

Improving Bisexual Lures for the Silver Y Moth *Autographa gamma* L. and Related Plusiinae (Lepidoptera: Noctuidae)

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The addition of synthetic eugenol and benzyl acetate to the known floral chemical and moth attractant phenylacetaldehyde synergized attraction of the silver Y moth *Autographa gamma*, an important noctuid pest. Traps baited with the ternary blend caught 2 to 6 times more *A. gamma* moths than traps baited with phenylacetaldehyde alone. Both female and male moths were attracted, supposedly in the natural sex ratio of the local population. More *A. gamma* were caught when the blend was formulated in dispenser types with higher release rates. Traps baited with the ternary lure in polyethylene bag dispensers caught 20% to 34% as many moths as were caught in traps baited with synthetic sex pheromone, suggesting that this improved bisexual lure could be efficient enough to yield a new tool for detection and monitoring of female and male *A. gamma*, for more reliable plant protection decisions.

The same ternary lure also improved trap catches of moths over phenylacetaldehyde alone for the plusiinae pests *MacDunnoughia confusa* (in Europe) and *Autographa californica* (in North America) and for the Noctuinae cutworm *Xestia c-nigrum* (in North America).

Keywords: Bisexual attractant, phenylacetaldehyde, benzyl acetate, eugenol, trapping, monitoring.

The silver Y moth (*Autographa gamma* L., Lepidoptera: Noctuidae) is an important polyphagous pest of vegetables and field crops in Europe, Asia and North Africa (Balachowsky, 1972), and it is generally controlled by insecticide sprays. For optimal timing of pesticide treatments detection of the occurrence of the pest and monitoring its presence is of utmost importance.

Pheromone traps are widely used for detection and monitoring of numerous pest moth spp. The female-produced pheromone of *A. gamma* is identified as (*Z*)-7-dodecenyl acetate and (*Z*)-7-dodecen-1-ol (Dunkelblum and Gothilf, 1983; Tóth et al., 1983). However, pheromone-baited traps will capture only males.

Traps capable of capturing females can provide more useful data, and may be advantageous for direct control purposes, in addition to detection and monitoring. For ex-

ample, 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). Most lures found to be useful in attract-and-kill or mass trapping strategies are attractive to females or both sexes, rather than exclusively males (Landolt, 1997; Gregg et al., 2018).

Phenylacetaldehyde (PHENAL) 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 *A. gamma* (Burgio and Maini, 1994; Plepys et al., 2002a,b; Tóth et al., 2010). However, its effect is relatively weak and according to the general experience of farmers, captures in traps baited with PHENAL are too low to allow for practical applications (M. Tóth et al., unpublished).

This present research was undertaken in the search for possible synergists which, when added to PHENAL, would yield catches high enough to draw plant protection decisions reliably. Starting point was provided by a chance finding, when we recorded in an experiment originally aimed at capturing other noctuids, that a four-component mixture of PHENAL + (*E*)-anethol (ANET) + eugenol (EUG) + benzyl acetate (BENZAC) caught a mean/trap of 104.6 vs. a mean/trap of 46.8 *A. gamma* in traps with PHENAL + ANET only ($P < 0.0001$ by Mann-Whitney U test, total caught in test 757 moths) (M. Tóth et al., unpublished). This suggested that EUG and BENZAC may have some positive influence on captures of *A. gamma*. This idea was further emphasized by catches of the closely related plusiine *MacDunnoughia confusa* Steph. in the same experiment: means of 64.6 vs. 27.8 in the four vs. the two-component mixtures, resp. ($P = 0.009$, total caught in test 462 moths) (M. Tóth et al., unpubl.).

The objective of the present study was to confirm whether the addition of EUG and/or BENZAC consistently improves catches of *A. gamma* in traps with lures releasing PHENAL, resulting in an improved multicomponent bisexual lure for the species. We also set out to investigate the effect of these lures on several other closely related plusiine noctuids as well.

Materials and Methods

Field tests

Tests aimed at comparing moth catches in traps were conducted at several sites in Hungary (*A. gamma* and *M. confusa*) and in the USA (*Autographa californica* Speyer) 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. Unless otherwise stated at each test site 5 blocks of traps were operated. Traps were inspected at several days' intervals (preferably twice weekly), when captured insects were recorded and removed.

In tests in Hungary, 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.csalomon-

traps.com. For killing captured insects, 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 catch container of traps.

Tests in the United States used the Universal Moth Trap, also known as the UniTrap (AgriSense Ltd., Pontypridd, UK). The trap design is one of a white bucket topped with a yellow funnel, and a green cover or lid suspended over the the funnel. VaporTape (2.5 by 2.5 cm) (Hercon Environmental, Emigsville, PA, USA) was placed in the bucket to kill captured insects.

Baits

Synthetic compounds applied in baits were obtained from Sigma-Aldrich Kft. (Budapest, Hungary). All compounds were >95% pure as stated by the supplier. The following dispenser types were used:

PE bag dispenser: 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 × 1.5 cm. The dispenser was attached to a plastic strip (8 × 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 and the dispensers were wrapped individually in pieces of alufoil. 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 100 mg/dispenser. In case of testing mixtures, compounds were loaded into the same dental roll in a single dispenser.

PE vial dispensers: bait dispensers were prepared by adding 100 mg amounts of synthetics into 0.7 ml polyethylene vials with lid (No. 730, Kartell Co., Italy, wall thickness ca 0.5 mm). After loading, the lid of the dispensers was closed and the dispensers were wrapped individually in pieces of alufoil.

PP syringe dispensers: the dispenser consisted of a ca 4 ml volume polypropylene tube, similar in shape to an injection syringe, which contained a 3 cm piece of dental roll (Celluron®, Paul Hartmann AG, Heidenheim, Germany). When preparing the baits 400 mg of the compounds was administered onto the dental roll through the larger opening at the end of the syringe, then this opening was closed. At the beginning of the experiment in the field the thin tube at the other end of the syringe was cut, and compounds could evaporate to ambient air through the resulting 4 mm ID hole.

Pheromone lures used were the commercial *A. gamma* lures available from CSALOMON® (PPI CAR HAS, Budapest, formulated on rubber dispenser).

Statistical analysis

As it is frequently found in field trapping experiments, the catch data (even after transformation) did not fulfil requirements for a parametric analysis. Therefore data were analysed by the non-parametric Kruskal–Wallis test. When the Kruskal–Wallis test showed significance ($P = 5\%$), 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. In this test we aimed at confirming the positive influence of the addition of BENZAC and EUG to PHENAL or the mixture of PHENAL plus ANET (preliminary chance finding mentioned in the Introduction). Treatments included (formulated in PE bag dispensers): PHENAL on its own, PHENAL + ANET, PHENAL + BENZAC + EUG and PHENAL + ANET + BENZAC + EUG, and at one of the sites unbaited controls.

The test was run parallel at two sites: Hódmezővásárhely, Csongrád county, June 7 – September 30, 2011 (maize field), and Dömsöd, Pest county, July 25 – November 11, 2011 (vineyard).

Experiment 2. The objective of the test was to check for the effect of BENZAC and EUG when added singly or together to PHENAL. Treatments included (formulated in PE bag dispensers): PHENAL on its own, binary mixtures of PHENAL with BENZAC or EUG, the ternary combination of PHENAL + BENZAC + EUG and unbaited controls.

The test was run parallel in maize fields at two sites: Agárd, Fejér county, May 28 – September 12, 2012, and Berettyóújfalu, Békés county, May 28 – September 8, 2012.

Experiment 3. In this test the performance of the ternary blend of PHENAL + BENZAC + EUG formulated in three different types of dispensers was compared. Treatments included PE vial, PE bag and PP syringe dispensers and unbaited controls. Although no measurements of release rates were performed, we supposed that PE vial dispensers were emitting at a lower rate than PE bag dispensers (because their wall was much thicker), and PP syringe dispensers were emitting faster than PE bag dispensers, since evaporated compounds could diffuse directly through the open hole.

The test was run parallel in maize fields at two sites: Szeged, Csongrád county, June 1 – October 23, 2012, and Vésztő, Békés county, June 1 - October 22, 2012.

Experiment 4. The objective of this experiment was to check the influence of the addition of BENZAC and EUG to PHENAL on the North American plusiine *A. californica*. Treatments included PHENAL, BENZAC or EUG on their own, all possible binary mixtures of the three compounds, the ternary mixture of the three compounds and unbaited traps. All lures were formulated in PE bag dispensers. The test was run in commercial fields of spearmint, near Toppenish, Yakima County, Washington, USA. The traps were maintained from 17 April to 15 May 2012, with traps checked once per week.

Experiment 5. In this test the performance of the ternary mixture of PHENAL + BENZAC + EUG lure (formulated in PE bag dispensers) was compared with that of synthetic *A. gamma* pheromone. The test was run in two consecutive years in a mixed orchard at Érd-Elviramajor (Pest county), August 14 – October 22, 2015 and June 1 – October 14, 2016. In each year four blocks of traps were operated.

Results

Several moth spp. belonging to Noctuidae, Pyralidae and occasionally other moth families were caught in the experiments conducted at different localities. In the present study we restrict our report only to catches of *A. gamma*, *M. confusa*, *A. californica* (all plusiinae moths), and on *Xestia c-nigrum* L. which was caught in large numbers in the test in the USA (Exp. 4). *Xestia c-nigrum*, is a noctuine and a cutworm. Catches of other moths will be reported elsewhere in the future.

In Exp. 1. highest mean catches of *A. gamma* were recorded in the traps baited with PHENAL + EUG + BENZAC and the quaternary mixture containing also ANET (Fig. 1A, B). Significantly lower catches were observed in traps baited with PHENAL only, or the PHENAL + ANET binary blend, although these catches were significantly higher than those in unbaited controls (Fig. 1B; on the other site no unbaited traps were operated).

M. confusa were captured in relatively low numbers at one of the sites only, where its catches showed a trend similar to the *A. gamma* catches (Fig. 1B).

In Exp. 2. highest mean catches of *A. gamma* were observed in traps baited with the binary mixture of PHENAL + EUG or the ternary mixture (Fig. 2A, B). Significantly lower catches were recorded in traps baited with PHENAL only or with the PHENAL + BENZAC blend, however, these catches were still higher than those in unbaited traps.

As for *M. confusa*, all binary and ternary combinations caught more than PHENAL only, while PHENAL still caught more than unbaited controls (Fig. 2B).

In Exp. 3 all baited treatments caught more *A. gamma* than unbaited controls (Fig. 3 A, B). Highest catches were recorded in traps baited with PE bag or PP vial dispensers,

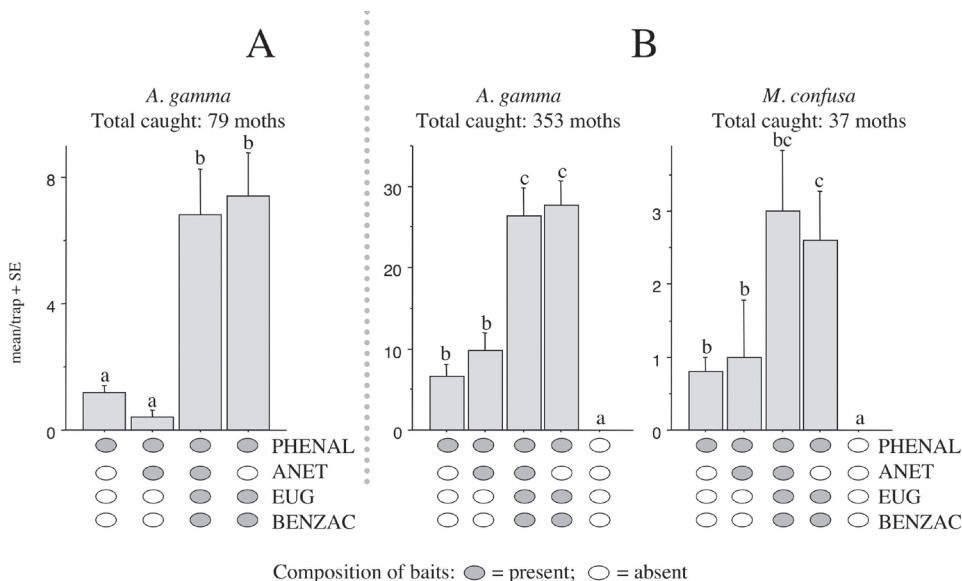


Fig. 1. Mean/trap catches of *Autographa gamma* and *MacDunnoughia confusa* in traps baited with phenylacetaldehyde (PHENAL), and its combinations with (*E*)-anethol (ANET), eugenol (EUG) and benzyl acetate (BENZAC) in Exp. 1. A = Hódmezővásárhely, B = Dömsöd. Columns with same letters within one diagram are not significantly different at $P = 5\%$ by Kruskal–Wallis, followed by pairwise comparisons with Mann–Whitney U test

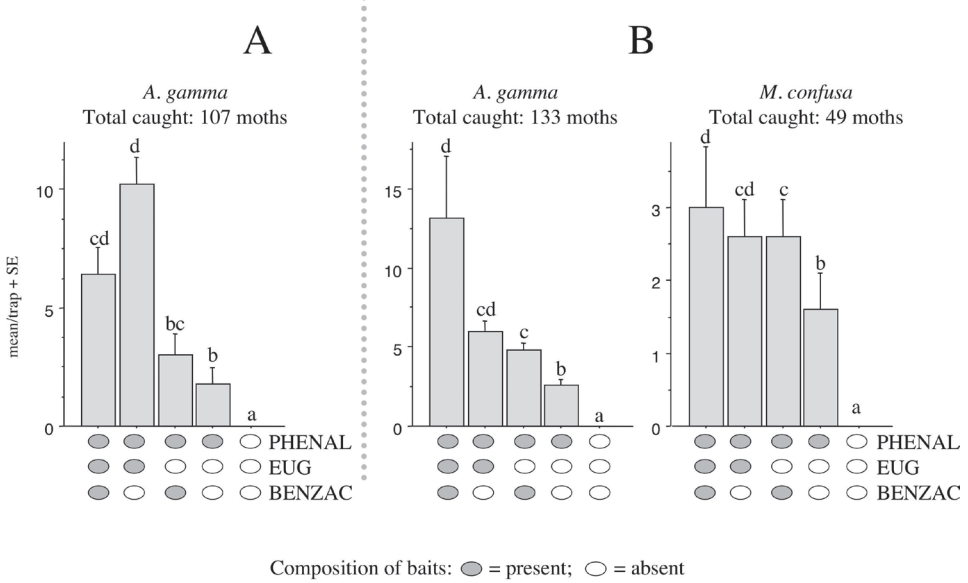


Fig. 2. Mean/trap catches of *Autographa gamma* and *MacDunnoughia confusa* in traps baited with phenylacetaldehyde (PHENAL), and its binary and ternary combinations with eugenol (EUG) and benzyl acetate (BENZAC) in Exp. 2. A = Agárd, B = Berettyóújfalu. For significance refer to Fig. 1

while those with PE vial dispensers caught significantly less. In this experiment only sporadic catches of *M. confusa* were recorded: a total of 3 or 5, 2 or 5, and 4 and 2 specimens (for PE bag, PP vial and PE vial, resp., at the sites Szeged and Vésztő).

In Exp. 4, all baited treatments caught significantly more *A. californica* than unbaited controls (Fig. 4). For this species a trend was observed of binary and ternary

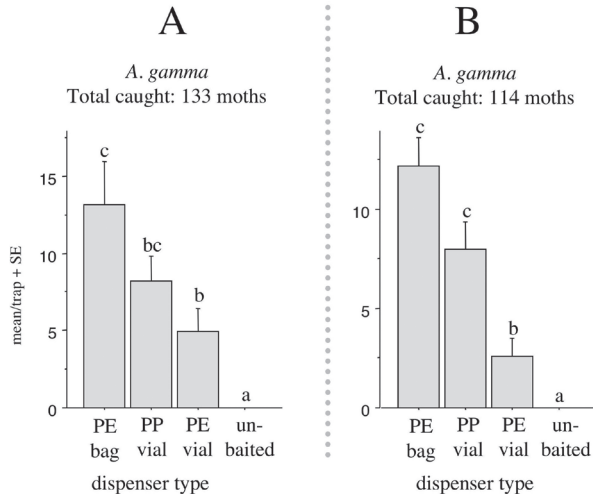


Fig. 3. Mean/trap catches of *Autographa gamma* in traps baited with the ternary combination of phenylacetaldehyde, eugenol and benzyl acetate in three different types of dispensers in Exp. 3. A = Szeged, B = Vésztő. For significance refer to Fig. 1.

Table 1

Mean/trap (\pm SE) catches of *Autographa gamma* in traps baited with the synthetic pheromone or with the bisexual lure in Exp. 5. in two consecutive years

Treatment	Mean/trap (+ SE)	
	2015	2016
Pheromone lure	280.0 \pm 28.2	205.8 \pm 38.8
Bisexual lure	96.5 \pm 16.0	42.0 \pm 4.0
P value (Mann-Whitney)	0.021	0.021
% of females (in bisexual lure)	45.3%*	73.2%
Total caught in test	1506	991

* based on 108 individuals, randomly selected from catch.

combinations catching more than PHENAL only (the difference being significant in PHENAL + BENZAC and the ternary blend). Of traps baited with the single compounds, BENZAC was catching less than PHENAL or EUG.

Catches of *X. c-nigrum* showed a trend similar to that of *A. californica*, with all baited treatments catching more than unbaited controls (Fig. 4). In *X. c-nigrum* significantly higher catches were recorded in traps with the ternary mixture than in all other binary or single compound baits. Again, traps baited with BENZAC caught less than all other baited treatments.

When comparing the performance of the ternary lure with that of synthetic *A. gamma* pheromone in Exp. 5, traps with the ternary lure caught significantly less (34.5% and 20.4% of mean/trap, in 2015 and 2016, resp.) of the moths caught in traps with synthetic pheromone, however, a sizeable proportion of the moths in the ternary lure were females (Table 1). Only males were caught in traps with the pheromone.

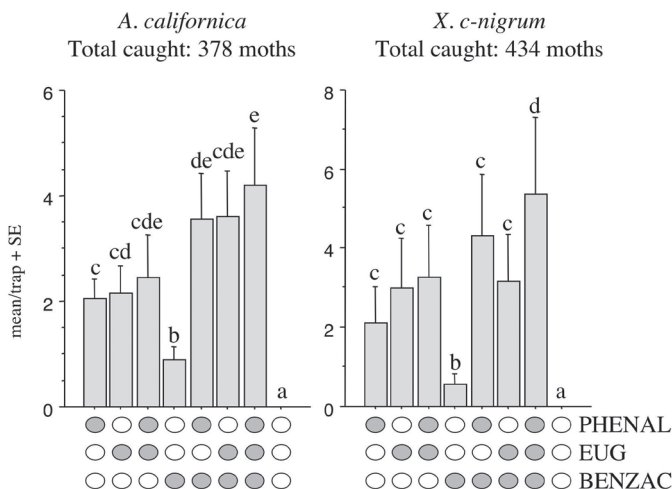


Fig. 4. Mean/trap catches of *Autographa californica* and *Xestia c-nigrum* in traps baited with phenylacetaldehyde (PHENAL), and its binary and ternary combinations with eugenol (EUG) and benzyl acetate (BENZAC) in Exp. 4. For significance refer to Fig. 1

Discussion

In the present study we confirmed field activity of PHENAL on *A. gamma*, as reported in earlier literature (Burgio and Maini, 1994; Plepys et al, 2002a,b; Tóth et al., 2010). Further, we confirmed that the addition of EUG and BENZAC to PHENAL resulted in increased catches of *A. gamma*: traps with the ternary lure invariably caught 2-6 times more than catches with PHENAL only.

When testing several dispenser types (Exp. 3), *A. gamma* showed a clear tendency to come in greater numbers to the types with supposedly higher release rates, PE bag and PP vial. In this respect *A. gamma* appeared to be similar to the closely related *A. californica*, which also preferred the highest release rate of a PHENAL lure (Landolt et al., 2001)

We expect that the level of catches in traps with the ternary blend in PE bag dispensers will be more reliable and already will reach levels suitable for detection and monitoring purposes. This idea is supported by the relatively high performance (20 to 34% of moths caught) of the ternary blend vs. synthetic pheromone, the latter which is the most powerful and sensitive detection tool for this pest species at present.

As opposed to traps with the pheromone (which caught only males), many females were caught in traps with the ternary blend. Sex ratios in ternary baited traps may have mirrored the natural sex ratio of the local populations present at the time of trapping. Future studies on whether the female percentages in the catch represent the actual sex ratio or are biased for some reason should be further studied for better management of *A. gamma*.

The fact that the new lure catches females in addition to males is of great importance, as protandry is a phenomenon occurring frequently in insects (Muralimohan and Srinivasa, 2008). Protandry should be easily observed with traps baited with a lure attractive to both males and females. The difference of the flight of females vs. males may have great significance in pest management. Timing of insecticide sprays to the flight of females could be more precise as it likely correlates better to the egg laying than would the catch patterns of males in pheromone traps, as shown with the codling moth *Cydia pomonella* L. (Knight and Light, 2004, 2005).

It appeared in the present study that of the two compounds evaluated with PHENAL, EUG had a more pronounced effect than BENZAC. However, further detailed studies are needed to unquestionably clarify the relative importance of EUG vs. BENZAC in the ternary blend. Both compounds are widespread odorants present in floral scent of many flowers, which may include plant species *A. gamma* adults feed on. To our knowledge BENZAC was shown to attract *A. californica* (Landolt et al., 2001) and *Trichoplusia ni* (Heath et al., 1992).

Apart from PHENAL, the attraction of *A. gamma* to the single compound methyl salicylate has been reported (Molleman et al., 1997). Despite the fact that we observed no or negligible attraction by *A. gamma* in many field tests in Hungary in which lures containing methyl salicylate were present (M. Tóth et al., unpublished), it may be advisable to investigate in the future whether combining methyl salicylate with the ternary lure described in this study yields any advantages.

It is noteworthy that the ternary blend described in the present study also worked well for the closely related plusiin *M. confusa*, catching 2-3 times more than PHENAL

only. Thus, this combination could form a starting point in the future for the development of a powerful bisexual attractant for *M. confusa* as well. The species is present in all Europe, and larvae are known to occasionally cause damage in the autumn, predominantly to ornamental plants (Balachowsky, 1972).

Results of tests of the present study on *A. californica* suggest that the addition of EUG and/or BENZAC could be worthwhile to study as bisexual attractants for additional species of pest plusiinae in North America as well. The ternary blend caught the greatest number of *A. californica* numerically, although in this case the binary combinations also performed well. Previous studies reported on several bisexual lure combinations for *A. californica*. Thus, when presented alone, PHENAL was strongly and BENZAC only weakly attractive, while catches were enhanced when PHENAL was presented with *cis*-jasmone or when BENZAC was presented along with benzaldehyde (Landolt et al., 2001). In another study more *A. californica* were caught when β -myrcene was presented with PHENAL or with BENZAC, compared with the compounds alone (Landolt et al., 2006). In a further study PHENAL and acetic acid presented together made a stronger lure than either compound alone (Landolt et al., 2013).

It may be well worth investigating the effect of combining EUG and/or BENZAC with these published attractants, in the hope of obtaining a more powerful lure for this important pest species in North America, as well as other general pest plusiinae such as *Trichoplusia ni* and *Anagrapha falcifera*.

The ternary blend described in this study may also be useful in the development of a bisexual lure for *X. c-nigrum*, as it caught significantly more than PHENAL only in the test performed in the USA. In earlier experiments conducted in Europe, more *X. c-nigrum* (both sexes) were caught in traps with the lure containing iso-amyl alcohol + acetic acid + isobutanol than in traps with PHENAL only (Tóth et al., 2010). This polyphagous noctuid pest has a cosmopolitan distribution, including Eurasia, North and South America and also some parts of Africa (Balachowsky, 1972).

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