Crop Protection 43 (2013) 79–88

Contents lists available at SciVerse ScienceDirect

Crop Protection

journal homepage: www.elsevier.com/locate/cropro

Effects of nitrogen fertilization on insect pests, their parasitoids, plant diseases and volatile organic compounds in *Brassica napus*

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ARTICLE INFO

Article history:
Received 25 May 2012
Received in revised form
31 August 2012
Accepted 3 September 2012

Keywords:
N fertilization
VOC emission
Meligethes aeneus
Ceutorhynchus obstrictus
Alternaria brassicae
Winter oilseed rape

ABSTRACT

Nitrogen (N) availability is a key factor influencing the yield of *Brassica napus* L. Thus, mineral fertilization is widely used to improve the quality and quantity of seeds. In this study, we conducted field experiments to determine the impact of nitrogen fertilization on *B. napus* pests, their parasitoids and plant diseases. The results showed that N treatment had an impact on the abundance of pollen beetles (*Meligethes aeneus* Fab.) and cabbage seed weevils (*Ceutorhynchus obstrictus* Marsh.) as well as dark spot disease (*Alternaria brassicae* (Berk.) Sacc.). Since pest abundance was not correlated with the flower and silique numbers, the feeding and oviposition sites, plant smell bouquets were analysed to determine potentially attractive or repellent volatile organic compounds. We detected 19 different compounds among which acetic acid and several lipoxygenase pathway products were emitted at higher levels from N-treated plants. Emission of a few other terpenoid compounds was correlated with the pest abundance in field conditions. Abundance of parasitoids of both pests was related to the host availability rather than to the fertilization treatment. Therefore, we suggest that plant chemical cues play a minor role in localization of hosts in close proximity to parasitoid. Dark spot disease levels decreased with increasing N availability, possibly reflecting enhanced emissions of acetic acid, a known antifungal volatile. This study demonstrates the effects of N fertilization on bud and flower volatile bouquets, which might play a role in *B. napus* insect pest host selection and in resistance to fungal plant diseases. Further studies are necessary to investigate the behavioural responses of insects to the changed volatile bouquets.

1. Introduction

Oilseed rape (*Brassica napus* L.) is a widely cultivated field crop in temperate climates (Blake et al., 2010) where its growth and development is most commonly limited by nitrogen (N) availability, especially at the outset of vegetative growth (e.g. Holmes, 1980; Rathke et al., 2006; Sieling and Kage, 2010). Conventionally, mineral fertilizers are applied to improve the yield and biochemical characteristics of this important agricultural crop. Fertilization with mineral N has been shown to increase plant size, height and inflorescence branching as well as seed protein content (Allen and Morgan, 1972; Blake et al., 2010; Grant et al., 2010; Holmes, 1980). However, due to economical and ecological reasons, the fertilization should be conducted carefully to apply only the amount necessary for optimal plant growth. Several studies have demonstrated that excessive N application, especially in *B. napus* cropping systems, often leads to N-leaching which causes soil and water pollution (Engström et al., 2011; Sieling and Kage, 2010). Application of only optimal fertilizer doses is also important for yield quality, since increasing N levels can result in decreased seed oil content (Grant et al., 2010). Although numerous research groups have focused on finding optimal N doses for improved yields and seed quality (e.g. Colnenne et al., 1998), very little is known about the impact of fertilization on *B. napus* insect pests and diseases or on other organisms associated with this cropping system. However, the interactions of *B. napus* with other organisms may considerably affect the yield as well as the quality of this crop throughout the growing season.

Among *B. napus* pests, the pollen beetle (*Meligethes aeneus* Fab., Coleoptera: Nitidulidae) and the cabbage seed weevil (*Ceutorhynchus obstrictus* Marsh., syn. *Ceutorhynchus assimilis* Payk.), (Coleoptera: Curculionidae) are the most widespread and persistent pests in the UK, Central, North and North Eastern Europe (Alford et al., 2003; Veromann et al., 2006b; Williams, 2010).

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http://dx.doi.org/10.1016/j.cropro.2012.09.001

Pollen beetle adults feed on pollen, destroy flower buds and lay eggs into the buds where their larvae feed on pollen. Seed weevils oviposit in developing siliques and their larvae consume seeds. The management of these *B. napus* pests is generally conducted by chemical pesticides, although their abundance could be efficiently controlled by their natural enemies, parasitoids (Büchi, 2002; Veromann et al., 2011; Walters et al., 2003). Parasitoids are insects, mostly from the order Hymenoptera, laying eggs on or inside the insects of other species. Their larvae develop and feed on the host, eventually killing it (Godfray, 1994). In Europe, the larvae of *M. aeneus* are parasitized by at least nine species of endoparasitoids. Among these, *Phradis interstitialis* (Thomson), *Phradis morionellus* (Holmgren), *Tersilochus heterocerus* (Thomson) and *Diospilus capito* (Nees) (Hymenoptera, Ichneumonoidea) are the most abundant and widespread (Nilsson, 2003). The most common parasitoids of *C. obstrictus* are larval ectoparasitoids belonging to the superfamily Chalcidoidea: *Trichomalus perfectus* (Walker), *Mesopolobus morys* (Walker) and *Stenomalina gracilis* (Walker) (Williams, 2003).

Among *B. napus* diseases, the most devastating are blackleg (*Leptosphaeria maculans* (Desm.) Ces.) and stem rot (*Sclerotinia sclerotiorum* (Lib.) de Bary). Additionally, the dark spot disease (*Alternaria brassicae* (Berk.) Sacc.) is a common fungal pathogen, especially on winter varieties (Giamoustaris and Mithen, 1997; Köhl et al., 2010). Disease control in conventional cropping systems is mainly conducted by preventive fungicide applications throughout the season, often without considering the presence or abundance of pathogens.

So far, it has been assumed that N usage is unlikely to have any direct effect on the abundance of the *B. napus* insect pests (Walters et al., 2003) and only a limited number of studies has focussed on the effects of N fertilization on parasitism level of some lepidopterans' and aphids' pests (Jansson, 2003; Kalule and Wright, 2002; Stadley et al., 2011). For instance, the amount of nitrogen supplied to *Brassica oleracea* var. *capitata* has been shown to increase the parasitism level of *Plutella xylostella* L. (Lepidoptera: Plutellidae) by *Diadegma insulare* (Cresson) (Hymenoptera: Ichneumonidae) (Jansson et al., 1991). However, fertilization changes plant architecture and inner microclimate of the crop, which might influence the searching efficiency of both the insect pests and their hymenopterous parasitoids (Walters et al., 2003). Additionally, plant architecture can have an impact on the distribution and accessibility of host larvae within the stand and therefore may affect the host location success of the parasitoids (Ulber and Fisher, 2006). There is some evidence of either negative or positive effects of N on stem rot disease and blackleg (Aubertot et al., 2003; Rathke et al., 2006; Söchting and Verreet, 2004), although the impact of fertilization on dark spot disease was non-significant in previous experiments (Söchting and Verreet, 2004).

Chemical communication between herbivores and their host plants depends on the plant and herbivore species and is generally based on multiple compounds (Blight et al., 1997). Cruciferous plants, such as *B. napus*, emit a complex mixture of biogenic volatile organic compounds (VOCs). Among them, isothiocyanates from foliage and floral compounds are known to be important cues in host selection by cruciferous pests and their parasitoids, aiding both finding and recognition of the host plant (Alford et al., 2003; Bartlet et al., 1993; Schiestl, 2010). Nitrogen fertilization could significantly affect the composition and levels of plant VOCs (Chen et al., 2010) and therefore also affect their attractiveness to pests. Our preliminary results on the effect of N show that there might be a correlation between the nutrient application and the abundance of pests, diseases and even parasitoids (Veromann et al., in press). Thus, it is necessary to conduct additional experiments to determine the factors involved in the pest and disease abundance, which could be further used to control the damage to the crop without the use of pesticides.

In this study we hypothesized that N fertilization affects the abundance of pests and parasitoids, as well as the spread of plant diseases, and that these potential modifications are associated with changes in the VOC bouquets of the plants. To test this hypothesis, we determined the VOC emissions of *B. napus* plants under laboratory conditions and compared these with the field data on abundance of insect pests, their parasitoids and plant diseases under different N treatments. The results of this study provide evidence that the composition of the VOCs emitted from *B. napus* plants is linked to the N fertilization effects on insect damage and dark spot disease.

2. Material and methods

2.1. Field experiment setup and yield estimation

The field studies were conducted in an experimental field of Jõgeva Plant Breeding Institute, Estonia (58°46′01″N, 26°24′27″E; elevation 74 m) in 2008 and 2009. The study site was ploughed and kept as a fallow for one growing season before starting this experiment. On the 23 April of each year, seven different N-fertilizer levels of 0, 60, 80, 100, 120, 140 and 160 kg of N (applied as NH_4NO_3 (Akron Group, Novgorod, Russia)) per hectare were applied on three randomized replicate plots of 10 m^2. Thus, the study design consisted of seven nitrogen treatments (hereinafter indicated as N0, N60, N80, N100, N120, N140, N160) for a total of 21 plots. Before sowing the seed in 2007 and in 2008 (for the 2008 and 2009 harvest, respectively), all the plots were fertilized with Kemira Power (Yara International ASA, Norway) 5–10–25 S Fe B complex fertilizer (300 kg ha^{-1}). In both years, a winter oilseed rape variety *B. napus* cv. *Silva* seeds were drilled on the 15 August in 2007 (for 2008 harvest) and on the 15 August in 2008 (for 2009 harvest) with seeding rate of 6 kg ha^{-1}. No insecticides or fungicides were applied.

The crop was harvested on the 7 September in 2008 and on the 11 August in 2009. The seed mass was measured for every plot by weighing the seeds at a moisture content of 7.5%. Thereafter, the yield per each plot was calculated as kg ha^{-1}. In addition, the number of siliques was counted. Silique counting was conducted at the pods ripening stage (plant growth stage BBCH 80–81 according to Lancashire et al., 1991) on three plants from each plot.

2.2. Quantification of pests, parasitoids and plant diseases

For estimation of egg laying activity and larval parasitization levels of *M. aeneus*, larvae were collected from *B. napus* flowers at stage BBCH 67–68 (at the end of full flowering) from five randomly chosen plants in each plot. Larvae were counted and second instar larvae were dissected in laboratory to determine their parasitization. Second instar is the last *M. aeneus* larval stage before dropping to the ground to pupate in the soil. Thus, by this time their parasitoids must have had found a suitable host to parasitize.

The establishment of damage and parasitization of *C. obstrictus* was assessed at stage BBCH 80–81 (beginning of pods ripening). Five siliques from the main raceme and five siliques from the third side branch were collected from five randomly chosen plants per plot and incubated in emergence traps in the laboratory as described in detail in Veromann et al. (2011). Four weeks later, emerged adult parasitoids or weevils' larvae were counted and identified. The exit-holes of larvae and parasitoids in all siliques were counted. Thereafter, all siliques were dissected and remains of any non-exited weevil larvae or the parasitoid pupae were noted. Finally, the mean number of emerged parasitoids, damaged siliques