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ORIGINAL ARTICLE

Catches of *Euxoa tritici* in pheromone traps for *Anarsia lineatella* are due to the presence of (*Z*)-5-decenyl acetate as an impurity

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Abstract

Traps baited with the synthetic pheromone of *Anarsia lineatella* Zeller (Lepidoptera: Gelechiidae) frequently captured also *Euxoa tritici* L. males (Lepidoptera: Noctuidae) in field tests in Hungary. As (*E*)-monounsaturated compounds are uncommon among sex attractants or pheromone components of Noctuidae, it was hypothesized that the *Euxoa* catches may have been due to impurities of the (*Z*) isomer in synthetic (*E*)-5-decenyl acetate, which is the major component in the pheromone lure of *A. lineatella*. Traps baited with synthetic (*Z*)-5-decenyl acetate captured large numbers of *E. tritici*, and the compound showed a clear dose–response effect. Reanalysis of the synthetic batch of (*E*)-5-decenyl acetate used in preparation of the *A. lineatella* lure showed the presence of 10% of the (*Z*) isomer. Traps baited with synthetic (*Z*)-5-decenyl acetate can be used in the future for detection and monitoring purposes of *E. tritici*, a widely distributed pest of cereals and other field crops. The compound also showed attraction of *Euxoa seliginis* Duponchel.

KEYWORDS

Euxoa seliginis, Gelechiidae, Lepidoptera, Lepidoptera, lure, monitoring, Noctuidae, phenology, sex attractant, synthetic pheromone, trapping, white-line dart

INTRODUCTION

The white-line dart, *Euxoa tritici* L. (Lepidoptera: Noctuidae), is a polyphagous pest of many field crops in Europe. The cutworms feed on various crops (e.g., alfalfa, lettuce, potato, beet, and other row crops (see: https://www.gov.mb.ca/agriculture/crops/insects/cutworms-field-crops.html) and also attack a wide range of low-growing plants, e.g., grapevines, shrubs, and young fruit trees in nurseries. They are generally 'surface-feeding' and browse mostly on the roots but exceptionally also ascend plants to attack the young shoots (Lafontaine, 1987; Gratwick, 1992; Alford, 2014). The univoltine *Euxoa* cutworms overwinter as young larvae in egg-shells, therefore they regularly feed on plants earlier than most of the bivoltine cutworms, thus their co-occurrence poses continuous damage risk from early spring throughout the vegetation period.

As larvae of the most numerous *Euxoa* species often appear together and their taxonomy is partly unclear (Fibiger, 1997; Mutanen, 2005), the economic importance of various species has not been fully clarified until now. In Europe, the *E. tritici* complex consists of three sibling taxa: *E. tritici*, *Euxoa nigrofusca* (Esper), and *Euxoa eruta* (Hübner), which are recently often considered as conspecific due to the lack of barcode differentiation (Huemer et al., 2019). However, further closely related and externally similar species occur, mostly in south-eastern and eastern parts of Europe; *Euxoa seliginis* (Duponchel) often co-occur with some members of the *E. tritici*-group in sandy areas of Hungary (Kovács, 1952, 1955). In Hungary, some *Euxoa* species, mostly *Euxoa temera* (Hübner), had serious outbreaks in the 1950's and 1970's (Szeőke, 1976; Mészáros, 1990) and their numbers have also increased in recent years (Nagy et al., 2020).

Cutworm populations are generally controlled by insecticide sprays (Bessin, 1997; Capinera, 2001). For optimal timing of pesticide treatments, the detection of the pest and its monitoring are of utmost importance. In the course of field trapping tests in Hungary using *Anarsia lineatella* Zeller (Lepidoptera: Gelechiidae) pheromone traps, we were surprised to regularly find specimens of *E. tritici* in the traps (Nagy et al., 2020). Because all specimens were males, this suggested a sex-attractant effect. The components of the *A. lineatella* synthetic pheromone lure are (*E*)-5-decenyl acetate (E5-10Ac) and (*E*)-5-decen-1-ol (E5-10OH) (Roelofs et al., 1975).

Among the sex pheromones or sex attractant components described from noctuids, compounds with an (*E*)-configured double bond are very rare (www.pherobase.com). On the other hand, (*Z*)-5-decenyl acetate (Z5-10Ac) has been found to be a sex pheromone/sex attractant constituent of several *Euxoa* spp. (www.pherobase.com). Consequently, this seemed to suggest that impurities of the (*Z*) isomer in synthetic E5-10Ac of the *A. lineatella* lure may be responsible for the attraction of *E. tritici*. The present study aimed at testing this hypothesis.

MATERIALS AND METHODS

Field experiments

Experiments aimed at catching *E. tritici* were conducted at several sites in Hungary using generally accepted methods (Roelofs & Cardé, 1977). Csalomon funnel traps (Plant Protection Institute, CAR, ELKH, Budapest, Hungary) were used, which have proved effective in trapping several large moth species (Tóth et al., 2000, 2010; Subchev et al., 2004); photos of the trap can be viewed at 'www.csalomontraps.com'. To kill captured insects, 1 × 1 cm of a household anti-moth insecticide strip (Chemotox; SaraLee, Temana Intl., Slouth, UK; active ingredient 15% dichlorvos) was placed in the traps.

Traps were arranged in blocks so that each block contained one trap of each treatment. Traps within blocks were separated by 8–10 m, and blocks were at least 30 m apart. At each site, five blocks of traps were operated. Traps were inspected every few days (preferably twice weekly), with captured insects recorded and removed.

Because the moths were killed with a slow-poison household anti-moth insecticide strip, they were mostly worn and often damaged, and the sibling taxa of the *E. tritici*-group were not separated despite the large number of prepared genitalia slides which show an overlapping variation, in agreement with the results of Mutanen (2005). However, the closely related *E. seliginis* was easily separated according to the external characters and the genitalia slides (Figures S1 and S2). The other sampled Noctuidae taxa were identified to species according to Varga (2012).

Baits

Synthetic Z5-10Ac applied in baits was a generous gift from the late H. Arn (Wädenswil, Switzerland) and had >98% purity by gas chromatography (GC). To prepare the baits, the required amounts were formulated on 4 × 10 mm pieces of rubber tubing (Taurus, Budapest, Hungary; no. MSZ 9691/6; extracted before use in boiling ethanol for 10 min, then also in methylene chloride overnight).

Pheromone lures of *A. lineatella* were commercial lures from the Csalomon trap family and contained 400 μg of E5-10Ac + 100 μg of E5-10OH on rubber dispensers. For preparing experimental lures, rubber of the same type and dimensions was used as in the commercial *A. lineatella* lures.

Experimental details

Experiment 1

The objective of this experiment was to check whether a range of synthetic Z5-10Ac doses evoked dose-dependent attraction of *E. tritici*, and how these catches compare with those of traps with the *A. lineatella* lure. Treatments included 1, 10, or 100 μg of Z5-10Ac, the *A. lineatella* lure, and unbaited controls.

The sampling site was an arable land surrounded by forest and grassland patches, northeast to Debrecen, Nyulas, Hajdú-Bihar County, Hungary (47°34.5'N, 21°35.3′E), 7 June–9 September, 2018. All lures were replaced on 9 July and 7 August.

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Experiment 2

This experiment was aimed at studying *E. tritici* catches using a high dose of Z5-10Ac and comparing it with the catches in traps with the *A. lineatella* lure. As the highest dose used in experiment 1 (100 μg) appeared the peak dose of the dose–response relationship, in this experiment a higher dose of 300 μg of Z5-10Ac was used and the *A. lineatella* lure.

Experiments were conducted simultaneously at Bocskaikert, Hajdú-Bihar County, Hungary (47°38.9'N, 21°39.1′E), 18 July–26 September, 2019, and Nagycsere, Hajdú-Bihar (47°31.4'N, 21°45.6′E), 15 July–26 September, 2019. The sampling site in Bocskaikert was situated in the margin of the suburban area, surrounded with home gardens, apple orchards, and mixed deciduous forests. The Nagycsere site was situated in a diverse landscape consisting of mixed deciduous forests, xeric sand and wet grasslands, extensive orchards, and arable lands.

Analysis of synthetic 5-decenyl acetate samples

To establish the composition of 5-decenyl acetate samples, GC electron impact ionization mass spectrometry (GCEI-MS) data were recorded on a Hewlett Packard 5890A GC, equipped with a 7673A autosampler, coupled to a Hewlett Packard 5972A mass selective detector equipped with a Rt-bDEXsm column, 30 m × 0.25 mm × 0.25 μm (Restek, Bellefonte, PA, USA). Oven program: from 60 to 200 °C at 2 °C per min. Injector and transfer line temperature 230 °C, injection volume: 1 μL, splitless (1 min). Helium (ultra-high purity grade) was used as the carrier gas (flow 1 mL per min).

Statistical analysis

Catch data did not fulfil the assumptions of parametric tests even after $\sqrt{(x+0.5)}$ transformation (Roelofs & Cardé, 1977), so they were analysed by non-parametric Kruskal-Wallis test followed by Mann–Whitney U test in case of significant differences. A paired t-test was used in comparing two treatments only in experiment 2. All statistical procedures were conducted using the software packages StatView v.4.01 and SuperANOVA v.1.11 (Abacus Concepts, Berkeley, CA, USA).

RESULTS

Field experiments

Experiment 1

All baited treatments caught *E. tritici* and catches were significantly higher in traps baited with the 10 and 100 μg doses of Z5-10Ac than in unbaited traps (Table 1). Catches showed a dose–response effect, with increasing doses catching increasing numbers of moths. *Euxoa tritici* catches in traps with 1 and 10 μg Z5-10Ac did not differ from catches in traps with the *A. lineatella* lure, whereas traps with 100 μg caught significantly more individuals.

Apart from *E. tritici*, another related species, *Euxoa seliginis* Duponchel, was also caught (Table 1). In this case, the 100 μg dose of Z5-10Ac caught significantly more than the other treatments except the 10 μg dose.

Significantly more *Acontia trabealis* Scopoli were caught in traps with 10 and 100 μg Z5-10Ac or with the *A. lineatella* lure (Table 1), compared with zero catch in control traps, and catches in traps baited with 10 μg dose of Z5-10Ac did not differ significantly from those in traps baited with the *A. lineatella* lure. Lower doses of Z5-10Ac caught fewer *A. trabealis*, indicating a dose–response relation (Table 1).

Anarsia lineatella were caught only in traps with the *A. lineatella* lure, significantly more than zero catch in the other treatments (Table 1).

Experiment 2

Similar numbers of *E. tritici* were caught in traps with a 300 μg dose of Z5-10Ac or with the *A. lineatella* lure at Nagycsere, whereas at Bocskaikert traps with Z5-10Ac

TABLE 1 Catches of moths in experiment 1: mean (± SE) number of moths per trap caught in traps baited with a dose range (1–100 μg) of (*Z*)-5-decenyl acetate (Z5-10Ac) or with an *Anarsia lineatella* commercial lure.

Lure	*Euxoa tritici*	*Euxoa seliginis*	*Acontia trabealis*	*Anarsia lineatella*
Anarsia lure	2.6 ± 0.6ab	0.2 ± 0.2a	29.4 ± 2.8c	14.0 ± 3.2b
Z5-10Ac (1 μg)	1.0 ± 0.5a	0a	6.8 ± 3.9ab	0.2 ± 0.2a
Z5-10Ac (10 μg)	5.8 ± 1.6bc	0.8 ± 0.4ab	8.6 ± 2.4bc	0a
Z5-10Ac (100 μg)	8.8 ± 2.5c	4.8 ± 2.3b	65.6 ± 18.1d	0a
Unbaited control	0a	0a	0a	0a
Total caught	91	29	552	71

Means within a column followed by the same letter are not significantly different (Mann–Whitney U-test: $P > 0.05$).

caught significantly more moths (Table 2). More *E. seliginis* were recorded in traps with Z5-10Ac, and the difference was significant in both sites (Table 2). Catches of *A. trabealis* in traps with Z5-10Ac or with the *A. lineatella* lure were low and similar. Again, *A. lineatella* were caught only in traps with the *A. lineatella* lure.

The phenology of the two species of *Euxoa* captured in traps differed between the two years. In 2018, *E. tritici* was collected from 19 June to 24 August whereas *E. seligninis* was not collected until 21 August. In 2019, *E. tritici* was captured from the date traps were set out (mid-July) until late August, whereas *E. seliginis* was not captured until 14 August in Bocskaikert and on 21 August in Nagycsere (Figure 1) and still swarmed at the end of the samplings in late September.

Analysis of synthetic 5-decenyl acetate samples

Gas chromatography-MS analysis of the synthetic batch of E5-10Ac used in the *A. lineatella* lure showed the presence of 10% of Z5-10Ac. The Z5-10Ac sample used in the field experiments had a confirmed isomeric purity of >98% (see 'Analysis of purity of 5*E*-decenac' in the Supporting Information).

DISCUSSION

Our results clearly show that Z5-10Ac is a sex attractant of *E. tritici* and *E. seliginis*. Nevertheless, the composition of pheromone emitted by *E. tritici* females is still unknown and may consist of more than just Z5-10Ac. Z5-10Ac frequently occurs among components of sex attractants or of sex pheromones in North American *Euxoa* spp. The compound on its own is reported to attract several North American species such as *Euxoa rockburnei* Hardwick, *Euxoa olivia* Morrison, *Euxoa campestris* Grote (Underhill et al., 1981), and *Euxoa mimallonis* Grote (Steck et al., 1982). It is one of the components in multicomponent lure mixtures for *Euxoa albipennis* Grote (Steck et al., 1979), *Euxoa basalis* Grote, *Euxoa pestula* Guenée (Steck et al., 1982), *Euxoa declarata* Walker (Underhill et al., 1981), *Euxoa idahoensis* Grote (Byers & Struble, 1987), *Euxoa ochrogaster* Guenée (Struble, 1981), and *Euxoa pleuritica* Grote (Steck et al., 1978), which belong to different species groups of this highly diverse genus (Lafontaine, 1987). This is

TABLE 2 Catches of moths in experiment 2: mean (± SE) number of moths per trap caught in traps baited with 300 µg of (*Z*)-5-decenyl acetate or with an *Anarsia lineatella* commercial lure, in Bocskaikert and in Nagycsere (Hungary)

	Euxoa tritici		*Euxoa seliginis*		*Acontia trabealis*		*Anarsia lineatella*	
Lure	Bocskai	Nagycsere	Bocskai	Nagycsere	Bocskai	Nagycsere	Bocskai	Nagycsere
Anarsia lure	11.2 ± 3.4	28.8 ± 6.1	6.2 ± 1.7	11.2 ± 3.3	0.4 ± 0.2	0.8 ± 0.4	7.8 ± 2.2	1.2 ± 0.6
Z5-10Ac	19.2 ± 3.1	25.0 ± 5.1	23.0 ± 1.3	32.8 ± 12.9	1.4 ± 0.5	1.2 ± 0.5	0	0
Total caught	152	269	146	220	9	10	39	6
t (d.f. = 1)	−2.294	0.657	−3.943	−2.990	−1.522	−0.704	3.949	2.103
P	0.026	0.52	0.0002	0.005	0.13	0.48	0.0004	0.054

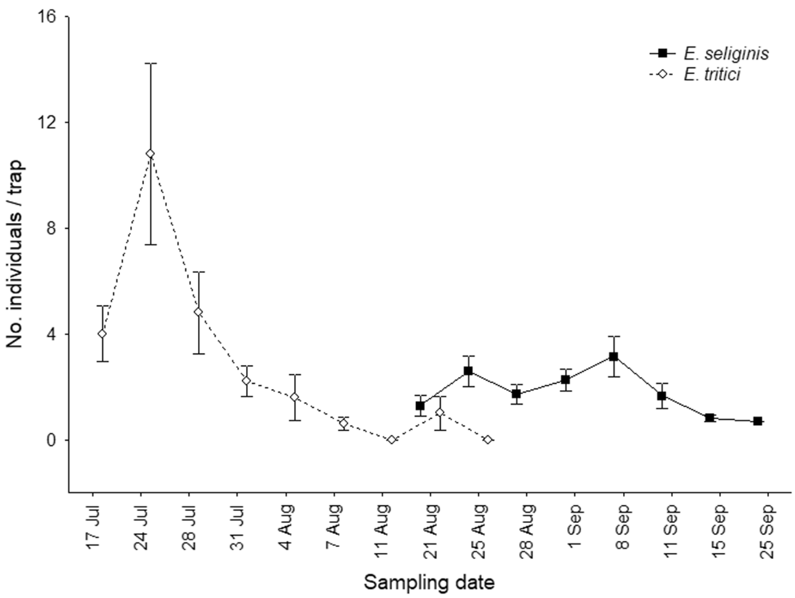

FIGURE 1 Temporal changes in the mean (± SE) number of *Euxoa tritici* and *Euxoa seliginis* per trap caught by volatile traps in Nagycsere in 2019.

the first report of Z5-10AC alone as a sex attractant of *E. tritici* and *E. seliginis*. However, Priesner (1988) previously reported that the combination of Z5-10AC and (*Z*)-3-dodecenyl acetate was attractive to *E. eruta*, considered by many to be conspecific with *E. tritici* (Huemer et al., 2019).

Among *Euxoa* spp. in Europe, the blend of Z5-10Ac with (*Z*)-7-dodecenyl acetate (Z7-12Ac) has been found to attract *Euxoa nigricans* L. (Szöcs et al., 1983) and *Euxoa hastifera* Donzel (Subchev et al., 1990). Our finding that Z5-10Ac is attractive to *E. tritici* and *E. seliginis* provides additional evidence for the role of this compound in *Euxoa* spp.

Captures of *E. tritici* in traps baited with the *A. lineatella* lure, as observed earlier (Nagy et al., 2020) and described in this study, were most probably due to the presence of Z5-10Ac as a 10% impurity in the E5-10Ac sample. This applies also to *E. seliginis* and *A. trabealis*, the other two noctuids captured.

The results of the present study confirmed literature data on Z5-10Ac as being a sex attractant of the non-pest *A. trabealis* (Arn et al., 1983; Szöcs et al., 1983), the third noctuid captured in field experiments of this study.

Comparing the amounts of Z5-10Ac in the *A. lineatella* lure (10% of a total of 400 μg of E5-10Ac sample/lure dispenser amounting to 40 μg of Z5-10Ac/lure) with catches in traps baited with a dose range of Z5-10Ac suggests that the presence of E5-10Ac in the *A. lineatella* lure did not significantly influence the catches of the *Euxoa* spp. The inertness of E5-10Ac is not surprising, as in this group of noctuids, no report is known in the literature on the occurrence of the (*E*) geometrical isomer in any species.

Results from experiment 1 provide evidence for a dose–response of *E. tritici*, as mean catches increased continuously with increasing dose. From 1 to 10 μg of Z5-10Ac the increase was significant, whereas mean trap catch increased further at 100 μg, but the difference was not significant. Likewise, in experiment 2, although catches were greater with 300 μg than with 40 μg (commercial *Anarsia* lure), significance was found only at Bocskaikert. This suggests that already at 10 μg the optimal dose response window (in the sense of Arn et al., 1985) has been reached, and doses above 10 μg of Z5-10Ac can be applied in lures for monitoring. In the present paper no measurement of actual release rates was performed from dispensers with the different dosages, and we acknowledge that ug loads may not exactly reflect the relative differences in release rates. Additional data are needed to determine the dose response for *E. tritici* to a broader range of Z5-10Ac doses.

Lures containing Z5-10Ac for *E. tritici* might be improved by the addition of other compounds if and when the sex pheromone(s) emitted by female *E. tritici* is determined. This is a task for future research.

The newly discovered sex attractant of *E. tritici* may become very useful in detection and monitoring of this species for plant protection purposes. These pests endanger a wide range of crops and vegetables, and even fruits, throughout the Palearctic region. The occurrence and the damage risk can be detected and evaluated by different types of traps such as blacklight, pheromone, and feeding attractant traps, of which pheromone traps are comparably the most cost-effective.

Furthermore, as examples from several moth families support, pheromones can also be used in mating disruption applications (Capinera, 2001; Witzgall et al., 2010; Smart et al., 2014). Species-specific traps can also provide detailed information on the less-known biology (e.g., population dynamics, phenology, etc.) and distribution of *E. tritici* sensu lato and some related species as *E. seliginis*. Furthermore, our study provides new data on the phenology of *E. tritici* which swarmed from late-June to the end of August in East Hungary in 2018, contrary to some earlier data (early July–August) (see 'macroheterocera.hu'). In the same time *E. seliginis* followed the general trend and swarmed from mid-August until the end of September in 2019. The large number (>100) genitalia slides prepared from the trapped specimens also confirmed the phenological differentiation between these related species (data not shown).

AUTHOR CONTRIBUTIONS

Antal Nagy: Conceptualization (equal); formal analysis (equal); methodology (equal); writing – original draft (lead). **István Szarukán:** Investigation (equal); writing – review and editing (equal). **Bjorn Bohman:** Methodology (equal); writing – review and editing (equal). **Szabolcs Szanyi:** Conceptualization (equal); methodology (equal); writing – original draft (equal). **Lajos Kozák:** Investigation (equal). **Arnold Szilágyi:** Investigation (equal). **Zoltán Imrei:** Investigation (equal); writing – review and editing (equal). **Jozef Vuts:** Investigation (equal); validation (equal). **Eszter Matula:** Investigation (equal). **Zoltán Varga:** Writing – original draft (supporting); writing – review and editing (equal). **Miklos Toth:** Conceptualization (lead); methodology (equal); writing – original draft (lead).

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CONFLICT OF INTEREST STATEMENT

The authors declare they have no conflict of interest.

DATA AVAILABILITY STATEMENT

Data available on request from the authors.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Figure S1. The male genitalia of *Euxoa tritici* vs. *Euxoa seliginis* show the following main differences: in the genital capsula (A, B, E, F), cucullus and corona in *E. seliginis* is broader, and the dorsal incision of the juxta is deeper than in *E. tritici*; the vesica seminalis (C, D, G, H) in *E. tritici* is proportionally longer and the basal diverticulum is proportionally smaller than in *E. seliginis*. (A) Genital capsula *Euxoa tritici* f. *eruta* HU Nagycsere, 12 km E Debrecen Slide VZ 11 420. (B) Genital capsula *Euxoa tritici* f. *nigrofusca* HU Vámospércs, 15 km E Debrecen Slide VZ 11 693. (C) Vesica *Euxoa tritici* f. *eruta* HU Nagycsere, 12 km E Debrecen Slide VZ 11 420. (D) Vesica *Euxoa tritici* f. *nigrofusca* HU Vámospércs, 15 km E Debrecen Slide VZ 11 693. (E) Genital capsula *Euxoa seliginis* HU Vámospércs, 15 km E Debrecen Slide VZ 10 033. (F) Genital capsula *Euxoa seliginis* HU Bagamér, 35 km SE Debrecen Slide VZ 11 659. (G) Vesica *Euxoa seliginis* HU Vámospércs, 15 km E Debrecen Slide VZ 10 033. (H) Vesica *Euxoa seliginis* HU Bagamér, 35 km SE Debrecen Slide VZ 11 659.

Figure S2. The female genitalia of *Euxoa tritici* vs. *Euxoa seliginis* show the following main differences: the papillae of ovipositor have in *E. tritici* much stronger and longer setae than in *E. seliginis*; the sclerotised lamellae in the ductus bursae in *E. seliginis* are proportionally thinner than in *E. tritici*; the bursa copulatrix is rounder in *E. seliginis*, with less prominent appendix bursae than in *E. tritici*. (A) *Euxoa tritici* f. *eruta* HU Bagamér, 35 km SE Debrecen Slide VZ 11743. (B) *Euxoa tritici* f. *eruta* HU Hajdúsámson, 15 km NE Debrecen Slide VZ 9918. (C) *Euxoa tritici* f. *eruta* Lectotype, coll. Corti (ex coll. Graslin), Switzerland, Wallis Slide VZ 7222. (D) *Euxoa seliginis* HU Bagamér, 35 km SE Debrecen Slide VZ 11 742. (E) *Euxoa seliginis* France, Camargue, coll. Corti (ex coll. Graslin).

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