

Bisexual lures and their comparison with synthetic sex attractants for trapping *Orthosia* species (Lepidoptera: Noctuidae)

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Abstract

Two bisexual lures have been developed using iso-amyl alcohol plus acetic acid combined with red wine extract (semisynthetic bisexual lure, SBL), or synthetic floral compounds (FLO) for trapping of noctuid moths with early spring activity of adults. *Orthosia* species were attracted by the tested semi-synthetic and synthetic lures indicating the possible role of the fermenting liquids and floral compounds in the feeding of these species. Bisexual lures attracted generally a lower number of individuals than the synthetic sex attractants of the given species, except for *O. cerasi* where FLO lures were more attractive than the sex attractant. Bisexual lures caught both sexes and females composed a sizable percentage of the catch. Since traps baited with different types of bisexual lures provided similar data on sex ratios, thus these presumably reflected the actual sex ratio of the given population. The use of these bisexual lures, especially of FLO in case of *Orthosia* spp., can be useful in surveys in which the capture of females and data on sex ratios are indispensable, for example in population dynamic and polyandry surveys. In contrast, in case of low population densities, the use of sex attractant lures seem to be still more efficient.

KEYWORDS

(E)-anethol, benzyl acetate, eugenol, floral bisexual lure, phenylacetaldehyde, volatile traps

1 | INTRODUCTION

The monitoring of population dynamics of pest species is essential in agriculture and forestry (Macgregor et al., 2019; Selikhovkin et al., 2018; Thomas, 2005). In the past decades, with the advent of sex pheromone research, synthetic sex pheromone or sex attractant-baited traps have been widely used for sampling of field populations of several hundreds of moth spp. (El-Sayed, 2020, <http://www.pherobase.com>), mainly for plant protection purposes.

However, since only one sex would respond to the stimulus of sex pheromones, such traps attract and capture only male moths. As a consequence, such traps can only provide biased and incomplete information on population trends. Therefore, research efforts have been recently increased, for the development of lures which would be appropriate to attract both males and females (Szanyi et al., 2017; Tóth et al., 2019, 2020).

In the trapping of Noctuidae species, an important breakthrough in the development of bisexual attractants was the characterization

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of iso-amyl alcohol and acetic acid originating from the identification of chemical components emanating from fermenting liquids (Landolt, 2000). These components merged in a synthetic lure were attractive to several noctuid species in North America (Landolt et al., 2007) and in Europe, as well (Tóth et al., 2010). In more recent studies in Hungary a more powerful semisynthetic bisexual lure (SBL) was developed, with the addition of red wine or red wine extract to iso-amyl alcohol plus acetic acid containing lures (Tóth et al., 2015). This new type of lures was capable to attract a great number of noctuid taxa in Hungary and Transcarpathia (the Ukraine), mainly species belonging to the subfamilies Acronictinae, Amphipyrinae, Xyleninae, Hadeninae and Noctuinae (Nagy et al., 2014, 2015; Szanyi et al., 2017).

Another type of noctuid feeding attractants originated from the old experience that the floral compound phenylacetaldehyde attracted many noctuid species to some extent (Cantelo & Jacobson, 1979; Creighton et al., 1973). The addition of some other synthetic floral compounds resulted in optimized bisexual attractants for numerous noctuids (Landolt et al., 2001, 2006; Nagy et al., 2021; Tóth et al., 2019, 2020). Based on these studies a four-component blend of floral compounds (FLO) was developed, consisting of phenylacetaldehyde, (*E*)-anethol, benzyl acetate and eugenol, which did attract significant numbers of mainly Plusiinae and Melicleptinae species (Nagy et al., 2014, 2015; Szalárdi et al., 2021; Szanyi et al., 2017).

The objective of this study was to test the efficiency and compare the performance of the above two bisexual lures with synthetic sex attractants on *Orthosia* species (Lepidoptera, Noctuidae, Hadeninae). *Orthosia* spp. are known as pests in orchards and forests, since their larvae feed on leaves of several forest and fruit trees (Bues et al., 1994, Cayrol, 1966). Thus, the detection and monitoring of *Orthosia* species would yield some practical significance in plant protection.

Adults of *Orthosia* species fly at early spring in Europe. Synthetic sex attractants have been characterized for *O. cruda* Den. & Schiff, *O. cerasi* Fabr. (= *stabilis* Den. & Schiff.), *O. incerta* Hufn., *O. gothica* L., and *Anorthoa* (*Orthosia*) *munda* Den. & Schiff. (Booij & Voerman, 1984; Tóth et al., 1992, 1993), and traps baited with these are used for trapping in Europe.

In a recent study, the SBL lure caught significantly less specimens of the *Orthosia* spp. than the respective sex attractants, although it caught regularly low numbers of both females and males (Szanyi et al., 2020). Since *Orthosia* are frequently observed to feed on catkins, the flowers of willow trees, it was thought to be worthwhile to test FLO, a phenylacetaldehyde-based floral attractant for this group of pests for better results.

Based on these preliminary surveys we targeted:

- to compare the efficiency of bisexual lures and respective sex attractants
- to test the attractivity of different lures on *Orthosia* spp. versus hibernated *Conistra* spp.

- to compare the biased vs. unbiased sex ratios in different lure types used
- to estimate the synchrony in sex ratios of different *Orthosia* spp.

2 | MATERIALS AND METHODS

2.1 | Field tests

Tests were conducted using tested methods in trapping experiments of the similar objectives (Roelofs & Cardé, 1977), at several sites in Hungary. Traps were arranged in a large circle, in random order, separated by 8–10 m. Traps were inspected at 3 or 4 days' intervals (preferably twice weekly), when captured insects were recorded and removed. At the same time, traps were moved one position forward in the circle to minimize positional effects.

As it was frequently found in field trapping tests, data did not fulfil statistical requirements for a parametric analysis, therefore data were analysed by the non-parametric Kruskal–Wallis test. If the Kruskal–Wallis test yielded significance ($p = 5\%$), then treatments were compared pairwise by the Mann–Whitney *U* test. All statistical procedures were conducted using the software packages StatView® v4.01 and SuperANOVA® v1.11 (Abacus Concepts).

2.2 | Traps

In the tests, funnel traps CSALOMON® VARL were used. These traps have routinely been used for trapping several noctuids (Tóth et al., 2010), including also *Orthosia* spp. (Szanyi et al., 2020); photos of the trap can be viewed at www.csalomontraps.com. For killing captured insects, a small piece (1×1 cm) of a household anti-moth insecticide strip (Chemotox® SaraLee, Temana Intl. Ltd; active ingredient 15% dichlorvos) was placed into the catch container of traps.

2.3 | Baits

The SBL lure tested was the same as described earlier (Szanyi et al., 2020; Tóth et al., 2015), with the active ingredients of iso-amyl alcohol, acetic acid and red wine (1:1:1).

The FLO lure contained synthetic phenylacetaldehyde, (*E*)-anethol, benzyl acetate and eugenol (1:1:1:1) (Szanyi et al., 2017).

Sex attractant lures were obtained commercially from the CSALOMON® trap family (PPI CAR ELKH), and with the same compositions as described previously (Booij & Voerman, 1984; Tóth et al., 1992, 1993): *O. cruda*: (Z)-11-hexadecenyl acetate plus (Z)-9-tetradecenyl acetate (20:1); *O. cerasi* (Z)-11-hexadecenal plus (Z)-9-tetradecenal (1:10); *O. incerta*: (Z)-11-hexadecenal plus (Z)-9-tetradecenal (100:1); *O. gothica*: (Z)-9-tetradecenyl acetate plus (Z)-9-tetradecen-1-ol plus (Z)-5-tetradecenyl acetate plus

(Z)-11-tetradecenyl acetate (20:20:1:1); *A. munda*: (Z)-11-hexadecen-1-ol plus (Z)-11-hexadecenal (20:1).

Exp. 2D: Julianna major, Budapest, February 27–April 15, 2020, mixed oak forest (Coordinates: 47.549012, 18,926,949).

2.4 | Experimental details

2.4.1 | Experiment 1

Treatments tested were the SBL lure, the FLO lure and the synthetic sex attractants of *O. cruda*, *O. cerasi*, *O. incerta*, *O. gothica*, *A. munda* and unbaited controls. From each treatment 4 traps (=replicans) were set out, giving a total number of 32 traps. The test was run from February 28 to April 11, 2019. The test was conducted at the Julianna major Experimental Station of the Plant Protection Institute of the Centre for Agricultural Research, Eötvös Loránd Research Network (PPI CAR ELKH, Budapest, Hungary), in a mixed oak forest (Coordinates: 47.549012, 18,926,949).

2.4.2 | Experiment 2

Treatments tested included the SBL lure, the FLO lure, the synthetic sex attractant of *O. cerasi* and unbaited traps. From each treatment 4 traps (=replicans) were set out, giving a total number of 16 traps. The test was run parallel at 4 sites:

Exp. 2A: Darvas, Hajdú-Bihar county, March 11–April 12, 2020, in the margin of a mixed oak forest (Coordinates: 47.130871, 21.320978).

Exp. 2B: Jánkmajtis, Szabolcs-Szatmár-Bereg county, March 1–April 18, 2020, in the margin of an oak forest (Coordinates: 47.934472, 22.683133).

Exp. 2C: Nyírbátor, Szabolcs-Szatmár-Bereg county, March 2–April 20, 2020, in the margin of a mixed willow-poplar forest (Coordinates: 47.829967, 22.167574).

3 | RESULTS

In the tests, the specimens of *Orthosia* spp. were the most abundant (ranging from 77% to 100%), with the exception of Exp. 2C (Figure 1), where the abundance of *Orthosia* (41%) was only second to that of *Conistra* spp. (59%). In this paper, we discuss results on *Orthosia* spp., while catches in detail, and results on *Conistra* spp. and other noctuids will be published elsewhere.

Orthosia cerasi was recorded in larger numbers at Exp.1, Exp. 2B and Exp. 2D (Figure 2). Traps baited with the *O. cerasi* sex attractant caught significantly more than unbaited traps, with the exception of Exp. 2B. The traps with the sex attractant caught only males. Both bisexual lures caught significantly more than unbaited. Most moths were recorded in FLO-baited traps, although the difference from SBL baited was significant only in one test (Exp. 2D), and from the sex attractant also in the same test (Exp. 2D). The low numbers of specimens caught in Exp. 2A and Exp. 2C were caught in FLO, SBL and the sex attractant, showing a similar tendency as larger catches did in the other experiments (Figure 2). Traps with the bisexual lures caught also many females, the female ratios in the catch ranged from 22% to 31% (FLO) and 27% to 37% in SBL. Female ratios of FLO and SBL within one test were remarkably similar (Figure 2). The remarkably higher abundance of *O. cerasi* in Exp. 1. did not have significant effect on sex ratio, comparing with Exp. 2B and 2D where lower abundances were observed.

Orthosia cruda was caught at Exp.1, Exp. 2B and Exp. 2D (Figure 2). In Exp. 1, the only test including the sex attractant of *O. cruda* it caught significantly more males than unbaited traps. Traps with the sex attractant caught only males. FLO- or SBL-baited traps caught significantly more specimens compared to the unbaited traps in all three experiments. FLO caught significantly more specimens than SBL in Exp. 1 and Exp. 2D, and caught similar mean numbers to

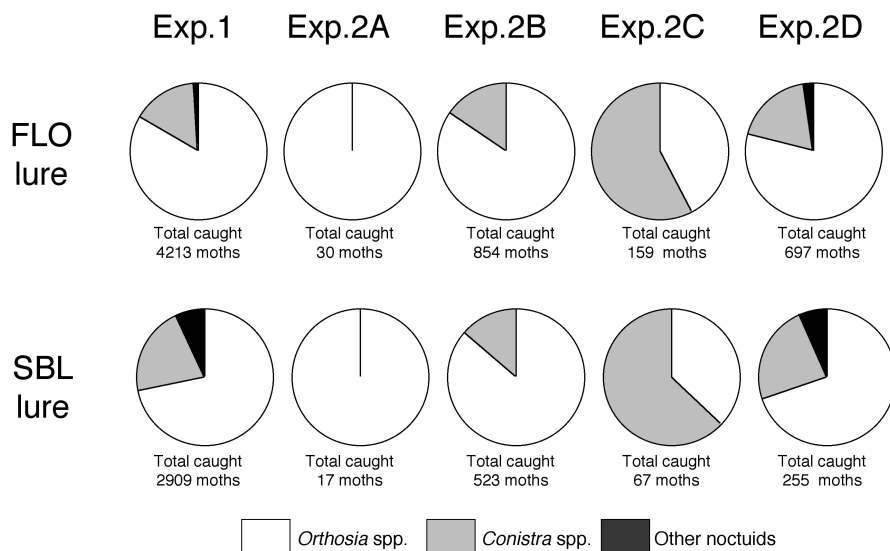


FIGURE 1 Abundance of noctuid specimens belonging to different taxa in the catch of the experiments

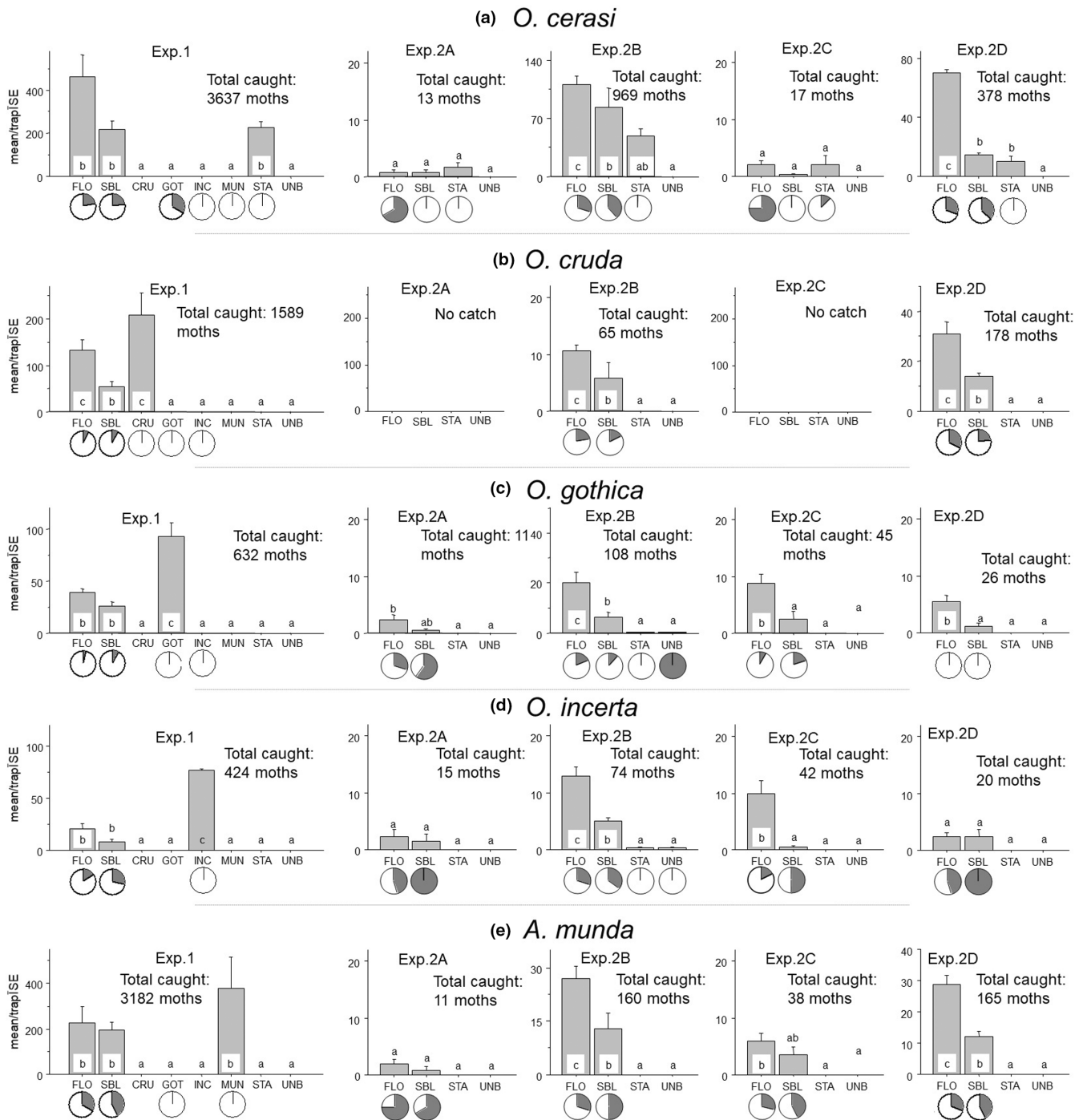


FIGURE 2 Mean/trap catches of our *Orthosia* species (a–e) in traps baited with bisexual lures FLO or SBL, with synthetic sex attractants (CRU = *O. cruda*, GOT = *O. gothica*, INC = *O. incerta*, MUN = *A. munda*, STA = *O. cerasi*) and in unbaited (UNB) traps. *O. cerasi* (=stabilis) (a): columns with same letter within one diagram not significantly different by Kruskal–Wallis test, followed by pairwise comparisons by Mann–Whitney *U* test ($P = 5\%$); *O. cruda* (b), *O. gothica* (c), *O. incerta* (d), *A. munda* (e): for significance refer to legends of Figure 1. Pie charts under the columns show sex ratio in the catch (white: males, grey: females)

the sex attractant (Exp. 1). The range of female ratios was from 8% to 33% in FLO and from 9% to 23% in SBL (Figure 2). Ratios of females in FLO and SBL within tests were similar.

Orthosia gothica catches were recorded in all experiments (Figure 2). In Exp. 1, where *O. gothica* sex attractant-baited traps were tested, they caught sizeable numbers of the species, significantly

more than FLO- or SBL-baited traps. The catch in the traps with the sex attractant were all males (Exp. 1). FLO treatment caught more *O. gothica* than SBL, with statistical difference in three out of five tests. Catches in experiments with relatively low numbers (Exp. 2A–D) were in FLO- and SBL-baited traps, showing similar tendencies unanimously, as relatively larger catches did in Exp. 1 (Figure 2). Female

ratios ranged from 4% to 21% (FLO) and 9% to 17% (SBL). The low catches in Exp. 2D (26 moths in total) caught in the two treatments were all males (Figure 4). Single male specimens were recorded in Exp. 1 (sex attractant of *O. incerta*) and Exp. 2B (sex attractant of *O. cerasi*), and a female specimen was caught in an unbaited trap in Exp. 2B (Figure 2).

Orthosia incerta catches were recorded in all experiments (Figure 2). The sex attractant caught significantly more males than any other treatments, where tested (Exp. 1). Sex attractant-baited traps caught only males (Exp. 1, Figure 2). FLO-baited traps caught numerically more *O. incerta* compared to SBL-baited ones in three out of four experiments (Exp. 1, Exp. 2B, Exp. 2C) with a significant difference in two cases (Exp. 2B and Exp. 2C). In Exp. 2A and Exp. 2D, FLO- and SBL-baited traps caught a few moth specimens, indicating a similar tendency to larger catches of the other experiments (Figure 2). Female ratios ranged from 17% to 31% (FLO) and 29% to 35% (SBL). In Exp. 2C, seven specimens of the 40 moths caught (18%) were female in FLO, and only a single female (50%) was recorded in SBL (Figure 2).

Some *Anorthoa munda* were trapped in all experiments with sizeable catches in Exp. 1 (Figure 2). The overall *A. munda* catches were the greatest in absolute numbers in Exp. 1, where the most specimens were collected from the *A. munda* sex attractant-baited traps, without significant difference from FLO- or SBL-baited traps. FLO treatment caught generally more than SBL with significant difference in Exp. 2B and Exp. 2D. In Exp. 2A, the small *A. munda* numbers in FLO and SBL treatments corresponded to the tendencies of the other experiments (Figure 2). Female *A. munda* ratios in catches were more or less similar in FLO- and SBL-treated traps in all tests ranging between 29%–34% and 43%–50%, respectively.

4 | DISCUSSION

In earlier studies in which both phenylacetaldehyde-based (floral) and iso-amyl alcohol-based (fermenting) bisexual lures were in parallel tested, moth species belonging to different genera or even subfamilies of Noctuidae usually showed a clear preference for the one or the other, being attracted in large numbers to the preferred lure, while in much lower numbers to the other type of lure (Nagy et al., 2014, 2015; Szanyi et al., 2017, 2019; Tóth et al., 2010). Supposedly, this was due to the feeding source preference of the studied species. In this respect, it is surprising that in this study *Orthosia* spp. responded well to both types of lures, whereas catches to FLO were generally higher (in many cases significantly so) than to SBL. This may suggest that although both types of stimuli were meaningful and important for the feeding of this group of insects, the phenylacetaldehyde containing floral scent feeding source might have been somewhat more important. This assumption seems to be supported by the old observation that *Orthosia* species are known as frequent visitors of willow catkins but occasionally they are attracted by saps of trees, as well (Bergmann, 1954; Ebert, 1998). A detailed study of feeding

sources of adult *Orthosia* moths should be conducted to support this indication.

When bisexual lures and sex attractant or pheromone lures were compared in previous studies, usually the sex-related stimuli proved to be much more powerful. In *Autographa gamma* L. (Plutellinae) for example, traps with a bisexual floral lure (optimized for the catch of the target species) caught on an average only 23% of the catch in pheromone-baited traps (Tóth et al., 2019), while in the *Helicoverpa armigera* Hbn. (Melipotriinae) only 30% (Tóth et al., 2020). Also, traps baited with SBL caught only ca 30% of the catch of the sex pheromone-baited traps in *Mamestra oleracea* L. (Olajos et al., 2019).

In this study, similar trends were observed in *O. cruda*, *O. incerta*, *O. gothica* and *A. munda*. In these cases, the sex attractant-baited traps captured 2–3 times more moths (although not always significant) than the FLO traps, which were the more efficient of the bisexual lures. The only exception was *O. cerasi*, where the FLO lures were more attractive than the sex attractant. After the first results we conceded that a confirmation of this phenomenon is needed. Therefore, we included only the sex attractant of the *O. cerasi* in the second year of the experiments of this study (Exp. 2). The results confirmed the finding of the Exp. 1.

An explanation of this result could be that either the sex attractant composition of *O. cerasi* is not yet optimized, or the sex-related response of this species is not as strong as in the other species. We have to consider that none of the components of the known *Orthosia* sex attractants has so far been verified for their presence in female-produced sex pheromones of the respective species, so whenever this is done in the future, new and better compositions could be characterized.

The bisexual lures attracted both sexes, and females composed a sizeable percentage of the moths caught. Females definitely need energy and vitamins to be able to produce viable eggs, thus it is not surprising that a feeding stimulus is essential to them. This practical advantage of the bisexual lures can be exploited in population biological surveys in which the survey of females is indispensable, for example in surveys of fertility and fertilization (e.g., polyandry, Rankin & Kokko, 2007; Torres-Vila et al., 2004; Tobin et al., 2012).

Female ratios in the two types of bisexual lures were strikingly similar within one site. This suggests that the relative responsiveness of the sexes was similar to the phenylacetaldehyde-based, and the iso-amyl alcohol-based types of lures. Thus, the sex ratios in these cases should reflect the actual sex ratio of the given population. However, to experimentally prove this, independent measurements of the natural sex ratios should be conducted in the future.

In conclusion, in this study FLO, a new, a more efficient bisexual lure of floral type can be used for sampling both sexes of *Orthosia* spp., especially in situations where the study of female specimens is indispensable. However, in most species, when the sensitive detection at low population densities is necessary, the synthetic sex attractant lures could be still more useful.

It is interesting to note that in analysing effluents of flowering and non-flowering *Salix fragilis* and *S. x rubens* twigs (Salicaceae),

the presence of none of the floral components of the FLO lure were found present (Kehl et al., 2010). In the future, the efficacy of the FLO lure could further be increased by the testing of compounds characterized by Kehl et al. (2010), for example 1,4-dimethoxybenzene and (*E*)- β -ocimene, which were reported by them as the most abundant components in flowering willow effluents.

AUTHOR CONTRIBUTIONS

Szabolcs Szanyi and Miklós Tóth conceived research. Szabolcs Szanyi, Antal Nagy, Júlia K. Jósvai, Zoltán Imrei and Miklós Tóth conducted experiments. Miklós Tóth analysed data and conducted statistical analyses. Szabolcs Szanyi, Zoltán Varga, Antal Nagy and Miklós Tóth wrote the manuscript. Szabolcs Szanyi and Miklós Tóth secured funding. All authors read and approved the manuscript.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

DATA AVAILABILITY STATEMENT

Raw data supporting the results in the paper was uploaded into the Zenodo open repository: DOI: 10.5281/zenodo.5879841; URL: <https://zenodo.org/record/5879841#YeiEWP70IPZ>; CITE: Szabolcs Szanyi, Zoltán Varga, Antal Nagy, Júlia K. Jósvai, Zoltán Imrei, & Tóth Miklós. (2022). Raw data of experiments on the Bisexual lures and their comparison with synthetic sex attractants for trapping *Orthosia* species (Lepidoptera: Noctuidae) (Version V1) [Data set]. Zenodo. <http://doi.org/10.5281/zenodo.5879841>

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