












Asian corn borer (*Ostrinia furnacalis* Gn., Lepidoptera: Crambidae): Attraction to a bisexual lure and comparison of performance with synthetic sex pheromone

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ABSTRACT

Host plant-derived semiochemicals are becoming the most promising attractants to lure corn borers to traps in the field. Following success with the European corn borer (*Ostrinia nubilalis*), a two-component blend bisexual lure (phenylacetaldehyde and 2-(4-methoxyphenyl)ethanol) of the host plant odor was

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tested in wind tunnel and field trapping experiments on the Asian corn borer (*Ostrinia furnacalis*) (ACB). To be able to compare the lure's performance with synthetic pheromone, a new route for the sex pheromone components (*Z*)-12-tetradecenyl acetate and (*E*)-12-tetradecenyl acetate was also developed, and the biological activity of the products was confirmed. The bisexual lure attracted both males and females of ACB in laboratory wind tunnel, and also in the field. Field trapping results indicated that traps with the bisexual lure attracted somewhat more ACB (both sexes) than pheromone baited traps, but this indication needs further confirmation. Traps baited with the bisexual lure may offer a new tool for monitoring ACB for practical purposes.

KEYWORDS

wind tunnel, field trapping, 2-phenylacetaldehyde, 2-(4-methoxyphenyl)ethanol, bisexual lure

INTRODUCTION

The Asian corn borer (ACB), *Ostrinia furnacalis* (Guenée), is the main pest of corn, *Zea mays* (L.), in East and Southeast Asia, including the Far East of Russia (Mutuura and Monroe, 1970). In addition to their great economic impact due to the decreased yield of many crops, representatives of the genus *Ostrinia* denote an eminent model of speciation and evolution of chemical communication (Lassance, 2010). The ACB is vicarious to the European corn borer (ECB), *Ostrinia nubilalis* (Hübner) and the two species share corn and sorghum as host plants (Ishikawa et al., 1999), but differ in dicotyledon hosts (He et al., 2006; Bourguet et al., 2014). Although these species were considered to be mostly allopatric at the Eurasian scale, with the ECB occurring in Europe and the ACB in Asia (Mutuura and Monroe, 1970), there are a few areas of sympatry in Russia and China (Wang et al., 2017). As a nocturnal insect that mates and oviposits at scotophase, the behavior of the ACB mostly relies on olfactory cues.

The ACB and ECB are believed to originate from the same parent progenitor, similar to the adzuki bean borer, *Ostrinia scapularis* (Walker), and evolved independently in Europe and Asia (Wang et al., 2017), adapting to corn feeding after the hostplant's introduction into Europe (Tenailon and Charcosset, 2011) and China (Ho, 1955) ca. 500 years ago. They convergently share some features related to their host agrotechnologies, such as positive geotaxis (Calcagno et al., 2017) and resistance to the toxins and antifeedants of maize (Campos et al., 1989; Kojima et al., 2010; Phuong et al., 2016). Screening for the first appearance of an insect of interest in an area in question provides a strong foundation for the selection of crop protection practices. Moth trapping in the field is an important step in planning and timing efficient crop protection practices (Smart et al., 2014).

Pheromone traps provide some estimate of pest abundance, but they attract only one sex, the males, for the species with female-produced sex pheromones, leaving female numbers as a matter of calculations and modeling. The correlation between pheromone trap captures and subsequent infestation has been reported in moths often (Ngollo et al., 2000; Mori et al., 2014; Ferracini et al., 2020). Nevertheless, other studies failed to draw the final level of infestation and yield losses from pheromone monitoring results (Jones et al., 2009; Miluch et al., 2013). Some studies report low catches of the ECB by pheromone



traps despite high catches by light traps obtained in the same area (Bereś, 2012; Cizej and Trematerra, 2017).

Females, especially mated females, ovipositing to the crop of interest, are directly related to the damage and losses in crop production. Host-derived semiochemicals attract not only mated females but also virgin females and males using the host plant as a mating site (Cantelo and Jacobson, 1979; Visser, 1986; Light et al., 2001). Thus, hostplant-derived lures may provide promising tools for monitoring pest insects in agro-ecosystems. Lures containing synthetic plant-derived compounds are generally called “bisexual lures,” as they attract both females and males, as opposed to pheromone lures, which in the case of most moths attract only males (Tóth et al., 2017; Nagy et al., 2021; Preti et al., 2021a, 2021b).

Observations made in the United States showed that small (~100–150 m²) areas covered with low (0.5–1.0 m high) and dense vegetation, mainly cereal vegetation, which are localized near cornfields, are selected by the ECB for mating as so-called “action sites” (Showers et al., 1976; DeRozari et al., 1977; Sappington and Showers, 1983; Sappington, 2005; Reardon et al., 2006). Analysis of the spatial distribution of the ECB in Europe (North Caucasus) revealed a fundamentally similar pattern of mating aggregations (Frolov and Trishkin, 1992). Thus, mating of the ECB is preceded by the concentration of the insect in small areas, where mating then occurs. Fewer results on the breeding biology of the ACB are available, but the situation is likely similar (Wang et al., 1994). It is obvious that the search for action sites begins with the discovery of cornfields, near which places for mating are sought. Because of crop rotations, the distance between overwintering sites (last year’s cornfields) and egg-laying sites (this year’s cornfields) can be quite significant.

Carefully assembled hostplant kairomone blends are highly potent lures for pest insects because their key components differ even for insects feeding on the same plant (Bruce and Pickett, 2011). A recently discovered bisexual lure attractant mixture composed of 2-phenylacetaldehyde (PAA) and 2-(4-methoxyphenyl)ethanol (4METH), previously identified as present in corn plant volatiles (Hammack, 1996), was successfully tested and characterized in field experiments with the ECB (Tóth et al., 2016).

The weak attractiveness of the sex pheromone in comparison to this recently described bisexual lure (Tóth et al., 2016; consisting of a PAA and 4METH blend) for the ECB was shown out in a study carried out in five European countries (Tóth et al., 2017).

Owing to the close taxonomic relatedness and similarity in feeding habits of the ACB and ECB, this study aimed to test the performance of the ECB bisexual lure for the ACB in controlled laboratory conditions and in field trapping experiments and to compare its performance to that of the synthetic ACB pheromone. A modified synthetic route to the ACB pheromone components was also developed.

MATERIAL AND METHODS

Insects

The laboratory culture of *O. furnacalis* was started from eggs provided by the China Institute for Plant Protection, Beijing, China. The year-round culture was kept inside a custom-built environmental chamber under a 16:8 photoregime and 25 °C (Frolov et al., 2019). Adults aged 3–5 days were tested in the wind tunnel.



Attractants

Two compounds, (*Z*)-12-tetradecenyl acetate (*Z*-12-14Ac) and (*E*)-12-tetradecenyl acetate (*E*-12-14Ac), have previously been identified in the sex pheromone of *O. furnacalis* (Klun et al., 1980; Ando et al., 1980).

The ACB sex pheromone was synthesized as follows (Fig. 1): propargyl alcohol was alkylated in liquid ammonia with 1-bromodecane to 2-tridecin-1-ol, which was then isomerized to 12-tridecin-1-ol by sodium amide in ethylenediamine. Alkylation of 12-tridecin-1-ol tetrahydropyranyl ether in liquid ammonia with iodomethane afforded 12-tetradecin-1-ol, the subsequent hydrolysis of which led to 12-tetradecin-1-ol. This acetylenic alcohol is converted to (*Z*)-12-tetradecene-1-ol by hydrogenation over a nickel boride catalyst. The first component of the pheromone was obtained by the acetylation of (*Z*)-12-tetradecene-1-ol. Aluminum hydride

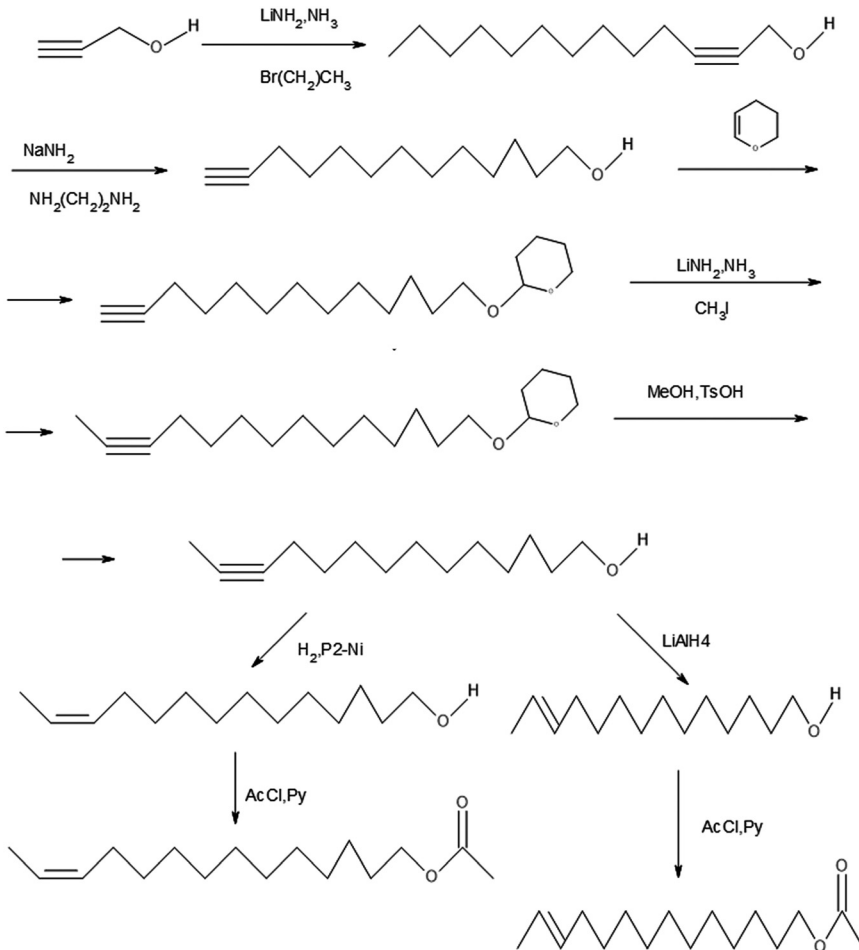


Fig. 1. Synthesis scheme of (*Z*)-12-tetradecenyl acetate and (*E*)-12-tetradecenyl acetate



reduction of 12-tetradecin-1-ol followed by acetylation of the resulting *E*-alcohol gives (*E*)-12-tetradecene-1-ol acetate, the second component of the pheromone of the ACB (Klun et al., 1980; Li and Schwarz, 1984; Kang et al., 1985). The structure of the obtained substances was supported by infrared spectroscopy and proton nuclear magnetic resonance spectroscopy. The purity of (*Z*)-12-14Ac was 95.9%, and that of (*E*)-12-14Ac was 97.2%, as determined by gas chromatography-mass spectrometry.

A 1:1 mixture of (*Z*)-12-14Ac and (*E*)-12-14Ac (ZE) (Klun et al., 1980) was used for field trapping and wind tunnel experiments. Dispensers for the field traps, bromobutyl rubber stoppers, were loaded with 1 mg of the pheromone blend and packed in multi-layer aluminum-plastic bags, which were stored in a freezer (-18°C) until use.

Pheromone blends in 3 doses of 10^{-7} , 10^{-6} and 10^{-5} mg were prepared in the laboratory from 10^{-2} g mL $^{-1}$ stock solution by tenfold serial dilutions in hexane (Lenreaktiv, CAS:110-54-3, Russia) and kept before use in the freezer at -18°C . Ten microliters of each solution was applied to the dispensers made of cigarette filter (2 mm thick transverse cuts) and dried in air until solvent evaporation to present to the moths in wind-tunnel testing. Pheromone gland extract was prepared by gently squeezing the female abdomens until the appearance of a drop of a liquid, after which the abdomen tip was cut with fine scissors and stored in hexane. This procedure was repeated several times to obtain a stock solution. Three female equivalent dosages were used as a lure in wind-tunnel experiments, which roughly corresponded to the 10^{-5} mg blend (Kou et al., 1992; Huang et al., 1998).

Bisexual lure dispensers (Tóth et al., 2017) for field trapping were composed of 100 mg of each component: PAA and 4METH applied to cotton dental roll (Celluron, Paul Hartmann AG, Heidenheim, Germany). In wind tunnel studies a 33 mg dose of the 1:1 PAA:4METH blend was used.

Wind tunnel

Only virgin moths of both sexes were used in laboratory tests.

Testing was performed as described previously (Shchenikova et al., 2020) under the following laboratory conditions: the dark period of zeitgeber time under 10 lx infrared light, ambient temperature of 21–23 $^{\circ}\text{C}$, and relative humidity of 75–80%. An airstream ($0.2\text{--}0.3\text{ m s}^{-1}$) was blown along the plexiglass tube with a diameter of 400 mm. The platform to release insects was placed 800 mm downwind from the odor dispenser. An ACB moth inside a glass Petri dish was placed on the releasing platform 5 min before the trial. The dish was opened at the same time as the dispenser was introduced into the tunnel. The proportion of individuals taking flight, upwind or downwind flight, and source contact, as well as the latent period of the flight reaction, were evaluated. Four series of experiments were conducted.

1. Control, the dispenser was filled with 10 μL of hexane, which was allowed to fully evaporate ($n = 52$; 33 males and 19 females); only data of 33 males were used for comparison with pheromone responses.
2. Synthetic pheromone blend of *O. furnacalis* at three doses of 10^{-7} ($n = 61$), 10^{-6} ($n = 60$), and 10^{-5} mg ($n = 63$) was tested on males.
3. Female pheromone gland extract in the dose of 3 female-equivalent was tested on males ($n = 62$).
4. Bisexual lure was tested on 22 females and 39 males ($n = 61$).



Field trapping

Field trapping was performed using the funnel traps CSALOMON[®] VARL (Tóth et al., 2017, <http://www.csalomontraps.com>). Three corn field locations were used to test bisexual lure versus synthetic pheromone and unbaited control traps during the 2019 and 2020 summer seasons: Khabarovsk Territory, near Khabarovsk, the Maritime Territory (Primorsky Krai), Ussurijsk District, settlement Timiryazevsky, and the Amur region, Tambovsky District, near vil. Sado-voe (Fig. 2).

Traps were supplied with insecticides (pieces of dog antiflea collars) to kill captured moths. Data for the Amur region were obtained only in the season of 2020; the experiment in 2019 failed because of heavy flooding in the area. To obtain more data on the comparison of the performance of pheromone versus the bisexual lure in the field, in 2020, all available CSA-LOMON[®] VARL devices were baited with pheromone or bisexual lure (no unbaited controls).

Traps were set up in randomized blocks of unbaited, bisexual lure, and sex pheromones spaced 30 m apart at about 1.5 m height above the ground. Inside blocks, traps were 5–10 m apart, depending on the local conditions (Fig. 3). Traps were initially mounted 50 cm above the ground and moved higher to the level of corn ear as the plants grew.

The traps were checked daily until the first appearance of moths in traps (to determine starting date of flight) and once two weeks later. The exact day of the trap examination sometimes varied slightly depending on the weather conditions. The dispensers were changed monthly. Field-trapped dry moths were preserved in a layer of cotton wool and transported to the All-Russian Institute of Plant Protection, Saint Petersburg, Russia, to check the correctness of species determination in the laboratory (performed by A.N. Frolov).

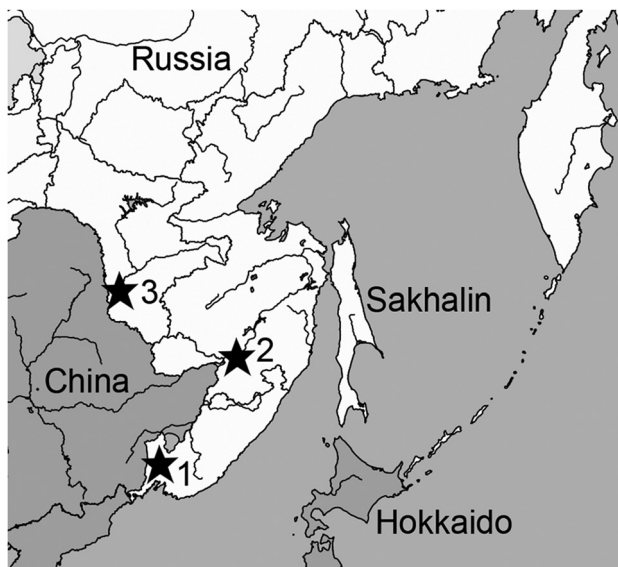


Fig. 2. Locations of field trapping tests. 1 - the Maritime Territory (Primorsky Krai) 43°51'31.8"N 131°57'30.7"E; 2 - Khabarovsk Territory, near city Khabarovsk, settlement Timiryazevsky 48°29'00.69"N 135°15'06.88"E; 3 - Amur region, Tambovsky District, near vil. Sadovoe 50°09'53.5"N 127°51'41.8"E



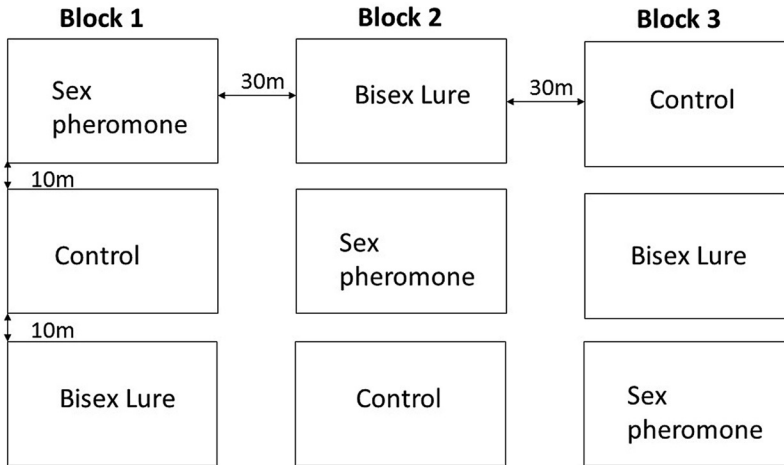


Fig. 3. Layout of traps in Khabarovsk and Ussurijsk areas. In Amur region only bisexual lure and sex pheromone baited traps were arranged in staggered formations

Data processing and statistics

Chi-square and z -test statistics were used to evaluate the data. To compare the attractiveness of pheromone versus bisexual lure traps, the results of field trappings were processed as follows: for each trap, the numbers of moths caught were summed over the season. Data were analyzed using the Student's t -test in the case of normally distributed data; if otherwise, the Wilcoxon signed-rank test or Mann–Whitney U -test were used. The Chi-square test was used to compare the frequencies. Statistical computations were conducted using PAST 4.03 (Hammer et al., 2001) and VassarStats online calculators: <http://vassarstats.net/>.

RESULTS

Laboratory experiments evaluating the ACB responses to synthetic sex pheromone blends and bisexual lures were performed before starting the field tests.

Wind tunnel

Wind tunnel testing showed a strong attraction of males to pheromone stimuli (Fig. 4A). In the control group of 33 male moths, only two of them took flight, but the direction was downwind, that is, away from the bait (Fig. 4A). Dose-response relationships showed a weak increase in the proportion of males taking flight, but they were statistically insignificant ($\chi^2 = 3.1987$, $df = 2$, $P = 0.2020$). A significant decrease in the latent period of the flight reaction with pheromone dose ($P < 0.05$, Student's t -test) was observed between the doses of 10^{-7} and 10^{-6} mg of the pheromone blend and did not change with further dose increase (10^{-5} mg) (Fig. 4B). The responses of males to the female pheromone gland extract were



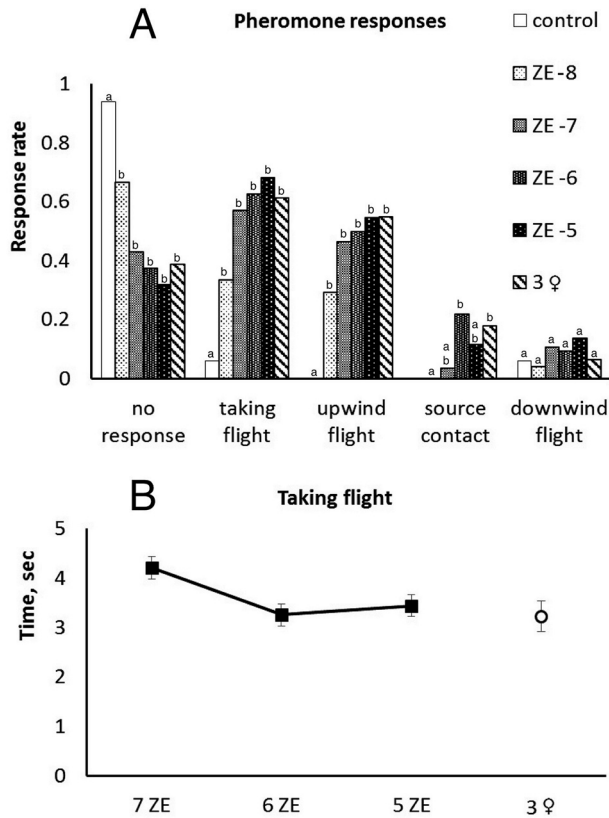


Fig. 4. Responses of male moths to different pheromone doses and female extract in the wind tunnel. A: the proportion of males showing the respective behaviors; B: latent period of taking flight responses. X-axis shows -lg (dose, mg) of synthetic pheromone blend, 3 ♀ = 3 female equivalent of pheromone gland extract. Columns with same lowercase letter within the same behavior are not significantly different at $P = 5\%$ by Chi square test

comparable with those to the synthetic pheromone blend of 10^{-6} and 10^{-5} mg on the dispenser (Fig. 4B).

The bisexual lure presented in the wind tunnel elicited responses in both male and female moths (Fig. 5). There were no significant differences between sexes in the frequency of taking flight or in upwind flight or source contacts. The latent period for the taking flight reaction was 4.8 ± 0.5 s in males and 4.7 ± 0.4 s in females. For all the responding moths, upwind flight was observed in 81% of the moths, and 44% reached the source of the odor.

In the control experiments, 16 of 19 females did not respond to the stimulus, and three moths took flight, two of which were downwind and one upwind (Fig. 5). Taking together the data obtained for males and females, more than 90% of moths did not respond to the control stimulus, and only one insect (1.9%) showed upwind flight.



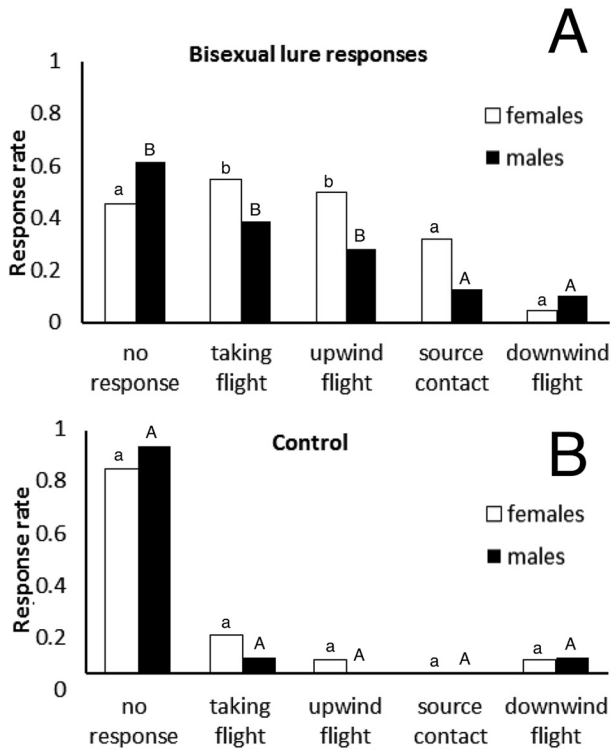


Fig. 5. Responses of male and female moths to bisexual lure in the wind tunnel. A: responses to bisexual lure; B: responses to control stimulus. Columns with same lowercase (females) and uppercase (males) letter within the same behavior are not significantly different at $P = 5\%$ by Chi square test

Field trapping

Field trapping resulted in catches of 95 *O. furnacalis* specimens, of which 71 moths were found in traps with bisexual lure, 19 moths (18 males and 1 female) in pheromone-baited traps, and five in unbaited control traps. The difference with the uniform distribution was statistically significant ($\chi^2 = 76.37$, $df = 2$, $P < 0.001$). Statistical analysis of block data revealed that traps with the bisexual lure caught more than pheromone-baited traps (Mann–Whitney U -test; $P < 0.01$) as well as control traps (Wilcoxon signed-rank test; $P < 0.001$) (Fig. 6, Table 1). Although pheromone traps seemed to catch numerically more than the unbaited controls (Fig. 6), the difference was statistically non-significant (Wilcoxon signed-rank test; $P > 0.05$).

Overall, males were trapped more often than females (69 vs. 26; $\chi^2 = 18.56$, $df = 1$; $P < 0.001$). Fisher's exact test calculations showed a significant preference for traps with bisexual lure over others ($P = 0.017$) for females, whereas males were trapped more often than females in traps with sex pheromones ($P = 0.009$).

Although no formal damage level measurements were done on the test sites, the fact that no damages were observed at any of the sites suggests that the population level of ACB was very low on the experimental sites.



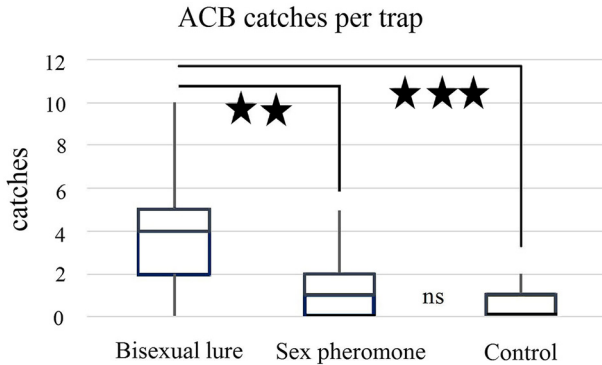


Fig. 6. Catches of *O. furnacalis* in traps baited with bisexual lure, with synthetic pheromone or unbaited. Boxplots show median, upper and lower quartiles, min and max of the samples. For control traps low quartile and median are equal to zero. Statistical differences: ** – $P < 0.01$; *** – $P < 0.001$, Mann–Whitney U -test

Table 1. Catches in blocks

Locality, year	Block	Bisex lure	Sex pheromone	Control
Khabarovsk Territory, 2019	1	7	0	0
	2	10	1	0
	3	0	1	1
The Maritime Territory, 2019	1	5	0	0
	2	1	0	0
	3	1	0	0
Khabarovsk Territory, 2020	1	3	0	1
	2	9	0	2
	3	10	1	1
The Maritime Territory, 2020	1	2	1	0
	2	2	0	0
	3	1	0	0
Amur region, 2020	1	4	2	–
	2	4	3	–
	3	5	5	–
	4	4	2	–
	5	3	3	–
Average		4.176470588	1.117647059	0.416666667
N		17	17	12

DISCUSSION

While the monitoring of pest insects with pheromone traps is in use for a great number of moth species (Witzgall et al., 2010; Prasad and Prabhakar, 2012), other semiochemicals have also been used for this purpose (Judd et al., 2017; Preti et al., 2021a, 2021b). The effectiveness of either lure should be tested separately for each species to choose the best formulation for agricultural use.



Laboratory wind-tunnel testing in the present study clearly indicated the attractiveness of the synthetic sex pheromone blend for male ACB moths, confirming the success of the novel route for its synthesis.

The high attractiveness of the pheromone blend in the wind tunnel (almost 70% of males responded to the pheromone stimuli) cannot directly be correlated with the number of males caught by pheromone traps in the field, which caught about 2.5 times fewer males than the bisexual lure. On the other hand, in the tunnel, males responded better to pheromones than to bisexual lures (55% vs. 28%, upwind flight, Fig. 4A and 5A). The inferior performance of pheromone traps in the field can be explained not only by the concurrent attraction of virgin males by calling females (Unnithan and Saxena, 1991; Kondo et al., 1993; Evenden et al., 2015; Frolov et al., 2020a, 2020b) but also by the fact that male moths temporarily lose or decrease the sensitivity to sex pheromones after mating (Gadenne et al., 2001; Fischer and King, 2008; Barrozo et al., 2011).

The attractiveness of the bisexual lure in the wind tunnel was not very high, with approximately 36% of moths responding, but worked roughly equally for both males and females. The number of females responding to the bisexual lure in the wind tunnel was relatively low, probably because only virgin females were used. The responses of mated females searching for oviposition sites are likely to be better (Anton et al., 2007; Saveer et al., 2012; Lemmen-Lechelt et al., 2018). The age of the highest responsiveness for males and females is yet unknown. Our preliminary data show that the best responses to the bisexual lure occur in younger virgin moths of both sexes (mated females were not tested) (Fig. 7). We have no data on the mating status of field-collected females in this study either.

In conclusion, both wind tunnel and field trapping data from the present study strongly suggest that the bisexual lure was attractive to ACB, and in this respect ACB appears to be similar to ECB (Tóth et al., 2016).

Although synthetic pheromone lures are widely used for ECB monitoring (Pélozuelo and Frérot, 2007), including in Europe (Kárpáti et al., 2016), there are indications that they can sometimes show unreliable trapping activity (Szócs and Babendreier, 2011). In field tests in Europe, the bisexual lure clearly surpassed the activity of synthetic ECB pheromones (both Z and E pheromonal strains) (Tóth et al., 2017; Frolov et al., 2020a, 2020b).

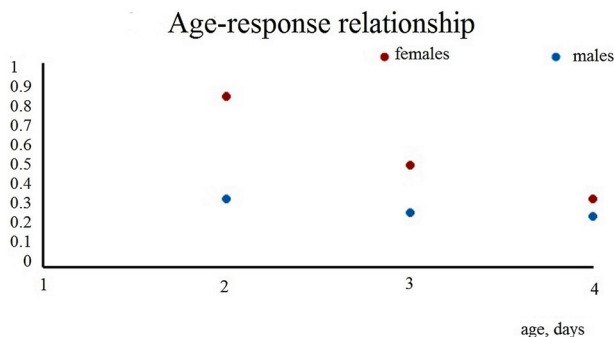


Fig. 7. Wind tunnel responses of virgin *O. furnacalis* males or females of different ages to the bisexual lure (mated females were not tested)



In the present study trapping data suggest that the bisexual lure may work better for catching ACB than the synthetic pheromone lure, making the case similar to that observed for ECB in Europe, however, due to the relatively low numbers captured this finding needs more detailed studies to be conducted in the future.

In any case, the bisexual lure appears to be a useful new addition to trapping tools already available to detect and monitor ACB. It definitely is a great advantage over the synthetic pheromone that traps with bisexual lure can be used for sampling the number of females in the population. Most likely, insecticide sprays timed to the flight pattern of females, instead of that of males, can be more precise and correlate better with egg-laying, rendering the sprays more effective (Knight and Light, 2005). A lure attracting both females and males can be used apart from monitoring as direct pest control, that is, in lure-and-kill techniques (Landolt et al., 1991; Camelo et al., 2007). The development of such control approaches against the ACB could provide an alternative to insecticide sprays, reducing both the amount of pesticide used and pesticide content in the crop.

Since the bisexual lure tested in this study was effective in attracting both the ACB and ECB, it would be tempting to hypothesize that it could also be active for other related pests or non-pest moths. Our preliminary data suggest that adzuki bean borer *O. scapulalis* antennae are sensitive to the main odorous compounds of corn PAA and 4METH, although their sensitivity is substantially lower than that of *O. nubilalis* feeding on maize (Shchenikova et al., 2020).

Besides the advantages of using plant-derived attractants, there are some possible disadvantages due to lower species specificity.

In Hungary, the bisexual lure was found to attract, in addition to the target ECB, the pyralid *Haritala (Pleuroptya) ruralis* (Scop.), which lives on nettle as a host plant (Nagy et al., 2019). Furthermore, some catches of the noctuids *Autographa gamma* (L.), *Macdunnoughia confusa* (Steph.), *Helicoverpa armigera* (Hbn.), and *Abrostola* spp. have also been reported. To diminish this problem, ACB bisexual traps were placed in the present study far inside from the field borders and lifted up when the plant would rise. Similar methods could greatly improve the percentage of target versus non-target insects in trappings for ECB in Europe (Nagy et al., 2019). In the Far Eastern tests of the present study the bisexual lure attracted a rather small number of honey bees, syrphid flies and butterflies which can be easily distinguished from ACB.

Conflicts of interest: The authors declare no conflict of interest.

Author contributions: A. F. and M. T. conceived the research. Authors A. F., A. S., O. S., I. G., M. Z., A. K., E. L., D. K., and V. K. conducted experiments. Author N. F. synthesized the pheromone components. Authors A. F., O. S., and M. Z. analyzed the data and conducted the statistical analyses. Author M. Z. wrote the original draft. Authors A. F. and M. T. edited the manuscript. Author A. F. secured funding. Author M. T. supplied the traps. All authors read and approved the manuscript.

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