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Development of a trap combining visual and chemical cues for the alfalfa longhorn beetle, *Plagionotus floralis*

Zoltán IMREI[1], Zsófia KOVÁTS[2], Teodora B. TOSHOVA[3], Mitko SUBCHEV[3], Krisztina HARMINCZ[4], István SZARUKÁN[4], Michael J. DOMINGUE[5], Miklós TÓTH[1]

[1]*Plant Protection Institute, Centre for Agricultural Research, Hungarian Academy of Sciences, Budapest, Hungary*
[2]*Eötvös Loránd University, Budapest, Hungary*
[3]*Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences, Sofia, Bulgaria*
[4]*University of Debrecen, Centre for Agricultural and Applied Economic Sciences, Debrecen, Hungary*
[5]*The Pennsylvania State University, Department of Entomology, University Park, PA, USA*

Abstract

We describe a trap comprised of both chemically and visually attractive stimuli for the alfalfa longhorn beetle, *Plagionotus floralis* (Pallas) (Coleoptera Cerambycidae Clytini), a pest causing increasingly serious damage each year in alfalfa fields in Central and Eastern Europe. Fluorescent yellow funnel traps caught significantly more *P. floralis* than traps with other colours, although in some tests non-fluorescent yellow traps also attracted more beetles than non-yellow traps. Fluorescent yellow reflects at a high intensity at wavelengths of 500 to 550 nm, which may account for the far better response of *P. floralis*. A ternary synthetic chemical lure of (*E*)-anethol, 1-phenylethyl alcohol, and 3-methyl-eugenol (TERN lure) generally increased the catches of *P. floralis* in the fluorescent yellow traps. 1-phenylethyl alcohol or 3-methyl eugenol used alone in fluorescent yellow traps caught significantly more *P. floralis* beetles than fluorescent yellow traps with no odour bait. The establishment of a threshold for fluorescent yellow traps with the floral attractant to monitor *P. floralis* populations would assist in decision making regarding the optimal application of agrotechnical measures. This protocol would improve plant protection practice with respect to both an economic and an environmental concern.

Key words: (*E*)-anethol, 1-phenylethyl alcohol, 3-methyl-eugenol, fluorescent yellow, attract, monitoring.

Introduction

In the past two decades the effects of visual and olfactory flower-related cues on attraction of many beetle species in several families have been elucidated, including chafers, (Coleoptera Scarabaeidae) (Imrei, 2003; Tóth *et al*., 2004a; Schmera *et al*., 2004; Vuts *et al*., 2008; 2010; 2012), click beetles (Coleoptera Elateridae) (Tóth *et al*., 2011), and leaf beetles (Coleoptera Chrysomelidae) (Tóth *et al*., 2006; Tóth *et al*., 2010). The family Cerambycidae includes the most significant group of anthophilous Coleoptera (Lovell, 1915a; 1915b in Linsley, 1959), and possible pollinators (Allison *et al*., 2004). Thus long horn beetles appear to be a suitable group to explore insect responses to floral compounds and visual cues.

Plagionotus floralis (Pallas) (Coleoptera Cerambycidae Clytini) is a Palaearctic species occurring in Middle and Eastern Europe to the Caucasus Mountains, as well as in Iran, the Middle East, Syria and Middle Siberia (Kaszab, 1971). The beetle flies from May to August, after which the larvae hatching from the eggs feed in the primary roots of alfalfa. The damage results in the death of the plants, especially in dry weather conditions (Mészáros, 1990; Toshova *et al*., 2010). *P. floralis* has a pest status in the Eastern part of Europe (Mészáros, 1990; Toshova *et al*., 2010; Bozsik, 2013), where agrotechnical measures against the pest, which usually prevent serious economic damage, are focused on ploughing of older fields and increased attention to crop rotation. A suitable method for sampling *P. floralis* would facilitate management by establishing a threshold, which would determine the optimal time for the application of agrotechnical measures. Such a protocol would lead to an improvement of plant protection practice with respect to both environmental and economic factors.

In the present paper we describe the development of a trap comprising both chemically and visually attractive stimuli for the alfalfa longhorn beetle, *P. floralis* starting from the results of unanticipated chance captures.

Materials and methods

Bait dispensers

For all experiments, polyethylene (PE) bag dispensers were used. For preparing the PE bag bait dispensers, a 1 cm piece of dental roll (Celluron®, Paul Hartmann AG, Heidenheim, Germany) was placed into a tight PE bag (layer thickness: 0.02 mm). The size of the PE bag was ca. 1.5 × 1.5 cm. The dispenser was attached to a plastic strip (8 × 1 cm) for easy handling when assembling the traps. For constructing the baits, neat compounds were administered onto the dental roll, and the opening of the PE bag was heat-sealed. Prior experience, mainly on scarab beetles (Coleoptera Scarabaeidae Cetoniinae), showed that the same chemicals in the bags did not lose activity during several weeks of field exposure. Thus, we decided that it was appropriate to renew the lures at 2- to 3-week intervals.

All dispensers were wrapped singly in pieces of aluminium foil and were stored at –30 °C until use. Synthetic compounds were obtained from Sigma-Aldrich Kft. (Budapest, Hungary). All compounds were > 95% pure as stated by the supplier.

Table 1. Field test sites.

	Site	Biotope	No. blocks	Period
Experiment 1	Debrecen, Hungary	alfalfa field	10	10 June-3 July, 2003
Experiment 2	Pilis, Hungary	mixed orchard	12	25 May-20 July, 2005
Experiment 3	Kismacs, Hungary	poppy seed field	12	24 June-12 July, 2004
Experiment 4	Vrazhdebna, Sofia, Bulgaria	alfalfa field	15	3 June-4 August, 2009
Experiment 5	Telki, Hungary	abandoned hillside with *Rosa*, *Crataegus* and *Prunus* bushes	12	11 June-28 July, 2003
Experiment 6	Telki, Hungary	abandoned hillside with *Rosa*, *Crataegus* and *Prunus* bushes	12	11 June-22 July, 2003
Experiment 7	Tárnok, Hungary	alfalfa field	12	22 May-19 July, 2004
Experiment 8	Vrazhdebna, Sofia, Bulgaria	alfalfa field	12	12 June-29 July, 2008
Experiment 9	Julianna major, Hungary	alfalfa field	12	18 May-26 July, 2004

Traps

Field tests were carried out with VARb3 modified funnel traps from the CSALOMON® trap family (www.csalomontraps.com) produced by the Plant Protection Institute (Hungarian Academy of Sciences, Budapest, Hungary) (Imrei *et al*., 2001; Schmera *et al*., 2004). The trap design, with upper funnels left unpainted (transparent) or painted in different colours (fluorescent yellow, yellow, light blue, white), has been shown to be effective in catching several beetle species, especially scarabs. For the reflectance spectra of the colours used, please refer to Schmera *et al*. (2004), Tóth *et al*. (2004a) and Jenser *et al*. (2010). The bait dispenser was suspended from the vertical plastic sheet, so that it hung in the middle of the funnel opening.

Field tests

Experiments were conducted at seven field sites in Hungary (2003-2005) and Bulgaria (2008-2009) (table 1). Traps were set up in a randomized complete block design. Traps were spaced 10-15 m apart within a block, and there was a distance of 40-50 m between blocks. Traps were inspected twice weekly, when captured insects were removed. Captured beetles were identified to species using the keys of Angelov (1995) and Kaszab (1971).

Experiment 1

The original objective of this preliminary experiment was to test the attraction of yellow colour to the click beetle, *Agriotes ustulatus* (Schaller) (Coleoptera Elateridae). The funnel traps with transparent or yellow upper parts were set up without chemical baits. In this paper we discuss only catches of *P. floralis*. Results for the original target species are not shown in the present paper.

Experiments 2, 3 and 4

The objective of these experiments was to test the colour preference of different beetle species without the presence of chemical bait. The treatments were funnel traps with transparent, white, blue, yellow or fluorescent yellow upper parts. In this paper we discuss only catches of *P. floralis*. Results for other species have been published in Tóth *et al*. (2005).

Experiment 5

Originally the objective of the experiment was to test the colour preference of *Cetonia aurata aurata* (L.) and *Protaetia cuprea* (F.) (Coleoptera Scarabaeidae) in the presence of their ternary floral bait (TERN lure) (Imrei, 2003), consisting of (*E*)-anethol, 1-phenylethyl alcohol, and 3-methyl-eugenol. The dispensers were prepared by administering a 200 µl amount of the synthetic blend (1:1:1 ratio) into a PE bag dispenser. The treatments consisted of differently coloured upper parts of the funnel traps deployed, including transparent, white, blue, ordinary yellow, and fluorescent yellow. In this paper we discuss only catches of *P. floralis*. Results for other species have been published in Tóth *et al*. (2005).

Experiment 6

The objective of the experiment was to compare the attraction of *P. floralis* to fluorescent yellow colour versus a transparent control, given the presence or absence of the TERN lure (Imrei, 2003). The dispensers were prepared as described in experiment 5. Traps with transparent or fluorescent yellow upper parts were tested.

Experiments 7 and 8

The objective of the experiment was to compare the relative importance of fluorescent yellow as a visual stimulus versus TERN lure (100 µl in a single dispenser) as chemical stimulus in the attraction of *P. floralis*. Thus there were four treatments in a 2 × 2 factorial design, including traps with transparent or fluorescent yellow upper parts and with or without the TERN lure.

Experiment 9

The objective of the experiment was to test the activity of single components of the TERN lure for the attraction of *P. floralis*. On the fluorescent yellow traps, treatments included (*E*)-anethol, 1-phenylethyl alcohol, and 3-methyl-eugenol and TERN lure (100 µl ea. in a single dispenser) or no bait. Transparent traps were also deployed with either the TERN lure or no bait.

Statistics

Since trap capture data generally did not fulfil the requirements for a parametric analysis, they were analysed by the non-parametric Kruskal-Wallis test. When Kruskal-Wallis indicated significant differences, pairwise comparisons by Mann-Whitney U test (Zar, 1999) were performed. All statistical procedures were conducted using the software packages StatView® v4.01 (Abacus Concepts Inc., Berkeley, CA).

Results

In experiment 1 all 191 *P. floralis* specimens caught came into traps with yellow upper funnels, while no *P. floralis* were caught in traps with transparent upper funnel ($P < 0.0001$ by Mann-Whitney U test).

In experiment 2 the largest catches were recorded in the traps with fluorescent yellow upper parts (table 2). The yellow traps caught a few *P. floralis* specimens, but these catches were not significantly different from the catches in traps with transparent, white, or blue upper parts which caught no *P. floralis*.

In experiments 3 and 4, fluorescent yellow traps caught significantly more *P. floralis* beetles than the other non-yellow colours (table 2). In experiment 3 the fluorescent yellow colour was significantly more effective than yellow, while in experiment 4 this same tendency was not significant. In experiment 4, yellow caught significantly more *P. floralis* than the non-yellow colours, and low catches (significantly higher than in transparent and blue) were recorded also in white traps (table 2).

In experiment 5, when all colour treatments were applied together in traps with the TERN lure, the fluorescent yellow traps caught significantly more *P. floralis* beetles than any other colour treatments (table 2). The yellow trap treatment was the only other one which attracted significantly more beetles than the transparent treatment.

In experiment 6 the fluorescent yellow traps with TERN lure caught significantly more *P. floralis* beetles than any of the other variations (table 3). In this case no *P. floralis* beetles were found in treatments lacking the TERN lure.

In both experiments 7 and 8, the fluorescent yellow colour in combination with the TERN lure caught numerically more *P. floralis* than fluorescent yellow traps with no lure, but the difference was not significant (figure 1). Both treatments with fluorescent yellow colour differed significantly from the catches of the transparent traps with or without the TERN lure (figure 1). In both experiments, a small number of *P. floralis* were caught in the transparent traps baited with TERN lure, while no beetles were caught in the unbaited transparent traps (the difference being significant only in experiment 7).

Table 2. Mean catches of *P. floralis* in traps of different colour (experiments 2, 3, 4 and 5).

Mean catch/trap ± S.E.	Pilis, 2005 experiment 2	Kismacs, 2004 experiment 3	Sofia, 2009 experiment 4	Telki, 2003 experiment 5[a]
Total catch:	23	36	772	108
Transparent	0.00 ± 0.00a	0.00 ± 0.00a	0.02 ± 0.02a	0.12 ± 0.09ab
White	0.00 ± 0.00a	0.05 ± 0.05a	0.63 ± 0.19b	0.29 ± 0.11bc
Blue	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a
Yellow	0.06 ± 0.06a	0.25 ± 0.1a	5.51 ± 1.26c	0.83 ± 0.32c
Fluorescent	1.38 ± 0.49b	1.50 ± 0.46b	13.28 ± 3.13c	3.39 ± 0.99d

Means with the same letter within one column are not different at $\alpha = 0.05$ by Kruskal-Wallis followed by pairwise comparisons by Mann-Whitney U test. [a]The same floral volatile bait was applied in all traps, which was the ternary combination of (*E*)-anethol, 1-phenylethyl alcohol and 3-methyl-eugenol.

Table 3. Mean catches of *P. floralis* in traps with fluorescent yellow or transparent upper parts in the presence or absence of the ternary floral lure of (*E*)-anethol, 1-phenylethyl alcohol and 3-methyl-eugenol (experiment 6). Total of *P. floralis* caught: 18 specimens.

Color of trap upper part	Floral olfactory lure	Mean catch/trap ± S.E.
Fluorescent	present	4.25 ± 1.31a
Transparent	present	0.25 ± 0.25b
Fluorescent	no	0.00 ± 0.00b
Transparent	no	0.00 ± 0.00b

Means with the same letter are not different at $\alpha = 0.05$ by Kruskal-Wallis followed by pairwise comparisons by Mann-Whitney U test.

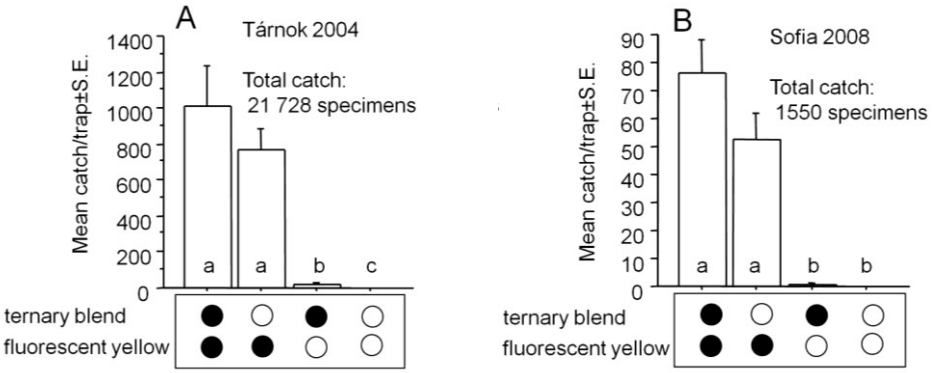

Figure 1. Mean captures of *P. floralis* in transparent or fluorescent yellow traps with or without the blend of (*E*)-anethol, 1-phenylethyl alcohol and 3-methyl-eugenol (TERN lure) (A: Experiment 7, B: Experiment 8). Means with the same letter within one diagram are not significantly different at $\alpha = 0.05$ by Kruskal-Wallis, followed by pairwise comparisons by the Mann-Whitney U test. Empty circles below the columns indicate absence, while full circles indicate presence of a stimulus in the given treatment.

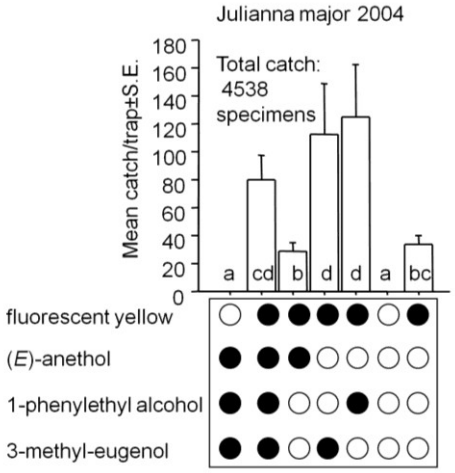

Figure 2. Mean captures of *P. floralis* in traps baited with (*E*)-anethol, 1-phenylethyl alcohol, 3-methyl-eugenol, or their ternary blend (TERN lure) with either fluorescent yellow or transparent upper parts, and in traps without a chemical lure (experiment 9). Means with same letter are not significantly different at $\alpha = 0.05$ by Kruskal-Wallis followed by pairwise comparisons by the Mann-Whitney U test. Empty circles below the columns indicate absence, while full circles indicate presence of a stimulus in the given treatment.

In experiment 9 the fluorescent yellow colour in combination with the TERN lure resulted in higher trap catches than the fluorescent yellow stimulus alone, but the difference was not significant (figure 2). Both treatments differed significantly from the catches of the transparent traps with or without TERN lure, which were both similarly ineffective, catching no beetles (figure 2). (*E*)-anethol in combination with the fluorescent yellow colour attracted numbers similar to fluorescent yellow traps with no bait. On the other hand, both 1-phenylethyl alcohol or 3-methyl eugenol in fluorescent yellow traps caught more beetles than fluorescent yellow traps with no bait. Fluorescent yellow traps with these single compounds were similar to the catch of the TERN lure.

Discussion

The chance finding of *P. floralis* in experiment 1 suggested that adults of this species were sensitive to yellow colour hues. A series of further experiments strongly suggested that from the two yellow hues tested, fluorescent yellow was definitely superior to bright yellow (table 2). In the reflectance spectra of these two colours, fluorescent yellow (which colour shows a greenish nuance) reflects at higher intensity in the wavelength range of 500 to 550 nm (Tóth *et al*., 2004a; Jenser *et al*., 2010). This difference in intensity may explain the far better response of *P. floralis* to fluorescent yellow in the present study. This response is parallel to the response of the European cherry fruit fly, *Rhagoletis cerasi* (L.) (Diptera Tephritidae) (Tóth *et al*., 2004b),

the vine thrips, *Drepanothrips reuteri* Uzel (Thysanoptera Thripidae) (Jenser *et al*., 2010) and two *Oxythyrea* sp. (Coleoptera Scarabaeidae) (Vuts *et al*., 2008; 2010) to these same two yellow hues.

In experiment 6 fluorescent yellow traps with the TERN lure (Imrei, 2003; Tóth *et al*., 2005) caught significantly more *P. floralis* than traps without the olfactory lure, suggesting that the presence of these chemicals added to the effect of the colour. Other experiments (7-8) comparing fluorescent yellow traps with or without the TERN lure invariably resulted in numerically higher catches in traps containing the chemical stimulus, despite the fact that statistical significance was not observed in most cases. This suggested that the attraction effect of the mixture of chemicals is often relatively low to *P. floralis*, while that of the visual stimulus is predominant.

In the ensuing experiment 9, which was specifically designed for studying the interaction of the chemical and the visual stimuli, it appeared that from the three synthetic compounds in the TERN lure, (*E*)-anethol was ineffective in increasing trap captures. Consequently attraction could probably be attributed to methyl eugenol and/or 1-phenethyl alcohol in the TERN lure.

To our knowledge no visual or chemical stimuli have previously been reported in the literature for the attraction of *P. floralis*. The intense attraction of *P. floralis* to fluorescent yellow most probably derives from their evolved behavioural response to the visual stimuli of flowers as feeding sites.

(*E*)-anethol (aromatic terpene), 1-phenylethyl alcohol (aromatic compound) and 3-methyl-eugenol (aromatic compound), the constituents of the TERN lure used in this study, have not been reported as constituents of an attractant of any longhorn beetle species before, but the same combination of compounds was shown to be highly attractive to cetoniine scarab beetles, *C. aurata aurata* and *P. cuprea* (Imrei, 2003; Tóth *et al*., 2005).

Currently we believe that a fluorescent yellow funnel trap baited with methyl eugenol and/or 1-phenethyl alcohol can be successfully deployed to sample *P. floralis* populations for agricultural uses. However, it may also be possible to further optimize the chemical composition of lures by testing other commonly occurring floral or specific pheromone compounds. The trap developed on the basis of the results described in the present paper already proved to be suitable for detection and monitoring purposes of *P. floralis* in Bulgaria (Toshova *et al*., 2010). In our opinion, management of *P. floralis* might be optimized in the future by using catch-based decision making, which would be evaluated on the basis of the mean number of specimens caught per year in such traps.

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Authors' addresses: Zoltán IMREI (corresponding author, e-mail: imrei.zoltan@agrar.mta.hu), Miklós TÓTH, Plant Protection Institute, Centre for Agricultural Research, Hungarian Academy of Sciences, Herman O. u. 15., H-1022 Budapest, Hungary; Zsófia KOVÁTS, Eötvös Loránd University, Budapest, Hungary; Teodora B. TOSHOVA, Mitko SUBCHEV, Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences, ul. Gagarin 2, BG-1113 Sofia, Bulgaria; Krisztina HARMINCZ, István SZARUKÁN, University of Debrecen, Centre for Agricultural and Applied Economic Sciences, Debrecen, Hungary; Michael J. DOMINGUE, The Pennsylvania State University, Department of Entomology, University Park, PA, USA.

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