



Crop management modifies the benefits of insect pollination in oilseed rape



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ARTICLE INFO

Article history:

Received 14 October 2014

Received in revised form 24 March 2015

Accepted 26 March 2015

Available online xxx

Keywords:

Cultivar

Ecological intensification

Ecosystem service

Fertilization

Honey bee

Interaction

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.

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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

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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 setup 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 (F_1) 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 dependency. 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 20'42", E11 56'60"). Soil characteristics of the experimental field were measured in 2011 (soil organic matter: 2.52%, total P: 822 mg P_2O_5 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 × 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 × 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 × 2 × 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 × 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 × 1 cm to support the plants against lodging and to protect the pods from bird predation.

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