Distribution of *Meligethes aeneus* (F.) and *M. viridescens* (F.) on cruciferous plants

Luule METSPALU[1], Ingrid H. WILLIAMS[2], Katrin JÕGAR[1], Angela PLOOMI[1],
Külli HIIESAAR[1], Peeter LÄÄNISTE[1], Eha ŠVILPONIS[1], Marika MÄND[1], Anne LUIK[1]

[1]Institute of Agricultural and Environmental Sciences, Estonian University of Life Sciences
Kreutzwaldi 1, 51014 Tartu, Estonia
E-mail: Luule.Metspalu@emu.ee

[2]Plant and Invertebrate Ecology Division, Rothamsted Research
Harpenden, Hertfordshire AL5 2JQ, UK

Abstract

The occurrence of *Meligethes aeneus* and *M. viridescens* (Coleoptera, Nitidulidae) on *Brassica rapa*, *B. napus*, *Erysimum cheiranthoides*, *Capsella bursa-pastoris*, *Thlaspi arvense* and *Sinapis arvensis* was investigated. *M. aeneus* first colonised winter *B. rapa*, but this plant species was not used for oviposition. *M. viridescens* appeared some weeks later on winter *B. napus* where it remained less abundant than *M. aeneus*. On spring *B. napus*, *M. aeneus* arrived at the field before buds were visible, but *M. viridescens* was not found. On *E. cheiranthoides*, adult *M. viridescens* were found but not their larvae or *M. aeneus*. In contrast, on *C. bursa-pastoris*, adult *M. aeneus* but not their larvae were found and no *M. viridescens* were found. *S. arvensis* was used for feeding and oviposition more by *M. viridescens* than by *M. aeneus*. Thus certain wild cruciferous plants are important food and oviposition resources for *M. aeneus* and *M. viridescens*.

Key words: *Meligethes aeneus*, *M. viridescens*, distribution, abundance, oilseed crops, cruciferous weeds.

Introduction

Pollen beetles *Meligethes aeneus* F. and *M. viridescens* F. are two of the most common pests on cruciferous crops throughout Europe. Both adults and larvae feed on pollen within buds and flowers, but can damage stamens, petals and ovaries of flowers, causing them to dry and fall leaving podless stalks (Williams, Free, 1978). The damage is greatest in the green and yellow bud stages of plant growth. When the buds on the main stem are damaged, more than half of the seed crop can be lost (Williams, Free, 1978; Nilsson, 1987; Williams, 2010).

In northern Europe, *M. aeneus* is the more common species and is found throughout spring and summer, whereas *M. viridescens* normally occurs later in the spring and usually makes up less than 10% of the populations of both beetles (Billqvist, Ekbom, 2001). Both species are univoltine. Adult beetles overwinter in the soil under herbaceous vegetation as well as in densely forested areas where they can often be aggregated (Nilsson, 1988).

When spring temperatures exceed 8–10°C, adult *M. aeneus* emerge from overwintering and at >12°C search for pollen in spring flowers nearby (Free, Williams, 1978; Borg, 1996). Longer-distance migrations are undertaken at >15°C (Free, Williams, 1979). *M. viridescens* need higher temperatures than *M. aeneus* to leave their overwintering sites. Both species are reproductively active for up to 2 months. Gravid females lay clutches of up to six or more eggs on buds (Ekbom, Borg, 1996). The larvae have two instars over 20–30 days (Nilsson, 1988). Full-grown larvae drop to ground, pupate in the soil, and emerge as young beetles about one month later.

Post- and pre-hibernation, adult *M. aeneus* and *M. viridescens* feed on the pollen of flowers from a large number of plant families (including the *Brassicaceae*, *Compositae*, *Labiatae*, *Rosaceae*, *Ranunculaceae* and *Umbelliferae*) (Free, Williams, 1978; Kirk-Spriggs, 1996; Williams, 2010) but they oviposit only in buds of the plant family

Brassicaceae (Free, Williams, 1978). Thus, they are generalists when searching for food but specialists when searching for oviposition sites. This specialism on *Brassicaceae* is determined largely by the chemistry of this plant family. Cruciferous plants, including *Brassica* spp., contain secondary metabolites, the glucosinolates, that are feeding and oviposition stimulants to many specialist phytophagous insects, but are deterrent to generalists (Chew, Renwick, 1995; Bartlet, 1996). On tissue damage, the glucosinolate breakdown products, such as the isothiocyanates, are also attractants to crucifer-specialist insects. Most cruciferous species contain more than one glucosinolate, but plants also contain substances that, in the case of certain species, can inhibit the development of pests. The acceptability of a particular cruciferous species to a particular crucifer-specialist insect, both for feeding and for oviposition, is determined not only by the range of glucosinolates present, but also by the balance between the stimulant and deterrent compounds (Chew, Renwick, 1995; Borg, 1996). In the majority of cases, the stimulant for feeding behaviour in the larvae corresponds closely to the oviposition stimulants, e.g. for oviposition of adults of *Pieris brassicae* L. and the feeding of caterpillars (Loon van et al., 1992).

The aim of the present study was to establish the temporal distribution and dynamics of *M. aeneus* and *M. viridescens* on three rape crops and four widely distributed cruciferous weeds of agricultural land in Estonia to establish whether the weeds provided feeding and oviposition resources for these beetles, thereby enhancing their potential as crop pests.

Materials and methods

Experimental design. The experiments were carried out in the experimental fields of the Estonian University of Life Sciences (Estonia, 58°18′ N, 26°41′ E) from May–July 2006. The rape crops: winter turnip rape (*Brassica rapa* var. *oleifera* subvar. *biennis* L.) (WTR, cultivar 'Credit'), winter oilseed rape (*Brassica napus* var. *oleifera* subvar. *biennis* L.) (WOR, cultivars 'Wotan' and 'Express') and spring oilseed rape (*Brassica napus* var. *oleifera* subvar. *annua* L.) (SOR, cultivar 'Maskot'). The cruciferous weeds: *Sinapis arvensis* L., *Capsella bursa-pastoris* (L.), *Thlaspi arvense* L. and *Erysimum cheiranthoides* L. The winter oilseed crops and weeds were sown in August 2005 in a randomized complete block design with four replicates of each of them. The plots (1 x 10 m) were separated by 0.5 m cultivated strips. The SOR plots (1 x 10 m),

sown in May 2006 were about 0.5 km from the crops of WTR, WOR and weeds, and were surrounded by wheat. The plots were sown at a rate of 200 viable seeds per m^2. The growth stages (GS) were recorded using the key of Lancashire et al. (1991).

Pollen beetle counts. Sampling for *Meligethes* spp. started at the beginning of the flowering of WTR (GS 50–51), early in May and finished when SOR was in 70–71 GS, at the end of July. Samples were taken twice a week from WTR, WOR and weeds and once a week from SOR in mid-morning throughout this period. For each plot, 20 plants were selected at random. The beating method (Williams et al., 2003) was used. The raceme of each rape plant was beaten three times over a plastic tray (28 x 22 x 9 cm) to dislodge *Meligethes* spp. beetles and larvae from the buds and flowers. As weeds were relatively low, with short branches, the whole plant was shaken. All pollen beetles and larvae were placed into separate labelled containers. Identification to species (Kirk-Spriggs, 1996), counting and averaging was carried out in the laboratory. To exclude the possibility of larvae not falling from the weed flowers and our mistaken conclusion that no eggs had been laid there, in addition to the beating, every time 20 blossoms were taken from all the weeds and replications and observed under the microscope at the laboratory.

Statistical analysis. In the statistical analysis the program *Statistica 7.0* was used. The mean numbers and their standard deviations (SD) of *M. aeneus* and *M. viridescens* in different test plants on different dates were calculated. The significance in changes of individual numbers over the fixed periods was tested by *One-Way Anova* and Tukey's Post Hoc Test. The significant levels are set on $P < 0.05$.

The WTR flowered from early to mid-May. The two WOR crops flowered from mid-May to mid-June (Figure 1). SOR flowered from late June to mid-July. Compared with the crop plants the flowering times of the weed plants were extended, mostly throughout May, June, and July.

Results and discussion

Meligethes aeneus. When observations started, WTR had reached GS 50–51 (green buds) WOR GS 39–50 and *C. bursa-pastoris*, *T. arvense* and *E. cheiranthoides* were in flower (Figure 1).

M. aeneus was numerous on the WTR but the number was constantly decreased during the flowering time. Each subsequent counting showed that the number was statistically significantly lower than during the previous counting ($F_{3,12} = 166.03$, $p = 0.000$) (Figure 2 A).
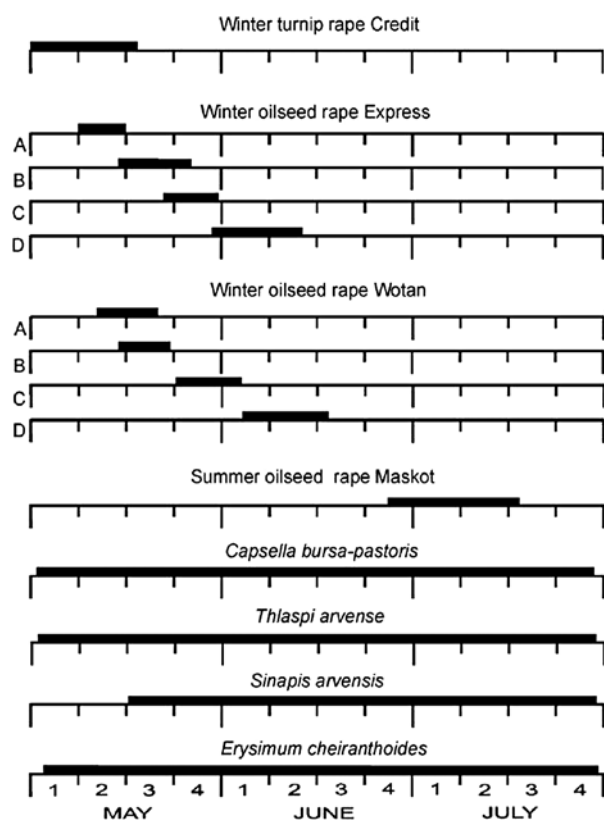
Note. A = primary raceme, B = secondary racemes, C = tertiary racemes, of oilseed rape, D = late flowering oilseed rapes on the edges of the experimental field.

Figure 1. Flowering times of cruciferous plant species observed

The first observation showed very few *M. aeneus* on the WOR cv. 'Express' (Figure 2 C) in bud. On cv. 'Mascot', which has a slower development compared with cv. 'Express', *M. aeneus* was not found at the first observation (Figure 2 B). As WOR started to flower and WTR was at the end of flowe-ring, the number of *M. aeneus* began to decrease on WTR and increase, to some extent, on WOR (see 16 May). While the main and side racemes were in flower, the abundance of *M. aeneus* changed on both cultivars a little. At the end of the flowering stage, they aggregated on the late-flowering plants and their numbers reached a high value, differing statistically significantly from the earlier number (Figure 2 B and C, 03 and 06 June). A figure representing the dynamics of the number of *M. aeneus* is rather similar in both the cultivars, however, on cv. 'Wotan' there were found slightly fewer beetles during each observation compared with the cultivar 'Express' (Figure 2 B and C). The number of *M. aeneus* increased significantly at the end of flowering on both WOR cultivars and changes in the numbers significantly depended on the time (cv. 'Express' – $F_{9,30} = 15.5$, $p = 0.000$ and cv. 'Wotan' – $F_{9,30} = 33.28$, $p = 0.000$). At the end
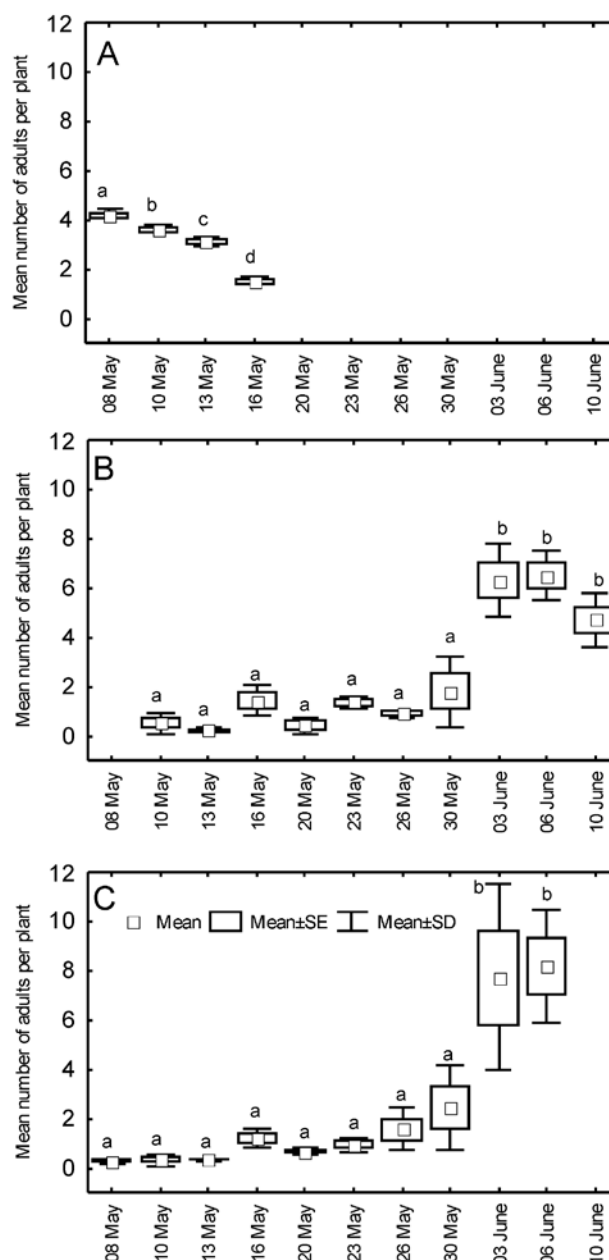

Note. Means followed by the same letter are not significantly different (Tukey HSD test, $p < 0.05$).

Figure 2. Mean number of adults *Meligethes aeneus* on winter turnip rape (*Brassica rapa*) cultivar 'Credit' (A), winter oilseed rape (*Brassica napus*) cultivars 'Wotan' (B) and 'Express' (C)

of May the larvae of *M. aeneus* were also found on the late-flowering plants of both WOR cultivars (03–10 June).

M. aeneus first appeared on SOR at GS 11–13 (1–3 leaves unfolded, 25 June) and hid in leaf axils. From end of June (GS 55 – individual flower buds visible but still closed, 27 June), the number of beetles increased, eggs and larvae were also found. Numbers of adults decreased from early July, and few individuals were found on plants in mid-July

(Figure 3). The changes in the abundance of *M. ae-neus* on spring oilseed rape cv. 'Mascot' significantly depended on the time ($F_{4,10} = 47.73$, $p = 0.000$).
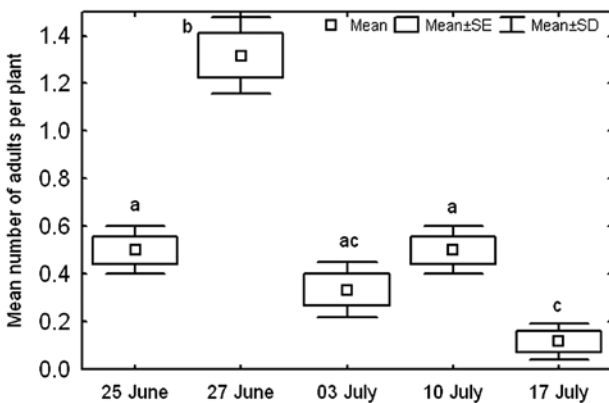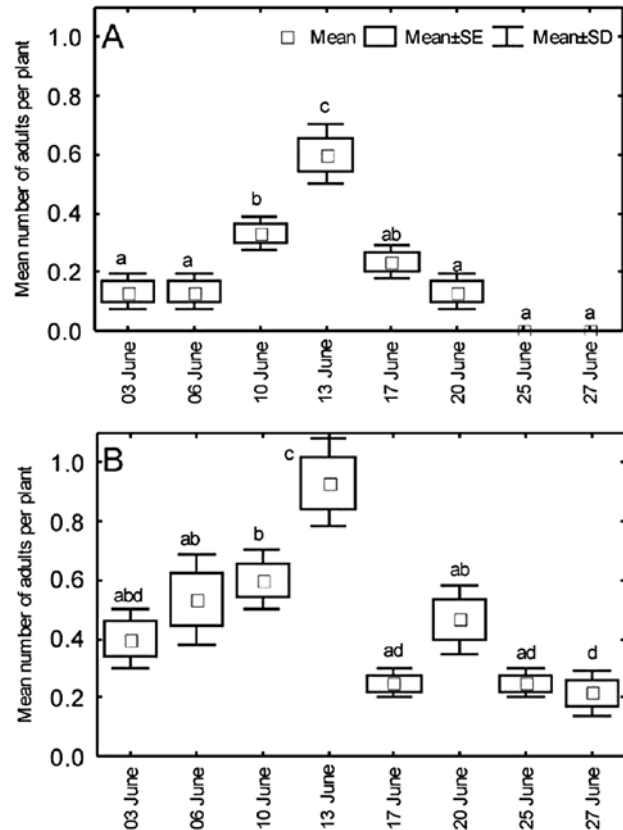

Figure 3. Mean number of adults *Meligethes ae-neus* on spring oilseed rape (*Brassica napus*)

On *C. bursa-pastoris*, *M. aeneus* appeared in early June (Figure 4 A). The number remained low throughout the observation period, increasing slightly in mid-June when late-flowering WOR plants finished flowering. After that the number decreased until by late June none remained. Changes in the numbers significantly depended on the time ($F_{7,16} =34.98$, $p = 0.000$). No *M. aeneus* larvae were found on *C. bursa-pastoris*.

Although *Sinapis arvensis* started flowering in mid-May, no *M. aeneus* were found on it until mid-June, and the numbers remained low (Figure 4 B). The increase in the number of beetles in mid-June on the *S. arvensis* occurred as late-flowering WOR plants at plot edges finished flowering. Thereafter, the numbers declined on the *S. arvensis* as SOR reached the flower bud stage. Change in the number of *M. aeneus* significantly depended on the time ($F_{7,16} = 15.09$, $p = 0.000$). A lot of larvae were found on *S. arvensis* at the end of June. No *M. ae-neus* were found on *T. arvense* or *E. cheiranthoides* throughout the study period.

Meligethes viridescens. From the beginning of May, a few *M. viridescens* appeared on WOR, but their number remained very low throughout the experimental period. The abundance of the species was, similarly to *M. aeneus*, lower, to some extent, on the cultivar 'Wotan' than on 'Express' (Figure 5 A and B). In the abundance of *M. viridescens* on both WOR cvs. there were significant differences between the observation days (cv. 'Wotan' $F_{8,16} = 24.89$, $p = 0.000$ and cv. 'Express' $F_{8,18} = 24.9$, $p = 0.000$).
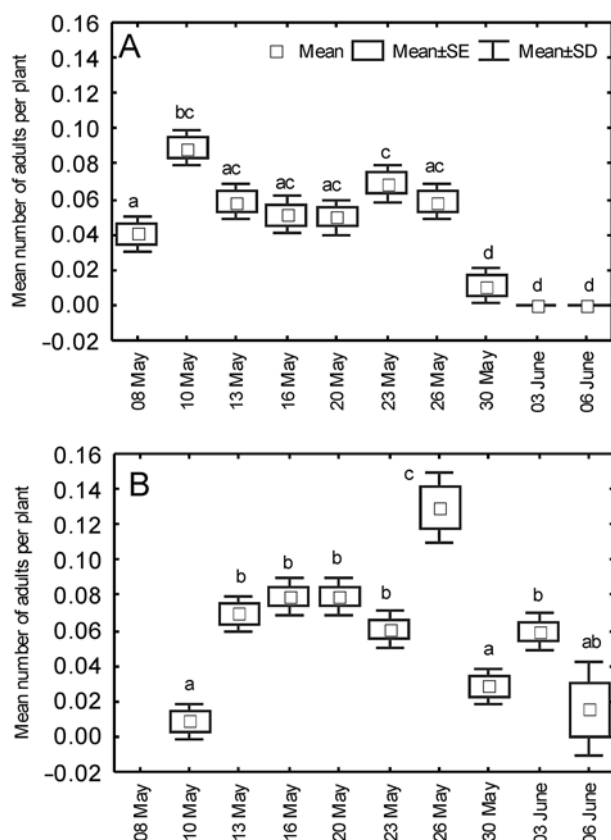

Note. Explanation under Figure 2.

Figure 4. Mean number of adults *Meligethes ae-neus* on *Capsella bursa-pastoris* (A) and *Sinapis arvensis* (B)

M. viridescens was most abundant on *S. ar-vensis* with a maximum average of more than four beetles per plant whereas larvae were also found. From the end of June, the number of beetles gradually decreased (Figure 6 A) and changes in the numbers significantly depended on the time ($F_{14,30} = 253.0$, $p = 0.000$).

On *E. cheiranthoides* (Figure 6 B), adults were present from early June, with a maximum in mid-June, followed by a decrease in late June, and changes in the numbers significantly depended on the time ($F_{6,14} = 32.66$, $p = 0.000$). No larvae were found on *E. cheiranthoides*. *M. viridescens* adults were not found on WTR, SOR, *C. bursa-pastoris* or *T. arvense*.

This study has shown that, the two most common pests of cruciferous crops, the pollen beetles *M. aeneus* and *M. viridescens*, differ markedly in their temporal distribution, abundance and utilisation of winter and summer rape crops and of four common and widespread cruciferous weeds of agriculture. *M. aeneus* appeared earlier in the year than *M. viridescens* being abundant throughout May, whereas *M. viridescens* did not become abundant until June. The phenology of *M. aeneus* appeared
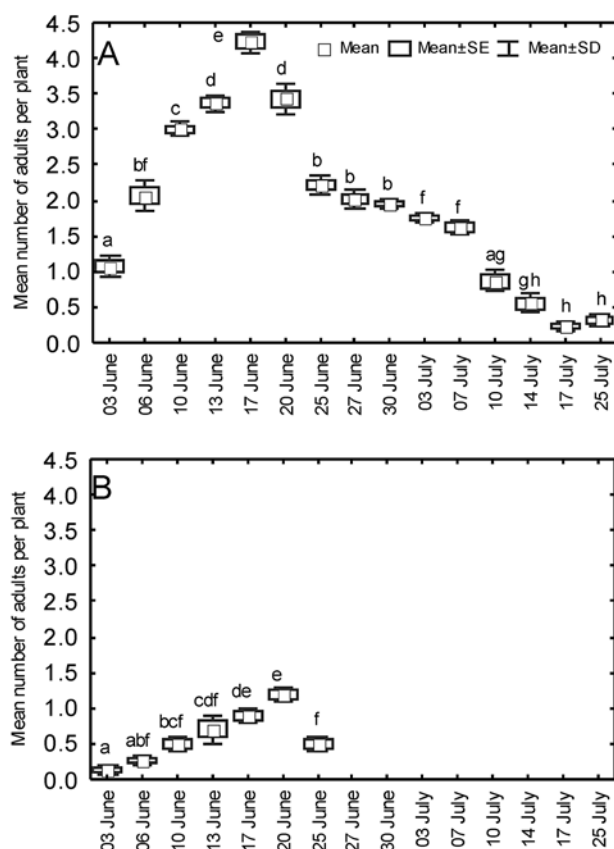
Note. Explanation under Figure 2.

Figure 5. Mean number of adults *Meligethes viridescens* on winter oilseed rape (*Brassica napus*) cultivars 'Wotan' (A) and 'Express' (B)

Note. Explanation under Figure 2.

Figure 6. Mean number of adults *Meligethes viridescens* on *Sinapis arvensis* (A) and *Erysimum cheiranthoides* (B)

better synchronised with the sequential flowering phenology of susceptible cruciferous crops studied than that of *M. viridescens*. Its early emergence from hibernation at a time when WTR, but few other plants, were in flower in the agricultural landscape, encouraged it to infest WTR in large numbers for maturation feeding on its pollen.

This is reported to take up to 3 weeks in the UK (Free, Williams, 1979) or 10–14 days at 15°C (Nilsson, 1988). It then moved to WOR where buds of a suitable size for oviposition, (2–3 mm; Borg, 1996) were already available. Here their numbers were lower than on WTR. The considerable increase in the number of *M. aeneus* and the emergence of its larvae in the samples in later flowering WOR plants indicates that the occurrence of such plants in a rape field provides the pest with the possibility to reproduce also on winter oilseed rape in the northern regions.

In late June, pollen beetles moved to SOR for oviposition. *M. viridescens* (adults and larvae) were found only on WOR and in low numbers. The synchrony between *M. aeneus* and WTR was probably enhanced in trial period by the exceptionally

warm spring temperatures, stimulating both beetle activity and plant growth. In late April, days above 12°C allowed beetle emergence from over wintering sites. In early May, most days were above the flight threshold temperature of *M. aeneus* (15°C). However, the warm spring weather also shortened the normal flowering period of WTR, thereby perhaps preventing oviposition/larval development by *M. aeneus* on this crop as suitable buds for oviposition were no longer available. The earlier development and infestation by pollen beetles of turnip rape compared to oilseed rape, whether autumn or springsown, has encouraged its use in trap crop systems to protect oilseed rape from infestation by insect pests (Hokkanen et al., 1986; Cook et al., 2007).

Both *Meligethes* species used *S. arvensis* for both feeding and oviposition. This conforms to Free and Williams (1978), who found *M. aeneus* larvae in the buds of *S. arvensis* growing amongst WOR in the UK, and Kirk-Spriggs (1996) who reported that Easton (unpublished) had reared both *M. aeneus* and *M. viridescens* from *S. arvensis*. However, in our study, *M. viridescens* was relatively more abundant on *S. arvensis* than *M. aeneus*. This suggests

that *M. viridescens* has a strong preference for this cruciferous weed over late-flowering WOR which was also in flower in mid-June, whereas the opposite was true for *M. aeneus*.

Further differences in host plant preference between the two species were evident with regard to their incidence on *C. bursa-pastoris* and *E. cheiranthoides*. *M. aeneus* used only the former and *M. viridescens* only the latter for feeding. Neither beetle species used either weed species for oviposition.

These differences in potential host plant resource utilisation for feeding and oviposition by the two species of *Meligethes* may partly result from their different responses to differences in host plant chemistry. Chemical cues of particular importance are various glucosinolates but other stimulants and deterrents are also known from the *Brassicaceae*. Of the different glucosinolates identified in oilseed rape, the most important are progoitrin, gluconapin and glucobrassicanapin (Etienne, Dourmad, 1994). They are found in all plant parts, but their quantities may vary considerably in different organs. At its initial growth stages, turnip rape has a high content of gluconapin and glucobrassicanapin, but later these decrease in the vegetative organs and are transferred into the generative organs. The content of gluconasturtin in turnip rape is relatively high in comparison to that in other *Brassicaceae* species. It is possible that the glucosinolates in WTR are phagostimulants for adult *M. aeneus* but deter oviposition.

Many studies have shown that olfactory and visual cues stimulate attraction and landing responses of *M. aeneus*. For example, yellow water traps baited with extracts of *B. napus* in methanol, or with isothiocyanates have been shown to attract *M. aeneus* (Free, Williams, 1978; Blight, Smart, 1999). However, in this study, beetles arrived at the field at a very early stage of SOR development before buds or flowers were present. Nilsson (1988) reported similar observations in Sweden. This suggests that at this stage the beetles were using olfactory rather than colour (yellow) cues to find their hosts (Ruther, Thiemann, 1997).

The responses of cruciferous pests in relation to cruciferous weed chemistry have been less well studied. Free and Williams (1978) found *M. aeneus* were attracted to yellow water traps bai-ted with extracts of *S. arvensis*. The attractiveness of *S. arvensis* for feeding and oviposition for both species may have been a response to its high glucosinolate content, particularly of sinapin and glucosinalbin (Booth et al., 1999). *S. arvensis* has also been shown to be a suitable food plant for both

adult and larval *P. chrysocephala*, an important winter pest of rape in Europe (Bartlet, Williams, 1991). *E. cheiranthoides* contains non-sulphur secondary metabolites such as cardenolides (Sachdev-Gupta et al., 1990) and cucurbitacin (Huang et al., 1993) which inhibit feeding in flea beetles (*Phyllotreta* spp.) and oviposition in cabbage butterflies (*P. rapae* and *P. brassicae*) (Renwick, 2001). The substances, including cucurbitacin B, have been used for the control of several plant pests (Renwick, 1996). *C. bursa-pastoris* contains the glucosinolates sinigrin and gluconapin. Sinigrin increases egg production in *Delia brassicae* Bouche (Francis et al., 2001). *C. bursa-pastoris* also contains alkaloids and saponins and deters feeding in *Phyllotreta* spp. (Nielsen, 1989) and *P. chrysocephala* L. (Bartlet, Williams, 1991). *Thlaspi arvense* is the host plant for *Pieris bryoniae* Hübner, *Pontia daplidice* L. and others but the reasons for its unsuitability for *M. aeneus* or *M. viridescens* species are not clear. We agree with Bartlet et al. (1999) that further work on the responses of pollen beetles to host plant chemistry is needed to enable us to understand more fully the chemical basis for their host plant selection and the mechanisms for the resistance of plants to their feeding and oviposition.

In conclusion, this study suggests that in Estonia, like in the UK (Free, Williams, 1978) and no doubt elsewhere in Europe, common and widespread cruciferous agricultural weeds provide important feeding and oviposition resources for *M. aeneus* and *M. viridescens*, enhancing their potential as pests of cruciferous crops.

Acknowledgements

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Meligethes aeneus (F.) ir *M. viridescens* (F.) paplitimas ant kryžmažiedžių augalų

L. Metspalu[1], I. H. Williams[2], K. Jõgar[1], A. Ploomi[1], K. Hiiesaar[1], P. Lääniste[1], E. Švilponis[1], M. Mänd[1], A. Luik[1]

[1]Estijos gyvybės mokslų universiteto Žemės ūkio ir aplinkos mokslų institutas
[2]Rotamstedo tyrimų centro Augalų ir bestuburių ekologijos skyrius

Santrauka

Tirta rapsinių žiedinukų *Meligethes aeneus* ir *M. viridescens* (Coleoptera, Nitidulidae) paplitimas ant *Brassica rapa, B. napus, Erysimum cheiranthoides, Capsella bursa-pastoris, Thlaspi arvense* bei *Sinapis arvensis* augalų. Nustatyta, kad *M. aeneus* pavasarį pirmiausia kolonizavo žieminį rapsiuką (*B. rapa*), tačiau ant šios rūšies augalų *M. aeneus* kiaušinėlių neaptikta. *M. viridescens* pasirodė keletą savaičių vėliau ant žieminio rapso (*B. napus*), tačiau jų buvo mažiau nei *M. aeneus* individų. Ant vasarinio rapso (*B. napus*) *M. aeneus* suaugėliai buvo nustatyti prieš pumpurų pasirodymą, o *M. viridescens* individų nebuvo aptikta. Ant *E. cheiranthoides* augalų nustatyti tik *M. viridescens*, o ant *C. bursa-pastoris* – tik *M. aeneus* suaugėliai vabalai, bet ne jų lervos. Garstukas (*S. arvensis*) buvo patrauklesnis *M. viridescens* nei M. aeneus, ant šios rūšies augalų *M. viridescens* maitinosi ir dėjo kiaušinėlius. Taigi, kai kurie laukiniai augalai (piktžolės) yra svarbus rapsinių žiedinukų *M. aeneus* ir *M. viridescens* mitybos ir dauginimosi šaltinis.

Reikšminiai žodžiai: *Meligethes aeneus*, *M. viridescens*, paplitimas, gausumas, aliejiniai augalai, kryžmažiedės piktžolės.