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Crop management modifies the benefits of insect pollination in oilseed rape

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ABSTRACT

In a factorial field plot experiment, high and low levels of inorganic nitrogen and of insect pollinators visiting the crop were manipulated and their combined effects on oilseed rape yield were quantified. A third factor was also included, testing whether different cultivars responded differently to the tested factors. Insect pollination was required to reach high yield and seed quality (oil content). Final benefits of pollination service were, however, greatly modified by cultivar, where the seed yield of the open-pollinated cultivar largely depended on insect pollination whereas the two hybrid cultivars did not. A near significant interaction between nitrogen input and insect pollination was also found, i.e. benefits to crop yield from insect pollination seemed to increase with decreased nitrogen levels. The differential response of the three cultivars suggested opportunities to use cultivars that are less dependent on insect pollination in landscapes where this service has been deteriorated. Increased access of nitrogen seems to partly compensate yield losses from poor insect pollination. Integrating conservation, environmental and agronomic sciences is therefore crucial to sustain agriculture productions through optimized management of agronomic inputs and biodiversity-based ecosystem services.

1. Introduction

Demand of agricultural products is predicted to double by year 2050 as a result of a growing world population and economic development ([Millennium Ecosystem Assessment, 2005](#)). An outstanding challenge is that future crop production systems will have to provide high yields, while minimizing negative impacts on the environment. Ecological intensification through the promotion of biodiversity and associated ecosystem services that directly support crop yields (e.g. through crop pollination, or biological pest control), has been proposed as a promising approach to handle this delicate balance ([Cunningham et al., 2013](#)). The adoption of ecological intensification is knowledge-intensive and requires interdisciplinary efforts to identify concrete options to replace external inputs with ecosystem services, and/or enhance services that support yield characteristics ([Bommarco et al., 2013](#)).

A factor that is often overlooked in the currently rapidly expanding research on ecosystem services linked to agriculture, is that different resources delivered to the crop plant, either as an ecosystem service provided by biodiversity (e.g. crop

pollination, or nutrient cycling), or as an external input applied by the farmers (e.g. chemical fertilizer, water), might interact in their relative contribution to crop yield (Klein et al., 2015). Instead, each single service is often studied in isolation, and its benefit to crop yield is most often implicitly considered as independent from the presence of other services, level of external inputs, and environmental conditions. This assumption may lead to double counting or underestimation of service benefits, and unrealistic management strategies in contrasting cropping systems (Seppelt et al., 2011). Context dependencies in general, and interactive benefits of multiple services in particular, remain poorly studied but are probably common (Boreux et al., 2013). A recent study has demonstrated, for instance, that the benefit to crop yield from animal-mediated pollination depends on pest control levels (Lundin et al., 2013).

Interactions between processes that occur below and above ground are particularly poorly investigated. Above ground, the importance of insect-mediated pollination for crop production is becoming increasingly acknowledged worldwide (Morandin and Winston, 2006; Klein et al., 2007). Although a deteriorated pollination service can lead to significant yield losses in many crops, this effect can vary considerably due to a number of abiotic and biotic factors (Bos et al., 2007; Boreux et al., 2013). For instance, Klein et al. (2015) found that water availability modified the effect of insect pollination on almond yield, i.e. drought reduced yield more in full pollinated plants than in the plants with pollinator exclusion. Hence, the availability of both soil resources and insect pollination are main direct determinants of reproduction in many flowering plants, but the degree to which each factor is limiting and how they can interact in their impact on plant reproduction is unknown for many species (Burkle and Irwin, 2009). The seed production of many plants depends, for instance, directly on nitrogen availability that determines resource allocation for growth and reproduction (Vaughton, 1991). Nutrients can, however, also strongly affect floral traits such as flower number or size, nectar production, and quality, which are important for attracting pollinating insects (Munoz et al., 2005; Burkle and Irwin, 2010).

The dependence of animal pollination for yield varies not only among crop species, but might also differ among cultivars of the same crop. The importance of assessing cultivar-specific responses to insect pollination has been highlighted for some time, but few studies have addressed this (Klein et al., 2007; Hudewenz et al., 2014). So far, breeding programmes have rarely aimed at changing, or even measuring insect pollination dependence in oilseed rape. There is therefore a lack of basic information on plant reproductive biology such as dependence on insect pollination for seed set, and nectar and pollen production for cultivars of most crops (Klein et al., 2007).

In the present study, an interaction in the benefit to yield of two resources in winter oilseed rape (*Brassica napus* L. var. *oleifera*) was tested: availability of insect pollination and nitrogen. With this set-up we tested the hypothesis that abundant availability of nitrogen can compensate yield losses due to poor insect pollination and vice versa. Oilseed rape yield has been shown to increase considerably with insect pollination, often by 20% or more (Free and Nuttal, 1968; Manning and Wallis, 2005; Sabbahi et al., 2005; Bommarco et al., 2012). However, there are also reports when no clear benefit has been found (Williams et al., 1987; Mesquida et al., 1988; Hayter and Cresswell, 2006). A possible explanation is a difference in yield response to insect pollination among cultivars (Hudewenz et al., 2014).

Our study provides a test of potential interactions in the combined benefits of insect pollination and nutrient availability for contrasting crop genotypes in an important annual crop. The existence of interactions among these factors has several practical implications (Klein et al., 2015). It will inform about the level of increases in yield quantity and quality that one can expect when promoting ecosystem services. It will also indicate the potential to buffer the impact of a deteriorated ecosystem service by changing agricultural management. The direction of interactions will further assist to identify options for ecological intensification through ecological replacement, i.e. where external inputs are replaced with ecosystem services in resource efficient cropping systems.

2. Methods

The experiment was performed on three cultivars of oilseed rape. The first cultivar (Catalina, Dekalb[®], Italy) was selected among the traditional open-pollinated cultivars. Unlike an inbred or a single-cross hybrid, each plant in a population will have a unique set of genes and will be genetically different from all other plants in the population (Gupta, 2007). Cultivars of this type were the only ones used in Europe until late 1990s. Recently, new cultivars have been developed that exploit hybrid vigour in the first offspring (F₁) from two crossed inbred lines. Amongst the hybrids one normal size (Excalibur; Dekalb[®], Italy) and one semi-dwarf type (PR45D01; Pioneer[®], Italy) were selected. Excalibur is considered a high yielding hybrid cultivar with high glucosinolate content, early maturity and moderate light leaf spot resistance. PR45D01 is also considered a high yielding cultivar, with relatively low glucosinolate content, and high resistance to lodging. Both hybrids had the male fertility restored and thus they produced viable pollen. Both traditional pedigree selection and hybridization aim at improving yield, but not at changing pollination dependence. These cultivars were commonly used in South Europe and were selected to represent current market alternatives to farmers. No information about their dependence on pollination was available.

The experiment was performed during the 2012 growing season in a field located at the Experimental farm of the University of Padova (Legnaro, Agripolis, elevation: 8 m, WGS84 N45 20⁰ 42⁰⁰, E11 56⁰ 60⁰⁰). Soil characteristics of the experimental field were measured in 2011 (soil organic matter: 2.52%, total P: 822 mg P₂O₅ kg⁻¹, C/N ratio: 15.5, pH 8.38). Preceding crop was winter wheat in 2010/2011. The crop was sown on the 24th of September 2011 with a seed density of 63 m⁻², an inter-row distance of 45 cm and seed distance within the row of 3.5 cm. A factorial split-plot design was adopted with three crossed factors: cultivar, nitrogen (N) and pollination treatment. The experimental field was composed of three blocks. Within each block three long crop strips (75 x 15 m) were created, one for each of the three cultivars. Each cultivar strip was split into two plots treated with two nitrogen levels (NO: control with no nitrogen application and N170: application of 170 kg N ha⁻¹) (Fig. 1). The N x cultivar parcels were separated by large corridor of bare ground (4 m wide) to allow harvest machinery passage. The insect pollination treatment was done by installing two pairs of cages one at each end of the parcel for a total of 72 cages. Each pair was composed of two adjoining cubic cages made of metallic tubes (each cage was 2 x 2 x 2 m). The cage pairs were placed on the vegetation in the early spring and covered four crop rows. Within each pair of cages crop density was regulated by removing plants in early spring to obtain an equal number of plants in each of the two neighbouring cages. On 21st of March 2012, well before the bloom onset, the entire experimental field was sprayed with lambda-cyhalothrin (9.48%) with a dose of 100 g l⁻¹ (Karate Zeon[®], Syngenta[®]) to suppress an attack of the pollen beetle (*Meligethes aeneus* F.). At the bloom onset between the 31st of March and the 2nd of April 2012, one of the two cages was closed with a plastic transparent net with a mesh of approximately 1 x 1 mm to exclude insect pollinators. To reduce micro-climatic and radiation differences between the open and the closed cage the roof of the open cage was covered with the same net. Several studies indicated that this type of net only slightly changes microclimatic conditions (e.g. Martin et al., 2013). Preliminary tests indicated that the diurnal temperature did not differ between closed and open cage. Only a difference in night minimum temperature was found where the open cage had c. 1 °C lower temperature than the closed cage for c. 2 h (one week of observation). The position of the open cages was alternated among plots between east and west relative to the closed cage to evaluate possible effects of differences in sun exposures (Fig. 1). Preliminary analyses showed that compass direction did not affect any of our response variables. Once the bloom ended, between 27th and 28th of April 2012 all nets were removed and the crop was left to ripen

under the same environmental conditions. During ripening, both cages were protected with a mesh size of 1 x1 cm to support the plants against lodging and to protect the pods from bird predation.

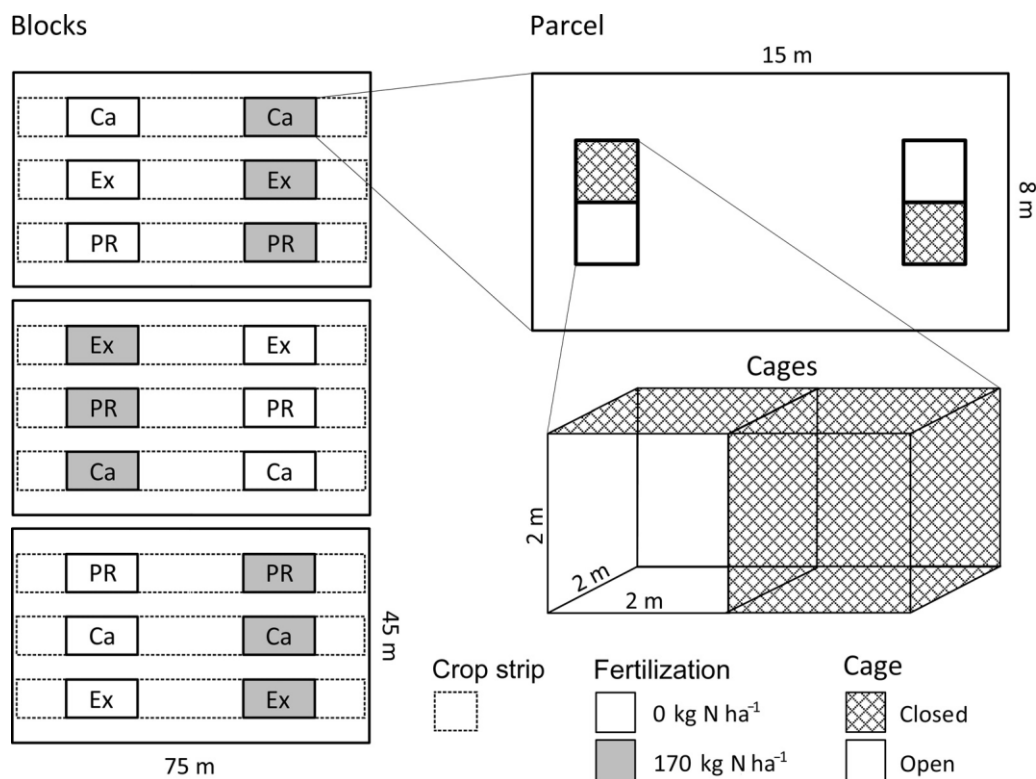


Fig 1. Scheme of the experimental design. The three cultivars Ca: Catalina, Ex: Excalibur, PR: PR45D01) were randomly sown in strips within each blok. Each of the cultivar strips was split into two parcels treated with two nitrogen levels (NO: no N application and N170: 170 kg N ha⁻¹). Two pairs of cages were placed within each parcel for the insect pollination treatment.

2.1. Visitation rate

Five honey bee hives were placed at 500 m from the experimental field to guarantee large visitation rates. The number was chosen because three colonies per ha are expected to provide optimal visitation rate in oilseed rape (Sabbahi et al., 2005). Between 17th and 21st April 2012 insect visitation rates were quantified in two occasions at the peak of bloom by counting the number of flower visits in the open cages. Insect visits were observed for 5 min per cage. The observations were made between 10:30 hours and 17:00 hours under sunny weather conditions with temperature above 17 ° C. The visitation rate refers to the total number of visits on all the plants in the open cage. As the observations were not carried out on single plants, it was not possible to estimate the potential differences between cultivars in geitonogamous selfing (multiple visits of the same insect on flowers belonging to the same plant).

2.2. Plant measurements

Once the crop was ripe (between the 18th and 20th of June 2012) an area of 1 m² was sampled in the middle of each cage. The entire above ground parts of the plants were harvested by cutting each plant 5 cm above the soil surface. Plants from each cage were placed in an open bag and placed in a ventilated greenhouse to dry for one week. After that the plants were

dried in oven at 65 °C for 24 h after which plants were threshed. Seed yield and total biomass were weighed. The weight of 1000 seeds was quantified by weighing 10 random samples of 100 seeds each taken from the total yield sample. Seed oil content was measured using the Twisselmann extraction method (Soxtec-Tecator™ equipment, FOSS®) (Zanetti et al., 2009). The average number of seeds per pod was assessed by randomly sampling 5 plants per cage. From each plant, 20 pods were collected starting from the tip of the main inflorescence and the number of seeds in each pod was counted. The number of pods per m² was further estimated by dividing the total seed yield by the average individual seed weight, and dividing this quantity by the number of seeds per pod and finally by the cage area. This was the only parameter that was not directly measured due to the very large samples that would have been needed. The number of pods was estimated using the number of seeds per pod from the main inflorescence and was therefore not representative of the whole plant. However, it can be considered a standardized relative measure that is useful to test for difference between the treatments.

2.3. Data analysis

The effects of cultivar, N and insect pollination treatment on the yield parameters described above were tested by using linear mixed models. The models included the three treatments and all the interactions as fixed effects while the random effects reflected the split-plot design (block/cultivar plot/N plot/cage pair). The model was estimated using the REML estimation method in the nlme package (Pinheiro and Bates, 2009) for R (R Core Team, 2012). The seed yield and oil content (%) were log-transformed before analyses. Just after the end of the bloom period a storm event damaged a part of the experimental plots. Hence, data from all the cages with the Excalibur cultivar from one block (n = 8) and two other cages ((i) Catalina, N: 170 kg, no pollination and (ii) Excalibur, N: 170 kg, open pollination) were removed. The analyses were thus based on data from 62 cages. For testing the effect of N and cultivar and their interaction on pollinator visitation rate the same model as described above was performed but without the pollination treatment. In total, data from the 36 open cages in two occasions were available.

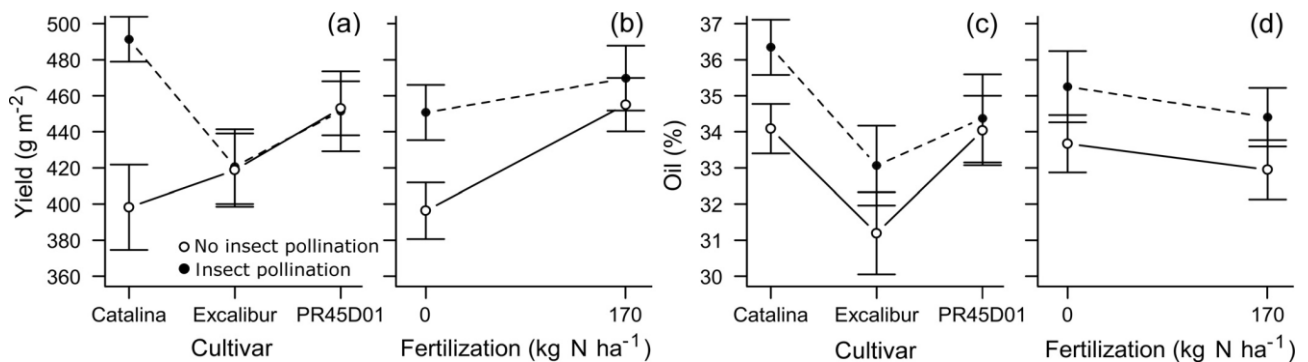


Fig. 2. Interaction between cultivar and insect pollination and between N and insect pollination for seed yield (a and b) and oil content (%) (c and d). The dots show mean values \pm SE.

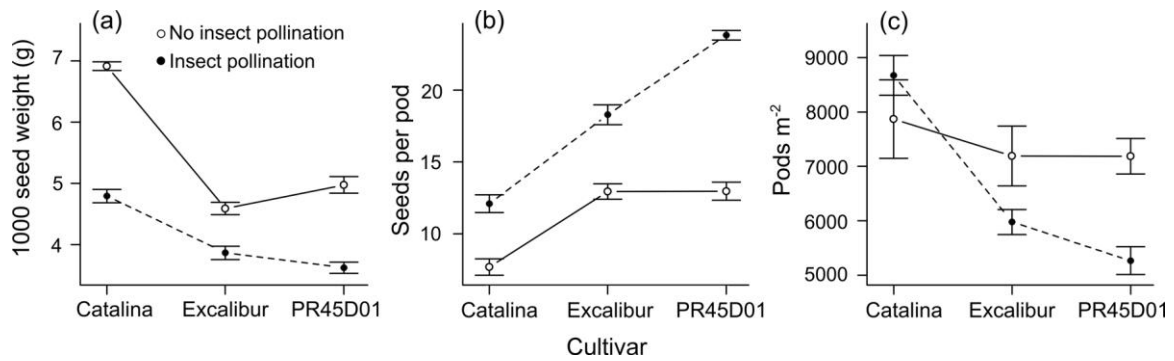


Fig. 3. Interaction between cultivar and insect pollination for (a) 1000 seed weight, (b) number of seeds per pod, and (c) estimated number of pods. The number of pods was estimated using the following formula: number of pods = seed yield/seed weight/number of seed per pod from the main inflorescence. The dots show mean values \pm SE.

3. Results

The honey bee was the most common pollinator accounting for 70%, wild bees (mostly *Andrena* spp. and *Lasioglossum* spp.) for 18%, and hover flies for 7% of the total visits. The other visits (rv5%) were made mainly by lepidopterans, coleopterans and dipterans. The mixed models showed no effect of cultivar or N on the visitation rate considering both the whole pollinator community and the single pollinator groups. Only the N treatment had a marginally significant effect on wild bees ($P = 0.077$), where the high N treatment received relatively larger number of visits than the low N treatment.

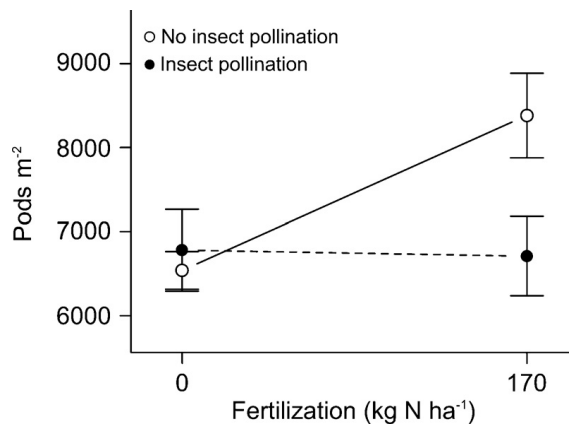


Fig. 4. Interaction between N and insect pollination for the number of pods. The number of pods was estimated using the following formula: number of pods = seed yield/seed weight/number of seed per pod from the main inflorescence. The dots show mean values \pm SE.

For seed yield, there was a significant cultivar \times pollination interaction ($P = 0.007$) indicating that insect pollination effect differed among cultivars. Insect pollination increased seed yield only for the open-pollinated cultivar (Catalina), while for the yield of the two hybrids did not increase with the presence of insects (Fig. 2a). The insect pollinated plants of Catalina produced on average 19% more seed yield compared to the plants inside the cages. For seed yield a marginal significant interaction between N and pollination was found ($P = 0.069$) where N fertilization partly compensated the lack of insect pollinators (Fig. 2b). The positive effect of pollination on oil content (%) (pollination, $P = 0.016$) was not affected by the cultivar (cultivar \times pollination, $P = 0.316$) (Fig. 2c). No interaction was found between N and insect pollination for oil content (N \times pollination, $P = 0.776$) (Fig. 2d).

Although seed yield of the hybrids was not affected by insect pollination, several yield components were strongly affected by insect pollination in all the cultivars. An interaction between pollination and cultivar was found for seed weight ($P < 0.001$), number of seed per pod ($P < 0.001$) and estimated number of pods m^{-2} ($P = 0.003$). Insect pollination created smaller seeds in all cultivars, but the effect was stronger for the open-pollinated line (Catalina) than for the two hybrids (Fig. 3a). The number of seeds per pod was also enhanced by insect pollination in all cultivars, but the effect was stronger for the semi-dwarf hybrid (PR45D01) (Fig. 3b). The estimated pod number increased with insect pollination in the open-pollinated cultivar and decreased in the two hybrids (Fig. 3c). The estimated number of pods increased with N in case of no insect pollination, but was not affected by N in the presence of insect pollinators (Fig. 4) (N x pollination, $P = 0.003$).

4. Discussion

The average yield difference of c. 600 kg ha^{-1} between pollinated and not pollinated plants at low nitrogen level is an interesting contrast for farmers. Our results show promise for identifying options for ecological replacement between ecosystems services and agronomic inputs emphasizing the economic importance of insect pollination for modern agriculture (Garibaldi et al., 2013). Several plant and yield characteristics were affected by insect pollination, cultivar and nutrient availability partly indicating how formation for the overall yield differed among treatments. The insect pollinated plants gave smaller seeds and increased the number of seeds per pod in all cultivars. This is in line with predictions and observations in several other plant species, where poorly pollinated plants invest more into the fewer seeds that are set from self- or wind-pollination when insect pollination is lacking (Jauker et al., 2012; Klein et al., 2015). One reason might be resource allocation, i.e. when a plant produces less seeds, more resources can be allocated to each individual seed increasing their weight significantly (Hudewenz et al., 2014). A general increase in oil content with insect pollination was also observed. Effects on seed quality from insect pollination are very poorly understood and barely investigated for plants in general. This quality parameter is directly linked to the market price and positive effects of insect pollination on oil content have been previously found in oilseed rape (Bommarco et al., 2012).

Final benefits to seed yield of insect pollination service were, however, greatly moderated by cultivar, where yield of the open-pollinated cultivar depended largely on insect pollination whereas the two hybrid cultivars did not. This is in line with a recent study comparing insect pollination dependence in different cultivars of oilseed rape (Hudewenz et al., 2014). The open-pollinated cultivar provided the overall highest yields. The scale of our experiment using a crop stand instead of isolated single plants and the lack of a full hand pollination treatment did not allow to investigate in details the mechanisms underpinning the cultivar differences in pollinator dependence. However, several possible explanations can be invoked. The observation of pollinator visits indicated that cultivar did not affect the visitation rate suggesting that the three cultivars presented similar attractiveness to pollinators. A possible explanation for the contrasting results between open-pollinated and hybrid cultivars may therefore relate to hybrid vigour. Hybrids are expected to produce a larger number of flowers that may compensate for poor cross pollination. This is partly indicated by the increase in the number of pods in absence of insect pollination exhibited by the two hybrids. In absence of xenogamy linked to insect activity, the two hybrids seemed to be able to allocate more resources in the production of flowers and pods. However, a recent study did not find the same response of open-pollinated and hybrid cultivars (Hudewenz et al., 2014), indicating that the pollinator dependency of a cultivar is not determined by its heritage (hybrid or open-pollinated cultivar). Rather, it seems more a cultivar-specific characteristic. Moreover, individuals in a field of hybrid plants are more genetically similar compared to plant individuals of an open-pollinated cultivar due to their breeding origin (Gupta, 2007) and therefore

probably the former benefit less from cross pollination. Another potential mechanism could be linked to differences in pollen availability, i.e. low rates of transfer of self- pollen (both autogamy and geitonogamy) in cultivars that are highly dependent on pollinators. Irrespective of pollen limitation, also post-fertilization processes such as high inbreeding depression may result in lower seed set in pollinator dependent cultivars. However, inbreeding depression is not considered a common problem in oilseed rape (Soengas et al., 2013).

The experiment indicates that insect pollination can, at least partly, compensate for low nitrogen inputs, providing interesting options to farmers to increase or maintain yield. We observed an increase in number of pods with no insect pollination only at high N levels. This observation may be explained by the compensatory capacity of the plants for low fruit and seed setting, which implies that a pollination deficit can be compensated by the development of larger number of fruits and seeds. The few studies testing interactions between nitrogen and insect pollination have found weak or no significant interaction in both wild and crop plants (e.g. Burkle and Irwin, 2010; Klein et al., 2015).

The results outline alternative options for farmers, agribusiness and society in deciding how to invest in management of ecosystem services, breeding, or external inputs such as chemical fertilizers. Increased access of nitrogen appears only to partly compensate yield losses from poor insect pollination, and to secure continued high yields one can choose to add more inorganic fertilizer in landscapes where pollination service is more degraded (Potts et al., 2010). However, nitrogen is a major production cost to the farmers. Up to 40% of the energy consumed to produce a crop is invested into inorganic nitrogen and this energy use contributes to climate change at a cost expected to increase. Furthermore, only about 40–50% of the applied nitrogen is actually harvested in many crops (Connor et al., 2011). Instead, nitrogen leakage to ground water, with ensuing eutrophication of streams, lakes and seas, has become a huge environmental problem worldwide (Compton et al., 2011). Much nitrogen is also lost by volatilization where arable soils emit nitrogenous gases that contribute to climate change and nitrogen deposition. Enhanced nitrogen use efficiency is therefore high on the policy agenda, both to decrease costs privately for farmers and for society. Choice of cultivar together with enhanced insect pollination appears to be an alternative path to strive towards high yield targets. The differential response of the three cultivars suggested opportunities to use cultivars that are less dependent on insect pollination in landscapes where the service has been deteriorated. In wild plants it is well known that adjustments of maternal investment in seed production can be strongly affected by both pollen and nutrient availability (Lloyd, 1980). The same can be expected for crop plants, although the relative importance of the two processes is likely to vary with cultivar. Integrating conservation, environmental and agronomic sciences is therefore crucial to sustain agriculture productions through optimized management of agronomic inputs and biodiversity-based ecosystem services.

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