Differences in colour preference among pollen beetle species (Coleoptera: Nitidulidae)

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Differences in colour preference among pollen beetle species (Coleoptera: Nitidulidae)

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Abstract

Pollen beetles (Coleoptera: Nitidulidae) are major pests of oilseed rape and other crucifers. Efficient and timely management of these pests can greatly be improved by effective monitoring of their spatial and temporal distribution. In field trials in Hungary, we have discovered striking differences in colour responsiveness among pollen beetle species: *Brassicogethes aeneus* F. 1775 (earlier *Meligethes aeneus*) and *B. viridescens* F. 1775 responded most strongly to fluorescent yellow traps, whereas *B. coracinus* Sturm 1845, *Fabogethes nigrescens* Sturm 1845 and *Meligethes atratus* Olivier 1790 were most attracted to blue or white traps. Differences in the spring flight period were also recorded, with *B. aeneus* and *B. viridescens* flying ca. 1 month earlier than the other three species. Further tests established that funnel traps having both fluorescent yellow and blue colour cues are the most efficient in attracting a wide range of pollen beetle species. On the other hand, fluorescent yellow traps can be used to detect and monitor *B. aeneus* specifically.

KEYWORDS
attract, *Brassicogethes*, colour, *Meligethes*, monitoring, trap

1 | INTRODUCTION

Pollen beetles (Coleoptera: Nitidulidae) are flower-visiting insects, with certain species causing damage to economically important crops via adult feeding on the pollen of unopened flower buds. This can lead to bud abscission and blind stalks, thereby preventing the growth of pods and leading to considerable seed yield loss (Seimandi-Corda et al., 2021). Females lay their eggs in flower buds, where the larvae feed on pollen. According to the genus-level taxonomic revision of the *Meligethinae* subfamily, where pest species occur, the former species complex of genera has been changed (Audisio et al., 2009). In this study, species names are used in accordance with these changes. *Brassicogethes aeneus* F. 1775 (earlier *Meligethes aeneus*) is a Holarctic species and a major pest of oilseed rape (*Brassica napus* L.) and other crucifers (Brassicaceae) in Europe (Sáringer, 1990; Audisio, 1993; Alford et al., 2003; Ekbom & Borg, 2011). The damage is especially significant in case of late flowering, when large migrations into the crop and egg laying happen before bud opening (Keszthelyi, 2016; Williams, 2010). Control of *B. aeneus* is currently achieved by insecticides (Mauchline et al., 2018). *B. viridescens* F. 1787 is also a pest of oilseed rape and *Brassica rapa* L. in Europe (Nolte & Fritzsche, 1952; Scherney, 1953; Albertini et al., 1988; Finch et al., 1990; Winfield, 1992; Zuranska et al., 1998; Hiiesaar et al., 2003; Marczali & Keszthelyi, 2003) and is now established in eastern North America, posing a risk to canola growing in this region (Mason et al., 2003). *B. aeneus* and *B. viridescens* can cause up to 70% yield losses in winter and spring oilseed rape in Europe (Nilsson, 1987). Furthermore, *Brassicogethes coracinus* Sturm 1845 is considered as an oilseed rape pest (Marczali & Keszthelyi, 2003; Nolte & Fritzsche, 1952; Scherney, 1953; Zuranska et al., 1998).

Chemical control of pollen beetles is only effective and environmentally more friendly if it is timed to their mass occurrence (Mauchline et al., 2018; Sáringer, 1990). Regrettably, because of the overuse of pyrethroid insecticides, resistant beetles have already appeared, spread and for now dominate in the main oilseed rape-growing areas of Europe. The spread of resistant pollen beetles highlights the need for more effective management strategies for oilseed rape pests (Slater et al., 2011). To locate their host plants, the beetles are attracted to the yellow colour of the flowers (Giamoustaris & Mithen, 1996) and to plant-derived volatiles, including isothiocyanates (Blight & Smart, 1999; Cook Bartlet, Murray & Williams, 2002; Cook et al., 2007). Detection, forecast and monitoring of the occurrence of pollen beetles are generally done by yellow water pan traps (Wasman, 1926; Möricke, 1953; Nolte, 1959; Görnitz, 1956; Fritzsche, 1957) or sticky yellow chromotropic traps (Büchs, 1993; Buechi, 1990; Ekbom & Borg, 1993; Mauchline et al., 2018; Skellern et al., 2017).

During the course of a spring field trapping trial, however, it was surprising to record sizeable numbers of pollen beetles in blue funnel traps (M. Tóth unpublished), which were originally designed for catching *Tropinota* (*Epicometis*) *hirta* Poda (Coleoptera: Scarabaeidae) (Tóth et al., 2004,2009). A detailed literature search on colour responses of pollen beetles revealed that besides the different hues of yellow, responses to white, blue or even green colours have also been reported (Table 1). As the listed studies reported on the colour preference of either *B. aeneus*, or *B. aeneus*/*B. viridescens*, or pollen beetles in general, this suggested that there may in fact be species-specific differences in the colour response of pollen beetles. This aspect of pollen beetle behavioural ecology, to our knowledge, was not studied previously, and thus the objective of the experiments presented here was to assess the field responses of pollen beetles to different colours with known reflectance spectra.

2 | MATERIALS AND METHODS

2.1 | Experimental design

Three trapping experiments focussed on pollen beetles were conducted in autumn-sown commercial oilseed rape fields, embedded in agricultural landscapes, in Hungary, using generally accepted methods (Roelofs & Cardé, 1977). At each site, traps were arranged in randomized blocks, with one trap of each treatment (=colour) in each block. Traps within blocks were separated by 8–10 m, and blocks were sited at least 30 m apart. Four blocks of traps were operated at each test site. Traps were inspected twice weekly, when captured insects were removed and taken into the laboratory for species identification, using the following morphological characters: (i) body length and shape, (ii) colour of body, legs and antennae, (iii) dorsal pubescence, (iv) clypeal margin, (v) shape of the elytra and scutellum, (vi) punctures on the body surface, (vii) number of teeth on the posterior margin of the forelegs, (viii) shape of the median lobe of male genitalia and (ix) shape, size and pigmentation of the ovipositor (Audisio, 1980).

Field tests deployed CSALOMON® VARb3 funnel traps (obtained from Plant Protection Institute, CAR ELKH, Budapest, Hungary), which have successfully been used for trapping beetle species (e.g. Imrei et al., 2001; Tóth et al., 2004). It is worth noting that coloured sticky sheets usually capture many non-target species, and the sticky material makes the determination of pollen beetle species difficult or even impossible. Water pan traps are also difficult to operate, as either the water is blown away in strong spring winds or is frozen over at this time of year in Hungary. The funnel traps, on the other hand, have the advantage over sticky sheets that they do not get saturated with captured insects and thus preserve their capture capacity for significantly longer, without losing sensitivity. A small piece (1 × 1 cm) of a household anti-moth insecticide strip (Chemotox® SaraLee, Temana Intl. Ltd, Slouth, UK; active ingredient 15% dichlorvos) was placed into the trap catch container to kill captured insects.

The inside surface of the upper panel parts (made of transparent plastic sheets) of the VARb3 traps was spray painted to different colours by PÁ-ME Bt. (Tamási, Hungary) using paints from Sericol Kft. (Budapest, Hungary) (painted surface: 19 × 32 cm). The following colours were compared: transparent (for control), white, blue, yellow and fluorescent yellow (Figure 1); this choice of colours was partially informed by previous studies (see Table 1) and influenced by their attractiveness to various insect species (e.g. Blaisinger, 1975; Rőth et al., 2016; Schmera et al., 2004). Reflectance spectra of the colours tested have been published before (Rőth et al., 2016; Schmera et al., 2004; Supplementary material). The traps were used as chromotropic traps, with no chemical lure added.

TABLE 1 Colours reported to be attractive (+) in the field for pollen beetles

Yellow	White	Green	Blue	Reference
+	–	–	–	Wasmann, 1926
+	+	–	–	Fritzsche, 1957
+	–	–	–	Nolte, 1959
+	+	–	–	Goos et al., 1976
+	–	–	–	Láska et al., 1986
+	–	+	–	Buechi, 1990
+	–	–	–	Finch, 1991
+	+	–	–	Košťál, 1992
+	–	–	+	Ekbom & Borg, 1993
+	+	–	–	Blight & Smart, 1999
+	–	–	–	Döring et al., 2012

2.2 | Experimental details

Experiment 1. The objective of this preliminary test was to compare the chromotropic response of pollen beetles to five treatment colours (white, blue, yellow and fluorescent yellow, plus transparent traps as control, Figure 1). Captured specimens were not identified to species. The experiment was run at one site in Komádi, Hajdú-Bihar county, Hungary (47.004168, 21.483931), from 6 April to 8 June 2004 (oilseed rape BBCH scale 32–83, Enz & Dachler, 1997).

FIGURE 1 CSALOMON® VARb3 funnel traps (Plant Protection Institute, CAR ELKH, Budapest, Hungary) used in field trials. The upper panels of the traps were (from left to right) yellow, fluorescent yellow, white, transparent and blue

FIGURE 2 CSALOMON® VARb3 funnel trap used in Experiment 3. One half of the upper panel of these traps was fluorescent yellow and the other half blue

Experiment 2. The objective of this test was to confirm preliminary results of Exp. 1. Pollen beetle specimens were separated to species (Audisio, 1980). The experiment was conducted simultaneously at two sites in Hungary: (1) Komádi, Hajdú-Bihar county, 1 April to 27 July 2005; and (2) Csárdaszállás, Békés county (46.863665, 20.937210), 31 March to 15 July 2005 (oilseed rape BBCH scale 32–97, Enz & Dachler, 1997).

Experiment 3. The test was aimed at studying whether the joint presence of the fluorescent yellow and blue colours (found most attractive in previous tests) influences the composition of pollen beetle species in the catch, that is, whether a multi-coloured trap can be used for the monitoring of all species showing sensitivity to its component colours. A 9.5 × 16 cm surface on each side of the upper panel was painted in fluorescent yellow and adjacent surface of the same size on each side in blue (Figure 2). The other two treatments were fluorescent yellow and blue traps. Beetles caught were separated to species (Audisio, 1980). The experiment was run simultaneously at two sites in Hungary: (1) Nadap, Fejér county (47.258195, 18.617044), 9 March to– 22 May 2007, and (2) Túrkeve, Jász-Nagykun-Szolnok county (47.103507, 20.740718), 31 March to 1 July 2007 (oilseed rape BBCH scale 32–97, Enz & Dachler, 1997).

2.3 | Statistical analysis

As it is frequently found in field trapping experiments, catch data (even after transformation) did not always fulfil requirements for a parametric analysis. Therefore, unless otherwise stated, pooled catch data over the sampling period for each trap were analysed by the non-parametric Kruskal–Wallis test. When the Kruskal–Wallis test showed significance, differences between treatments were analysed by pairwise comparisons with Mann–Whitney *U* test ($p = 0.05$).

All statistical procedures were conducted using the software packages StatView® v4.01 and SuperANOVA® v1.11 (Abacus Concepts, Inc.,).

3 | RESULTS

In the preliminary Experiment 1, a large number of pollen beetles (not determined to species) were captured. We noted marked differences between the first and second half of the test period in the catches of traps painted in different colours (Figure 3a). In the first half of the test period (4 April to– 5 May, Figure 3b), mean catches of blue and white traps did not differ from those of control traps, whereas yellow and fluorescent yellow traps caught more pollen beetles than control traps. The highest catches (differing from all other treatments) were recorded in fluorescent yellow traps, whereas catches of white, blue and yellow traps did not differ from each other.

In the second half of the test period (8 May to 6 June, Figure 3c), white and blue traps caught significantly more pollen beetles than all other treatments (not differing from each other), whereas yellow and fluorescent yellow traps did not catch more than control traps.

At both sites in Experiment 2, fluorescent yellow traps caught significantly more *B. aeneus* than all other treatments, although traps painted in the other three colours also caught more than control traps (Figure 4). Most beetles were caught in the first half of April (Komádi; Figure 5) and in the end of April and early May (Csárdaszállás).

Brassicogethes viridescens catches showed different distribution patterns between the two sites. At Komádi, similarly high catches were recorded in fluorescent yellow, white and blue traps, and catches of yellow traps did not differ from the transparent control (Figure 4).

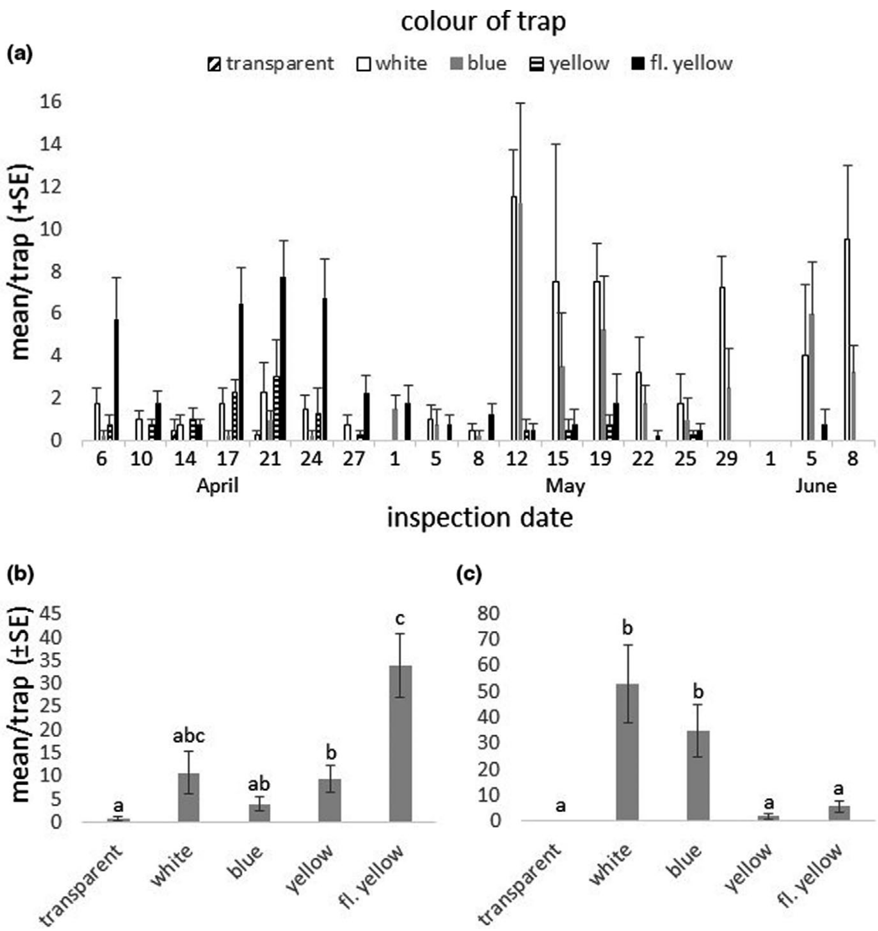

FIGURE 3 Mean catches of pollen beetles in white, blue, yellow, fluorescent yellow and transparent traps during two consecutive periods of Experiment 1 at the Komádi site. A = seasonal distribution of catches (total caught 616 beetles); B = mean catches between April 4 and May 5 (total caught 235 beetles) and C = mean catches between May 8 and June 6 (total caught 381 beetles). Columns with same letter are not significantly different within one diagram by Kruskal–Wallis followed by Mann–Whitney *U* test ($p < 5\%$)

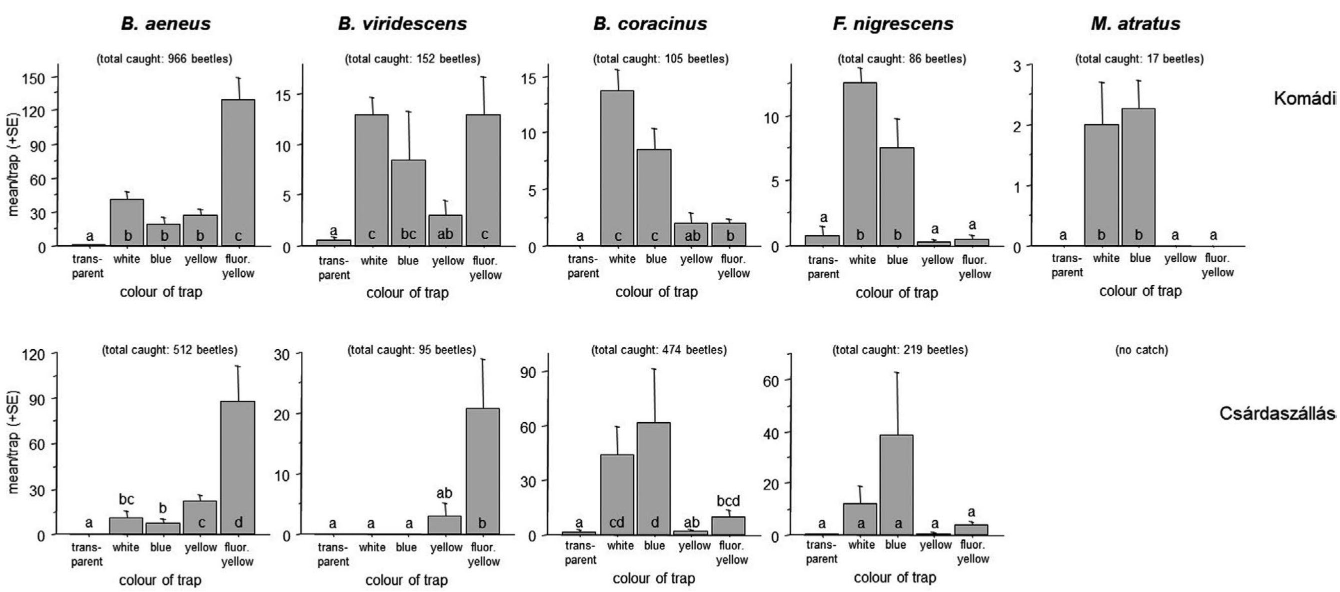

FIGURE 4 Mean catches of pollen beetle species in white, blue, yellow, fluorescent yellow and transparent traps in Experiment 2 at the Komádi and Csárdaszállás sites. For significance, refer to Figure 2

However, at Csárdaszállás, only fluorescent yellow traps caught significantly more than all other treatments; catches in yellow traps were only numerically higher than in other treatments (Figure 4). Most beetles were captured in early April, with a second peak in June (Komádi; Figure 5) and in the end of April and early May (Csárdaszállás).

Brassicogethes coracinus catches showed similar general tendencies at both sites (Figure 4), with significantly higher catches in white and blue traps than in those with the other colours. Yellow traps did not catch more than control traps. Most beetles were recorded throughout May (Komádi; Figure 5) and in the second half of May (Csárdaszállás).

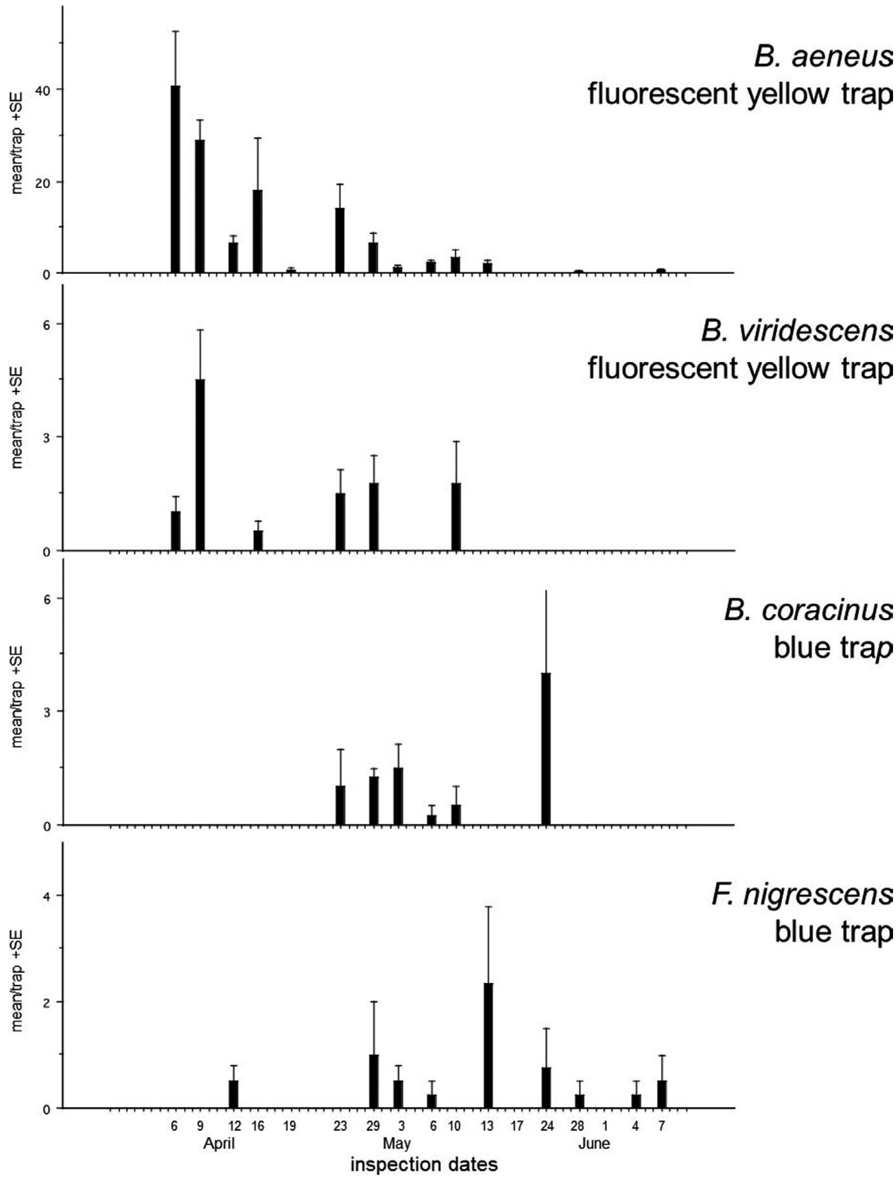

FIGURE 5 Seasonal flight patterns of pollen beetles at the Komádi site (Experiment 2) in fluorescent yellow traps (*B. aeneus*, total caught 514 beetles; *B. viridescens*, total caught 52 beetles) and blue traps (*B. coracinus*, total caught 34 beetles; *F. nigrescens*, total caught 30 beetles)

Catches of *Fabogethes nigrescens* Sturm 1845 showed similar tendencies at both test sites (Figure 4), with higher catches in white or blue traps than in other traps; however, this difference was only significant at the Komádi site. Most beetles were recorded in the middle of May at both sites (Komádi; Figure 5).

Finally, *Meligethes atratus* Olivier 1790 was caught in low numbers only at Komádi. Catches in blue and white traps were significantly higher than by any other colours (Figure 4). Beetles were caught at the end of April and in May.

In Experiment 3, most pollen beetles (all species together) were recorded in traps having both blue and fluorescent yellow surfaces (Figure 6) as compared to the single colours, although the difference between fluorescent yellow and fluorescent yellow + blue traps was not significant at the Nadap site.

Of the single species, more *B. aeneus* was caught by fluorescent yellow + blue traps compared to blue traps (Figure 6). Catches in fluorescent yellow + blue traps did not differ significantly from those in fluorescent yellow traps at either site.

As for *B. viridescens*, catches in fluorescent yellow + blue traps were higher than in traps with single colours (Figure 6, Nadap). At the Túrkeve site, traps painted fluorescent yellow (alone or in combination with blue) caught numerically more *B. viridescens* than blue traps, but the differences were not significant.

B. coracinus catches in blue or fluorescent yellow + blue traps were higher than in traps with fluorescent yellow colour only (Figure 6), which difference was significant only at Nadap.

Similarly, more *F. nigrescens* were caught in blue traps (with or without the fluorescent yellow colour) than in traps painted in fluorescent yellow only (Figure 6), but the difference was significant only at Nadap.

4 | DISCUSSION

We hypothesized that the surprising difference in colour responses of pollen beetles between the first and second half of the preliminary Experiment 1 trial period might be due to the differing colour

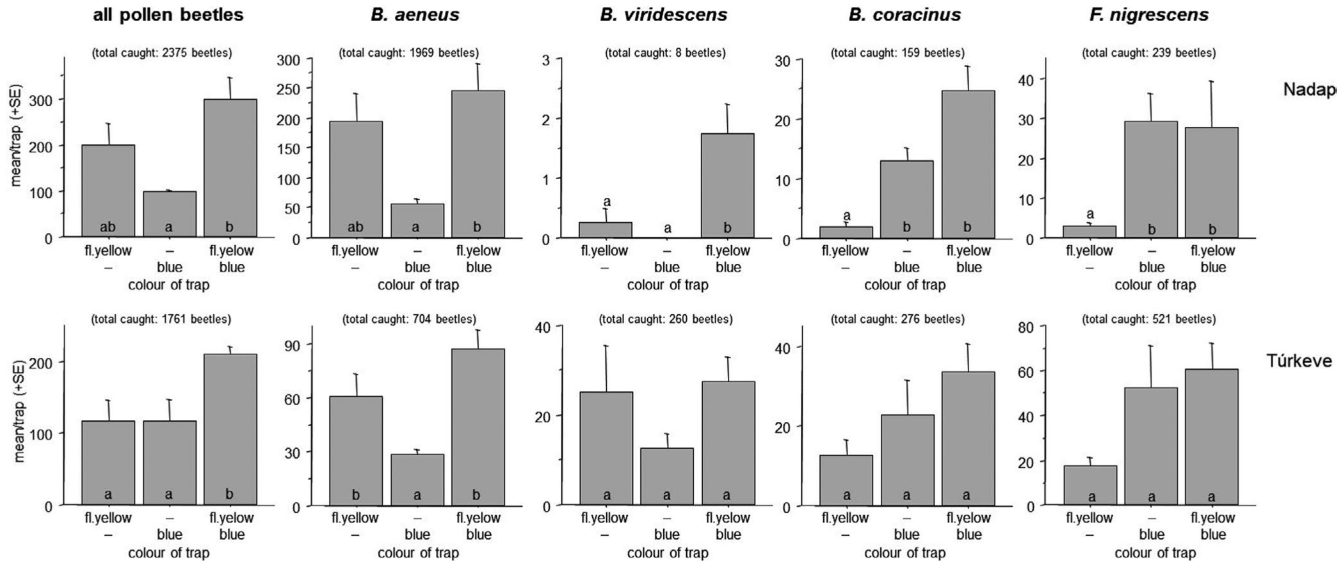

FIGURE 6 Mean catches of pollen beetle species in traps painted fluorescent yellow or blue, or having both colours together, in Experiment 3, at the Nadap and Túrkeve sites. For significance, refer to Figure 2

preferences of a range of species active in distinct parts of the flight season. This assumption was supported by subsequent tests focussing on species-specific patterns in colour preference: *B. aeneus* and *B. viridescens* were most attracted to fluorescent yellow, whereas *B. coracinus*, *F. nigrescens* and *M. atratus* preferred blue and white traps.

There is a relatively little body of knowledge available on the species composition of pollen beetle assemblages because collected individuals are either simply not determined to species or are automatically assumed to be *B. aeneus*. However, Karltorp and Nilsson (1981) found the number of *B. viridescens* to be 10% of the total number of pollen beetles collected on oilseed rape, whereas according to Nolte and Fritzsche (1952) and Fritzsche (1957), the number of *M. viridescens* may exceed the number of *M. aeneus* towards the end of the vegetation period. Based on observations of pollen beetle swarming phenology over 4 years (Marczali & Keszthelyi, 2003), *B. aeneus* was the dominant species, the proportion of all identified adults varying between 66% and 80%. The ratio of other species increased during the vegetation period but remained below that of *B. aeneus*, which was the most common (occurred in 100% of all samples), followed by *B. coracinus* (78%), *B. viridescens* (50%), *F. nigrescens* (29%) and *M. atratus* (21%). Finally, the most frequent companion species of *B. aeneus* in oilseed rape, white mustard and poppy fields in the Czech Republic was found to be *B. viridescens*, followed by *Clypeogethes subaeneus* Sturm 1845, *B. coracinus* and *Meligethes carinulatus* Förster 1849 (Tóth et al., 2013). According to Liu et al. (2021), an evolutionary shift in the host associations of pollen beetles from the Rosaceae to the Brassicaceae has taken place. *M. atratus* larvae develop in flowers of the Rosaceae, in particular *Rosa* and *Rubus*, whereas those of *F. nigrescens* develop in Fabaceae flowers, such as *Trifolium*, *Onobrychis*, *Ononis* and *Lotus* (Audisio et al., 2009). On the other hand, *B. aeneus* and *B. viridescens* are strictly associated for larval development with species in the Brassicaceae (Audisio et al., 2009; Metspalu et al., 2011).

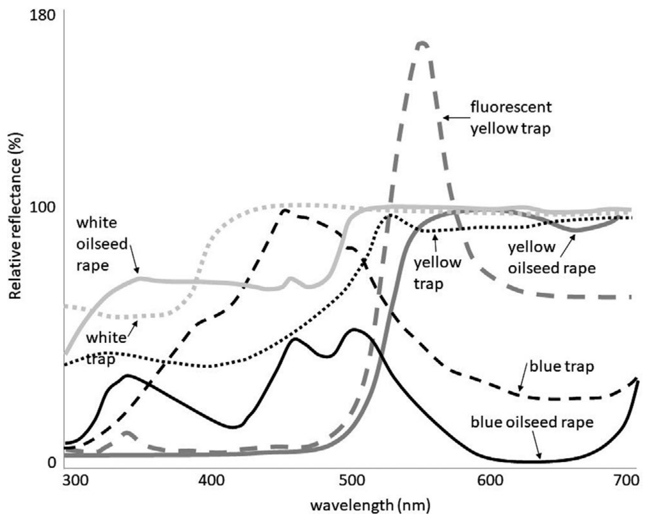

FIGURE 7 Reflectance spectra of yellow, white and blue oilseed rape petals, and VARb3 funnel traps with upper panels painted in fluorescent yellow, yellow, white and blue. Spectra were redrawn based on Cook et al. (2013), Rőth et al. (2016) and unpublished data (see supplementary material)

The reasons for the observed preferences among the range of colours tested in closely related pollen beetle species are unclear. It could be that the different species are sensitive to different regions of the oilseed rape petal colour reflectance spectrum. The visual system of the brassica specialist *B. aeneus* is tuned to perceiving the yellow petal colouration of their host plants for host location (Döring et al., 2012). In fact, both oilseed rape flowers and fluorescent yellow traps exhibit a high reflectance above 530 nm, but the petals reflect more intensely in the orange and red regions (Figure 7). Interestingly, the mean spectral sensitivity curve of *B. aeneus*, as defined by the electroretinogram (ERG) technique, peaks at 540 nm (green receptor; Döring et al., 2012), coinciding with the reflectance maximum of fluorescent yellow traps, which indicates that these traps provide an optimal visual stimulus for *B. aeneus* and perhaps also for *B. viridescens*. In addition, it is more likely that their landing behaviour is coupled to a neural mechanism called colour-opponent mechanism, with antagonistic input from the green versus a short-wavelength (blue or UV) photoreceptor. In fact, the role of this process in colour choice of *B. aeneus* is suggested by Döring et al. (2012), with positive input from the green receptor and negative input from the blue receptor. According to Chittka (1996), optimal colour opponent systems are all those which comprise of two opponent processes with weighting factors differing strongly from one another; the green–blue photoreceptor opposition was also shown to be the case in aphid colour preference (Döring et al., 2009). It can be suggested that the yellow colour of the traps will have excited the green receptor but not the antagonistic blue receptor of these beetle species and that therefore, based on a colour opponent mechanism, the behavioural response to yellow was strong. The same mechanism would then lead to a preference for yellow flowers.

The preference of *B. coracinus* for white traps may be explained by the relatively high reflectance (60%–70%) of this colour in the ultraviolet (UV) region (i.e. in the 300–400 nm range; Figure 7). As with all studied insects (Briscoe & Chittka, 2001), *B. aeneus* possesses UV receptors and is attracted to objects with high UV reflection (Cook et al., 2013; Döring et al., 2012) based on which it can be supposed that *B. coracinus* is also UV sensitive, hence its preference for white traps. Indeed, UV sensitivity may also provide an alternative explanation for the attraction of *B. coracinus*, *F. nigrescens* and *M. atratus* to white, as well as blue, traps, the latter of which also exhibit a UV component (up to 50%) and which might be sufficient to evoke beetle behavioural activity. More speculatively, oilseed rape flowers dyed blue (Cook et al., 2013) reflect in similar regions as blue traps (but less intensely; Figure 7), which perhaps explains the preference of *F. nigrescens* and *M. atratus* for blue traps. [Here, the example of artificial blue oilseed rape petals (Cook et al., 2013) is used only as an approximation for the reflectance of similarly coloured flowers.] ERG spectral sensitivity curve measurements will also prove invaluable in shedding more light on colour sensitivity of these two species.

Besides catching *B. aeneus* and *B. viridescens* in this study, fluorescent yellow VARb3 funnel traps are suitable for population monitoring of *Plagionotus floralis* Pallas and *Pseudovadonia livida* F. (Coleoptera: Cerambycidae) (Toshova, Anatashova, Tóth & Subchev, 2010; Toshova et al., 2016), and the rose chafers *Oxythyrea funesta* Poda and *O. cinctella* Schaum (Coleoptera: Scarabaeidae) (Vuts et al., 2008,2012). It can be speculated that these species also bear photoreceptors sensitive at the 540 nm region, hence their strong attraction to fluorescent yellow traps. VARb3 blue traps efficiently catch the scarabs *E. hirta* and *Tropinota squalida* Scop. (Coleoptera: Scarabaeidae) due to the strong attraction of these species to the hue of blue that the upper panel of this trap was painted (Schmera et al., 2004; Tóth et al., 2009). They might have photoreceptors sensitive in the 450–480 nm (blue) region of the electromagnetic spectrum, together with UV-sensitive receptors, as suggested for *B. coracinus*, *F. nigrescens* and *M. atratus*. VARb3 blue traps are also attractive for *Cetonia a. aurata* L. and *Potosia cuprea* Scop. (Coleoptera: Scarabaeidae), but here the visual cue needs to be complemented with an olfactory attractant for maximum catches (Lohonyai et al., 2018; Vuts et al., 2010). This indicates interactions between these modalities during signal processing in the central nervous system as opposed to, for example, *E. hirta*, where the dominance of visual stimuli on behavioural outputs related to attraction is observed (Schmera et al., 2004). Studies by Blight and Smart (1999) suggest that interactions between visual and olfactory stimuli may occur in *B. aeneus* in an additive manner, where attraction to yellow sticky traps was enhanced by 1.7–3.3 times by the addition of a blend of isothiocyanates. It may be possible to find a chemical attractant to be used in fluorescent yellow VARb3 traps for pollen beetle monitoring, with the promise to optimize a blend of compounds that synergizes the effect of the colour (Jönsson et al., 2007; Thöming et al., 2020; Tóth et al., 2015).

From a practical point of view, if the aim is to detect and monitor *B. aeneus*, that is, the most abundant oilseed rape pest pollen beetle, fluorescent yellow funnel traps can be used, whereas if the aim is to catch multiple species, traps with a fluorescent yellow–blue colour combination perform better. The latter colour stimulus could also be used for biodiversity monitoring, similar to, for example, Ikemoto, Kuramitsu, Sueyoshi, Seguchi & Yokoi (2021). As well as providing a powerful visual stimulus, the funnel traps used in this study have the advantage over sticky sheets that they do not get saturated with captured insects and thus preserve their capture capacity for significantly longer, without losing sensitivity. It should also be noted that the biology of pollen beetle species is very similar, so in the event of rapid spring temperature rises, these minimal differences in swarming phenology can disappear and the species can appear and damage at the same time.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTION

Author 2 and author 10 conceived research. Author 1, author 5, author 6 and author 7 conducted experiments. Author 8 and author 9 contributed material. Author 3, author 4 and author 10 analysed data and conducted statistical analyses. Author 1, author 3, author 9 and author 10 wrote the manuscript. All authors read and approved the manuscript.

DATA AVAILABILITY STATEMENT

Raw trap catch data have been archived at Pollen beetle trap colour preference (figshare.com).

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