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# An Improved Female-targeted Semiochemical Lure for the European Corn Borer *Ostrinia nubilalis* Hbn.

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The addition of synthetic 4-methoxy-2-phenethyl alcohol to the known attractant phenylacetaldehyde synergized attraction of the European corn borer *Ostrinia nubilalis*, the blend invariably catching 3 to 5 times more than phenylacetaldehyde on its own. Highest catches were recorded by the 1:1 blend. Both females and males were attracted, supposedly in the natural sex ratio of the local population. This improved bisex *O. nubilalis* attractant could be more efficient and more suitable for detection and monitoring purposes than previously know lures, making possible to draw more reliable plant protection decisions.

**Keywords:** bisex attractant, phenylacetaldehyde, 4-methoxy-2-phenethyl alcohol, trapping, monitoring.

The European corn borer (*Ostrinia nubilalis* Hbn., Lepidoptera: Pyralidae) is an outstandingly important pest of maize worldwide. It was originally present in temperate regions of Europe and Asia, but it later was introduced into North America as well. In Europe it causes regular damages in maize-growing countries and it is generally controlled by insecticide sprays (Szőcs and Babendreier, 2011). For optimal timing of pesticide treatments detection of the occurrence of the pest and monitoring its presence is of utmost importance.

For detection and monitoring purposes pheromone traps are widely used in numerous pest moth spp. The female-produced sex pheromone of *O. nubilalis* has been identified (Klun and Brindley, 1970; Klun and Junk, 1977) and pheromone traps are offered to growers by many commercial companies. However, pheromone-baited traps will capture only males. Traps capable of capturing females could provide more useful data. For example, based on female catches giving information on the female flight pattern, more accurate estimates can be given on the timing of oviposition, and thus result in more precise pest control decisions (Wall, 1989; Witzgall et al., 2010).

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Phenylacetaldehyde (PAA) is recognized as broadly attractive to Lepidoptera (Creighton et al., 1973; Cantelo and Jacobson, 1979; Meagher 2001), and has been reported to attract also both sexes of *O. nubilalis* (Maini and Burgio, 1990; Burgio and Maini, 1994). However, its effect on *O. nubilalis* is relatively weak and according to general experience of farmers captures in traps baited with PAA are too low to allow for practical applications (Tóth et al., unpublished).

This present research was undertaken in the search for possible synergists which, when added to PAA, would yield catches high enough to draw reliable plant protection decisions. The chemicals tested were selected based on their occurrence in the scents of maize silk (Hammack, 1996) or of flowers visited by pest moths (Haynes et al., 1991; Heath et al., 1992; Landolt and Smithhisler, 2003; Guédot et al., 2008; Landolt et al., 2014) and their performance in past field tests as moth attractants (Landolt et al., 2001, 2006, 2011; Meagher and Landolt, 2008; Tóth et al., 2010). We evaluated chemicals in combination with PAA and in comparison with PAA.

## Materials and Methods

### *Field tests*

Tests were conducted at several sites in Hungary using accepted methods in trapping experiments of the same nature (Roelofs and Cardé, 1977). Traps were arranged as 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. At each test site 5 blocks of traps were operated. Traps were inspected at some days' intervals (preferably twice weekly), when captured insects were recorded and removed.

### *Traps*

In the tests, funnel traps CSALOMON® VARL were used. These traps have routinely been used for trapping several moth species of larger size (Tóth et al., 2000, 2010; Subchev et al., 2004); photos of the trap can be viewed at www.csalomontraps.com. For killing captured insects, a small piece ($1 \times 1$ cm) of a household anti-moth insecticide strip (Chemotox® SaraLee, Temana Intl. Ltd, Slouth, UK; active ingredient 15% dichlorvos) was placed into the catch container of traps.

### *Baits*

Synthetic compounds applied in baits were obtained from Sigma-Aldrich Kft. (Budapest, Hungary). All compounds were $>95\%$ pure as stated by the supplier. For preparing the bait dispensers, a 1 cm piece of dental roll (Celluron®, Paul Hartmann AG, Heidenheim, Germany) was placed into a tight polyethylene bag made of 0.02 mm linear polyethylene foil. The dimensions of the polyethylene sachets were ca. $1.5 \times 1.5$ cm. The dispenser was attached to a plastic strip ($8 \times 1$ cm) for easy handling when assembling

the traps. For making up the baits, compounds were administered onto the dental roll and the opening of the polythene bag was heat-sealed. Earlier experience showed that the bait did not loose from its activity during several weeks of field exposure (Tóth et al., 2010); hence we decided that it was safe to renew the lures at 4-week intervals. The dose of single compounds was generally 100 mg/dispenser (with the exception of Exp. 3; please refer to description of treatments in Exp. 3 later in this section). In case of testing mixtures, compounds were loaded into the same dental roll in a single dispenser.

*Experimental details*

Experiment 1. In this preliminary screening test the addition of potential synergists to PAA was tested. The following combinations were added:

AD1 = (*E*)-cinnamaldehyde + *ß*-caryophyllene

AD2 = 4-oxoisophorone

AD3 = *ß*-ionone + methyl salicylate + *ß*-caryophyllene

AD4 = 4-methoxyphenethyl alcohol + (*E*)-cinnamyl alcohol + (*E*)-anethol

The test was run parallel in maize fields at two sites: Balmazújváros, Hajdú-Bihar county, June 8–October 6, 2013, and Ófehértó, Szabolcs-Szatmár-Bereg county, May 31–September 27, 2013,

Experiment 2. This was a subtraction test where single components of the combination AD4 found synergistic in Exp. 1 were subtracted in order to pinpoint the activity to a single component or a binary mixture. Apart from PAA, the following mixtures were tested: PAA + 4-methoxyphenethyl alcohol (4METH) + (*E*)-cinnamyl alcohol (CINNOH) + (*E*)-anethol (ANET); PAA + 4METH; PAA + 4METH + CINNOH; PAA + 4METH + ANET; PAA + CINNOH + ANET; and unbaited controls.

The test was run parallel in maize fields at two sites: Újkígyós, Békés county, May 27–September 2, 2014, and Mezősas, Hajdú-Bihar county, May 24 – September 6, 2014,

Experiment 3. The objective of this test was to compare combinations of PAA + 4METH in different ratios vs. activity of the single compounds. Treatments included PAA + 4METH in 10:1, 1:1 and 1:10 ratios, PAA or 4METH alone, and unbaited controls.

The test was run parallel in maize fields at two sites: Mezősas, June 1 – September 11, 2015, and Látókép, Hajdú-Bihar county, June 1 – September 3, 2015,

*Statistical analysis*

The catch data from field trapping tests were transformed using $(x+0.5)^{1/2}$ to normalize distributions (Roelofs and Cardé, 1977) and were analyzed by ANOVA. If the ANOVA yielded significance, then treatment means were separated by Games–Howell test (Games and Howell, 1976; Jaccard et al., 1984). Where one of the treatments caught no insects the Bonferroni–Dunn test (Dunn, 1961) was used to check that mean catches in other treatments were not significantly different from zero catch (see also Figure legends). Inspection dates when no moths were caught by any of the traps were excluded from analyses.

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

# Results

In Exp. 1 at both sites and in both sexes most numerous *O. nubilalis* catches were recorded in traps containing PAA + AD4, which caught significantly more than PAA alone in case of males (Fig. 1A–B). Addition of all other variations had no significant effect when compared to PAA alone in both tests and in both sexes. Unbaited controls caught no moths. Catches of baited treatments other than treatment with AD4 were not significantly different from zero catch (recorded in unbaited controls), with the exception of male catches (all treatments at Balmazújváros) and female catches in PAA alone and PAA + AD3 at Balmazújváros (Fig. 1A–B).

In Exp. 2 all treatments containing 4METH showed invariably a tendency of catching more than PAA alone, the difference being significant in the binary PAA + 4METH (both sexes, both sites); in the quaternary PAA + 4METH + CINNOH + ANET (males both sites, females at Mezősas), and the ternary PAA + 4METH + CINNOH and PAA + 4METH + ANET (both sexes at Mezősas) (Fig. 2A–B). The ternary blend PAA + CINNOH + ANET caught similar mean numbers as PAA alone (both sexes, both sites). Unbaited controls caught no moths, and catches of all baited treatments were superior (both sexes, both sites).

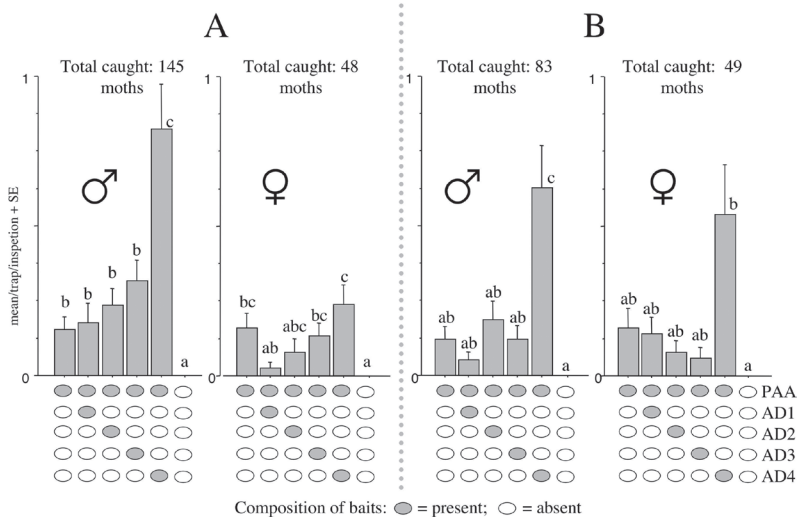


Fig. 1. Mean catches of male and female *O. nubilalis* in traps baited with phenylacetaldehyde (PAA), its blends with potential synergist candidates added, and in unbaited traps. (AD1 = (*E*)-cinnamaldehyde + *ß*-caryophyllene; AD2 = 4-oxoisophorone; AD3 = *ß*-ionone + methyl salicylate + *ß*-caryophyllene; AD4 = 4-methoxyphenethyl alcohol + (*E*)-cinnamyl alcohol + (*E*)-anethol). Balmazújváros (A) and Ófehértó (B), 2013 (Exp. 1). Columns with same letters within one diagram do not differ significantly at P = 5% by ANOVA, Games–Howell, Bonferroni–Dunn

In Exp. 3 again all binary lures containing PAA + 4METH together showed a tendency of catching more than PAA alone (males: all 3 ratios in both tests; females: 1:1 ratio in both tests, 1:10 ratio at Látókép); or 4METH alone (males: all 3 ratios in both tests; females: 1:10 and 1:1 ratios in both tests, 10:1 ratio at Látókép) (Fig. 3A–B). Single components did not catch significantly more than unbaited (with zero catch), on the other hand, all treatments with binary mixtures caught more than unbaited controls (both sexes, both sites).

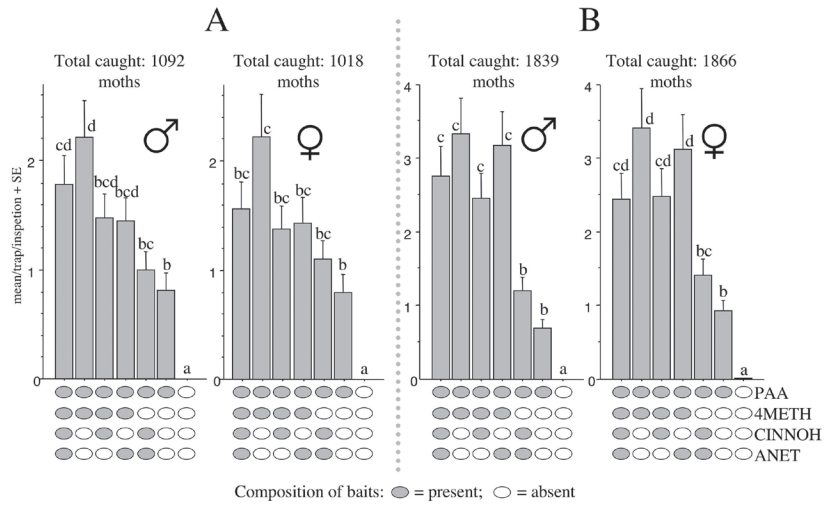


Fig. 2. Mean catches of male and female *O. nubilalis* in traps baited with phenylacetaldehyde (PAA), its binary, ternary and quaternary blends with 4-methoxyphenethyl alcohol (4METH), (*E*)-cinnamyl alcohol (CINNOH) and (*E*)-anethol (ANET), and in unbaited control traps. Újkigyós (A) and Mezősas (B), 2014 (Exp. 2). For significance refer to Fig. 1

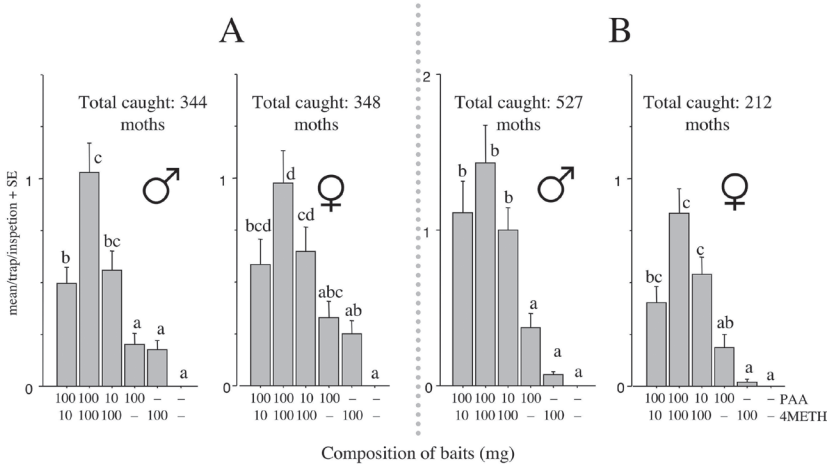


Fig. 3. Mean catches of male and female *O. nubilalis* in traps baited with phenylacetaldehyde (PAA) or 4-methoxyphenethyl alcohol (4METH), their binary mixtures in three ratios and in unbaited control traps. Mezősas (A) and Látókép (B), 2015 (Exp. 3). For significance refer to Fig. 1

In the course of all three experiments a number of moths other than *O. nubilalis* were also captured, with some noctuids predominating (i.e. *Autographa gamma* L., *Mac-Dunnoughia confusa* Steph., *Abrostola* spp., etc.) and some pyralids (i.e. *Pyrausta costalis* F. or *Haritala ruralis* Scop.). A report on catches of these non-target species will be published in detail elsewhere.

# Discussion

In the present study we confirmed field activity of PAA on *O. nubilalis*, as reported in earlier literature (Maini and Burgio, 1990; Burgio and Maini, 1994). The farmers's experience of PAA catching low numbers was also confirmed since in many of our experiments the catch with PAA was not significantly greater than zero catch in unbaited traps.

The synergistic activity of the addition of 4METH was clearly demonstrated in the present study. The effect was visible for both sexes of *O. nubilalis*. Catches in traps baited with blends of PAA + 4METH were invariably catching 3 to 5 times more than PAA on its own. We trust that the level of catches in traps with the blend will be more reliable and already will reach levels suitable for detection and monitoring purposes.

Sex ratios in PAA + 4METH baited traps were supposedly mirroring the natural sex ratio of the local populations. At least we detected no deviation from sex ratios in traps with only PAA in any of our tests. Future studies on whether the female percentages in the catch represent the actual sex ratio or are biased for some reason should be a priority for better management of *O. nubilalis*.

The fact that the new lure is catching also females is of great importance. Protandry is a well-known phenomenon in case of insects (Muralimohan and Srinivasa, 2008) and it could be observed well with traps baited with a lure capable to attract both male and female specimens. The difference of the flight of females vs. males may have great significance in pest management. Timing of the insecticide sprays to the flight of females could be more precise as it evidently correlates better to the egg laying than catch patterns of males recorded in pheromone traps (Knight and Light, 2004, 2005).

To our knowledge field attraction of 4METH has been described so far only for beetles. It is a component (together with indole) of the floral attractant of *Diabrotica barberi* Smith and Lawrence (Coleoptera: Chrysomelidae) (Metcalf et al., 1995). In *Epicometis hirta* Poda (Coleoptera: Scarabaeidae) the addition of 4METH to the floral attractant consisting of (*E*)-cinnamyl alcohol + (*E*)-anethol was synergistic (Vuts et al., 2010).

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