# Pollination of two oil-producing plant species: Camelina (*Camelina sativa* L. Crantz) and pennycress (*Thlaspi arvense* L.) double-cropping in Germany

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## Abstract

Camelina and pennycress are two annual oil-producing plant species that have recently gained attention as biofuel feedstock crops. Prior to commercial production, information on their breeding and pollination system is essential to ensure sustainable management. We conducted pollination experiments and observed flower visitors in an experimental double-cropping system in southern Germany. We found that common camelina varieties were mainly self-pollinated and yield of one variety seemed to benefit from insect visitation, whereas pennycress was predominantly wind pollinated. Camelina showed higher overall visitation rates by insects than pennycress. Flies and wild bees visited both crop species, but honey bees visited camelina only. We conclude that both oil crop species produce yield without pollinators but offer foraging resources for different insect taxa at times when few other crops and native plants are flowering.

*Keywords:* biofuels, Brassicaceae, breeding system, ecological sustainability, ecosystem services, honey bees, wild bees

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## Introduction

Nowadays agriculture not only provides goods for human food consumption but also feedstock for the bioenergy sector. Ever increasing worldwide energy consumption and demand for renewable energy forms has lead to a steep rise of biofuel feedstock production in agriculture (United Nations, 1998; BP, 2012; OECD, 2012). In temperate regions main biofuel feedstock crops are corn (*Zea mays* L.), rapeseed (*Brassica napus* L.), and soy (*Glycine max* (L.) MERR.) (FAO, 2008; UNEP, 2009). The expansion of their cultivation for biofuel feedstock production and its ecological sustainability is, however, questionable. Major areas of concern are indirect land-use change (iLUC), simplification of landscapes, rivalry of food and fuel production, the use of agro-chemicals and fertilizers, and detrimental effects on biodiversity (Lüdeke-Freund *et al.*, 2012). Therefore, the search for alternative cultivation systems and biofuel feedstock species is ongoing.

Camelina (*Camelina sativa* L. Crantz) and pennycress (*Thlaspi arvense* L.) are two species recently considered for biofuel feedstock production in temperate regions. Both are gaining importance as their oil has been

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proven suitable for biodiesel (Moser *et al.*, 2009; Moser, 2010) and biokerosene production (Shonnard *et al.*, 2010). Other applications are in the cosmetic, biolubricant, and in the culinary sector (the last for camelina only) (Pilgeram *et al.*, 2007; Cermak *et al.*, 2013).

Camelina and pennycress can be both cultivated in a double-cropping system with other crops. Double-cropping is a cultivation method, where two crops are consecutively produced and harvested on the same land in the same year. While it is common practice in the (mid-) southern United States (especially with wheat followed by soy; Marra & Carlson, 1986; Crabtree *et al.*, 1990), it is less established at higher latitudes as in Germany. A reason is that the second crop may not mature due to the short remainder of the growing season at higher latitudes, unless it is a very fast growing early maturing crop (Shapiro *et al.*, 1992; Gesch & Archer, 2013) or harvested premature, i.e. for biomass production (Karpenstein-Machan, 2001; Heggenstaller *et al.*, 2008). Camelina and pennycress can, however, potentially reach maturity as a second crop in a double-cropping system in Germany due to a very short vegetation cycle of 80–120 days. Double-cropping increases the period of time in which the land is used for crop production. This increases the overall production and economic returns and minimizes the problem of food displacement and effects of indirect land-use change (Lüdeke-Freund

et al., 2012). Further advantages of double-cropping are the protection of soils from wind and water erosion through vegetation cover, the improvement of soil structure and the reduction in nitrate leaching into deeper soil layers (Schmidt, 2000; Cherr et al., 2006; Heggenstaller et al., 2008). Furthermore, double-cropping diversifies the system on a temporal scale through the introduction of another crop species. Disadvantages of double-cropping include additional work load for the farmer, risk of crops not reaching maturity due to climatic constraints, risk of lower yields of subsequent crops due to delayed sowing dates, and/or decreased moisture levels affecting germination and emergence of the second crop (Marra & Carlson, 1986; Shapiro et al., 1992).

As the cultivation of camelina and especially pennycress at a larger scale is still at its beginning many questions of how to grow the crops ecologically sustainable remain open. One central question regards the species' breeding and pollination systems. This information is important, because crop yields may depend on or benefit from pollination services by insects (Klein et al., 2007). Therefore, the breeding systems of camelina and pennycress have to be understood to ensure a reliable crop production. This knowledge is needed as a basis for decision-making on pollination management and its potential optimization. Furthermore, mass flowering of the two species may retract flower visitors of endangered wild plant species (Holzschuh et al., 2011), or may supply additional flower resources to insects, especially later in the year (Westphal et al., 2003, 2009). Hence, it is important to assess the flower visitors and possible impacts of camelina and pennycress cultivation in a double-cropping system on pollination services and wild pollinator functional diversity.

Camelina and pennycress are commonly assumed to be self-pollinated, often based on flower morphology, but systematic studies are lacking (camelina: Fruwirth, 1906; Plessers et al., 1962; Zubr, 1997; Mulligan, 2002; Vollmann et al., 2005; pennycress: Knuth, 1908; Mulligan, 1972, 2002; Best & McIntyre, 1975; Al-Shehbaz, 1986). Flower visitors of pennycress are listed by Knuth (1908), Mulligan & Kevan (1973), and Al-Shehbaz (1986); however, no conclusions about the breeding system are drawn. Studies explicitly addressing the breeding system are lacking for both species. Thus, the objective of this study was to assess the breeding and pollination systems of both plant species.

## Material and methods

### Crop species and the double-cropping system

Camelina and pennycress are summer and winter annuals belonging to the mustard family (Brassicaceae). They possess a short vegetation cycle of 80–120 days and produce oil rich seeds. Camelina is 30–100 cm tall with light to dark yellow flowers and pear-shaped fruits. It is native to Eurasia (Francis & Warwick, 2009) and naturally found as a weed in agricultural fields and in dry ruderal habitats (Rothmahler, 2002). Pennycress is 10–50 cm tall, has small white flowers and winged silicles containing 10–16 seeds. It originated in Eurasia (Mitich, 1996) and grows naturally on nutrient-rich, loamy fields, and ruderal habitats (Rothmahler, 2002). Until recently, both species were mainly considered weeds in crop production systems. However, camelina was grown for its oil and grain in Europe as early as the late neolithic, and especially during the Bronze and Iron Age (Kroll, 1994). During the Middle Ages cultivation declined and continued only sporadically until the modern times (Kroll, 1994).

The short vegetation cycle and strong competitiveness as weeds makes both species candidates for cultivation in a low input double-cropping system, which can be incorporated into existing crop rotations. In temperate regions, this can either be in summer from late June to September as the second crop of the system after the cultivation of winter cereals like barley (Fig. 1) or during winter from September to June as the first crop preceding corn or soy (Lüdeke-Freund et al., 2012; Gesch & Archer, 2013). The latter is difficult to realize for camelina in Germany, due to a too short growing season left for the second crop following camelina. This study was conducted in the summer double-cropping system, which is feasible in Germany for both species.

We chose three common varieties of camelina to test for variety effects within one species (Klein et al., 2007). The target varieties were Ligena (Deutsche Saatenveredelung, Germany), Celine (French variety from the United States, obtained from Cropland Biodiesel), and Calena (Bayrische Saatenveredelung, Germany). As of yet, no official varieties exist for pennycress. Therefore, only one line (Spring 32 from Western Illinois University, USA; European lines were not available) was used and variety effects could not be tested.

### Study area

The experiments were conducted from June to September 2011 in Dundenheim, Offenburg, Baden–Württemberg, southern Germany (48°27′13.85″N, 7°49′08.14″E, 144 m asl.). Mean annual temperature and precipitation are 10.83 °C and 492.37 mm, respectively, based on data of 25 years (mean monthly temperature and precipitation for the study period June–September: 18.80 °C and 104.87 mm, respectively) (Deutscher Wetterdienst, 2012). Each variety/line was hand sown at rates of 0.3–0.5 g m$^{-2}$ in field strips of 5 × 18 m on June 20th, 2011. The land was previously cultivated with strawberries. The field was ploughed and harrowed before broadcasting the seeds, which were mixed with 2 kg of sand for better distribution. To ensure proper soil contact a roller was used afterward. No fertilizer was applied and the use of agrochemicals against pests and diseases was not deemed necessary. The site was chosen due to warmer climatic conditions and a longer vegetation period compared to northern Germany as time availability for growing an additional crop may be a critical issue later in the year.
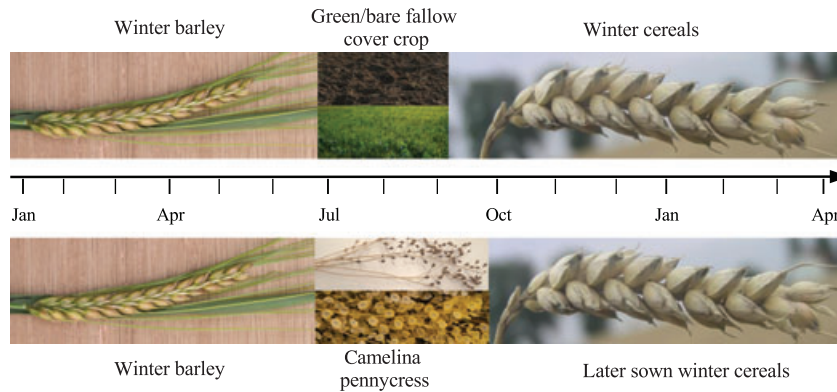
Winter barley Green/bare fallow cover crop Winter cereals

Jan    Apr    Jul    Oct    Jan    Apr

Winter barley    Camelina pennycress    Later sown winter cereals

**Fig. 1** Commonly used crop rotation (upper panel) and the proposed summer double-cropping system with winter cereals and camelina and pennycress (lower panel) adapted for Germany. Pictures of barley and wheat modified from Rasbak (2004), Filippov (2010).

## Pollination experiment

We studied the breeding system (degree of autonomous self-pollination = autogamy, wind pollination = anemogamy and insect pollination = zoogamy) by conducting the following pollination treatments:

1 Open pollination (OP)
2 Autonomous self-pollination (SP)
3 Wind pollination (WP)
4 Hand cross-pollination with pollen from a different plant individual of the same variety ($CP_s$)
5 Hand cross-pollination with pollen of another variety ($CP_o$, for camelina only).

Each treatment comprised 10 plant individuals, totaling 150 plant individuals for all camelina varieties (50 plants per variety) and 40 plant individuals for pennycress. Treatments were conducted on a plant scale during five and six consecutive days starting with nonflowering plants on July 30th until August 3rd for camelina and July 30th until August 4th 2011 for pennycress, respectively.

In the open pollination treatment (OP), plant individuals were marked without any further manipulations and insects had access to flowers. For the self-pollination (SP) treatment, plant individuals were bagged with air permeable bags (Crispac bags, 330 × 750 mm for camelina, 100 × 175 mm for pennycress, small punching Super micro SM, diameter 0.5 mm, Baumann Saatzuchtbedarf, Waldenburg, Germany), excluding pollen carried by wind or insects, but allowing autonomous self-pollination including geitonogamy. For WP, insects were excluded by gauze bags, allowing autonomous self-pollination and wind-transported pollen to enter (Klein *et al.*, 2003). We used mesh gauze (Rantai S48, mesh size 0.8 × 0.8 mm, thread size 0.19 mm) bags of 45 × 75 cm for camelina and 20 × 24 cm for pennycress, which were closed around the stem by stapling. To keep insects from climbing up the stem into the bags through the remaining gap we placed sticky glue (Aurum Insekten Leim, Neudorff GmbH KG, Emmerthal, Germany) onto the stem. Hand cross-pollination was conducted daily by transferring pollen with a pin from fresh flowers of other plant

individuals of the same variety to all newly opened flowers of the focal plant individuals ($CP_s$). To test for variety effects of the pollen source (same vs. different variety) on pollination success in camelina we conducted hand cross-pollination with pollen of a different variety ($CP_o$). Focal plant individuals were pollinated with pollen of the neighboring variety: Ligena was fertilized with pollen from Celine, Celine with pollen from Calena, and vice versa. The plant individuals of both hand cross-pollination treatments were also covered with Crispac bags to exclude wind and insect pollination after flower opening before daily treatments. However, self-pollination could not be excluded.

To keep bags from touching plant parts, especially flowers, and to rule out insect pollination from the outside (in WP), all bags were stabilized with wire.

After treatment was completed, bags and newly emerging flowers and flower buds were removed to ensure maturing fruits derived from our treatments. All flowers/fruits in the experiment were counted and plants left for ripening. Fruits were harvested on September 1st for camelina and August 30th/31st for pennycress. Seeds of 10 pods per plant individual were counted to calculate the mean number of seeds per pod. All seeds per plant individual were counted (Seed counter 'Contador' with feed container No.1, for rape seeds and small seeds, Pfeuffer GmbH, Germany), dried at room temperature, and weighed (Sartorius Model BP 1200 toploader balance, Sartorius, Germany).

## Flower visitor observations

We studied the pollination systems by observing the flower-visiting community. Therefore, five rounds of transect walks were conducted for camelina (two per variety for Celine and Calena, one for Ligena) and two rounds for pennycress. Transects were 1.5 m from the field edge, resulting in three transects of 15 m length and 0.7 m width per field, which were repeatedly sampled over a period of 30 min. Sampling was done on sunny days with temperatures above 18 °C and low wind speeds ($<2.5$ ms$^{-1}$). All observed flower-visiting insects were caught in vials with ethyl acetate in the

field and stored in 80% ethanol in the lab. Identification was done to species level for the main functional groups honey bees (*Apis mellifera*), wild bees, and syrphid flies (Syrphidae) using Amiet *et al.* (1999, 2001, 2007) and Bothe (1996). True bugs (Hemiptera), other flies (Diptera), and all other groups were identified to morphospecies. Mean number of captured insect individuals per transect walk were used for comparison among plant species.

### Data analyses

Analyses were based on plant individuals. Yield in terms of fruit set (FS), mean number of seeds per pod (SpP), mean number of seeds per open flower (SpOF), and mean seed weight per open flower (SWpOF) were used as response variables to compare pollination treatments.

The number of open flowers (OF) correlated with the type of bags used for the treatment (low in treatments with Crispac bags, high with gauze or no bag, camelina: $P = 0.0001$, pennycress: $P = 3.19e\text{-}06$) suggesting a bag or microclimate effect (Fig. S1).

As the number of OF influences the number of pods that can potentially develop, the response variable developed pods (DP) may show a mixture of treatment and bag effect. Therefore, fruit set (FS), calculated as:

$$FS = DP/OF \tag{1}$$

was used resulting in a response variable clean of bag effects.

Pollination treatment may not only influence the number of fruit set, but also pod filling, i.e. number and weight of seeds in pods. Thus, pollination success (PS) was defined as the product of DP and mean number of seeds per pod (SpP):

$$PS = DP * SpP \tag{2}$$

However, because DP, as a term based on different numbers of open flowers, may be affected by a bag effect we used FS instead, resulting in the mean number of seeds set per open flower (SpOF):

$$SpOF = (DP/OF) * SpP \tag{3}$$

This term is independent of the flower actually setting fruit or not, but reflects the mean number of seeds produced by one flower.

To further take into account weight difference resulting from the treatments mean individual seed weight (ISW) was added to Eqn (3), resulting in the following:

$$SWpOF = (DP/OF) * SpP * ISW \tag{4}$$

SWpOF is the mean collective seed weight produced by one flower.

The effect of the pollination treatment on fruit set and pollination success was analyzed using a one-way ANOVA with subsequent Tukey test (glht function in library multcomp, R version 2.15.0 for Linux) (Hothorn *et al.*, 2008; R Development

Core Team, 2012). Response variables used were FS, mean number of seeds per pod (SpP), mean number of seeds per open flower (SpOF) and mean seed weight per open flower (SWpOF). SpOF and SWpOF are adequate units to answer the question of the study, as they are independent of any unequally distributed number of open flowers and also reflect the quality of pollination. For camelina we analyzed the data pooled for all varieties, as also the varieties on a separate basis.

In the camelina variety Ligena three plant individuals (one SP, two WP) and in pennycress one plant individual (WP) died during the experiment and were excluded from the analyses.

As the experimental pollination treatments partly intermix the different pollination regimes SP, WP, and insect pollination (IP), we estimated the effect of the last on the mean number of seeds per open flower and mean seed weight per open flower using the following model:

$$y_i = P_i + e_{ij}, \tag{5}$$

where y seeds per open flower or seed weight per open flower
  P pollination treatments (Crispac bags, gauze bags, no bag)
  e error term

The different bagging types used for the pollination treatments allow for different pollination regimes. With no bags ( =OP treatment) SP, WP, and insect pollination (IP) is possible. The gauze bags ( =WP treatment) allow SP and WP, whereas the Crispac bags ( =SP treatment) only allow SP.

Rewriting Eqn (5) in matrix notation and replacing treatments by pollination regimes gives the equation:

$$y = X'b + e \tag{6}$$

where y vector (of seeds per OF or seed weight per OF)
X incidence matrix linking observations with unknowns (b)
b vector of unknowns (SP, WP, IP)
e error term.

X has then to be set up for the three constituent components SP, WP, and IP, instead of experimental treatments. As an example, for a plant under OP (no bag) the correspondent line in X is 1 1 1 indicating SP, WP, and IP, whereas a plant with a gauze bag leads to 1 1 0.

Once X has been set up, the least squares solution for b is straight forward:

$$\hat{b} = (X'X) - 1 * X'y \tag{7}$$

The obtained estimates were then added together and set at 100%, representing the naturally available pollination. Subsequently, the contribution of each pollination regime in percent was calculated.

### Results

#### Breeding system of camelina

Our experiments showed that camelina is mainly self-pollinated. Across all treatments and the three varieties, a total of 9527 flowers opened of which 7936 developed

and 1626 did not develop to pods. Fruit set was significantly higher in the open and hand cross-pollination treatment with pollen of another variety compared to the wind pollination treatment, but fruit set from self-pollination did not differ from that in open pollination (Fig. 2a). The mean number of seeds per pod, the mean number of seeds per open flower as well as mean seed weight per open flower (in the following section referred to as number of seeds per pod, number of seeds per open flower, and seed weight) were all significantly higher in the open compared to the wind and self-pollination treatments (Fig. 2b–d). The different cross-pollination treatments with pollen of a different or the same variety did not differ between each other for any of the response variables.

All varieties presented highest fruit set, number of seeds per pod, number of seeds per open flower as well as seed weight when open pollinated (Table S1). Fruit set of the variety Ligena differed among treatments in the overall model, however, we found no significant differences between the pairwise treatment combinations. The number of seeds per pod, number of seeds per open flower as well as seed weight did not significantly differ among pollination treatments (Table S2).

Fruit set of the variety Celine was significantly lower in the wind compared to the open and self-pollination treatments (Table S2). However, fruit set of self-pollinated flowers did not differ from open-pollinated flowers. The number of seeds per pod of open-pollinated flowers did not differ from the wind- and self-pollinated flowers, but was significantly higher in the open pollination treatment compared to both hand cross-pollination treatments. The number of seeds per open flower was significantly higher in the open compared to the wind and both hand cross-pollination treatments, but the self-pollination treatment did not differ from the open pollination treatment (Table S2). Furthermore, the seed weight was higher in the open pollination treatment than under hand cross-pollination with pollen of the same variety and wind pollination, but not higher compared to the self-pollination treatment.

None of the pollination response variables of the variety Calena were significantly different among the pollination treatments (Table S2).

In all varieties, autonomous self-pollination contributed highest to the number of seeds per open flower and seed weight. The individual contribution of wind and insect pollination varied among varieties (Table S4).
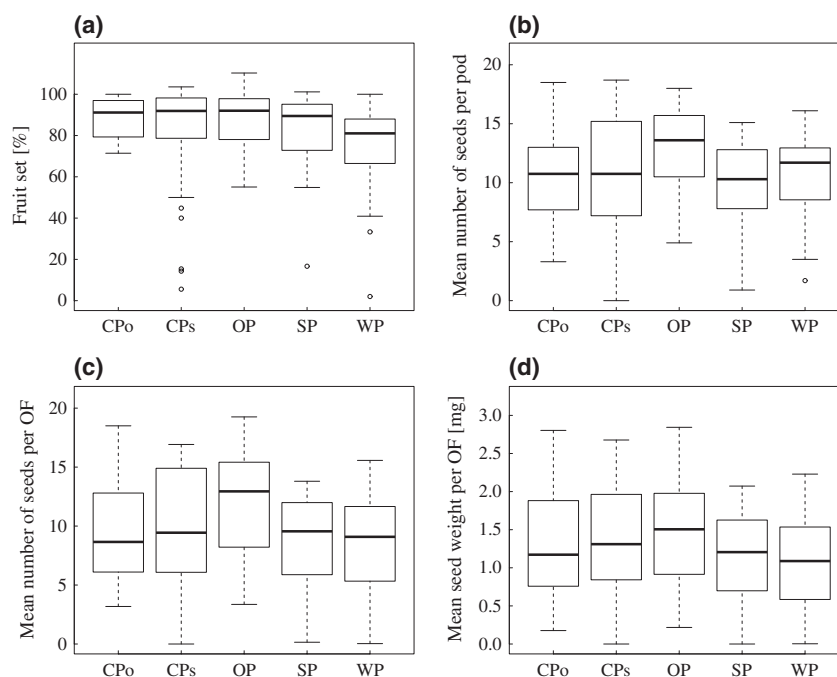


Fig. 2 (a) Fruit set, (b) mean number of seeds per pod, (c) mean number of seeds per open flower, and (d) mean seed weight per open flower as a function of the pollination treatments hand cross-pollination with pollen of a different variety (CP$_o$), hand cross-pollination with pollen of the same variety (CP$_s$), open (OP), self- (SP), and wind pollination (WP) for camelina (pooled across varieties). The horizontal line in each box is the median, the box defines the hinge (25–75% quartile), and the vertical line represents the sample minimum and maximum within max. 1.5 times the hinge. Points outside this interval are represented as dots. P-values are <0.05 between WP-CP$_o$ and WP-OP in (a), OP-CP$_o$, OP-CP$_s$, OP-SP, and OP-WP in (b), OP-CP$_s$, OP-SP and OP-WP in (c) and OP-SP and OP-WP in (d).

*Breeding system of pennycress*

Our experiments revealed that pennycress is mainly wind pollinated. A total of 3008 flowers opened during the experiment, out of which 2212 developed into mature pods, whereas 775 flowers did not. Fruit set and number of seeds per pod were significantly lower in the self-pollination than in all other pollination treatments (Fig. 3a and b; Table S3). Furthermore, hand cross-pollinated flowers produced significantly less seeds per pod than open-pollinated flowers (Fig. 3b). The number of seeds per open flower as well as seed weight was also significantly lower in the self-pollination treatment than in all other pollination treatments (Fig. 3c and d).

Wind pollination contributed 61% to the number of seeds per open flower, whereas self- and insect pollination contributed 29% and 9%, respectively. For seed weight the estimated contribution was 29%, 58%, and 13% for self-, wind, and insect pollination, respectively (Table S4).

*Flower-visiting insects of camelina and pennycress*

In total, we found 29 species visiting camelina and 19 visiting pennycress (Table 1). The mean number of flower-visiting insects per transect walk was higher on camelina (24.6 individuals) than on pennycress (16.5 individuals). Main functional flower-visiting taxa were honey bees, *Apis mellifera*, wild bees of the genera *Lasioglossum* (sweat bees) and *Hylaeus* (face masked bee), and syrphid flies (*Syriphidae*). Overall, the flower visitor community did not differ by genera, but by species between the two plant species (Fig. 4). Only four flower visitor species were found in both crop species (Table 1). Honey bees were only present in camelina, but not in pennycress (Fig. 4). The most dominant sweat bee species was *L. malachurum* in camelina and *L. calceatum* in pennycress. Syrphid flies were mainly found in camelina, while other fly species were more abundant on flowers of pennycress.

## Discussion

We studied the breeding and pollination system of camelina and pennycress in a double-cropping system. By doing so we filled one of several ecological knowledge gaps that need to be answered before a new agricultural crop growing concept for biofuel production can be successfully established and considered sustainable (Lüdeke-Freund *et al.*, 2012).
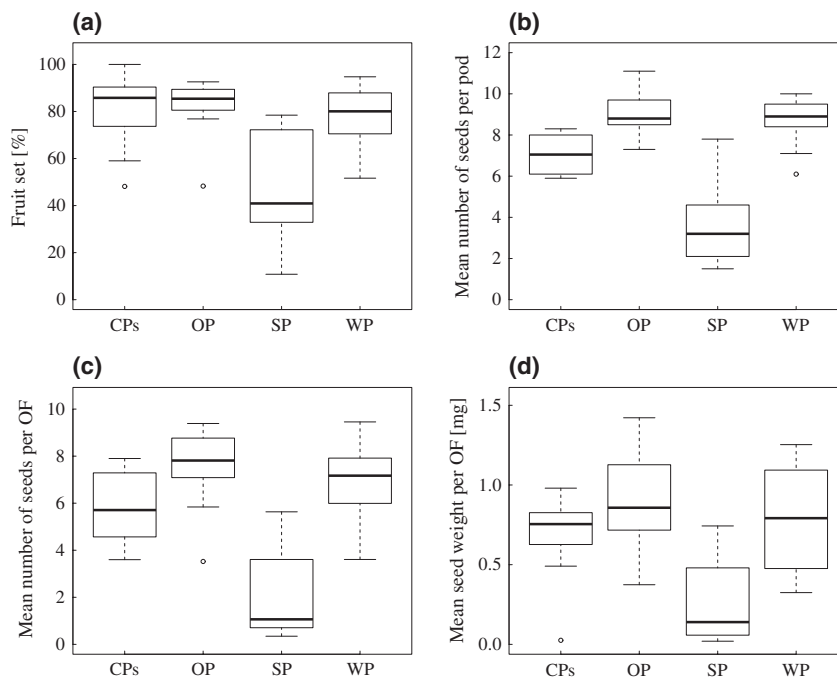


**Fig. 3** (a) Fruit set, (b) mean number of seeds per pod, (c) mean number of seeds per open flower, and (d) mean seed weight per open flower as a function of the pollination treatments hand cross-pollination with pollen of the same variety (CP$_s$), open (OP), self- (SP), and wind pollination (WP) for pennycress. The horizontal line in each box is the median, the box defines the hinge (25–75% quartile), and the vertical line represents the sample minimum and maximum within max. 1.5 times the hinge. Points outside this interval are represented as dots. Self-pollination is significantly different ($P < 0.05$) from all other treatments across all response variables. In addition, in (b) the contrast OP-CP$_s$ is significantly different ($P < 0.05$).

**Table 1** Species list with total number of individuals and means of flower visitors from transect walks in camelina and pennycress (total duration of transect walks 5 × 30 min and 2 × 30 min, respectively). The category 'others' contains a total of seven species of which five were present in camelina, three in pennycress, and one in both

| Order | Family | Species | Camelina | | Pennycress | |
|---|---|---|---|---|---|---|
| | | | Sum | Mean | Sum | Mean |
| Hymenoptera | Apidae | *Apis mellifera* | 57 | 11.4 | 0 | 0 |
| | Andrenidae | *Andrena* sp.1 | 1 | 0.2 | 0 | 0 |
| | Halictidae | *Lasioglossum calceatum* | 5 | 1.0 | 8 | 4.0 |
| | | *Lasioglossum malachurum* | 19 | 3.8 | 0 | 0 |
| | | *Lasioglossum morio* | 0 | 0 | 1 | 0.5 |
| | | *Lasioglossum pauxillum* | 1 | 0.2 | 0 | 0 |
| | | *Lasioglossum subhirtum* | 2 | 0.4 | 0 | 0 |
| | | *Lasioglossum tricinctum* | 1 | 0.2 | 0 | 0 |
| | | *Lasioglossum zonolum* | 1 | 0.2 | 0 | 0 |
| | | *Halictus* sp. 1 | 1 | 0.2 | 0 | 0 |
| | Colletidae | *Hylaeus annulatus* | 0 | 0 | 0 | 0 |
| | | *Hylaeus communis* | 2 | 0.4 | 4 | 2.0 |
| | | *Hylaeus punctatus* | 0 | 0 | 1 | 0.5 |
| | Pompilidae | Gen. sp. 1 | 0 | 0 | 1 | 0.5 |
| | Chalcidoidea | Gen. sp. 1 | 1 | 0.2 | 0 | 0 |
| Diptera | Syrphidae | *Epeodes corrolae* | 1 | 0.2 | 0 | 0 |
| | | *Eristalis* sp. 1 | 1 | 0.2 | 0 | 0 |
| | | *Helophilus trivittatus* | 1 | 0.2 | 0 | 0 |
| | | *Sphaerophoria* sp. 1 | 5 | 1.0 | 0 | 0 |
| | | *Syritta pipiens* | 2 | 0.4 | 1 | 0.5 |
| | | Gen.sp. 1 | 1 | 0.2 | 0 | 0 |
| | Other (Brachycera) | Fam. gen. sp. 1–5 | 0 | 0 | 5 | 2.5 |
| | | Fam. gen. sp. 6–7 | 2 | 0.4 | 0 | 0 |
| Hemiptera | | Fam. gen. sp. 1 | 0 | 0 | 1 | 0.5 |
| | | Fam. gen. sp. 2–5 | 11 | 2.2 | 0 | 0 |
| Coleoptera | Coccinellidae | Gen. sp. 1 | 0 | 0 | 1 | 0.5 |
| | | Gen. sp. 2 | 1 | 0.2 | 0 | 0 |
| | Nitidulidae | *Brassicogethes aeneus* | 0 | 0 | 4 | 2.0 |
| Other | | | 7 | 1.4 | 4 | 2.0 |
| Total | | | 123 | 24.6 | 33 | 16.5 |

## Breeding system of camelina

Our pollination experiment identified a breeding system dominated by autonomous self-pollination for camelina, thereby supporting earlier statements in the literature (Fruwirth, 1906; Knuth, 1908; Plessers *et al.*, 1962; Zubr, 1997; Mulligan, 2002; Vollmann *et al.*, 2005). Recently, Walsh *et al.* (2012) reported low out-crossing rates of 0.09–0.28%, also pointing to self-pollination. Our data, pooled for all varieties, indicate that camelina benefits from insect pollination with better filled pods – both for seed number and weight. However, this effect was not significant on a variety scale. All varieties presented highest fruit set, number of seeds per pod, number of seeds per open flower as well as seed weight per open flower when open (insect)-pollinated. However, the difference of open pollination to self-pollination was not statistically significant. The estimated contribution of insect pollination to yield of 12–36% is substantial, but
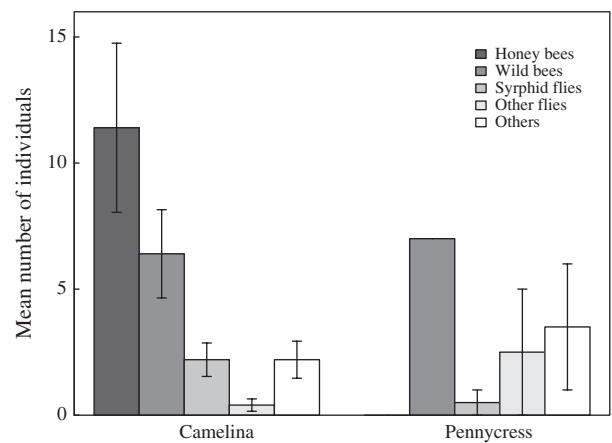


**Fig. 4** Mean number of flower-visiting individuals per transect walk in camelina and pennycress with SE (total duration of transect walks 5 × 30 min and 2 × 30 min, respectively). For pennycress, wild bees SD = 0, therefore SE = 0.

may be by chance. The positive effect of insect pollination on yield in the pooled data was driven by an open pollination benefit found in the variety Celine. However, yield was only significantly higher in the open than in the wind pollination treatment, but not higher than in the self-pollination treatment. Therefore, Celine seems to benefit a little from insect pollination, whereas Ligena and Calena do not. There was no effect on pollination success depending on pollen source, i.e. whether the pollen came from the same or another variety.

Although insects were visiting our experimental open pollination treatment, their visitation frequency may have been too low for a significant effect. An additional open pollination treatment with supplemental hand pollination and hand pollination treatments with different pollination intensities may help to understand the role of pollen limitation. To further elucidate how much pollinating insects can contribute to camelina yield, a cage experiment with high stocking rates of pollinators such as honey bees or solitary bees should be considered in future research (see Jauker *et al.*, 2011 for a cage experiment on rape pollination).

Other biofuel crops, such as rapeseed and soy, also produce yield without the contribution of insects, but may benefit from insect pollination (Erickson, 1975; McGregor, 1976; Steffan-Dewenter, 2003; Chiari *et al.*, 2005; Sabbahi *et al.*, 2005; Klein *et al.*, 2007; Durán *et al.*, 2010; Bommarco *et al.*, 2012; Milfont *et al.*, 2013). Other studies, however, found little evidence of insect pollination benefits to yield (Erickson, 1975; Williams *et al.*, 1986, 1987; Mesquida *et al.*, 1988). These contrasting results may be caused by differences in insect pollination benefits among varieties (Erickson, 1975; Williams, 1978; Kevan & Eisikowitch, 1990; Hudewenz *et al.*, 2013).

The estimated contribution of wind pollination varied greatly among varieties in our experiment. While wind pollination was high for Ligena and low for Calena, Celine showed negative estimates. This resulted from lower values in the wind than self-pollination treatment. In the experimental wind pollination treatment, both, self- and wind pollination were possible. Therefore, one would expect similar or higher values for the treatment wind than self-pollination. This indicates high variability among plant individuals per treatment. Future research should therefore increase the number of replicated plant individuals. However, even though the extent of contribution from wind pollination remains unclear, our results show a clear dominance of self-pollination for all three camelina varieties.

### Breeding system of pennycress

For pennycress, this study revealed a breeding system strongly dominated by wind pollination, which is contrary to findings in the literature stating self-pollination as the main mode of pollination (Knuth, 1908; Best & McIntyre, 1975; Al-Shehbaz, 1986; Warwick *et al.*, 2002). Best & McIntyre (1975) reported high yield after covering flowers to prevent cross-pollination. However, they did not state which bagging material was used. Possibly it allowed pollen transported by wind to pass through. Knuth (1908) and Al-Shehbaz (1986) suggested self-pollination based on flower morphology, with Knuth (1908) additionally mentioning floral features serving for cross-pollination.

Contrary to our expectations, pollination success was lower under hand cross-pollination than under open pollination in both, camelina and pennycress. This is likely due to incomplete hand cross-pollination, i.e. too little amounts of pollen transferred to the stigma for maximum seed set, yet enough to set fruit (Winsor *et al.*, 1987). Because pennycress pollen is not sticky (personal observation), it was challenging to transfer high pollen amounts to the stigmas. Therefore, it is difficult to draw final conclusions about pollen limitation from our experiment and future experiments should consider a cross-pollination treatment of freely accessible flowers experiencing supplementary pollination on top of insect pollination.

The estimates for pennycress showed the highest contribution to pollination success by wind pollination, whereas self-pollination contributed half as much and insect pollination played a minor role.

### Consequences of production for insects and future research directions

Several bioenergy crops, including the two annual oil crop species presented here, can produce commercial yield without insect pollination. However, their production may still have major consequences for native and managed pollinating insects. These may include positive and negative effects resulting from mass flowering to alterations of landscapes. Mass-flowering crops can support important pollinator groups (Westphal *et al.*, 2003, 2009; Diekötter *et al.*, 2013), but can conversely threaten the reproductive success of native plant species flowering at the same time by drawing away pollinators (Holzschuh *et al.*, 2011). Vegetation cover during winter, as in the winter cultivation of pennycress (Lüdeke-Freund *et al.*, 2012), may positively affect populations of beneficial insects, such as pollinators or natural enemies of pests (Booij & Noorlander, 1992). On the other hand, agricultural pests and diseases may also benefit, by having additional resources to survive the winter (Sumner *et al.*, 1995).

We suggest a number of research directions as a result of this study. An investigation of the impacts of

camelina and pennycress cultivation on native plant species and flower visitor populations is lacking at the landscape scale. The effect of cultivation on the reproduction of native plants and other crop species should be tested by assessing which species are still flowering in the period of cultivation and by comparing visitation rates of pollinators to flowering wild plant species in the presence and absence of a double-cropping system. Furthermore, the amount of pollen and nectar available from camelina and pennycress and their utilization by pollinators should be quantified.

We conclude that, although production of biofuel feedstock from both crop species is little dependent on pollinators, they offer foraging resources for different insect taxa at times when few other crops and native plants are flowering.

## Acknowledgements

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## Supporting Information

Additional Supporting Information may be found in the online version of this article:

**Fig. S1**. Number of open flowers as a function of the pollination treatments hand cross-pollination with pollen of a different variety ($CP_o$, camelina only), hand cross-pollination with pollen of the same variety ($CP_s$), open (OP), self- (SP), and wind pollination (WP) for (a) camelina (pooled across varieties) and (b) pennycress.

**Table S1.** Means and SE of (a) fruit set, (b) mean number of seeds per pod, (c) mean number of seeds per open flower, and (d) mean seed weight (mg) per open flower per pollination treatment hand cross-pollination with pollen of a different variety ($CP_o$), hand cross-pollination with pollen of the same variety ($CP_s$), open pollination (OP), self-pollination (SP), and wind pollination (WP) for the camelina varieties Ligena, Celine, and Calena.

**Table S2.** P-values from *post hoc* Tukey test between the pollination treatments for (a) fruit set, (b) mean number of seeds per pod, (c) mean number of seeds per open flower, and (d) mean seed weight per open flower for the camelina varieties Ligena, Celine, and Calena.

**Table S3.** P-values from *post hoc* Tukey test between the pollination treatments for (a) fruit set, (b) mean number of seeds per pod, (c) mean number of seeds per open flower, and (d) mean seed weight per open flower for pennycress.

**Table S4**. Contribution of the pollination regimes self- (SP), wind (WP), and insect pollination (IP) to the mean number of seeds per open flower (SpOF) and mean seed weight per open flower (SWpOF) in percent for the camelina varieties Ligena, Celine, and Calena, and the pennycress line spring 32.