2014; Bommarco et al., 2012; Lindström et al., 2016; Stanley et al., 2013; Zou et al., 2017). There is no accepted explanation for such a high variability, which may result from farming practices (Marini et al., 2015), plant varieties (Hudewenz et al., 2014), or variation in pollinator communities (Rader et al., 2015). The major pollinators also depend strongly on the study being honeybees (*Apis mellifera*), bumblebees, wild bees or hoverflies (Garratt et al., 2014; Lindström et al., 2016; Zou et al., 2017b). Factors affecting OSR yields include pollinator visitor rate (Bartomeus et al., 2014; Woodcock et al., 2013), nearby honeybee hives (Lindström et al., 2016), and bee diversity (Zou et al., 2017). In addition, the measurements used to estimate the effects on OSR yields varied between studies, from being a small part of the OSR plant (Stanley et al., 2013), total seed production per plant (Hudewenz et al., 2014; Zou et al., 2017), or a set of OSR plants from either small (< 2 m<sup>2</sup>, Araneda Durán et al., 2010; Bommarco et al., 2012; Bartomeus et al., 2014) or large (> 50m<sup>2</sup>, Lindström et al., 2016) field section (in this latter study, only the contribution by honeybees was investigated). So far, to our knowledge, no study has ever quantified the effect of pollinators on yield at field scale for oilseed rape.

Here, we use, for the first time, a systemic approach by quantifying the effect of insect pollination on OSR yields, at both field scale and individual plant scale, combining field scale yields and field scale assessments of pollination. We used both empirical data obtained for 151 fields and experimental manipulation of pollination in 101 fields. The yield estimates from both methods were compared with pollinator abundance and diversity, obtained by trapping in the fields. The OSR focus fields were distributed along gradients of landscapes with varying concentration of meadows, semi-natural habitats and organically farmed fields, which are all known to affect pollinator abundance and diversity (Holzschuh et al., 2008; Kennedy et al., 2013; Steffan-Dewenter et al., 2002; Woodcock et al., 2013). By using landscape gradients, we aimed to maximise the variation in the pollinator community to be able to quantify its effects on yield and identify the main pollinators involved. By measuring various fecundity traits of the OSR plants, such as the fruiting success, seeds per pods, seed unit weight and seed mass, we also identified the traits that were most affected by pollinators. Finally, we quantified the relative contributions of insects (large versus small), wind and self-pollination at plant level. Our experimental design, changing pollinator abundances using landscape variations as well as using pollinator exclusion, allowed us to i) quantify the effect of pollinator rich landscapes on pollination rate, and ii) quantify the contribution of pollination by insects at plant (grain biomass per plant) and field (yield) scales.

## 2. Material and methods

### 2.1. Study site, experimental fields and landscape context

Pollinator exclusion experiments and farming surveys were conducted between 2013 and 2016 in the LTSER “Zone Atelier Plaine & Val de Sèvre”, a 450 km<sup>2</sup> study site located in the south of Deux-Sèvres district (Fig. 1a), central western France (Fig S.A, <http://www.za.plainevalsevre.cnrs.fr/>, Bretagnolle et al., 2018). Only winter OSR is grown in the LTSER, representing about 8% of the agricultural area (Fig. 1a). Experiments were conducted directly in commercial farm fields. Since we were interested in quantifying insect pollination in OSR fields under normal conditions, we did not request any modification of the farming practices. We used a moving window to randomly select 1 km<sup>2</sup> squares (Fahrig et al., 2011) that represented density gradients of three environmental features: semi-natural habitats (hedgcs and forest

fragments), meadows, and organically farmed fields (obtained from the French parcel register 2014). All these landscape features are known to strongly influence the abundance of pollinators (Kennedy et al., 2013) and were mapped in the GIS LTSER (Bretagnolle et al., 2018). Within the selected squares, an OSR focus field was then chosen, if present (usually, there was only one OSR field). On average, OSR fields were at 365 m (48 to 1152 m) from the nearest OSR neighbour. Field size ranged from 0.65 ha to 28.5 ha (mean 6.3 ha). The selected fields had similar soil types according to the IGCS soil map (available at <https://www.geoportail.gouv.fr/>). In 93% of the fields the soil was calcareous and in the rest the soil was red (with some clay).

A first set of 151 OSR fields (27 in 2013, 45 in 2014, 48 in 2015, and 31 in 2016) was used for an empirical assessment of the effects of pollinator abundance and diversity on crop yield at the field scale. No field was used twice in the four years. We interviewed the farmers, owners of the fields, to collect information on practices (fertilizer, pesticides, and OSR variety) and yield at the end of each cropping season (during winter). A second set of 101 fields (15 in 2013, 29 in 2014, 27 in 2015, and 30 in 2016) was used for pollinator exclusion experiments, of which 66 were also in the first set. The two sets differed because some farmers refused the survey or refused permission for the exclusion experiment. There were 28 varieties of OSR, mainly restored hybrid (88.7%) and conventional (11.1%). All OSR varieties in this study could be self-pollinated or cross-pollinated.

### 2.2. Experimental treatments

Six individual OSR plants were selected in each field at two positions: one at the field edge and one at 20 m from the edge in the field core. These two positions were selected to assess whether the distance from semi-natural habitats affected the pollination by insects (Woodcock et al., 2016). For each individual OSR plant, three (2013), two (2014) and four (2015–16) secondary branches were selected for pollinator exclusion treatments (Fig. 1c). There were different numbers of branches in each year because we tested different exclusion treatments. The branches were selected so as to be at the same flowering stage and adjacent or opposite to each other. The various exclusion treatments allowed self-pollination (SF), wind-pollination (W), small-bodied (SP) and large-bodied (LP) insect pollinators. One of the branches was used as a control (570 branches in total) where all flowers could be pollinated in any way (insects, wind and self-pollination, “LP + SP + W + SF”). A second branch was enclosed in a small mesh bag (0.6 mm mesh size, 517 branches), for which the flowers could only be self-pollinated or wind pollinated (“W + SF”). In 2013, 2015 and 2016, a third branch was enclosed in large mesh bag (3 mm mesh size, 403 branches), allowing self-pollination and pollination by wind and small insects (“SP + W + SF”). Finally, in 2015 and 2016, a fourth branch was enclosed in a gas-permeable Osmolux bag (Pantek, France) (272 branches), excluding all except self-pollination (“SF”). In 2013 only, each treatment was replicated for each plant (i.e. two controls, two large and two small mesh treatments per plant). The branches were bagged before onset of flowering and plants were visited weekly to adjust the bags, lifting them upwards to cover new or future flowers while releasing those flowers that had faded. The bags were completely removed after the last flower had faded. The operations were carried out gently to avoid as far as possible any effect on pod development (Wragg and Johnson, 2011). Branches were collected five days before the harvest. In 2015 and 2016, the rest of plant was also collected to estimate the total plant biomass and total seed biomass. In 2016, six further OSR plants were collected, three from the edge and three at 20 m from the edge, from each of the 44 fields monitored that year, to estimate the effect of pollinators on the total plant production (see Appendix A in Supplementary material for sample sizes and treatments for each year).

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