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Use of entomopathogenic nematodes and UV-reflecting mulches to control the cabbage fly *Delia radicum* L. on turnip cabbage

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1. Introduction, Knowledge, Objectives

One of the most important insect soil pests of *Brassicae* crops in Europe and North America is the cabbage root fly, *Delia radicum* (Diptera: Anthomyiidae) (Finch & Collier, 2000). One very promising and environmentally save method to control in particular soil dwelling pest insects are entomopathogenic nematodes (EPN). Recent progress in mass production, prolonging shelf life and application methods have reduced costs and improved efficacy as well (Stock, 2005). Different greenhouse and field experiments demonstrated that in particular *Steinernema feltiae* was efficient in infecting and reducing the number of *D. radicum* larvae comparable to that of chemical insecticides (Georgis et al., 2006). Another promising mean to prevent pests from colonizing host plants or selecting oviposition sites is interfering with their visual orientation. Among these, UV-reflecting mulches have been already proven to deter certain pests such as aphids, whiteflies or thrips from settling on host plants and mitigated initial infestation (Greer & Dole, 2003). By orientating towards its host plant *D. radicum* apparently utilizes two searching mechanisms: for longer distances it relies on volatile cues from host plants, and for shorter distances on plant visual stimuli (Prokopy, 1983). So it seems to be reasonable attempting to interrupt the searching behavior of *D. radicum* by such a visual barrier.

2. Material and Methods

Preparation of plants and plots

The experiments were conducted in 2014 on two different locations in Ahlum and Ruthe (Lower Saxony, Germany) with two sets of plants per each location. Preparation of plots and arrangement of the experiments on both locations were kept identical. Turnip cabbage (*Brassica oleracea* var. *gongylodes* L.) of the variety “Lech” were arranged in a random block design with four plots per block. Each plot was 7.5 m x 1.2 m in size with 1 m distance between blocks. Ten weeks old turnip cabbage plants were arranged in four rows (30 plants per row) in each plot and planted in Ruthe on 14.05.2014 (first set) and on 02.07.2014 (second set), in Ahlum on 07.05.2014 and 25.06.2014 the first and second set respectively. Plants were harvested eight weeks after planting on all locations.

Treatments

1. control (water), 2. negative control “SpinTor” (Spinosad, 12 ml/1000 plants diluted in approx. 2 l/m^2 and sprayed 1-2 days before planting), 3. Nematodes – *S. feltiae*

("Nemaplus" from e-Nema, Germany, 3 applications, 180000 nematodes/plant), 4. UV-reflecting mulch ("Mil Black LDPE 60" (aluminum-metallized, Star Metal Planting Inc., Escondido, USA).

EPN were applied early in the morning to reduce negative effects of UV exposure and desiccation upon detecting first laid eggs of *D. radicum* followed by two more applications after 10 and 20 days. For detection of laid eggs felt collars (Olbis, Lausanne, Switzerland) were used. In control and UV-reflecting mulch plots five of such collars were placed on plants in the same row and observed two times in the week. For the final evaluation ten plants of each plot were taken. Plants that had felt collars were excluded from the final evaluation. Additionally, approx. 10 cm^3 of the soil with roots around the plant were taken, roots were washed, the water-soil suspension sieved with two sieves (2 and 1.4 mm mesh), and consequently the pupae and larvae were counted. Afterwards the root damage for each plant was visually estimated on a scale from 1 to 4 (Root Damage Index - RDI): 1: no visual damage; 2: superficial feeding scars (root cortex undamaged); 3: deep scars (taproot intact); 4: severe damage on the tap root, plant dead.

Statistics

For statistical evaluation the software R version 3.2.1 (http://www.R-project.org) was utilized. Numbers of pupae and larvae were log+1-transformed, and a linear mixed model was utilized with set, location, treatment and their interactions as effects, followed by ANOVA. For evaluation of root damage the mean RDI with linear mixed model (location, set, treatment, and their interaction) was used, followed by ANOVA. The number of detected eggs was analyzed treating each date separately by generalized linear model, followed by ANOVA and F-test (depending on graph used in results). For graphical illustration the package gglot2 was used (Wickham H, 2009).

3. Results

Number of larvae and pupae

By analysis of variance significant differences were demonstrated among the sets (F-value: 15, p=0.001) as well as between locations (F-value: 66.9, p<0.0001), in addition significant differences were shown among treatments (F-value: 52.9, p<0.0001). Moreover, the differences among the sets were significantly different depending on the treatment (F-value: 2.09, p=0.03) and the efficacy of the treatments was significantly dependent on the location (F-value: 13.3, p<0.0001) (Fig 1a). Among all utilized methods the most efficient one was the chemical insecticide "SpinTor". It reduced the number of pupae and larvae as compared to control 5-fold in Ahlum and 4.5-fold in Ruthe. The degree of pest reduction demonstrated by EPN was on the other hand inconsistent and location dependent. So in Ahlum EPN caused approx. 3.3-fold reduction of pupae and larvae over two sets compared to control, whereas in Ruthe EPN reduced the number of pupae and larvae 1.4-fold over both sets (Fig. 1a). The use of UV-reflecting films also led to lower numbers of larvae and pupae of *D. radicum*. The degree of reduction in Ruthe was 4.2-fold, in Ahlum 1.9-fold.

Root damage

ANOVA revealed significant differences in RDI among sets (F-value: 21.1, p=0.0006), and treatments (F-value: 14.9, p<0.0001). Moreover the differences among the sets were dependent on the location (F-value: 18.7, p=0.0009). Plants treated with “SpinTor” showed the least damaged roots. EPN effects were inconsistent, on one hand EPN reduced RDI in Ahlum in both sets (1.3- and 1.1-fold reduction respectively), whereas in Ruthe a reduction in RDI occurred only in the second set. UV-reflecting mulches decreased the root damage over all locations and sets, whereby the highest degree of reduction was accomplished in Ruthe in the second set and was 1.8-fold (Fig. 1b).

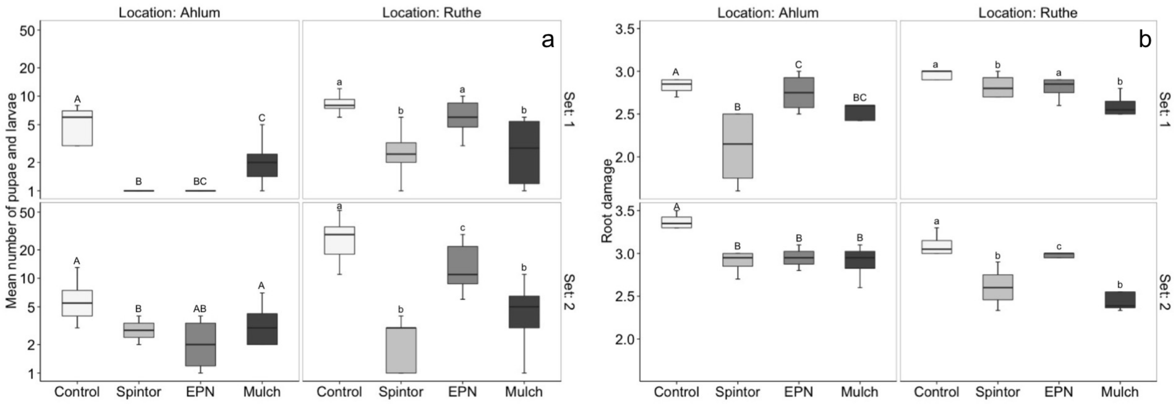

Fig. 1: Mean numbers of pupae and larvae (a) and mean root damage (b) on turnip plants in Ahlum und Ruthe counted after root washing (n=10, box plots followed by the same letter are not significantly different at P < 0.05).

Egg laying behavior

Only control and mulch plots were equipped with felt collars. In Ruthe UV-reflecting mulches reduced number of eggs on some dates significantly, but overall only tendency for a reduction effect could be stated. The maximum degree of egg reduction was detected on 26.06.2014 and was approx. 3.8-fold (Fig. 2a). In Ahlum UV-reflecting mulches did not affect egg laying of *D. radicum* (Fig. 2b).

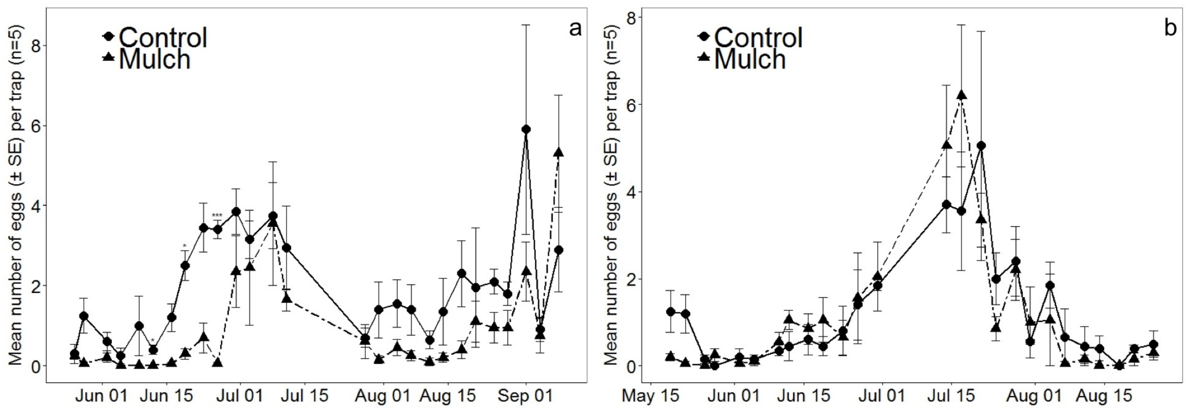

Fig. 2: Mean number of *D. radicum* eggs (two sets combined) counted in felt collars on turnip cabbage plants in Ruthe (a) and in Ahlum (b). Means followed by an asterisk are significantly different from each other (ANOVA, F-test). *** P < 0.001,** P < 0.01, * P < 0.05.

4. Discussion

Among the different treatments the insecticide “SpinTor” always showed the highest efficacy for controlling larvae of *D. radicum*. It significantly reduced the numbers of pupae and larvae, which resulted in less root damage, in both locations among all sets. Similar high efficacy of this compound was demonstrated for controlling *D. radicum* or onion maggots *Delia antiqua* in cabbage and cauliflower (Ester et al., 2003; Nault et al., 2006). However, latest reports are also showing an increasing level of resistance to this insecticide at certain locations (Beck, 2014). *S. feltiae* were able to reduce the numbers of pupae and larvae, however the efficacy was inconsistent varying between locations and sets. The possible reason might be the soil type in Ahlum that was more suitable for nematodes. *S. feltiae* localizes hosts by active search and dense or heavier soils hamper moving, which can result in lesser parasitism rates (Stuart et al., 2008). On the other hand lighter or sandy soils enhance nematodes’ host localization. Other factors for possible variability in EPN efficacy such as EPN sensibility to desiccation and UV-radiation (Fenton et al., 2002), seemed to be of minor relevance since EPN were always applied in the morning hours avoiding strong UV-radiation and plants were always watered prior to applying nematodes, to provide optimal levels of soil moisture. Moreover, the timing of first application which could also be crucial was chosen one week after detection of first eggs to achieve that nematodes encounter the most susceptible L_2 and L_3 instars (Nielsen, 2003). Regarding the reduction of RDI *S. feltiae* was efficient in reducing root damage only in Ahlum but not in Ruthe, which can be correlated with the higher remaining mean numbers of pupae and larvae in Ruthe.

The UV part of natural solar radiation is relevant for herbivorous insects mainly for stimulation of "take-off" and orientation in space (Kumar & Poehling, 2006), whereas for host plant search contrasts between dark soil and green plant is an important stimulus (Döring et al., 2004). Consequently, it has been shown with some herbivorous insects such as thrips or psyllids that artificial reflection of sun light from soil surface by reflecting mulches may interrupt host plant search (Summers & Stapleton, 2002, Croxton & Stansly, 2014). Information about species from the order *Diptera* in this regard is rather scarce. There was one similar experiment conducted with *Platyparea poeciloptera* Schrank (Diptera, Tephritidae) (Koch, 2011) showing that the use of UV-reflecting mulch indeed can repel egg deposition of females and reduce the number of developed pupae to a similar low level as chemical insecticides.

In the present experiment the influence of the UV-reflecting mulch influenced the population dynamics of *D. radicum* irregular. In Ruthe significant reduction in egg deposition could be observed, whereas in Ahlum UV-reflecting mulch only slightly lowered numbers of eggs, however the root damage in mulch plots was lower as compared to control throughout the entire experiment. Overall reflecting mulches seem to be an interesting tool for cabbage fly control.

5. Conclusions

In conclusion the studied options for *D. radicum* control EPN and reflective mulches show some potential but there is need for further optimization in the frame of an integrated control system.

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