**Meligethes aeneus** oviposition preferences, larval parasitism rate and species composition of parasitoids on *Brassica nigra*, *Raphanus sativus* and *Eruca sativa* compared with on *Brassica napus*

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**Highlights**

- Oviposition rate of the pollen beetle varies with plant species.
- The species composition of pollen beetles' parasitoids varies with plant species.
- *Brassica nigra* has a potential to reinforce the natural control of the pollen beetle.
- *Brassica nigra* could be used as a parasitoid bank.

**Graphical Abstract**

**Abstract**

The trap crop strategy is based on host plant discrimination by pests and their parasitoids, which may respond differently to various host plant cues, thus affecting their respective population distributions. We conducted a three-year study to compare the responses of the most damaging pest of oilseed rape (*Brassica napus* L.), the pollen beetle (*Meligethes aeneus* Fab.), and its hymenopteran parasitoids to various potential trap crops: *Brassica nigra*, *Raphanus sativus* var. *oleifera* Pers. and *Eruca sativa* Mill. with that to *B*. *napus*. We recorded their abundance, oviposition preferences and the species composition of the parasitoids.

Our results show that oviposition rates of the pollen beetle and its parasitoids as well the species composition of the parasitoids varies with plant species. We discuss the potential of these plant species, especially *B*. *nigra*, to enhance the natural control of the beetle by fostering several parasitoid species. The species composition of the parasitoids on different host plants compared with on *B*. *napus* is presented for the first time. In addition to trapping pests, the trap crops could also act as parasitoid banks, enhancing natural control of the pest through providing suitable hosts for natural enemies, without increasing the population growth of the next generation of pests.
1. Introduction

One of the most important principles of integrated pest management (IPM) is the prevention and/or suppression of harmful organisms, encouraging the use of non-chemical methods and target-specificity to control pest abundance (BiPRO, 2009). One of the tools that addresses these principles is the use of a trap crop strategy for the pests and their naturally-occurring biocontrol agents i.e. the natural enemies of pests, including parasitoids.

As phytophagous insects locate the crop by responding behaviorally to different visual and olfactory cues, manipulation of these cues and hence of pest behavior can be used to reduce or avoid pest damage to the crop. Trap cropping aims to reduce pest colonization in the main crop by attracting pests to areas of trap crop planted close to the main crop (Hokkanen, 1991; Cook et al., 2007a,b, 2013). If the plant species used as a trap crop is also attractive to parasitoids and the percentage of parasitism is high enough to control the population size of their host insect, there is no need to destroy the trap crop or to treat it with insecticides. Under these circumstances, the trap crop can also perform as a parasitoid bank and support the increase of diversity and abundance of beneficial arthropods.

Oilseed rape (Brassica napus ssp. oleifera L.) (Brassicaceae) is the third most widely grown crop in the European Union (FAO, 2013). In Estonia, the area grown has increased 82-fold over the past 20 years reaching 86,700 hectares in 2012 (Statistics Estonia, 2013). This increase has supported the population growth of crucifer-specialist pests. One of the most damaging pests throughout Europe is the pollen beetle (Melitgethes aeneus (Fabricius) (Coleoptera: Nitidulidae)) (Alford et al., 2003; Cook and Denholm, 2008; Ekborg, 2010; Veromann et al., 2006a,b,c, 2008; Williams, 2010). Adult pollen beetles feed on pollen from plants belonging to different families (Free and Williams, 1978; Fritzsche, 1957; Williams, 2010), but oviposit only in buds of brassicaceous plants (Ekborg and Borg, 1996; Free and Williams, 1978; Nilsson, 1989) although they have behavioral preferences for some Brassica species over others (Buechi, 1990; Ekborg and Borg, 1996).

Generally, the abundance of pollen beetles is controlled by applying synthetic insecticides (Thieme et al., 2010), which may not solve the pest problem (Hokkanen, 2000) and can even increase it (Veromann et al., 2008). Another problem is the development of pyrethroid resistance in pollen beetles (Hansen, 2003, 2008; Heimbach et al., 2006; Cook and Denholm, 2008; Thieme et al., 2010; Tillikainen and Hokkanen, 2008). Further, pesticides have a detrimental effect on parasitoids that are essential enemies of many crop pests and may act as keystone species in ecosystems (Murchie et al., 1997; Thies et al., 2003; Veromann et al., 2011).

In Europe, the key species of parasitoids controlling the abundance of pollen beetle are Phratis interstitalis Thomson, Phratis morionellus Holmgren, Tersilochus heterocerus Thomson (Hymenoptera: Ichneumonidae) and Diaspius capito Nees (Hymenoptera: Braconidae) (Nilsson, 2003). Of these, adults of T. heterocerus and P. morionellus commonly colonize the crop at the same time – at the beginning of flowering of either spring or winter oilseed rape varieties (Ulber and Nitzschke, 2006; Ulber et al., 2010; Williams, 2006) while D. capito is a multivoltine species which gains more importance when spring varieties start to flower (Miczulski, 1967; Nilsson, 2003). Tersilochus heterocerus, P. morionellus and D. capito oviposit into small larvae within buds and large second instar larvae in open flowers (Röber and Blunck, 1920; Nilsson, 2003; Osborne, 1960). Diaspius capito is mainly distributed in northern Europe and more common on spring oilseed rape (Hokkanen, 2008; Nilsson, 2003; Veromann et al., 2006a,b). The average parasitism percentage of pollen beetle larvae varies between 25% and 50% in Europe (Ulber et al., 2010), but can reach 90% (Ulber et al., 2006). The abundance of this pest can be effectively lowered with a parasitism rate of 30–40% (Hokkanen, 2008). In Estonia, a parasitism rate of 48% has been reported (Veromann et al., 2013), although in conventional cropping systems it is more usually under 4% (Veromann et al., 2009).

The potential of trap cropping to reduce insecticide treatments and to avoid damage caused by pollen beetles in oilseed rape has been intensively studied (Buechi, 1990; Cook et al., 2006; Cook and Denholm, 2008; Ekborg and Borg, 1996; Veromann et al., 2012) as has the potential of parasitoids to control the pest (Ekborg, 2010; Ferguson et al., 2003; Hokkanen, 1991, 1989, 2006; Jönsson et al., 2004; Kromp and Kraus, 2006; Nilsson, 2003; Nilsson and Ahman, 2006; Nilsson and Andreasson, 1987; Nitzsche and Ulber, 1998; Osborne, 1960; Ulber et al., 2010). However, potential trap crops other than B. rapa have received only minor attention so far (Hokkanen et al., 1986; Kovács et al., 2013; Veromann et al., 2012) and the potential of parasitoids has only been investigated separately on plant species other than B. napus (Billqvist and Ekborg, 2001a,b). The effects of potential trap crops on the efficiency and species composition of parasitoids remain unexplored.

In this study, we hypothesized that cruciferous plants differ in their attractiveness for oviposition to pollen beetle adults and their larval parasitoids. To test this hypothesis, we compared the oviposition preferences of the pollen beetle and its parasitoids for the potential cruciferous host plants Brassica nigra (L.) W. D. J. Koch (syn. Sinapis nigra L.), Raphanus sativus L. var. oleiformis Pers. and Eruca sativa Mill. (syn. Eruca vesicaria (L.) Cav.) with that for spring oilseed rape.

2. Materials and methods

2.1. Study area and experimental design

Studies were carried out in an experimental field of the Estonian University of Life Sciences, Tartu, between summers 2009 and 2011. The experiment was laid out in a randomized complete block design with three replicates of each plant species: B. napus, R. sativus, B. nigra and E. sativa. Each plot was 1 × 5 m with 1 m wide bare soil buffer zone around each plot. Neither fertilizers nor pesticides were applied.

2.2. Plant material

Plots were sown on 7 May 2009, 12 May 2010 and 9 May 2011 at 250 seeds per m². In 2009, seeds of B. nigra and E. sativa cv. ‘Poker’ of Gavris Plant-breeding and Seed-production Company were purchased from Hansaplant LLC seed company and seeds of B. napus spring variety cv. ‘Maskot’ of Svalöf Weibull AB (the company’s current name is Lantmännen SW Seed AB) and R. sativus cv.’Bille’ of GSS Saatzucht Salzmünde GmbH, E. sativa were obtained from the seed collection of the Estonian University of Life
Sciences. The cultivar of *B. napus* used in this study is early to medium maturing with medium height. The *R. sativus* cultivar has pale violet flowers. In 2010 and 2011, seeds from the previous year’s study were sown.

2.3 Insect sampling

To estimate pollen beetle oviposition activity, the buds and flowers of ten randomly chosen plants were collected from each plot at full flowering stage (GS 64–67; Lancashire et al. 1991; Table 1), dissected in the laboratory and all larvae counted.

To determine the parasitisation rate of the pollen beetle larvae, second instar larvae i.e. the last larval stage before dropping to the ground to pupate in the soil, were dissected under a stereoscopic microscope (Olympus SZ-CTV). Different numbers of specimens were dissected depending on plant species: 912 from *B. napus*, 1140 from *B. nigra*, 140 from *E. sativa* and 599 from *R. sativus*. Parasitoid larvae and eggs were counted and the percentage parasitism was calculated. Parasitoid larvae and eggs were identified to species using the key by Osborne (1960).

2.4 Statistical analyses

To test the statistical significance of plant species and year on the flower supply and on the number of beetle larvae per plant, the Poisson model considering fixed effects of year and plant species and random effect of replicate was applied (GLIMMIX Procedure). In parasitism rate analyses the logistic model with the same factors was used. The same models only without year effect were used to compare the flower supply, number of beetle larvae per plant and parasitism rate between plant species for each year. The modeling results are presented as least square means (± standard error). The species composition of the larval endoparasitoids of the beetle was compared with the Fisher exact test. Spearman correlation analysis was used to study the relationship between flower supply, number of beetle larvae and their parasitism rate. The analyses were conducted with SAS 9.1 software (SAS Institute, Cary, NC, USA). All results were considered statistically significant at *P* < 0.05.

3. Results

Plant species had a significant influence on the abundance of pollen beetle larvae over the three-year study period (*F* = 14.07, *df* = 3, *P* = 0.0001) but year as a factor had no influence (*F* = 2.93, *df* = 2, *P* = 0.069). The effect of plant species was also significant each year (2009: *F* = 33.07, *df* = 3, *P* < 0.0001; 2010: *F* = 16.52, *df* = 3, *P* = 0.0009; 2011: *F* = 9.09, *df* = 3, *P* = 0.0059). For oviposition in 2009, *B. nigra* and *B. napus* were preferred over *E. sativa* and *R. sativus* (*P* < 0.01; Fig 1); in 2010, the greatest number of beetle larvae per plant was found from *B. napus*, more than from *B. nigra* and *E. sativa*, which had the lowest infestation rate, differed also from *B. nigra* and *R. sativus* (*P* < 0.001). In 2011, the abundance of beetle larvae was similar on *B. napus*, *B. nigra* and *R. sativus*, with significantly fewer on *E. sativa* (*P* < 0.01).

The number of flowers per plant varied with plant species (*F* = 9.35, *df* = 3, *P* = 0.0002), with significantly more on *B. nigra* (*t* = –2.92, *df* = 30, *P* = 0.0065) and fewer on *E. sativa* (*t* = 2.32, *df* = 30, *P* = 0.027; Fig. 2) than on *B. napus*. The number of flowers per plant also differed with year (*F* = 9.35, *df* = 3, *P* = 0.0002).

The parasitism rates of beetle larvae differed with plant species (*F* = 23.54, *df* = 3, *P* = 0.0001) but not year (*F* = 1.77, *df* = 2, *P* = 0.19).

Biological control by parasitoids was the most efficient on Brassica species, especially on *B. nigra*, where the parasitism rate was higher than on all other plant species (*P* < 0.05) and a similar tendency was followed each year, even though no significant differences were found (Fig. 3).

Flower supply affected the abundance of beetle larvae only weakly (*B. nigra*: *r* = 0.22, *N* = 90, *P* = 0.037; *B. napus*: *r* = –0.15, *N* = 90, *P* = 0.14; *E. sativa*: *r* = –0.33, *N* = 90, *P* = 0.0017 and *R. sativus*: *r* = 0.049, *N* = 90, *P* = 0.65); also no statistical support was found to indicate that the parasitism rate depends on host abundance on most plant species (*B. nigra*: *r* = 0.014, *N* = 75, *P* = 0.90); *B. napus*: *r* = 0.067, *N* = 84, *P* = 0.54; *E. sativa*: *r* = 0.19, *N* = 46, *P* = 0.20); however, on *R. sativus* this relationship was moderate (*r* = 0.37, *N* = 51, *P* = 0.0083). In addition, no significant correlations between parasitism rate and flower supply were found (*B. nigra*: *r* = –0.047, *N* = 75, *P* = 0.69), *B. napus*: *r* = 0.11, *N* = 84, *P* = 0.32; *E. sativa*: *r* = –0.23, *N* = 46, *P* = 0.13; *R. sativus*: *r* = –0.18, *N* = 51, *P* = 0.20) on all studied plant species.

In total, 805 hymenopteran parasitoids of four species: *T. heterocerus*, *P. morionellus*, *D. capito* and one unidentified species, named the “healthy-fat-one”, were detected from beetle larvae during the three year study (Fig. 3). The most common species was *T. heterocerus*, comprising over 50% of all parasitoids found during the study. Nearly one quarter of parasitoids was identified as *P. morionellus* and 17% as *D. capito*. In total, six parasitoid larvae of the “healthy-fat-one” type remained unidentified but over the study they formed less than 1% of all parasitoids.

Species composition of parasitoids differed significantly with plant species (χ² = 305.97, *df* = 9, *P* < 0.0001; Fig. 3) and with year (χ² = 309.50, *df* = 6, *P* < 0.0001); the most abundant species in 2009 and 2011 was *T. heterocerus*, in 2010 *P. morionellus*. *Diosilus capito* was present each year, but formed less than 25% of all parasitoids.
each year. The unidentified “healthy-fat-one” was present only in 2009 (Fig. 3). During the study all four parasitoid species were present on *B. napus*, *T. heterocerus*, the most abundant parasitoid species, was absent on *E. sativa* and the “healthy-fat-one” on *B. nigra* and *R. sativus* (Fig. 3). The largest number of parasitoids was recorded from *B. nigra*, followed by *B. napus*, with a few only from *E. sativa* and *R. sativus*.

Over the years, *P. morionellus* has exploited its potential heavily on *B. napus*, where it formed 50% of all parasitoids on this plant species, *D. capito* on *E. sativa* (50%) and *R. sativus* (79%) but *T. heterocerus* on *B. nigra* (75%).

4. Discussion

Visual and olfactory cues are both important in host plant location by phytophagous insects, including the pollen beetle (Blight and Smart, 1999; Cook et al., 2007a,b, 2013; Döring et al., 2012; Giamoustaris and Mithen, 1996). In this study we found, that, for oviposition, the beetle preferred *B. napus*, *B. nigra* and *R. sativus* over *E. sativa*. This concurs with results from laboratory trials that also showed the beetle preferred *B. nigra* and *B. napus* over *E. sativa* (Ekomb, 1998; Ekomb and Borg, 1996), confirming differences in attractiveness within the same plant family. But, as no differences between *B. napus* and *B. nigra* were detected, our results contrast with the laboratory studies of Borg (1996), Ekomb and Borg (1996) and Ulber and Thieme (2007), all of whom reported significantly fewer larvae on *B. nigra* than on *B. napus*. On the other hand, the only study conducted in field conditions has shown *B. nigra* to be considerably more attractive than *B. napus* to overwintered pollen beetle adults for feeding as well as for oviposition (Veromann et al., 2012). Also the attractiveness of *R. sativus*, which was similar to that of *Brassica* species, contrasts with the laboratory studies of Ekomb (1998), who found species from the genus *Brassica* to be more attractive to the beetle than crucifers from other genera.

Although pollen beetles are known to respond to yellow color during host location (Blight and Smart, 1999; Cook et al., 2006,
species in our study was be the dominant species attacking the pollen beetle in Finland abundant on (2001) and Veromann et al. (2006d) who found parasitoid species: this concurs with Husberg and Hokkanen (2001), P. can speculate that olfactory cues associated with the host and used attracted to the plants infested by pollen beetle larvae (Jönsson et al., 2004) but we can hypothetically assume that the beetle distinguishes between plant species firstly by their chemical composition, and uses visual cues secondarily to locate the most suitable host. For flying insects, olfactory cues are paramount as these can be detected in flight whilst avoiding excessive energy cost and predators (Pyke et al., 1977).

The parasitism rates of pollen beetle larvae were investigated on B. nigra, E. sativa and R. sativus for the first time. Compared to previous studies from several European countries where a parasitism rate exceeding 50% has been reported on B. napus (Austria: Kromp and Kraus, 2006; Finland: Hokkanen, 2006; Germany: Nitzsche and Ulber, 1998; Sweden: Nilsson, 1989; Switzerland: Büchi, 2002; UK: Williams, 2006), rates in the current study were considerably lower. On B. napus, it varied from 16.5 up to 33.6% over the years, which is smaller than was reported before in Estonia (48%, Veromann et al., 2013), but higher than found in conventional fields in Estonia (0–16%) (Veromann et al., 2006a,b). The variation of parasitism rates between other plant species was even higher: from 0% up to 38%. Spatial variation of parasitism rate depends on several different factors such as the availability of nectar as food source in concurrence with certain species, flower morphology, parasitoids’ mandibles, ability to locate food sources (Gilbert and Jervis, 1998; Idris and Graffius, 1995; Jervis et al., 1993; Lee and Heimpel, 2003; Wäckers 2005) as well as on the enhanced fecundity of females which also depends on plant species (Idris and Graffius, 1995). Therefore, parasitism rate is highly conditioned by the local factors. In the current study parasitoids were supported most on B. nigra, whereas on E. sativa and R. sativus the parasitism rate was up to 10-fold lower than on Brassica species. This is also confirmed by Kaasik et al. (2013) who found higher parasitism rates of pollen beetle on Brassica species compared to another alternative host plant, S. alba. Therefore, based on our results, we conclude that parasitoids are attracted more to Brassica species than to other crucifer genera even when the latter support the development of pollen beetle larvae.

The species composition of the endoparasitoids of pollen beetle larvae on different host plant species were investigated for the first time. During the study, three key species of endoparasitoids were found: T. heterocerus, P. morionellus and D. capito; these are the most common and widely distributed species throughout Europe (Nilsson, 2003; Ulber et al., 2010). The host seeking behavior is similar in all three parasitoid species: they seek for hosts from buds and open flowers (Williams and Cook, 2010). The host finding success of female T. heterocerus on B. nigra were predominantly more effective than that of female P. morionellus and D. capito. These differences might be linked to their host searching behavior, as T. heterocerus explores flowers despite host abundance (Ferguson et al., 2003; Jönsson et al., 2004) but P. morionellus is attracted to the plants infested by pollen beetle larvae (Jönsson and Anderson, 2008). Larvae parasitized by T. heterocerus constituted over 75% of all infested larvae on B. nigra, thus, we can speculate that olfactory cues associated with the host and used by P. morionellus were less effective than the more visual search by T. heterocerus. On B. napus, P. morionellus outnumbered other parasitoid species: this concurs with Husberg and Hokkanen (2001) and Veromann et al. (2006d) who found P. morionellus to be the dominant species attacking the pollen beetle in Finland and Estonia, respectively. In general, the most abundant parasitoid species in our study was T. heterocerus, because it was extremely abundant on B. nigra. This was interesting, as in northern Europe, T. heterocerus is outnumbered by P. morionellus and D. capito on B. napus (Jönsson et al., 2004; Kaasik et al., 2013; Ulber et al., 2010; Veromann et al., 2006a–d) which were also found from all plant species in the current study. This indicates that P. morionellus and D. capito can efficiently locate alternative host plants. On non-brassica species, E. sativa and R. sativus, D. capito was the most efficient parasitoid species.

Thus, it is possible that T. heterocerus could more effectively locate suitable hosts on a host plant other than oilseed rape and therefore exhausted almost all (80%) of its oviposition potential on this alternative host plant. Less than 20% of all T. heterocerus specimens were found on B. napus indicating its lower attractiveness either due to unfavorable host abundance or better recognition of B. nigra than B. napus. Also, it may be possible that they were more attracted to volatiles emitted by B. nigra. In that case, it might be possible to use B. nigra to attract this parasitoid species and therefore to promote biocontrol in certain areas from where it could spread to oilseed rape fields. As this study showed the parasitoids of the pollen beetle to be density independent, they might spread to other plant species in case of limited host larvae availability on B. nigra.

5. Conclusion

Our study shows that B. nigra has potential to reinforce the natural control of the pollen beetle. Not only was it attractive to this pest as an oviposition site, but it provided suitable hosts for parasitoid species not prevalent on oilseed rape. In addition to trapping pests, B. nigra could also act as a parasitoid bank, providing habitat for beneficial insects.

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References

Billqvist, A., Ekborn, B., 2001b. Effects of host plant species on the interaction between the parasitic wasp Dialius capito and pollen beetles (Meligethes spp.). Agric. Forest Entomol. 3 (2), 147–152.


