

Development of a synthetic floral lure for pollen beetles (Coleoptera: Nitidulidae)

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ABSTRACT

Pollen beetles (Coleoptera: Nitidulidae) rank among the most important pests of oilseed rape (*Brassica napus*). For their timely detection in early spring, yellow sticky or water pan traps are used; however, it has been suggested that the addition of chemical lures to attractive visual cues could improve trap efficacy. During the course of field trials in Hungary, we have developed a 3-component synthetic floral lure consisting of (*E*)-anethol + (*E*)-cinnamyl alcohol + (*E*)-cinnamyl acetate, which attracted large numbers of pollen beetles into large capture-capacity fluorescent yellow funnel traps. There was no apparent difference between the pollen beetle species *Brassicogethes aeneus* F. 1775 (earlier *Meligethes aeneus*), *Brassicogethes viridescens* F. 1775, *Brassicogethes coracinus* Sturm 1845 and *Fabogethes nigrescens* Sturm 1845 in their responses to the 3-component lure, which can therefore be used to trap all of them. Funnel traps with the new ternary floral lure were more efficient in catching beetles than those with lures containing 2-phenethyl isothiocyanate, a previously described plant-derived attractant for pollen beetles. However, the effect of the addition of the isothiocyanate to the ternary blend was not completely clear from these experiments and thus requires further studies.

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KEYWORDS

monitoring, floral lure, trap, pollen beetle, oilseed rape

INTRODUCTION

Pollen beetles (Coleoptera: Nitidulidae) are major pests of oilseed rape (*Brassica napus* L.) and other crucifers (Brassicaceae) in Europe, parts of North Africa and Asia, and North America (Williams, 2010). Based on studies with *Brassicogethes aeneus* F., 1775 (earlier *Meligethes aeneus*), to locate their larval host plants, pollen beetles are attracted to the colour of *B. napus* flowers (Giamoustaris and Mithen, 1996) and to plant volatiles, including isothiocyanates (Blight and Smart, 1999; Cook et al., 2002). Females lay their eggs in the flower buds and eat the pollen, which leads to bud abscission and blind stalks, thereby preventing the growth of pods and leading to considerable seed yield loss, particularly in the more damage-susceptible spring-sown crops (Cook et al., 2007; Keszthelyi, 2016; Seimandi-Corda et al., 2021).

Chemical control of pollen beetles is currently achieved by insecticides (Mauchline et al., 2018), which is only effective and environmentally less damaging if timed to the mass occurrence of the beetles (Sáringner, 1990; Mauchline et al., 2018). An undesired side-effect of the use of pyrethroids in oilseed rape fields is their negative impact on pollinators, e.g. honey bees (Dworzańska et al., 2020). To reduce harmful insecticide effects on pollinating insects, detection, forecast and monitoring approaches for the occurrence of pollen beetles have been developed and are generally done by yellow water pan traps, sticky chromotropic yellow traps (Mauchline et al., 2018) and non-sticky fluorescent yellow or blue funnel traps (Vuts et al., 2022). The efficacy of chromotropic traps may be improved with the addition of chemical lures, which was demonstrated earlier with isothiocyanate-containing lures (Blight and Smart, 1999). However, no detection or monitoring trap with semiochemical lure was available for growers at the time when the present research was started.

The present research was initiated by chance findings. First, in a trapping trial originally aimed at catching *Tropinota (Epicometis) hirta* Poda (Coleoptera: Scarabaeidae), we recorded a surprisingly high number of pollen beetles: a mean of 193.3 in traps with the *T. hirta* lure vs. 79.0 in traps without the lure ($P = 0.049$), suggesting that the lure attracted them (M. Tóth and I. Szarukán, unpublished). The *Epicometis hirta* lure contained a blend of (*E*)-anethole [1-methoxy-4-((*E*)-1-propenyl)-benzene] (abbreviated later as ANET) and (*E*)-cinnamyl alcohol [(*E*)-3-phenyl-2-propen-1-ol] (abbreviated later as CINNOH) (Tóth et al., 2004).

Second, in a field screening test originally aimed at catching noctuid moths (Lepidoptera: Noctuidae), traps baited with a blend of ANET + CINNOH + (*E*)-cinnamaldehyde [(*E*)-3-phenyl-2-propenal] (abbreviated later as CINNAL) + (*E*)-cinnamyl acetate [(*E*)-3-phenyl-2-propenyl acetate] (abbreviated later as CINNAC) + phenylacetaldehyde (2-phenylacetaldehyde) (abbreviated later as PHENAL) caught a mean of 1,051.3 pollen beetles vs a mean of 84.6 beetles ($P = 0.021$) in traps with PHENAL only (M. Tóth and I. Szarukán, unpublished). This again suggested that ANET and one or more of the cinnamic compounds were responsible for pollen beetle attraction.

The objective of the present research was to confirm field activity of the above floral compounds on pollen beetles, to optimize blend composition and to compare the performance



of the floral blend with that of 2-phenethyl isothiocyanate (PHENETH), previously described as a synthetic, larval host plant-derived field attractant for pollen beetles (Blight and Smart, 1999).

MATERIAL AND METHODS

Field tests

Tests aimed at catching pollen beetles were conducted in oilseed rape fields at several sites in Hungary, using generally accepted methods (Roelofs and Cardé, 1977). Traps were arranged in blocks so that each block contained one trap of each treatment. Traps within blocks were separated by 8–10 m, and blocks were sited at least 30 m apart. 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 the body, legs and antennae, (iii) dorsal pubescence, (iv) clypeal margin, (v) shape of the elytra and scutellum, (vi) punctures on the body surface, (vii) the number of teeth on the lateral margin of the forelegs, (viii) shape of the median lobe of male genitalia and (ix) shape, size and pigmentation of the ovipositor (Audisio, 1980). According to the genus-level taxonomic revision of the Meligethinae subfamily, the former species complex of genera has been changed (Audisio et al., 2009). In this paper, species names are used following these changes.

Field tests deployed the CSALOMON[®] funnel trap types VARb3 or VARL, which have successfully been used for trapping several beetle species (e.g. Imrei et al., 2001; Tóth et al., 2004; photos of the traps can be viewed at www.csalomontraps.com). Since pollen beetles were known to be attracted to yellow colour (Blight and Smart, 1999), the outside surfaces of the funnel traps used were painted in fluorescent yellow (for reflectance spectrum, refer to Róth et al., 2016; Vuts et al., 2022). 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.

Chemicals used in lures were obtained from Sigma-Aldrich Kft. (Budapest, Hungary) and were >95% pure as stated by the supplier.

Lure dispensers were polyethylene bag dispensers (PE bag) as described earlier (Imrei et al., 2001; Tóth et al., 2003a, 2004). On preparing the lures, 100 µL of a compound was administered onto the cellulose roll inside the polyethylene sachet, after which it was heat-sealed. In the case of multicomponent mixtures, compounds were loaded into a single dispenser. PE bag dispensers have successfully been used to dispense various floral compounds to capture beetles (Imrei et al., 2001; Tóth et al., 2003a, 2011), moths (Tóth et al., 2010, 2014, 2020) and lacewings (Tóth et al., 2009a). Since earlier experience showed that PE bag lures can start to lose efficacy after a month in the field, lures were exchanged to new ones at monthly intervals.

Statistical analysis

As it is frequently found in field trapping experiments, the catch data (even after transformation) did not fulfil requirements for parametric analysis. Therefore, unless otherwise stated, data were analysed by the non-parametric Kruskal-Wallis test. When the Kruskal-Wallis test showed significance ($P < 0.05$), differences between treatments were analysed by pairwise comparisons with Mann-Whitney U test.



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

Experimental details

Experiment 1. The objective of this preliminary test was to confirm the importance of ANET, CINNOH, CINNAC and CINNAL in pollen beetle attraction (see Introduction for background). Captured pollen beetle specimens were not identified to species. The experiment was run at Kápolnásnyék, Fejér county, Hungary, April 7 – May 9, 2006, with 4 blocks of fluorescent yellow VARb3 funnel traps. Treatments included i) a quaternary blend of ANET, CINNOH, CINNAL and CINNAC, ii) their ternary combinations from which one of the compounds was subtracted, and iii) unbaited control traps.

Experiment 2. The objectives of this test were i) to confirm the activity of the most attractive ternary floral blend from Exp. 1., ii) to study the effect of subtracting cinnamic compounds singly from this ternary blend, iii) to compare the performance of the ternary floral blend with that of the known attractant PHENETH, and iv) to study the influence of the addition of PHENETH to the ternary floral blend. Treatments included i) the ternary blend of ANET + CINNOH + CINNAC, ii) binary blends with CINNOH or CINNAC subtracted from the ternary blend, iii) the quaternary blend of ANET + CINNOH + CINNAC + PHENETH, iv) PHENETH alone and v) unbaited control traps. To see whether there are species-specific differences in pollen beetle responses to the different treatments, pollen beetle specimens caught were separated into species. The experiment was conducted simultaneously with 5 blocks of fluorescent yellow VARL funnel traps at two sites in Hungary: Exp. 2A – Nadap, Fejér county, March 21 – April 22, 2007, and Exp. 2B – Túrkeve, Jász-Nagykun-Szolnok county, March 14 – July 1, 2007.

Experiment 3. The objective of the tests was to confirm the results of Exp. 2 by comparing the ternary floral blend with the quaternary blend containing also PHENETH and with PHENETH alone, and with unbaited control traps. Captured specimens were not identified for species. Treatments included i) the ternary blend of ANET + CINNOH + CINNAC, ii) the quaternary blend of i) with PHENETH added, iii) PHENETH on its own, and iv) unbaited control traps. The experiment was run simultaneously at 3 sites in Hungary using 6 blocks of fluorescent yellow VARL traps at each site: Exp. 3A – Kápolnásnyék, Fejér county, May 19 – July 4, 2008, Exp. 3B – Nadap, Fejér county, March 17 – May 2, 2008, and Exp. 3C – Debrecen, Hajdú-Bihar county, April 7 – July 2, 2008.

RESULTS

In Exp. 1., a total of almost three thousand pollen beetles (not determined to species) were captured (Fig. 1). The greatest mean catch was recorded in traps baited with the quaternary blend and with the ternary blend from which CINNALD was missing. Lower catches were observed with other ternary combinations (although the catch of the blend without ANET was



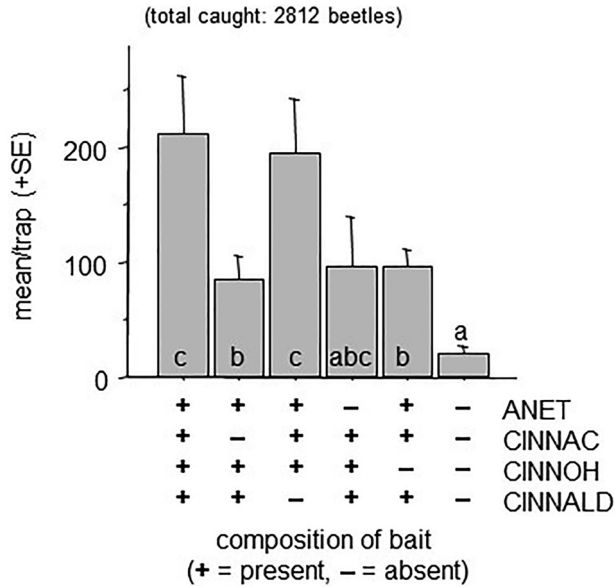


Fig. 1. Mean catches of pollen beetles (not separated to species) in traps baited with ternary and quaternary mixtures of ANET, CINNAC, CINNOH and CINNALD in Exp. 1. (Kápolnásnyék, 2006). Means with same letter within the diagram are not significantly different at $P = 5\%$ by Kruskal-Wallis test, followed by pairwise comparisons with Mann-Whitney test. ANET: (*E*)-anethole, CINNAC: (*E*)-cinnamyl acetate, CINNOH: (*E*)-cinnamyl alcohol, CINNALD: (*E*)-cinnamaldehyde

not significantly different from that of the best blends). All baited traps caught more than unbaited traps, except for the ternary blend without ANET.

In Exp. 2., pollen beetle specimens caught were determined to species (Fig. 2). *B. aeneus* catches showed similar distribution patterns between the two test sites (Fig. 2). All treatments containing lures caught significantly more than unbaited traps. All lures containing floral compounds caught more than those loaded with PHENETH only, except for the ANET + CINNAC binary lure in Exp. 2A. Catches with binary, ternary or quaternary lures were uniform, except the quaternary lure at the Túrkeve site (Fig. 2, Exp. 2B), which captured significantly more than all other lures.

Brassicogethes viridescens F. 1787 was caught in sizeable numbers only at the Túrkeve site (Fig. 2), and its catches showed a similar pattern to that of catches of *B. aeneus*, all treatments catching more than unbaited traps and all lures containing floral compounds catching more than PHENETH alone.

All traps with lures at the Nadap site caught more *Brassicogethes coracinus* Sturm 1845 than unbaited traps, and all lures containing floral compounds caught more than PHENETH (except for the ANET + CINNAC binary lure) (Fig. 2). Traps at the Túrkeve site (with ca. one order of magnitude lower total catches than at Nadap) baited with PHENETH on its own and with ANET + CINNAC did not catch more *B. coracinus* than unbaited traps. Similarly high catches were recorded in traps with the binary ANET + CINNOH, the ternary ANET + CINNAC + CINNOH and the quaternary combination containing PHENETH.



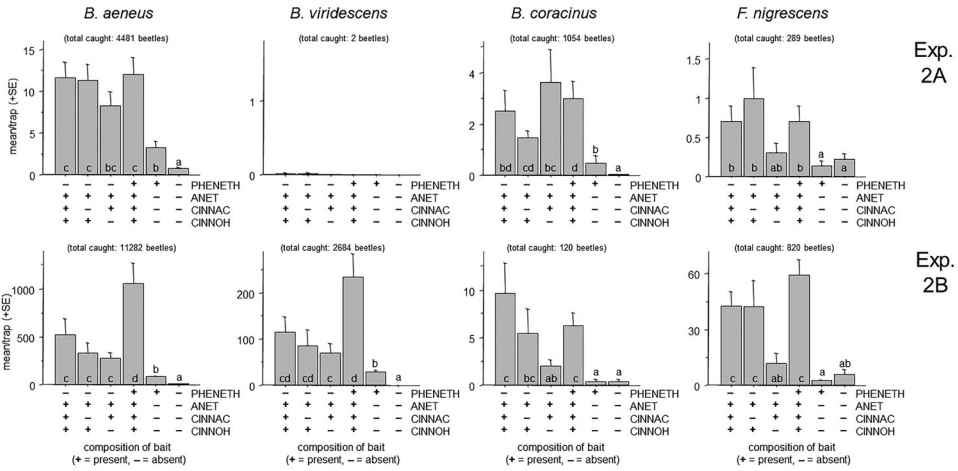


Fig. 2. Mean catches of pollen beetle species in traps baited with binary, ternary and quaternary combinations of PHENETH, ANET, CINNAC and CINNOH in Exp. 2. Exp. 2A = Nadap, 2007; Exp. 2B = Túrkeve, 2007. For significance, refer to Fig. 1. PHENETH: 2-phenethyl isothiocyanate

Catches of *Fabogethes nigrescens* Sturm 1845 showed similar trends at both test sites (Fig. 2). Traps with the PHENETH lures only and those with the binary ANET + CINNAC lure caught similarly low numbers as did unbaited traps. Uniformly high catches were recorded in traps with the binary ANET + CINNOH, the ternary ANET + CINNAC + CINNOH and the quaternary combination containing PHENETH.

In Exp. 3, more pollen beetles (all species together) were recorded in traps baited with PHENETH on its own than in unbaited traps at all 3 test sites (Fig. 3). Traps baited with the ternary ANET + CINNAC + CINNOH combination and with the quaternary lure containing

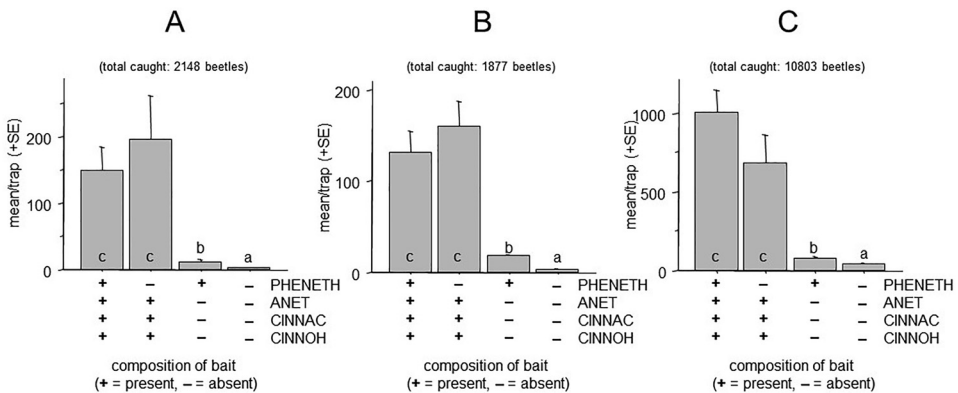


Fig. 3. Mean catches of pollen beetles (not separated to species) in traps baited with ANET, CINNAC, CINNOH, PHENETH and their mixtures in Exp. 3. (A = Kápolnásnyék, 2008; B = Nadap, 2008; C = Debrecen, 2008). For significance, refer to Fig. 1



PHENETH caught much more pollen beetles than traps with PHENETH on its own, and there was no significant difference between the catches of traps with the ternary and quaternary lures.

DISCUSSION

Here we show that a four-component lure comprising ANET, CINNOH, CINNALD and CINNAC in a ratio of 1:1:1:1 is an attractant of pollen beetles. It seems from subtraction tests that CINNALD is not necessary for full activity and can thus be omitted from the blend without apparent loss of activity. In some cases, the omission of either the acetate or alcohol cinnamic compound from the ternary blend was without any negative effect on lure performance, which may suggest their redundant role. Further clarification awaits future studies; however, at present, the ternary blend of ANET + CINNOH + CINNAC can be used as a powerful floral attractant for pollen beetles. The previously described, larval host plant-derived field attractant PHENETH was less active on its own than the ternary blend, the effect of the addition of which not being completely clear from these experiments and thus requires further studies; it increased the attractiveness of the ternary blend numerically in several experiments, whereas significantly only in Exp. 2 for *B. aeneus*.

The four pollen beetle species captured in this study showed remarkable similarities in their responses to the blends of floral compounds and/or the isothiocyanate PHENETH. In earlier studies, they showed marked differences in their colour preferences, *B. aeneus* and *B. viridescens* responding best to fluorescent yellow, while *B. coracinus* and *F. nigrescens* to blue or white (Vuts et al., 2022), which might reflect differing host plant preferences. Their similar behavioural responses to the compounds tested in the present study may indicate a more general role for floral volatiles emitted by a range of visited species, which serve as non-specific stimuli indicating feeding sites (=flower). This can be advantageous for growers, as although *B. aeneus* is usually the dominant pest, significant damage can be attributed to other pollen beetle species (e.g. Williams, 2010). However, considerably less literature data is available on other species, because they are either simply not examined, or all collected individuals are automatically counted as *B. aeneus*. Observations of pollen beetle species' swarming in four consecutive years at Keszthely revealed that *B. aeneus* was dominant (66–80%), and the proportion of *B. coracinus*, *B. viridescens* and *F. nigrescens* increased during the vegetation period but remained below that of *B. aeneus* (Marczali and Keszthelyi, 2003). Based on the time of their appearance and the frequency of their occurrence, these authors suggest that the latter three species can be also considered as rape pests. In the same study, *B. aeneus* was found to be the commonest species (occurred in 100% of all samples), followed by *B. coracinus* (78%), *B. viridescens* (50%), *F. nigrescens* (29%) and *M. atratus* (21%) (Marczali and Keszthelyi, 2003). Species ratios in the trap catch of the present study were as follows: Nadap site (Exp. 2A) – *B. aeneus* (77%), *B. coracinus* (18%), *B. viridescens* (0.04%), *F. nigrescens* (4.96%); Túrkeve site (Exp. 2B) – *B. aeneus* (75.7%), *B. coracinus* (0.8%), *B. viridescens* (18%), *F. nigrescens* (5.5%).

All floral compounds tested in the present study are relatively widespread across many plant families (Knudsen et al., 1993). They also frequently play a role in insect chemical communication. ANET on its own or in mixtures has been described as an attractant of flower-feeding scarabs (Coleoptera: Scarabaeidae) (Allsopp and Cherry, 1991; Cherry et al., 1996; Leal et al., 1994; Tóth et al., 2003b; Vuts et al., 2010), a glaphyrid beetle (Coleoptera: Glaphyridae) (Vuts



et al., 2014), a flower-visiting longhorn beetle (Coleoptera: Cerambycidae) (Imrei et al., 2014), and even a yponomeutid and a noctuid moth (Lepidoptera: Yponomeutidae; Noctuidae) (Bengtsson et al., 2006; Tóth et al., 2020). CINNOH has been reported as an attractant of scarabs (Coleoptera) (Donaldson et al., 1990; Tóth et al., 2003b, 2009b), a chrysomelid (Coleoptera: Chrysomelidae) (Bruce et al., 2011), flower-visiting *Diabrotica* spp. (Coleoptera: Chrysomelidae) (Hammack, 2001) and several pollinator ants (Hymenoptera: Formicidae) (De Vega et al., 2014), and it is a pheromone component of a bug (Heteroptera) (Aldrich et al., 1979). CINNAC is an attractant for several *Oxythyrea* spp. (Coleoptera: Scarabaeidae) (Donaldson et al., 1986), and CINNALD in combination with ANET strongly attracts a click beetle species (Coleoptera: Elateridae) (Tóth et al., 2011). These data clearly show that insects commonly exploit these compounds to locate flowers, the attraction of pollen beetles described in the present study adding to this picture. Interestingly, none of the four volatiles are known from *B. napus* flowers (e.g. Jakobsen et al., 1994), so they may be connected to nectar plant location in pollen beetles, which are generalist flower visitors.

We conclude that the floral lure containing ANET + CINNOH + CINNAC greatly enhances pollen beetle catches, thereby supplementing the attractiveness of the fluorescent yellow colour of VARb3 funnel traps described by Vuts et al. (2022). However, the effect of the addition of PHENETH to the ANET + CINNOH + CINNAC blend needs clarifying to decide if it significantly increases pollen beetle catches; until then, the use of the ternary lure is suggested. Based on the results of Exp. 1 of this study and Tóth et al. (2015), the interaction of the chemical cue (lure) and the visual cue (colour) is synergistic. Also, the ternary blend is more attractive than isothiocyanate-containing lures for pollen beetles, and it appears to be not worthwhile to combine the two types of chemical lures.

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