Optimization of a Chemical Attractant for *Epicometis* (*Tropinota*) *hirta* Poda

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Z. Naturforsch. **59 c,** 288–292 (2004); received June 16/August 25, 2003

In field trapping tests in Hungary cinnamyl alcohol (3-phenyl-2-propen-1-ol) and *trans*-anethole [(1-methoxy-4-(1-propenyl)benzene)] attracted significantly more adult *Epicometis* (*Tropinota*) *hirta* (Coleoptera, Scarabaeidae, Cetoniinae) when presented together in the same bait compared to the single compounds. Best attraction was recorded by a 1:1 mixture. Addition of other common floral scent compounds, *i.e.* 3-methyl eugenol, 4-methoxy-cinnamaldehyde, anisylacetone, β-ionone, cinnamyl acetate, cinnamic aldehyde, eugenol, indole, 2-phenylethanol or phenylacetaldehyde did not influence catches. The binary cinnamyl alcohol/*trans*-anethole bait described in this study is recommended for use in traps of *E. hirta* for agricultural purposes.

Key words: Epicometis (*Tropinota*) *hirta*, Cinnamyl Alcohol (3-Phenyl-2-propen-1-ol), *trans*-Anethole [(1-Methoxy-4-(1-propenyl)benzene)]

Introduction

In Hungary, *Epicometis* (*Tropinota*) *hirta* Poda (Coleoptera, Scarabaeidae, Cetoniinae) is the most important pest cetoniin causing damage through feeding on the reproductive parts of flowers of many plants, which are orchard and ornamental trees and bushes and other plants of agricultural importance (Endrödi, 1956; Homonnay and Homonnayné-Csehi, 1990). Occasionally, the pest can damage even cereals (Hurpin; 1962, Homonnay and Homonnayné-Csehi, 1990). The species is widespread in large parts of Eurasia, from the Mediterranean to the Middle East and Central Asia (Hurpin, 1962).

The attraction of flower-visiting insects by flowers is frequently influenced by chemical cues (Kevan and Baker, 1983). Through field screening of synthetic floral scent compounds potent attractant combinations have been discovered for pest scarabs, as exemplified among others by the case of the Japanese beetle (*Popillia japonica* Newman) (Coleoptera, Scarabaeidae) (Ladd and McGovern, 1980; Ladd *et al.*, 1981). Such synthetic attractants can be used as baits in traps or by other methods in practical control of the target pest (Potter and Held, 2002).

In preliminary studies we found that *E. hirta* was significantly attracted to traps baited with cinnamyl alcohol (3-phenyl-2-propen-1-ol) or with *trans*-anethole [(1-methoxy-4-(1-propenyl)benzene)] on all testing occasions (Tóth et al., 2003). In several of the screening tests, some attractive activity of 2-phenylethanol or cinnamyl acetate was also observed (Tóth et al., 2003). The present study was undertaken to investigate whether the attractive activity of the above compounds can be enhanced when presented together, or in combination with other floral scent compounds known to be attractive towards other beetle spp., in order to attempt the optimization of a chemical attractant for *E. hirta*.

Materials and Methods

Baits

Throughout the experiments polythene bag bait dispensers were used. A 1 cm piece of dental roll (Celluron®, Paul Hartmann, Heidenheim, Germany) was put into a tight polythene bag made of 0.02 mm linear polyethylene foil. The dispenser was attached to a 8 cm × 1 cm plastic handle for easy handling when assembling the traps. For making up the baits, the required amount (between 0.2 to 0.02 ml) of each compound was administered onto the dental roll, and the opening of the polythene bag was heat sealed. Dispensers were wrapped singly in pieces of alufoil and were stored at −30 °C until use. Synthetic compounds were obtained from Sigma-Aldrich (Budapest, Hungary). All compounds were > 95% pure as stated by the suppliers.

0939–5075/2004/0300–0288 $ 06.00

Traps

The traps used were the standard VARb2 funnel traps used by the Budapest laboratory for catching scarab spp. (Fig 1). The trap consisted of an opaque plastic funnel (top opening outer diameter: 13 cm; funnel hole diameter: 3 cm; height of funnel: 16 cm), under which a transparent plastic round catch container (ca 1 l capacity) was attached by a rubber band. The top funnel opening was enlarged by attaching transparent plastic sheets to the trap body, so that the inner diameter of the top funnel opening became ca 20 cm. The bait dispenser was suspended from the plastic sheet, and it hung in the middle of the funnel opening, at ca 1 cm higher than the level of the upper edge of the funnel. A small piece (1 cm × 1 cm) of household anti-moth strip (Chemotox®, Sara Lee, Temana Intl. Ltd, Slouth, UK; active ingredient 15% dichlorvos) was placed into the catch container as a killing agent for captured insects.

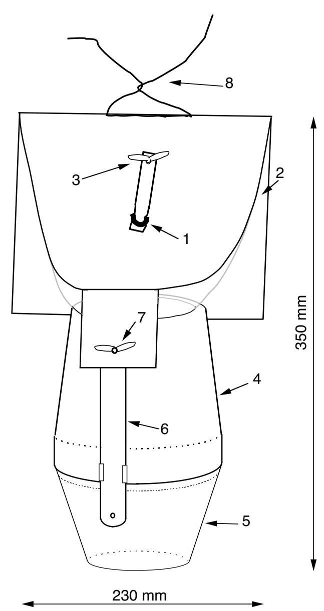

Fig. 1. Diagram of funnel trap (codename VARb2) used in the tests. 1 = Bait dispenser; 2 = transparent plastic sheet used to enlarge the funnel opening; 3 = clip holding the bait in place on the inside of the enlarged funnel opening; 4 = opaque plastic funnel; 5 = transparent plastic catch container; 6 = side lath holding funnel and catch container together; 7 = clip holding side lath and plastic sheet together; 8 = hanging wire.

Trapping experiments

Trapping experiments were conducted at three sites in Hungary: in cherry orchards at Halásztelek (Pest county) and Esztergom (Komárom-Esztergom county), and in a non-agricultural area (meadow with *Crataegus* and *Sambucus* bushes) at Agárd (Fejér county). Traps were set up in blocks. Each block was comprised of one of each treatment (= bait). The distance of traps within a block was 5–10 m. The distance between blocks ranged between 50–100 m. When inspecting the traps, captured insects were recorded and removed. Unless otherwise indicated, traps were inspected twice weekly and old baits were replaced by new ones at two to three weeks intervals. Traps were set up at the soil surface, on sunny places, attached to poles. The large opening of the funnel with the bait was ca. 35 cm above ground level.

Details of single experiments: Exp. 1: Halásztelek, April 14–May 7, 1998, 4 blocks of traps; Exp. 2: Halásztelek, May 12–June 2, 1998, 4 blocks of traps; Exp. 3: Agárd, May 12–June 2, 1998, 9 blocks of traps; Exp. 4: Halásztelek, April 27–May 30, 1998, 5 blocks of traps; Exp. 5: Agárd, May 5–25, 1999, 10 blocks of traps; Exp. 6: Halásztelek, May 5–25, 1998, 8 blocks of traps; Exp. 7: Agárd, April 12–May 3, 2001, 10 blocks of traps; Exp. 8: Agárd, May 3–17, 2001, 10 blocks of traps; Exp. 9: Esztergom, March 28–April 19, 2002, 10 blocks of traps; Exp. 10: Esztergom, April 19–May 14, 2002, 10 blocks of traps.

Statistical procedure

For the numerical analyses we pooled samples from different trap-emptying occasions. For each analysis, we attempted to use a parametric test. However, when a data set did not satisfy the assumptions of a parametric test checked by Kolgomorov-Smirnov test (Zar, 1999) and Levene test (Statsoft, 2000), then data were transformed (*i.e.* $\sqrt{x}$) to obtain normality. When even transformed data did not meet the assumptions of a parametric test, non-parametric statistics were used. Parametric ANOVA (Zar, 1999) was performed without any data transformation in experiments 1, 2, 3, 5 and 6; while in experiment 10, square root-transformed data were used. If parametric ANOVA showed significant differences, LSD test (Statsoft, 2000) was used for comparisons of the means. In experiment 9, both the original and transformed data set violated the assumptions of a parametric

ANOVA, therefore, non-parametric Kruskal-Wallis ANOVA (Zar, 1999) was performed. In experiments 4, 7 and 8, only two treatments were compared, and due to homogeneity problems the Mann-Whitney test (Zar, 1999) was used. All statistical tests were performed by the STATISTICA® computer program (Statsoft, 2000).

Results and Discussion

Since in our previous studies cinnamyl alcohol was significantly attractive in each experiment (Tóth *et al.*, 2003), we chose this compound for a basis and tested the influence of the addition of other attractant candidates to this basic bait. In a preliminary test (experiment 1) numerically higher catches than with cinnamyl alcohol alone were recorded only when *trans*-anethole was added (mean numbers caught: cinnamyl alcohol: 18.0 beetles; *trans*-anethole: 9.0 beetles; 1:1 mixture of cinnamyl alcohol and *trans*-anethole: 28.5 beetles). In the same test mixtures (ratio 1:1) of cinnamyl alcohol with the structurally related cinnamyl acetate or cinnamic aldehyde caught a mean of 2.8 and 8.5 beetles, respectively. Probably due to the preliminary nature of this test data showed large variations and there were no significant differences between the captures (parametric ANOVA, details not shown). Still, based on these preliminary results it appeared that it was worthwhile to continue the study of the influence of the addition of *trans*-anethole to cinnamyl alcohol in more detail.

In experiment 2 the mixture of cinnamyl alcohol and *trans*-anethole caught a mean of 22.5 *E. hirta* beetles which was significantly higher than captures by any of the two compounds tested singly (cinnamyl alcohol: 0.5 beetles; *trans*-anethole: 1.0 beetles, respectively) ($p < 0.001$ by LSD test; parametric ANOVA: $df = 2$, $F = 7.299$, $p = 0.013$). When testing different ratios or the two compounds (experiment 3), the 1:1 ratio caught significantly higher numbers than the 10:1 or 1:10 ratios, or the single compounds (Fig. 2). There was no significant difference between catches by the 10:1 or 1:10 ratios and the single compounds. We concluded that a blend containing equal amounts of cinnamyl alcohol and *trans*-anethole would be more attractive than the pure compounds, and used this mixture as a basis in future optimization tests.

In subsequent tests the influence of eugenol (2-methoxy-4-propylphenol) was studied, since

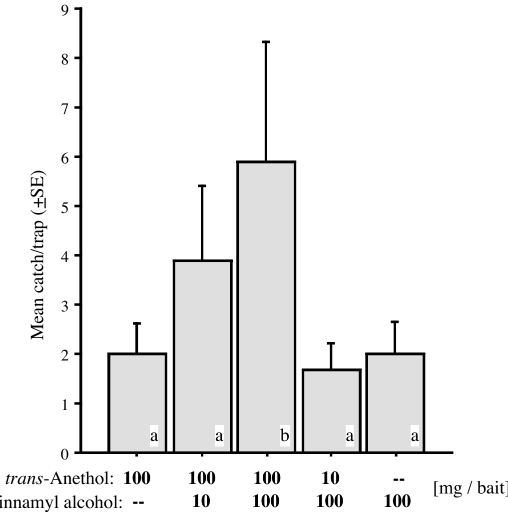

Fig. 2. Captures of adult *E. hirta* in traps baited with cinnamyl alcohol, *trans*-anethole and their mixtures in different ratios (experiment 3). Columns with same letters are not significantly different ($p < 0.05$, LSD test, parametric ANOVA: $df = 4$, $F = 5.125$, $p = 0.002$).

this compound has been widely known as an attractant component among scarab beetles (Ladd and McGovern, 1980; Ladd *et al.*, 1981; Klein and Edwards, 1989; Reed *et al.*, 1991; Benyakir *et al.*, 1995; Allsopp and Cherry, 1991; Donaldson *et al.*, 1986, 1990), and also because in previous screening tests in some cases the mixture of cinnamyl alcohol and eugenol caught numerically more *E. hirta* than cinnamyl alcohol alone (Tóth *et al.*, 2003).

In experiments 4 and 5, the addition of eugenol in varying ratios to the binary mixture of cinnamyl alcohol and *trans*-anethole was without effect (Table I).

In the following tests (experiments 6–10) a number of further attractant candidate compounds were tested, added singly or as sets to the basic cinnamyl alcohol/*trans*-anethole mixture. Among the compounds tested, 2-phenylethanol showed some attractive activity towards *E. hirta* in one preliminary test (Tóth et al., 2003). Phenylacetaldehyde, 3-methyl eugenol, anisylacetone [4-(4-methoxyphenyl)-2-butanone], *β*-ionone [4-(2,6,6-trimethyl-1-cyclohexen-1-yl)-3-buten-2-one] and geraniol [(*E*)-3,7-dimethyl-2,6-octadien-1-ol] have been described as attractants or attractant components for several scarabs (Allsopp and Cherry, 1991; Donaldson *et al.*, 1986, 1990; Ladd and

Table I. Catches of *E. hirta* in traps baited with binary and ternary mixtures of cinnamyl alcohol, *trans*-anethole and eugenol.

Bait [mg]			Mean catch of *E. hirta*	
trans-Anethole	Cinnamyl alcohol	Eugenol	Exp. 4[a]	Exp. 5[b]
100	100	1	n.t.	2.56
100	100	5	n.t.	4.44
100	100	25	n.t.	5.89
100	100	100	6.25	n.t.
100	100	–	5.50	4.33

[a] Mann-Whitney test: $U = 6.5$, $Z = -0.433$, $p = 0.665$.
[b] Parametric ANOVA: $df = 3$, $F = 0.581$, $p = 0.632$.
n.t. = Not tested in this particular experiment.

McGovern, 1980; Ladd *et al.*, 1981; Leal *et al.*, 1994), or have been found by us to attract several related cetoniins in Hungary (results to be published in detail elsewhere). 4-Methoxy-cinnamic aldehyde and indol are widespread floral compounds and are known to be attractive among others towards *Diabrotica* spp. (Coleoptera, Chrysomelidae) (Metcalf, 1994; Metcalf *et al.*, 1995). In experiments 6–10 the addition of none of the compounds or compound sets showed significantly greater catches of *E. hirta* than the basic cinnamyl alcohol/*trans*-anethole mixture (Table II).

In conclusion, the best performance was observed in the present study by the 1:1 cinnamyl alcohol/*trans*-anethole mixture for catching *E. hirta*. Both compounds are known flower volatiles (Knudsen et al., 1993) and may play a role in host finding of the adult beetles. Among scarabs, cinnamyl alcohol has been found to be attractive towards the cetonins *Pachnoda* spp., and *Oxythyrea* spp. and the rutelins *Anomala transvaalensis* Arrow and *Adoretus tessulatus* Burmeister (Donaldson *et al.*, 1986, 1990). *Trans*-anethole has previously been reported to be attractive to the scarabs *Anomala marginata* Robinson (Scarabaeidae, Rutelinae), *Trigonopeltastes delta* Forster (Scarabaeidae, Melolonthinae) (Cherry *et al.*, 1996) and *Eupoecila australasiae* Donovan (Scarabaeidae, Cetoniine) (Alsopp and Cherry, 1991). This compound is also the major component (together with phenethyl propionate and geraniol) of the food lure of *Anomala octiescostata* Burmeister (Scarabaeidae, Rutelinae) (Leal *et al.*, 1994). To the best of our knowledge, the binary mixture of cinnamyl alcohol and *trans*-anethole has not been described elsewhere as a bait combination for other scarab spp.

The optimal *E. hirta* attractant reported in the present study shows some prespective as a bait for

Table II. Catches of *E. hirta* in traps baited with the mixture of cinnamyl alcohol and *trans*-anethole and other candidate attractant compounds.

Bait [mg]; added to a mixture of cinnamyl alcohol and *trans*-anethole								Mean catch of *E. hirta*				
ANIS	BION	PHENOH	EUG	PHENAL	INDOL	3METEUG	PARA	Exp. 6[a]	Exp. 7[b]	Exp. 8[c]	Exp.9[d]	Exp.10[e]
–	–	–	–	–	–	–	–	42.8	32.6	15.2	25.2	15.4
100	100	100	100	–	–	–	–	40.7	n.t.	n.t.	n.t.	n.t.
100	–	–	–	–	–	–	–	35.4	n.t.	n.t.	n.t.	n.t.
–	100	–	–	–	–	–	–	26.0	n.t.	n.t.	n.t.	n.t.
–	–	100	–	–	–	–	–	46.6	n.t.	n.t.	n.t.	n.t.
–	–	–	100	–	–	–	–	37.4	n.t.	n.t.	n.t.	n.t.
–	–	100	100	100	100	–	–	n.t.	58.4	n.t.	n.t.	n.t.
–	–	100	100	100	100	100	100	n.t.	n.t.	8.2	n.t.	n.t.
–	–	100	–	100	–	100	–	n.t.	n.t.	n.t.	42.1	18.3
–	–	–	100	–	100	–	100	n.t.	n.t.	n.t.	16.2	n.t.
–	–	100	–	100	–	–	–	n.t.	n.t.	n.t.	n.t.	24.1

[a] Parametric ANOVA: $df = 5$, $F = 0.627$, $p = 0.680$.
[b] Mann-Whitney test: $U = 40.5$, $Z = 0.718$, $p = 0.473$.
[c] Mann-Whitney test: $U = 31$, $Z = -1.436$, $p = 0.151$.
[d] Kruskal-Wallis ANOVA: $H = 0.547$, $p = 0.761$.
[e] Parametric ANOVA: $df = 2$, $F = 0.591$, $p = 0.561$.
n.t. = Not tested in this particular experiment. The basic bait of 1:1 cinnamyl alcohol and *trans*-anethole was dosed at 100 mg ea.
ANIS = anisylacetone [4-(4-methoxyphenyl)-2-butanone]; BION = β-ionone [4-(2,6,6-trimethyl-1-cyclohexen-1-yl)-3-buten-2-one]; PHENOH = 2-phenylethanol; EUG = eugenol; PHENAL = phenylacetaldehyde; INDOL = indole; 3METEUG = 3-methyl eugenol; PARA = 4-methoxy-cinnamaldehyde.

practical use. Its application appears to be the more promising, since in a sample of ca 200 beetles captured in a monitoring trap ca 60% were females (unpublished). However, since visual cues represent another important factor of attraction for flower-visiting insects (Kevan and Baker, 1983), for the development of a potent trapping device visual sensitivity of the target insect should also be taken into consideration. Development of a potent trapping device incorporating both chemical and visual cues for *E. hi*rta is under way.

Acknowledgements

The present study was partially supported by OTKA grant T 37569 of the Hungarian Academy of Sciences. The authors are greatly indebted to Á. Szentesi for useful advices in the preparation of the manuscript.

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