Identification and field evaluation of (E)11,13tetradecadienal as sex pheromone of the strawberry tortrix (Acleris comariana)

Svensson, Glenn P.; Tönnberg, Victoria; Sigsgaard, Lene

Published in:
Journal of Applied Entomology

DOI:
10.1111/jen.12619

Publication date:
2019

Document version
Publisher's PDF, also known as Version of record

Document license:
CC BY

Citation for published version (APA):
INTRODUCTION

Acleris (Hübner) is a relatively large genus in the moth family Tortricidae with approximately 250 described species, including several important pests. A female-produced sex pheromone has been identified in four species: the yellowheaded fireworm (A. minuta; Schwarz, Klun, Hart, Leonhardt, & Weatherby, 1983), the western blackheaded budworm (A. gloverana; Gray, Shepherd, Gries, & Gries, 1996), the eastern blackheaded budworm (A. variana; Gries et al., 1994), and the yellow tortrix (A. fimbriana; Thunberg) (Liu & Meng, 2002). Females of all four species produce the same gland constituent, (E)-11,13-tetradecadienal (E11,13-14:Ald), to attract conspecific males. For both A. gloverana and A. variana, (E)-11,13-tetradecadienol (E11,13-14:OH) and (E)-11,13-tetradecadienyl acetate (E11,13-14:OAc) are also produced by females and elicit antennal response in males, but addition of these to E11,13-14:Ald does not enhance attraction in field trapping experiments (Gray et al., 1996). In contrast, addition of (Z)-11,13-tetradecadienal (Z11,13-14:Ald) to E11,13-14:Ald increases trap catches of A. gloverana (Gray et al., 1996). For A. fimbriana, E11,13-14:OAc increases attraction of males, whereas E11,13-14:OH has an inhibitory effect on males when combined with E11,13-14:Ald (Liu & Meng, 2002). Finally, four Acleris species are attracted to...
The strawberry tortrix (*A. comariana* Lienig and Zeller) is widely distributed in Eurasia. This moth is a key pest in strawberry production in southern Sweden and Denmark (Lindhardt et al., 2003; Swedish Board of Agriculture, 2015). The species is the most commonly found tortricid on strawberries (Alford, 1984; Bovien & Thomsen, 1950; Solomon et al., 2001; Stenseth, 1982). In a recent survey conducted in organic and conventional Danish strawberry fields, about 95% of the collected tortricid larvae were identified as *A. comariana* (Sigsgaard et al., 2014). It has earlier been reported as a pest of strawberry in northwest Europe (Stenseth et al., 2003; Swedish Board of Agriculture, 2015). The species is a highly variable species in bivoltine, and in Sweden and Denmark the spring generation flies in June-July and the summer generation flies in August-October. Females lay their eggs underneath the leaves of the strawberry plant. Eggs overwinter and larvae of the spring generation often hatch at the time of flower petiole development. Feeding on flowers results in either aborted fruits or small, malformed fruits, which are generally rejected by consumers. Today, visual assessment is used to monitor larval densities of *A. comariana* in local fields, which is time-consuming and limits the possibility to reliably track population fluctuations over multiple years. Here, we report the identification of the sex pheromone of *A. comariana*, and development of a highly efficient trap lure, which can greatly facilitate detection and monitoring of this pest in strawberry production.

## 2 | MATERIALS AND METHODS

### 2.1 | Collection and rearing of moths

Strawberry leaves infested by *A. comariana* were collected from a 16 ha commercial field near Lockarp (55°31′N, 13°03′E), and a 2 ha commercial field near Flädie (55°44′N, 13°03′E), Skåne Province, Sweden, during May and August 2018. The leaves were arranged into bouquets tightly wrapped with a plastic bag, just slightly open in the top. The still attached leaf petioles were placed in water so that the leaves could remain fresh meanwhile the larvae continued their development. Leaves and larvae were transferred to transparent Plexiglas cages (30 cm × 30 cm × 60 cm), with a fine mesh net on the back side, and placed in a climate chamber at 20°C, 65% r.h., and a 17:7 L:D photoperiod. Pupae were separated by sex and kept in separate plastic boxes until adult emergence.

### 2.2 | Chemicals

Compounds previously identified from other *Acleris* species were purchased from Pherobank (Wijk bij Duurstede, The Netherlands) and included $\text{E11,13-14:Ald}$ (chemical purity 91%; isomeric purity >99%), $\text{Z11,13-14:Ald}$ (chemical purity 96%; isomeric purity >99%), $\text{E11,13-14:OAc}$ (chemical purity 86%).

## 2.3 | Observation of female calling behaviour and extraction of pheromone glands

Female *A. comariana* (1–3 day old, $n = 9$) were placed individually in transparent plastic jars and monitored at red light illumination during the whole scotophase, but no extrusion of the pheromone gland could be observed. Without knowledge about the calling period, the initial strategy was to perform extraction 2–3 hr into the scotophase. Abdominal glands of 1–3 day old females were dissected in ultrapure (>99%) heptane (Merck, Darmstadt, Germany) (>5 µl/gland) at 22°C for 20 min. Five, seven and eight glands were pooled in separate batches and stored at −18°C until used in electrophysiological and chemical analyses. Additional extractions were performed 1 hr before the end of the photophase as well as 4 hr into the scotophase, but these only contained 1–2 glands.

## 2.4 | Electrophysiology

Gas chromatography coupled with electroantennographic detection (GC-EAD) was used to identify compounds in *A. comariana* female gland extracts that can be detected by conspecific males. The tip of the antennae of 1–4 day old males were cut off, and the head with antennae mounted to a PRG-2 EAG probe (10× gain) (Syntech, Kirchzarten, Germany) using Blågel conductive gel (Cefar, Malmö, Sweden). For these analyses, aliquots of 0.4 female equivalents of gland extract, and blends of synthetic $\text{E11,13-14:Ald}$, $\text{Z11,13-14:Ald}$, $\text{E11,13-14:OH}$ and $\text{E11,13-14:OAc}$ (1 ng of each compound), were injected into an Agilent 7890A gas chromatograph (Agilent Technologies, Palo Alto, CA, USA), equipped with a polar HP-INNOWax column (30 m × 0.25 mm × 0.25 µm film thickness; J&W Scientific, Agilent Technologies, Santa Clara, CA, USA), or a non-polar HP-5 column (30 m × 0.32 mm × 0.25 µm film thickness; J&W Scientific) in splitless mode. Hydrogen was used as carrier gas (flow rate: 1 ml/min), and injector temperature was 250°C. The column effluent was split at a 1:1 ratio between the flame ionization detector (FID) and the EAD. Oven temperature was maintained at 80°C for 1 min after injection, then increased by 5°C/min or 10°C/min to 220°C, with a final hold of 10 min at this temperature. The column effluent passed through a heated transfer line set at 255°C and was mixed with charcoal-filtered and humidified air before reaching the antennal preparation, which was placed 1 cm from the glass tube outlet. Simultaneous FID and EAD signals were recorded using the GC-EAD Pro Version 4.1 software (Syntech). In total, 17 antennal preparations were used in these analyses.

## 2.5 | Chemical analyses

Compounds in gland extracts that elicited a response in antennae of males were analysed by gas chromatography coupled with mass spectrometry (GC-MS). Extracts were injected into an Agilent...
thermic reference compounds. Rate: 1 ml/min), and injector and transfer line temperatures were 250 EAD recordings. In all analyses, helium was used as carrier gas (flow oven temperature in these analyses was programmed as for the GC–MS analyses of extracts showed that the retention time and mass spectrum of the active compound matched that of synthetic (*) was identified as (E)-11,13-tetradecadienyl in subsequent analyses with gas chromatography coupled to mass spectrometry.

7890A gas chromatograph equipped with a non-polar HP-5MS column (30 m × 0.25 mm × 0.25 µm film thickness; J&W Scientific) and linked to an Agilent 5975C mass-selective detector. Some analyses were performed on a Hewlett-Packard 6890 gas chromatograph (Hewlett-Packard Co., Palo Alto, CA, USA) equipped with a polar HP-INNOWax column (same dimensions as for the GC-EAD analyses), and linked to a Hewlett-Packard 5975 mass-selective detector. The oven temperature in these analyses was programmed as for the GC-EAD recordings. In all analyses, helium was used as carrier gas (flow rate: 1 ml/min), and injector and transfer line temperatures were 250 and 280°C, respectively. Active compounds were identified through comparison of retention times and mass spectra with those of synthetic reference compounds.

2.6 | Field experiments

To check the attraction of A. comariana males to the geometric isomers of 11,13-tetradecadienyl, trapping experiments were conducted in the strawberry field near Lockarp during September 2018. Test compounds were dissolved in heptane, and 100 µl of a solution was applied on red rubber septa (11 × 5 mm, #224100-020; Wheaton Science Products, Millville, NJ, USA) to be used as lures in traps. Each test solution contained 1% of the antioxidant 3-tert-Butyl-4-hydroxanisole (BHA) to stabilize the active ingredient. Transparent plastic delta traps (Csalomon, Budapest, Hungary) with sticky inserts were suspended 1 m above ground from white plastic fence poles (Grangården, Sweden). Five replicates per treatment were used in all experiments. Traps were separated by 10 m within a replicate. The distance between replicates was 10 m in the first experiment, and 50 m in the second and third experiment, and the distance between experiments was at least 100 m. For the last two experiments, three replicates were placed after each other along a row, and the last two replicates in parallel with these. For each experiment, traps were checked twice per week, captured moths were counted, and inserts replaced if needed. After each check, a trap was moved one step within the row to further reduce positional effects on catches.

In the first experiment, performed 7th to 21st of September, lures included either 100 µg of £11,13-14:Ald or only heptane solvent (control). A second experiment was performed 7th to 14th of September, where traps were baited with various ratios of £11,13-14:Ald and Z11,13-14:Ald (100:0, 75:25, 50:50, 25:75 and 0:100) with a total dose of 100 µg per septum. Finally, a third experiment was performed 14th to 28th of September and tested £11,13-14:Ald at 0.1, 1, 10, 100 and 1,000 µg per septum. The traps in this last experiment stayed in the field for 2 weeks, but catches decreased drastically during the second week due to strong winds and low temperatures and many traps were lost, so only catch data from the first week could be included in the statistical analysis. All statistical tests were based on mean weekly catches per trap, which were log (x + 1) transformed to approach normal distribution of data. A t test was used to compare catches for the first experiment, whereas a one-way ANOVA, followed by multiple comparisons adjusted according to the Bonferroni post hoc test, was used to compare catches among treatments for the last two experiments. All tests were performed using SPSS Version 19 (SPSS Inc., Chicago, IL, USA).

3 | RESULTS

All moths emerging in the laboratory (~150 individuals) were identified as A. comariana, showing the dominance of this species in strawberry fields in southern Sweden. A subsample of 36 specimens were examined in detail (genital preparation) to confirm species identity using Razowski (2001) and Svensson (2006), and vouchers were saved. At least six colour morphs were observed according to the classification by Turner (1968): grey-brown, grey-black, brown-black, marbled-brown, melanic and plain-brown.

The GC-EAD analyses of gland extractions from 2–3 hr into the scotophase revealed a single compound that consistently elicited a strong response from antennae of males (Figure 1). Subsequent GC-MS analyses of extracts showed that the retention time and mass spectrum of the active compound matched that of synthetic £11,13-14:Ald, and the activity of this compound was confirmed in further GC-EAD analyses (Figure 2). The compound was not detected in gland extracts from earlier or later time periods, but these contained few glands. The Z-isomer of the aldehyde was also found to be EAD-active (Figure 2). The E- and Z-isomers could not be resolved on the HP-INNOWax column, and only partially resolved on the HP-5 column using different temperature ramps (0.036 min at 5°C/min, 0.028 min at 10°C/min, and 0.022 min at 15°C/min), making it impossible to distinguish if the antennal response upon stimulation with gland extract was caused by presence of the E-isomer alone or both isomers. The presence of the Z-isomer, however, could not be confirmed in any of the pooled extracts analysed by GC-MS. Finally, there was no antennal response upon stimulation...
with synthetic $\text{E}_{11,13-14}:\text{OH}$ or $\text{E}_{11,13-14}:\text{OAc}$ (Figure 2), and these compounds were not detected in any of the extracts.

In the first field experiment, traps baited with 100 µg of $\text{E}_{11,13-14}:\text{Ald}$ captured on average 93 males per week, whereas a single male was found in a control trap ($t = 25.10$, $df = 18$, $p < 0.001$, Figure 3). In the second experiment, there was no significant difference in attraction of males to the pure isomers of the aldehyde or blends of these ($F = 1.30$, $df = 4$, $p = 0.31$, Figure 4). Finally, the dose–response experiment showed that catches increased with increasing dose ($F = 165.48$, $df = 4$, $p < 0.001$), and 100 µg and 1,000 µg attracted significantly higher numbers of males compared to 10 µg (Figure 5). The same six colour morphs were found in the traps as observed for the individuals emerging in the climate chamber. No other moth species were captured in significant numbers in any of the three experiments.

FIGURE 2 Flame ionization detector (FID) and electroantennographic detector (EAD: male Acleris comariana antennae) responses to synthetic reference compounds (1 ng of each) analysed on a HP-5 column: (E)-11,13-tetradecadienal (A), (Z)-11,13-tetradecadienal (B), (E)-11,13-tetradecadienol (C) and (E)-11,13-tetradecadienyl acetate (D)

FIGURE 3 Mean catch (±SEM) per week of male Acleris comariana in traps baited with 100 µg of (E)-11,13-tetradecadienal or heptane solvent (control) in a strawberry field near Lockarp, Sweden, 7–21 September 2018 ($n = 10$). Bars with different letters indicate significantly different catches [$t$ test on log($x + 1$)-transformed data: $p < 0.001$]

FIGURE 4 Mean catch (±SEM) per week of male Acleris comariana in traps baited with various ratios of (E)-11,13-tetradecadienal and (Z)-11,13-tetradecadienal (total dose of 100 µg) in a strawberry field near Lockarp, Sweden, 7–14 September 2018 ($n = 5$). No significant differences in catches among treatments were observed [ANOVA on log($x + 1$)-transformed data followed by multiple comparisons according to the Bonferroni post hoc test: $p > 0.05$]

4 | DISCUSSION

Infestation by A. comariana is an increasing problem in strawberry fields in southern Sweden and Denmark, where this moth is a major pest (Sigsgaard et al., 2014; Svensson, Törnberg & Sigsgaard, unpubl. data). Current methods to control populations of this species include treatment with either pyrethroids or Bacillus thuringiensis, which is not sufficiently efficient, and pheromone-based methods could provide an efficient and sustainable pest management strategy. In this study, we report $\text{E}_{11,13-14}:\text{Ald}$ as the female-produced sex pheromone of A. comariana. No other compound in gland extracts elicited a significant antennal response in males (Figure 1). The Z-isomer of the aldehyde was also found to be antennally active (Figure 2), and attracted similar numbers of males as the E-isomer in the ratio experiment (Figure 4), indicating that $\text{Z}_{11,13-14}:\text{Ald}$ may also be part of the sex pheromone of A. comariana, but it could not
be detected in gland extracts in this study. From a practical point of view, isomeric purity of the aldehyde is not critical for efficient monitoring of the species. Traps baited with 100 and 1,000 µg of (E)-11,13-tetradecadienal in a strawberry field near Lockarp, Sweden, 14–21 September 2018 (n = 5). Bars with different letters indicate significantly different catches (ANOVA on log(x + 1)-transformed data followed by multiple comparisons according to the Bonferroni post hoc test: p < 0.001)

Figure 5: Mean catch (±SEM) per week of male Acleris comariana in traps baited with various doses of (E)-11,13-tetradecadienal in a strawberry field near Lockarp, Sweden, 14–21 September 2018 (n = 5). Bars with different letters indicate significantly different catches (ANOVA on log(x + 1)-transformed data followed by multiple comparisons according to the Bonferroni post hoc test: p < 0.001).

In summary, we have identified (E)-11,13-14:Ald as the sex pheromone of the strawberry pest A. comariana, and showed that this compound is attractive to males. For monitoring of this pest in strawberry fields, a bait containing 100 µg of (E)-11,13-14:Ald with 1% of BHA as stabilizer provides an efficient trap lure for at least two weeks. The present study is a first step in the development of a pheromone-based monitoring program aiming at tracking the flight
phenology of the spring and summer generations of the species and correlating catch data with estimates of larval density, and possibly also levels of damage, to establish a tool for decision making on direct control measures. Also, future studies should evaluate the potential for direct population control of *A. comariaena* via pheromone-based mating disruption.

**ACKNOWLEDGEMENTS**

We thank the farmers Mats Olsson and Gerth Einarsson for letting us work in their fields, and Bengt-Ake Bengtsson for helping us with species identification of moths. Financial support was provided by Swedish farmers’ foundation for agricultural research (SLF) and the Swedish Research Council for Environment, Agricultural Sciences and Spatial Planning (FORMAS).

**AUTHORS’ CONTRIBUTIONS**

All authors conceived research, GPS and VT conducted experiments and contributed material, GPS analysed data, performed statistical analyses and wrote the manuscript. All authors secured funding, and read and approved the manuscript.

**ORCID**

Glenn P. Svensson [https://orcid.org/0000-0001-8112-8441](https://orcid.org/0000-0001-8112-8441)

Lene Sigsgaard [https://orcid.org/0000-0001-6478-5079](https://orcid.org/0000-0001-6478-5079)

**REFERENCES**


**How to cite this article:** Svensson GP, Tönnberg V, Sigsgaard L. Identification and field evaluation of (E)-11,13-tetradecadienal as sex pheromone of the strawberry tortrix (Acleris comaria). *J Appl Entomol*. 2019;143:535–541. https://doi.org/10.1111/jen.12619