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Original scientific paper

Effects of insecticides intended for *Ceutorhynchus napi* Gyll. control in oilseed rape on ground beetles

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SUMMARY

The effects of insecticides that are commonly used for conventional and integrated oilseed rape (OSR) management on ground beetles were studied. Monitoring of harmful species showed that only insecticides intended against *Ceutorhynchus napi* should be applied. There were no differences in beetle numbers and phenology of settling of *C. napi* in the OSR fields that received different management practices.

The type of OSR management has a primary and significant impact on ground beetles abundance. Early in the spring, ground beetles settled more massively on the non-tilled OSR field with abundant weed cover and mulch on soil surface. However, there were no significant differences in species richness between the OSR fields managed differently. A total of 22 species were recorded. Early in the spring, the granivorous ground beetles *Amara aenea* (47.3%) and *Harpalus distinguendus* (32.5%) were dominant.

When insecticides were applied, immigration of ground beetles began, so that their adverse effect was minimal. In both management systems the number of ground beetles and their diversity increased after spraying. In conclusion, no significant harmful effects of the insecticides on ground beetles were detected in OSR fields managed in two different ways.

Keywords: Insecticides; Ground beetles; Rape stem weevil; Oilseed rape

INTRODUCTION

Ground beetles (Carabidae) are a numerous group of zoophagous and phytophagous species that are very sensitive to habitat conditions. They can therefore be used for indicating changes in the environment and especially in agricultural fields that are generally exposed to disturbances (Cole et al., 2002, 2005; Lővei & Sunderland, 1996; Rainio & Niemelä, 2003). In agrobiocenosis, the type of field management plays a very important role in species composition and abundance since different management practices cause significant environmental changes to which carabids react (Büchs, 2003).

Ground beetles belong to the most important invertebrate predators in arable crops. They are particularly numerous in oilseed rape, where conservation of their populations is gaining in importance (Büchs & Alford, 2003). In the context of sustainable agriculture, the maintenance and enhancement of carabid diversity is of major interest (Booij, 1994).

Insecticides may be harmful to carabid populations and there is an awareness that these predators should be protected in order to accomplish their full potential for biological control (Luff, 1983). Adverse effects of insecticides may occur in the field, depending on the time of insecticide application and coinciding presence of carabids.

On the effect of insecticides may also influence the phenology and carabid species composition which may vary considerably over space and time (Makarov, 1994; Pozsgai & Littlewood, 2014; Thiele, 1977). It is assumed that an early spring application of insecticides in oilseed rape is not harmful because there are no carabids (Büchs, 2003). It implies a dependence of carnivorous species among them on prey that is either absent or at very low density early in the spring. On the other side, there is plenty of food for seed predator species in early spring, so that their presence may be endangered. Seed predator species are important for reducing frequent weeds (Klimeš & Saska, 2010; Saska, 2008)

Considering the 6 most significant insect pest species of oilseed rape, farmers usually apply insecticides to control the pollen beetle *Meligethes aeneus* F., as well as the stem weevils (*Ceutorhynchus napi* Gill. and *Ceutorhynhus pallidactylus Mars.*) but rarely *Dasineura brasicae* Winn. (Williams, 2010). In contrast to the north of Europe, some regions of Central and Eastern Europe often have rape stem weevil as the more important pests (Bozsik, 2010; Bucur & Roşca, 2011; Juran et al., 2011; Sivčev et al., 2015; Williams, 2010). This could have significant implications for predators because massive incidence of *C. napi* sometimes requires the use of insecticides up to 3 weeks ahead of insecticide treatments intended against *C. pallidactylus* or *M. aeneus*, which are most frequently applied (Büchs, 1998) . Thus, harmful effects of insecticides may vary as a result of their different timing of application.

Due to species habitat preferences and composition, insecticides may be expected to have different effects in fields managed differently. Insecticide application is based mainly on the intensity of pest attack and rarely considers also the presence of beneficial organisms, such as predators. Therefore our aim was to explore the ground beetle fauna in different crop management systems at the time of early application of insecticides aimed to control rape stem weevil. Little is known about which species of ground beetles are present at the time of insecticide application in oilseed rape and thus potentially endangered. Considering that different crop management systems imply the application of different insecticides our intention was to examine potential changes in ground beetle assemblage.

MATERIAL AND METHODS

The experiment was conducted on two commercial winter oilseed rape (OSR) fields located in northern Serbia (N45 57.280 E19 37.554). The OSR fields were managed in accordance with conventional and integrated production practices. Soil was prepared by ploughing in the conventional field, while the integrated field was disk harrowed, leaving a mulch cover from straw remains and a dense weed cover of *Stelaria media* (L.) Vill. and *Capsella bursa pastoris* (L.) Medik. There was 30.5 OSR/m^2 and <1 weed/m^2 in the conventional field, while the integrated field had 35 OSR and 22 *C. bursa pastoris* and 20 *S. media* plants/m^2. *Lamium purpureum* L. was also found in patches and at a much lower density.

Insecticides were applied according to estimates based on monitoring pests by 4 yellow water traps (YWT) per field. The YWTs were raised above crop canopy in order to catch flying imigrating ground beetles and pests. Pest monitoring with the YWTs revealed that only *C. napi* exceeded a threshold for insecticide treatment. Infestation of the two differently managed fields was similar. There was no significant difference in the number of *C. napi* infesting the conventional (174.75±26.60) and integrated fields (181.75±71.40), t(6)

=.184 , p = . 860. Maximum pest immigration occurred in the period between 12 and 23 of March. Insecticides were applied on 25 March at the OSR developmental stage BBCH 22-25. Besides *C. napi* adults at the egg laying stage, *C. pallidactylus* were also present, only in much lower numbers (the ratio was 8:1), and some adults of pollen beetles *M. aeneus* at the beginning of immigration. The conventional cropping system included an insecticide treatment with chlorpyrifos + cypermethrin (500 g/ha a.i. + 50 g/ha a.i.), while cypermethrin (40 g/ha a.i.) was applied as part of the integrated cropping system. A standard tractor sprayer with 300 l water/ha was used. In accordance with standard local practice a total of 148 kg/ha N, 80 kg/ha P, and 80 kg/ha K was additionally applied on the conventional field and half of that amount in the integrated field.

Ground beetles were sampled in 8 pitfall traps (diameter 110 mm) arranged in a line at the center of each field. The distance between neighboring pitfall traps was 50 m. Insects were collected every 1-2 weeks from January onwards.

Means were represented with ±s.d. The significance of differences in the mean number of trapped specimens and species richness under each insecticide treatment was tested with the paired t-test. The independent samples t-test was used to analyze the differences between the abundance and richness of species in the two OSR management systems. Statistical analysis was performed using IBM SPSS 21.0 for Windows (IBM, 2013).

RESULTS

Ground beetle population in both OSR fields was very weak prior to and at the time of insecticide treatments aimed to control *C. napi*. The post-insecticide treatment period was characterized by rapid population increase, greater in the field practicing integrated management. There was a significant difference in the mean number of ground beetles found in pitfall traps between the integrated (41.75±16.10) and conventional fields (19.88±6.06), t(14) =3.598 , p = .003.

However, species richness was similar in the two OSR fields managed in different ways. A total of 22 ground beetle species were found in both fields (Table 1). Of these, 13 species were found in the conventional field, while 19 carabid species were detected in the integrated field. There was no significant difference in the mean number of ground beetle species found per pitfall trap between the integrated (6.00±1.60) and conventional field (6.13±2.10), t(14) =.134, p = .895.

Of a total of 493 specimens found in pitfall traps in both fields, *A. aenea* (47.3%) and *H. distinguendus* (32.5%) accounted for the largest part with 393 specimens (Table 1) . The abundance of these two species was greater in the field with integrated management. Some less numerous species, such as *Poecilus cupreus* and *Poecilus sericeus,* clearly showed that they preferred plowed field (Table 1).

Table 1. Ground beetles (Carabidae) assemblages recorded with pitfall traps in different management systems, 12 March – 27 April 2011 (Con = conventional; Int = integrated)

Species	Con	Int	Total
Amara aenea De Geer	42	191	233
Amara consularis Duftschmid	1	1	2
Amara familiaris Duftschmid	0	7	7
Amara similata Gyllenhal	4	6	10
Anchomenus dorsalis Pantoppodian	13	16	29
Metalina properans Stephens	2	1	3
Calathus ambiguous Paykull	0	1	1
Calathus fuscipes Goeze	0	1	1
Harpalus anxius Duftschmid	1	1	2
Harpalus dimidiatus Rossi	0	1	1
Harpalus distinguendus Duftschmid	68	92	160
Harpalus pumilus Sturm	0	2	2
Harpalus pygmaeus Dejean	0	1	1
Harpalus rufipes De Geer	3	0	3
Harpalus signaticornis Duftschmid	0	3	3
Harpalus zabroides Dejean	0	1	1
Ophonus azureus Fabricius	0	1	1
Poecilus cupreus Linnaeus	7	0	7
Poecilus punctulatus Schaller	6	2	8
Poecilus sericeus Fischer von Waldh.	10	4	14
Poecilus versicolor Sturm	1	0	1
Zabrus tenebrioides Goeze	1	2	3
Total	159	334	493

The post-treatment period showed the same trend of increasing abundance and species richness of carabids in the OSR fields managed as integrated and conventional systems (Figure 1). Dependent time series of means for carabids abundance and species variety were compared using 30 March as the baseline date because no carabids were trapped in the earlier sampling period.

The period before insecticide application (12-23 March, BBCH 22-25) was characterized by a very low activity density on the ground surface with only 1 specimen of *H. distinguendus* found in both fields. However, colonisation of the OSR fields started during that period and flying specimens of *Amara* species and *Harpalus distinguendus* were sampled by YWTs.

After insecticide treatment, continuous flight activity and increased ground activity were detected in both OSR fields (BBCH 50-51) (Figure 2). Five days after treatment there was an increase in the number of ground beetles in pitfalls, while flying activity was of the same intensity, which indicates that the applied insecticides caused no harm to ground beetles. Over a period of 5 to 12 days after treatment, the population of ground beetles was increasing and both kinds of traps registered their maximum ground level activity and maximum flight (OSR, BBCH 55-57) (Figure 2). Flying of both spring breeders was terminated after 12 April about 3 weeks after the start.

During the flight period, 79 specimens were trapped in the 8 YWTs mounted in the OSR fields. Of that number, *Amara* were the most numerous (64 specimens - 81%), namely: 44 *Amara familiaris*, 13 *Amara aenea* and 7 *Amara similata*. The YWTs also trapped 15 *Harpalus distinguendus* beetles. The mean number of ground beetles in YWTs was similar in the fields managed as conventional (9.25 ±15.86) and integrated systems (10.50±17.02, t(6)=. 107, p=.918).

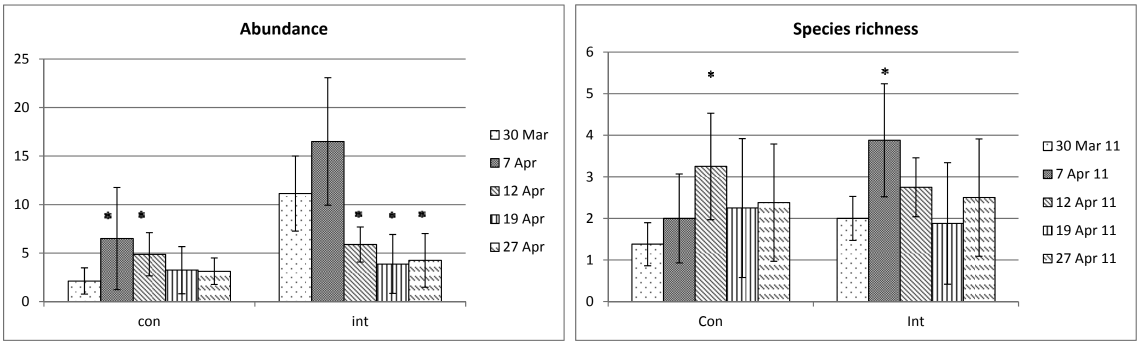
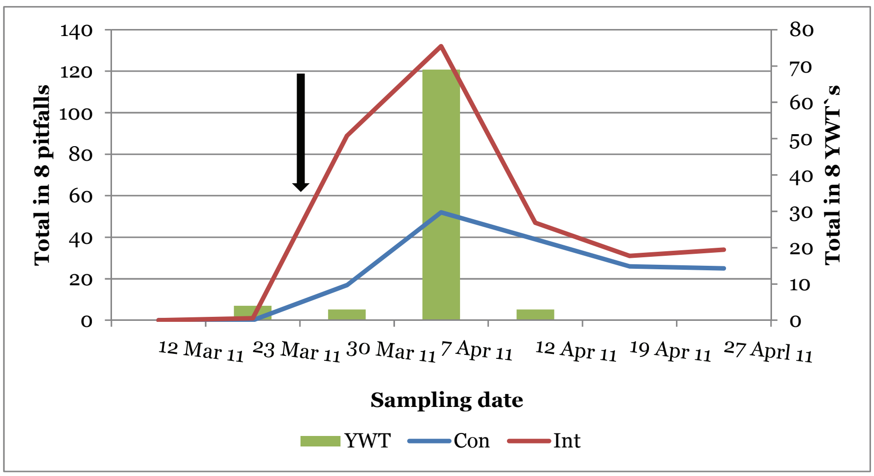


Figure 1. Means and paired t-test comparisons of carabid activity, density and species richness in OSR fields managed as conventional (Con) and integrated (Int) systems, using 30 March as baseline (* indicates significant differences p<0.05)



Insecticide application

Figure 2. Activity of ground beetles detected with pitfall and yellow water traps

The species of both genera are seed predators and their predominance is associated with the presence of dense cover of *S. media* and *C. bursa-pastoris* plants, particularly in the integrated field (Table 1). *S. media* overwinter as full-grown plants which bloom and form seeds very early in the season. Early presence of this weed was primarily responsible for the intensive colonisation by seed predatory ground beetles. *C. bursa-pastoris* was in full bloom, as well as *S. media*, around 7 April (BBCH 55-57), when aerial and ground level activities reached maximum. During intensive weed blooming, the activity density was significantly different between the integrated (16.500±6.5683) and conventional fields (6.500±5.2644), t(14)=3.360, p=.005. However, over the following period from 12 April (BBCH 60-61) to 19 April (BBCH 63-65), flight ceased completely and ground level activity decreased to a level where the mean number of carabids per pitfall trap did not differ significantly between the conventional (3.25±2.435) and integrated fields (3.88±5.027), t(14)=.316, p=.756

DISCUSSION

Ground beetles can be good bioindicators of disturbances in the environment due to different human activities (Rainio & Niemelä, 2003). This widespread insect group is vulnerable to the effects of agricultural management practices in association with various chemical agents, such as pesticides, heavy metals, etc. (Kotze et al., 2011).

The side effects of insecticides on beneficial organisms depend on a large number of factors, but essentially mostly on the coincidence of pests, predators and spraying timing (Alford et al., 2003).

Insecticides from different chemical groups are toxic to Carabidae (Lee et al., 2001; Prasifka et al., 2008; Tooming et al., 2014). In contrast to laboratory conditions where insecticides show their full adverse effect, toxicity to ground beetles is not fully manifested under field conditions (Alford et al., 2003). It is not known precisely which ground beetle species may be endangered, or the full scale of impact of insecticides on their biodiversity. For example, species that are active at night may be protected from direct exposure at the time of insecticide application, which may result in their lower mortality.

In oilseed rape, direct exposure of epigaeic predators to insecticides is relatively weak, owing to dense layers of vegetation that protect them from contamination; this is especially so in the later stages of crop growth when insecticides are applied to combat pests such as brassica pod midge and cabbage seed weevil (Büchs, 2003).

It has been shown that insecticide seed treatments of oilseed rape may have severe adverse effects on epigaeic predators such as ground beetles, because even predatory species such as *Poecilus cupreus* are partly phytophagous and will also feed on seeds (Buchs et al., 1991).

The analysis of the ground beetle community in our trial showed that granivorous species were dominant during early spring. Their settlement is largely influenced by the method of land cultivation and presence of mulch and weeds. Carnivorous species are sporadically present and, excepting *Anchomenus dorsalis*, colonize an area later. The dominance of granivorous carabids suggests that a large number of weed plants, such as *S. media* and *C. bursa pastoris,* is very important in determining the phenology and composition of carabid communities. Those weeds successfully overwinter and, very early in the spring, provide seeds that feed very mobile granivorous ground beetles that can fly in search of a suitable place for feeding and reproduction.

From our results we have seen that it is the integrated management system, due to its way of soil cultivation and large quantities of mulch, i.e. the remains of straw and weeds from previous vegetation, that creates a very attractive environment for seed predators such as *Amara aenea* and *Harpalus distinguendus.* Therefore, any potential negative effect of that management system will occur in this group of predators which feed on seeds and appear early in the spring when there is no animal prey for carnivores.

On our integrated field (not plowed, with mulch and weed cover) carabid abundance was twice as great as it was on the conventional field. Such a high number of carbids on the OSR fields early in the spring can be an indication of potentially harmful effect of insecticides. Our results clearly show that in the event of a necessity to control *C. napi* this damage would be small because the pest is suppressed very early at the beginning of colonization of granivorous ground beetles. However, application of insecticides to suppress *M. aeneus* and *C. pallidactylus* may occur as long as 3 weeks after treatment of *C. napi* (Büchs, 1998). Therefore, it infers that harmful effects of insecticides could occur on weedy and mulched OSR fields, where *M. aeneus* and *C. pallidactylus* are being suppressed because the time of insecticide application coincides with the maximum activity of granivorous ground beetle species. Later applications of insecticides would be harmful if coinciding with the reproductive period of the predators, when they are highly active in the field.

Such applications may have long-lasting effects on predator population development because reproduction and egg laying are disrupted (Büchs, 2003).

Diversifying agro-ecosystems with refuge habitats may buffer the negative consequences of insecticide application on carabids (Lee et al., 2001). Our data highlight the importance of undisturbed refuge because the most numerous are *Amara* species and *H. distinguendus*, which fly over into fields to settle. These species could also fly off and quickly leave contaminated fields or remain in refuge habitats and thus avoid insecticides (Alford et al., 2003).

Re-establishment of predator populations in fields following pesticide application is clearly an important issue, which may be assumed to be achieved by re-invasion from adjacent areas and subsequent repopulation by in-field survivors (Büchs, 2003).

The dominance of *Amara* species (*similata* and *ovata*) in most of Europe's rapeseed fields is attributable to the increased presence of weeds resulting from missing herbicide treatments (Büchs, 2003)

Amara species are important seed predators that prefer seeds of *Stellaria media* (L.) Vill., *Capsella bursa-pastoris* (L.) Med. (Saska, 2008) (Klimeš & Saska, 2010). These weeds were dense and dominant in our integrated field. Since there was no animal food available in that period it is clear that an abundance of mulch and seed bank from the autumn and weed species with mature seeds in the spring caused differences in the abundance of ground beetles between the two differently managed OSR fields. *S. media* overwinters as a well developed plant and it is the first weed that forms seeds. Carabids feed on seeds that have just fallen onto the soil but also on those that had dropped into soil previously, which further emphasizes the importance of reducing the number of weeds in fields (Martinková et al., 2006). In search of seeds, carabids even climb plants, especially grasses and *Umbelliferae* (Hůrka, 1996).

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Delovanje insekticida namenjenih suzbijanju *Ceutorhynchus napi* Gill. u usevu uljane repice na karabide

REZIME

Ispitivan je efekat insekticida u konvencionalnoj i integralnoj proizvodnji i zaštiti uljane repice. Monitoring štetnih vrsta je pokazao da se insekticidi moraju primeniti samo u suzbijanju *Ceutorhynchus napi*. Brojnost imaga i fenologija naseljavanja *C. napi* se nije razlikovala na različitim poljima uljane repice.

Pokazalo se da način gajenja uljane repice ima primarni uticaj na brojnost karabida jer su se one u značajno različitom broju naselile na različito gajenim poljima uljane repice. Rano u proleće, karabide su u najvećem broju naselile neorano polje pokriveno korovima i malčom od slame. Međutim, nije bilo značajnih razlika u bogatstvu vrsta koje su naselile ova dva polja uljane repice. Ukupno je registrovano 22 vrste karabida. U rano proleće dominantne su granivorne vrste *Amara aenea* (47.3%) i *Harpalus distinguendus* (32.5%).

U vreme kada su primenjeni insekticidi, počinjala je imigracija karabida tako da je njihova brojnost bila mala a stoga je i štetni efekat insekticida bio minimalan. Posle prskanja je došlo do porasta broja karabida i njihovog diverziteta na oba polja sa različitim načinom gajenja. Na osnovu toga se može zaključiti da nema značajnog štetnog delovanja insekticida na karabide kada se suzbija *C. napi* u poljima uljane repice u konvencionalnoj i integralnoj proizvodnji.

Ključne reči: Insekticidi; Karabide; Velika repičina pipa; Uljana repica