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Published in:
Journal of Applied Entomology

2023

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

Received: 2 January 2023 | Revised: 28 February 2023 | Accepted: 1 March 2023

DOI: 10.1111/jen.13115

ORIGINAL CONTRIBUTION

JOURNAL OF APPLIED ENTOMOLOGY WILEY

Identification of (*E*)- and (*Z*)-11-tetradecenyl acetate as sex pheromone components of the currant pest *Euhyponomeutoides albithoracellus*

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Funding information
Swedish Farmers' Foundation for Agricultural Research, Grant/Award Number: O-20-20-452

Abstract

The currant bud moth *Euhyponomeutoides albithoracellus* is a destructive pest in black currant orchards in Northern Sweden and Finland. The larvae feed on the buds, and at high densities, the species can cause severe yield losses. Sex pheromone components of the bud moth were identified via solvent extraction of excised female pheromone glands, analyses by gas chromatography with electroantennographic detection and gas chromatography–mass spectrometry and field trapping experiments. Antennae of males responded strongly and consistently to two compounds in extracts, identified as (*E*)-11-tetradecenyl acetate and (*Z*)-11-tetradecenyl acetate. Weaker and less consistent responses were observed to the corresponding alcohols, (*E*)-11-tetradecenol and (*Z*)-11-tetradecenol, and tetradecyl acetate. Field tests showed strong attraction of bud moth males to a 1:1 blend of (*E*)-11-tetradecenyl acetate and (*Z*)-11-tetradecenyl acetate. Adding the alcohols to the binary acetate blend reduced trap catches drastically, whereas tetradecyl acetate had no statistically significant impact on male attraction when added to that binary blend. Finally, testing different compositions of the binary acetate blend revealed highest catch in traps baited with a 25:75 or 50:50 ratio of the *E*:*Z* acetate isomers. The identification of sex pheromone components of the bud moth contributes to developing sustainable control of this pest via monitoring and mating disruption with sex pheromone.

KEYWORDS
currant bud moth, currant pest, field trapping, pheromone gland analysis, *Ribes nigrum*, Yponomeutidae

1 | INTRODUCTION

Commercial production of currants (*Ribes* spp) in the Nordic countries has decreased during the last decades (www.fao.org), and one reason for this decline is the damage caused by destructive insect pests and the limited possibilities to control these due to stricter EU regulations on pesticide use. Three moth species cause major damage in both conventional and organic production of black currant *Ribes nigrum* (L.): the currant shoot borer *Lampronia capitella* (Clerck) (Prodoxidae), the currant clearwing moth *Synanthedon tipuliformis*

(Clerck) (Sesiidae) and the currant bud moth *Euhyponomeutoides albithoracellus* Gaj (syn. *Kessleria rufella* (Tengström)) (Yponomeutidae). The female-produced sex pheromones of *L. capitella* and *S. tipuliformis* have been identified (Löfstedt et al., 2004; Priesner et al., 1986), and commercial lures are available for monitoring these moths in currant orchards. The pheromone for *E. albithoracellus* has not yet been identified, but pheromone lures aimed for the large fruit-tree tortrix *Archips podana* (Scopoli) (Tortricidae), which contain a 1:1 mix of (*E*)-11-tetradecenyl acetate (*E*11-14:OAc) and (*Z*)-11-tetradecenyl acetate (*Z*11-14:OAc) (Persoons et al., 1974), can be used for efficient monitoring of this pest (e.g. Peltotalo & Touvinen, 1986), indicating that the chemical composition of the sex pheromones of the two species is similar.

In northern Sweden and Finland, *E. albithoracellus* is considered a major pest on currants (Hellqvist, 1981; Tuovinen, 1989; Tuovinen et al., 2008). The life history of this nocturnal species is well studied (Heikinheimo, 1978; Hellqvist, 1981, 1990). The flight occurs in June–July, and mated females lay eggs on the leaves of *Ribes* spp. The young larva feeds on leaves for a short period and then enters a developing shoot bud in a leaf axil where it continues feeding and then hibernates. In early spring the following year, the larva exits the emptied hibernation bud and moves to another bud, which is also consumed. Usually, the larva continues feeding on the leaves and racemes of an emerging shoot, before it drops to the ground where it pupates in a cocoon at the time when currants start to flower (Heikinheimo, 1978). As each larva destroys 2–3 buds, high population densities of the pest can result in great yield losses (Hellqvist, 1981). The damage caused by *E. albithoracellus* is similar to that of *L. capitella*, and the two species are often found in the same orchard.

Pyrethroids have previously been used in early spring to kill young bud moth larvae when they disperse from their hibernation buds, but such insecticide application is difficult due to the wet soil conditions in the fields during this period. The routine applications with endosulfan or fenpropatrin, that formerly were carried out against the gall mite *Cecidophyopsis ribis* (Westwood) shortly before the flowering of black currant, also had some effect on larger larvae (Hellqvist, 1981). However, since 2010 all pyrethroids are banned within EU for use in black currant orchards, and there is thus an urgent need for alternative cost-effective and environmentally safe pest control methods. Targeting adults would be beneficial because it will limit the initial damage caused by young larvae. An optimized sex pheromone would facilitate monitoring of bud moths in currant orchards, and trap catch data could be a useful tool in integrated pest management (IPM) to get information about presence, flight phenology and abundance of the species, which will aid in decisions about optimal use of pesticides. The pheromone could also potentially be used for population control by mating disruption. We here report the identification of sex pheromone components of *E. albithoracellus* by electrophysiological and chemical analyses of compounds produced from the terminal abdominal gland of female moths and field trapping experiments to demonstrate their behavioural activity.

2 | MATERIALS AND METHODS

2.1 | Collection and rearing of moths

Black currant twigs infested by *E. albithoracellus* were collected at Sikfors, Sweden (65°3′N, 21°11′E), in March–April and sent to Lund. Twigs were placed in 500 mL glass jars filled with water to keep them fresh during the development of moth larvae. The twigs were transferred to transparent Plexiglass cages (30 × 30 × 60 cm) with a fine mesh net on the back side and placed in a climate room at 22°C, 65% r.h. and 20:4 L:D photoperiod. Larvae were allowed to feed on fresh twigs until pupation. The pupae were removed from their cocoons, separated by sex on the basis of genital characters, and kept in separate plastic boxes until adult emergence. Moths of 1–4 days of age were used in all analyses.

2.2 | Chemicals and dispensers

The compounds *E*11-14:OAc, *Z*11-14:OAc, (*E*)-11-tetradecenol (*E*11-14:OH) and (*Z*)-11-tetradecenol (*Z*11-14:OH) (>95% chemical purity; >98% isomeric purity) were obtained from Pherobank (Wijk bij Duurstede, The Netherlands), whereas tetradecyl acetate (14:OAc) (>99% chemical purity) was purchased from Sigma-Aldrich (Burlington, MA, USA). Red rubber septa (11 × 5 mm, #224100–020; Wheaton Science Products, Millville, NJ, USA) were used as lures in the field trapping experiments.

2.3 | Extraction of female pheromone glands

The terminal abdominal segments, including the ovipositor, were dissected ca. 2 h into the scotophase. Glands were placed in a micro vial including 5–10 μL of ultrapure (>99%) heptane (Merck, Darmstadt, Germany) and left for 30 min at room temperature, after which the extract was transferred to a new vial and stored at −18°C until used for electrophysiological or chemical analyses.

2.4 | Electrophysiological analyses

Gas chromatography coupled with electroantennographic detection (GC-EAD) was used to identify compounds in *E. albithoracellus* female gland extracts that elicited antennal response in conspecific males. In these analyses, 1 μL of gland extract or a blend of synthetic candidate compounds including *E*11-14:OAc, *Z*11-14:OAc, *E*11-14:OH, *Z*11-14:OH and 14:OAc (1 ng/μL each) was injected into an Agilent 7890A gas chromatograph (Agilent Technologies), with hydrogen as carrier gas (velocity: 51 cm/s; flow rate: 1.8 mL/min) and an injector temperature set at 225°C. Columns used were either a medium-polar HP-INNOWax (30 m × 0.25 mm ID, 0.25 μm film thickness) or a non-polar HP-5 (30 m × 0.32 mm ID, 0.25 μm film thickness) (J&W Scientific, Folsom, CA, USA). Column temperature was maintained

at 80°C for 1 min and then increased to 210°C at a rate of 10°C/min and held for 10 min. The antennal preparation consisted of the head with both antennae and was mounted to a PRG-2 EAG probe (10× gain) (Syntech, Kirchzarten, Germany) using conductive gel (Blågel, Cefar, Malmö, Sweden). Charcoal-filtered and humidified air was blown over the antennae from a glass tube outlet positioned at 5 mm distance from the preparation. The effluent from a column was split 1:1 with half the sample going to the flame ionization detector (FID) and the other half to the antennal preparation after passing through a heated transfer line set at 230°C. In total, 20 antennal preparations gave reliable results in the GC-EAD recordings (each preparation was used only for 1–2 runs). Data were analysed using GC-EAD Pro Version 4.1 (Syntech, Kirchzarten, Germany).

2.5 | Chemical analyses

Analyses of pheromone gland extracts of *E. albithoracellus* and synthetic reference compounds were performed on an Agilent 5977B mass-selective detector coupled to an Agilent 8890 gas chromatograph equipped with an HP-INNOWax column (dimensions as above). Oven temperature was kept at 80°C for 1 min and then increased to 230°C at a rate of 10°C/min and held for 15 min. Injector and transfer line temperatures were 250°C and 280°C, respectively, and helium was used as the carrier gas. The compounds eliciting antennal responses in GC-EAD recordings were identified through comparison of their retention times with those of synthetic reference compounds (see above).

2.6 | Field trials

The first trapping experiment was performed 13th July – 2nd August 2004 in an abandoned black currant orchard in Sörfors, Sweden (63°52′N, 20°01′E), to investigate the activity of pheromone candidate components that had elicited antennal response in GC-EAD analyses. Septa were loaded with different combinations of *E*11-14:OAc, *Z*11-14:OAc, *E*11-14:OH, *Z*11-14:OH and 14:OAc (100 μg/compound) or solvent only for control traps. In 2005, a second experiment was performed in the same orchard 22nd June – 26th July to investigate if different amounts (10, 30, 100 and 300 μg) of 14:OAc added to a 1:1 mixture of *E*11- and Z11-14:OAc (100 μg/compound) would affect attraction of males. Finally, a third experiment was carried out 8th June – 21st July 2022 in an active black currant orchard in Rödupp, Sweden (66°30′N, 22°46′E), to analyse attraction of males to different ratios (10:90, 25:75, 50:50, 75:25 and 90:10) of *E*11- and Z11-14:OAc (total dose 100 μg). Synthetic blends were prepared in hexane (2004–2005) or heptane (2022), and 100 μL solutions were added to septa. Delta traps (laboratory-made or purchased from CSalomon, Plant Protection Institute, Hungarian Academy of Science, Budapest, Hungary) were used and hung on branches at ≈1 m height. In each experiment, five replicates were used, separated by at least 20 m, and within a replicate, traps were randomized and set 5 m apart (2004–2005) or 10 m apart (2022) in a row of bushes. Traps were checked twice per experiment, and sticky inserts replaced if needed. Traps were moved one position within the row after each check in the experiments 2004–2005, but not in 2022.

2.7 | Statistical analyses

No males were trapped in control traps in the first experiment, and this treatment was excluded from the statistical analysis. Catches per trap were pooled and log $(x+1)$ transformed before applying one-way ANOVA, followed by multiple comparisons adjusted according to the Bonferroni post hoc test, to compare catches among treatments. All significance tests were performed using SPSS Version 27 (SPSS Inc., Chicago, IL, USA).

3 | RESULTS

3.1 | Electrophysiological analyses

In the GC-EAD analyses of gland extracts using the INNOWax column, antennae of male *E. albithoracellus* showed strong and consistent response to two compounds eluting close to each other (Figure 1a). Weaker and less consistent responses were observed to

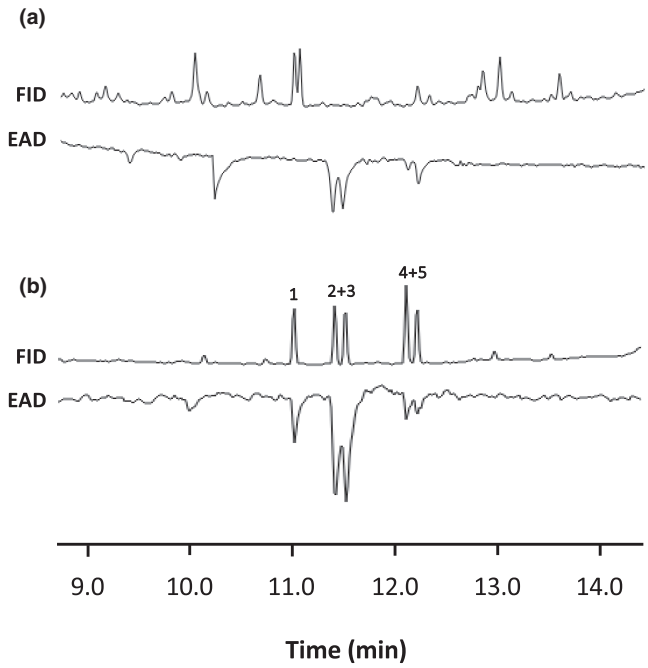

FIGURE 1 Gas chromatography with flame ionization (FID) and electroantennographic detector (EAD: male *Euhyponomeutoides albithoracellus* antennae) responses to (a) an aliquot of a 0.2 female equivalent of pheromone gland extract or (b) synthetic reference compounds (tetradecyl acetate (1), (*E*)-11-tetradecenyl acetate (2), (*Z*)-11-tetradecenyl acetate (3), (*E*)-11-tetradecenol (4) and (*Z*)-11-tetradecenol (5); 1 ng per compound). The analyses were performed using HP-INNOWax column.

two later eluting compounds with similar retention times. In addition, an antennal response was occasionally observed to a fifth compound eluting earlier than the other gland constituents. However, none of these compounds could be identified in the subsequent chemical analyses because the amounts present in the extracts were below the detection limit of the GC–MS. Based on the elution pattern of antennally active gland constituents, and the fact that male bud moths are attracted to the *A. podana* lure (Peltotalo & Touvinen, 1986), we hypothesized that the two compounds eliciting strongest antennal response were *E*11-14:OAc and *Z*11-14:OAc, that the later eluting compounds were the corresponding alcohols, *E*11-14:OH and *Z*11-14:OH and that the early eluting compound was 14:OAc, which has been reported as a sex pheromone component in other ermine moth species. Thus, additional GC-EAD analyses were performed using a synthetic blend including these five compounds as stimulus to confirm their activity.

All candidate compounds were shown to be electrophysiologically active, and their retention times matched the corresponding antennal responses observed in the analyses of gland extracts (Figure 1b). When stimulated with the synthetic blend, the responses to the unsaturated acetates were consistently higher than the responses to the corresponding alcohols. In addition, 14:OAc was found to elicit a strong antennal response, although a similar response was only observed inconsistently when antennae were stimulated with gland extracts, indicating that the compound was not produced by females in amounts eliciting any EAD response (Figure 1a). The response amplitudes for *E*11-14:OAc and *Z*11-14:OAc were similar when antennae were stimulated with gland extract and synthetic compounds (Figure 1a,b), indicating that these isomers were produced in similar amounts by female moths. Additional GC-EAD and GC–MS analyses using an HP-5 column showed similar results, although separation of the isomers of 11-14:OAc and 11-14:OH was very poor on this column (data not shown).

3.2 | Field trials

In the first experiment, we observed significant differences in catches among treatments ($F = 18.72$, df = 5, $p < 0.001$). High numbers of *E. albithoracellus* males were attracted to traps baited with a 1:1 blend of *E*11- and *Z*11-14:OAc, as well as to traps baited with this binary blend in combination with 14:OAc (Figure 2). Very few males were trapped when the lure contained only one of the Δ11 acetate isomers. In addition, trap catches were drastically reduced when the corresponding alcohols were present in a lure in the same amounts as the acetates. The average catch was numerically >60% higher when 14:OAc was added to the binary acetate blend (Figure 2), but the difference between that treatment and the binary blend alone was not statistically significant. A second trapping experiment was performed to further investigate the potential synergistic effect on male attraction when adding different amounts of 14:OAc to the binary acetate blend. Again, no significant effect of 14:OAc on trap catches was observed ($F = 1.32$, df = 4, $p > 0.05$, Figure 3). Finally,

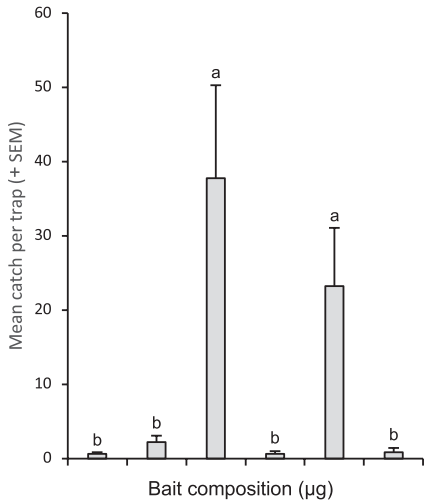

FIGURE 2 Catch of male *Euhyponomeutoides albithoracellus* in traps baited with different blends of candidate pheromone compounds. The field trial was performed in 2004 in a black currant orchard at Sörfors, Sweden. Bars with different letters indicate significantly different catches (ANOVA on log($x+1$)-transformed data followed by multiple comparisons according to the Bonferroni post hoc test: $p < 0.01$).

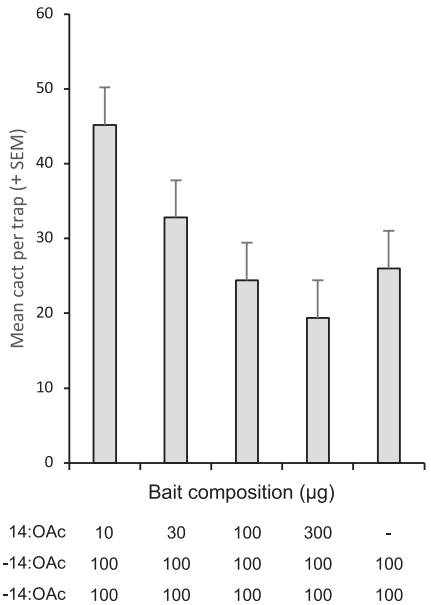

FIGURE 3 Catch of male *Euhyponomeutoides albithoracellus* in traps baited with different amounts of tetradecyl acetate in combination with a 1:1 blend of (*E*)-11-tetradecenyl acetate and (*Z*)-11-tetradecenyl acetate. The field trial was performed in 2005 in a black currant orchard at Sörfors, Sweden. 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: $p > 0.05$).

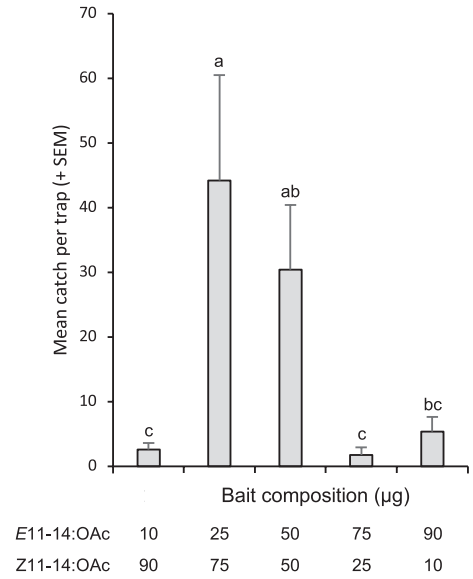

FIGURE 4 Catch of male *Euhyponomeutoides albithoracellus* in traps baited with different relative ratios of (*E*)-11-tetradecenyl acetate and (*Z*)-11-tetradecenyl acetate. The field trial was performed in 2022 in a black currant orchard at Rödupp, Sweden. Bars with different letters indicate significantly different catches (ANOVA on log($x+1$)-transformed data followed by multiple comparisons according to the Bonferroni post hoc test: $p<0.05$).

the third experiment revealed significant differences in attraction of males to different compositions of the binary acetate blend ($F = 8.46$, df = 4, $p<0.001$). Large numbers of males were captured in traps baited with 25% or 50% of the *E* isomer, whereas the other ratios attracted significantly fewer males (Figure 4).

4 | DISCUSSION

The results from our electrophysiological and chemical analyses, and trapping experiments, show that the main components of sex pheromone of *E. albithoracellus* are *E*11-14:OAc and *Z*11-14:OAc. Strong antennal responses to both acetate isomers were observed in GC-EAD analyses using female gland extracts and synthetic reference compounds (Figure 1). In addition, the first field trial revealed that both isomers are needed for attraction of conspecific males, and subtraction of either isomer resulted in a drastic trap catch reduction (Figure 2). Antennal responses to *E*11-14:OH and *Z*11-14:OH were also observed, but these were weaker and less consistent compared to the responses to *E*11- and *Z*11-14:OAc. Adding the alcohols to the binary acetate blend resulted in almost complete loss of attraction. We cannot, however, exclude the possibility that the alcohols are still part of the sex pheromone of *E. albithoracellus* and that the strong inhibitory effect observed was caused by using excessive amounts or skewed relative ratios of the alcohol isomers in relation to the acetate isomers.

Strong antennal response was also observed to 14:OAc during GC-EAD analyses with a blend of synthetic candidate compounds, but when antennae were exposed to gland extracts, a response at the retention time matching this compound was inconsistent. The second field test showed that adding different amounts of 14:OAc to the binary acetate blend did not cause any statistically significant differences in attraction of males to traps (Figure 3). An explanation for the strong antennal response, but lack of behavioural effect, to 14:OAc in male *E. albithoracellus* is thus unclear, but may be because of activation of the receptors for the unsaturated pheromone components on the male antenna, which has been observed in other ermine moth species (Löfstedt et al., 1990). Attraction of males to lures containing 14:OAc has not been reported for other species of the genus *Euhyponomeutoides* (www.pherobase.com), and so far, reports of 14:OAc as a primary sex pheromone component or a synergistic secondary component are restricted to small ermine moths of the genus *Yponomeuta* (e.g. Löfstedt et al., 1986, 1991).

The third experiment testing different relative ratios of *E*11- and *Z*11-14:OAc showed that male *E. albithoracellus* were highly attracted to lures containing 25% or 50% of the *E* isomer, whereas much fewer males were captured in traps baited with the other blends tested (Figure 4). The chemical analyses of gland extracts from individual females revealed that the *E. albithoracellus* sex pheromone is produced in minute amounts. Neither acetate isomer could be detected by FID, and the relative ratios of the compounds in the extracts could thus not be established. Our GC-EAD and catch data, however, suggest that females produce a pheromone that is indeed close to the 1:1 blend of *E*- and *Z*11-14:OAc used in lures for monitoring of *A. podana* (Persoons et al., 1974), which Tuovinen (1989) found useful also for trapping of *E. albithoracellus*.

Our identification of female-produced sex pheromone components of *E. albithoracellus* is the first such study from the genus *Euhyponomeutoides*. A screening study in Japan by Ando et al. (1981), testing attraction of moth species to various lures, reported catches of male *Euhyponomeutoides trachydeltus* (Meyrick) in traps baited with *Z*11-14:OAc, but data on pheromone or sex attractant composition for other congeners are lacking. In a broader phylogenetic context, *E*11- and *Z*11-14:OAc are common sex pheromone components in lepidopterans, and confirmed activity of these compounds in field tests has been reported from species in the families Cosmopterigidae (Bestmann et al., 1993), Crambidae (e.g. Klun et al., 1973), Noctuidae (e.g. Burns & Teal, 1989), Pyralidae (e.g. Wakamura et al., 1999), Tortricidae (e.g. Roelofs & Arn, 1968) and Yponomeutidae (e.g. Löfstedt & van der Pers, 1985).

Damage caused by bud moth larvae is a major problem for currant growers in northern Sweden and Finland, whereas recent monitoring suggests that the species is absent in currant orchards in Norway (O. Anderbrant, unpubl. observations). With stricter EU regulations on pesticide use, there is an urgent need for alternative control methods for currant pests, including *E. albithoracellus*.

Today, monitoring of the species is performed using lures aimed for *A. podana* (Persoons et al., 1974). Based on the results from this study, there is no need to develop a more specific pheromone lure for monitoring of the currant bud moth, and growers can use the *A. podana* lure for this purpose. The next step in the implementation of pheromone-based methods in IPM of *E. albithoracellus* is to evaluate mating disruption as an efficient control tactic for this pest. Kivijärvi et al. (2005) performed small-scale mating disruption experiments in organic currant orchards in Finland using high densities of the *A. podana* lure. Experiments were performed in 2 years using different types and densities of dispensers. The results from that pilot study, where only part of the crop area was treated, are difficult to evaluate, however, and treatment of whole fields is needed to study the efficacy of mating disruption for control of *E. albithoracellus*.

AUTHOR CONTRIBUTIONS

Glenn P. Svensson: Data curation; formal analysis; methodology; writing – original draft; writing – review and editing. **Olle Anderbrant:** Data curation; funding acquisition; writing – review and editing. **Elisabeth Öberg:** Data curation; writing – review and editing. **Erling V. Jirle:** Data curation; writing – review and editing. **Sven Hellqvist:** Conceptualization; data curation; writing – review and editing. **Christer Löfstedt:** Conceptualization; methodology; writing – review and editing.

ACKNOWLEDGEMENTS

We thank Ann-Kristin Isaksson for collecting and sending black currant branches to Lund, Peter Henriksson for allowing us to perform trapping experiments in his black currant orchard, and Christian Olsson and Göran Birgersson for their initial analyses of bud moth gland extracts. This study was supported by a grant from the Swedish Farmers' Foundation for Agricultural Research (No. O-20-20-452) to OA.

CONFLICT OF INTEREST STATEMENT

The authors declare that they have no conflict of interest.

DATA AVAILABILITY STATEMENT

The trap catch data have been deposited in the DORIS database at the following link: https://snd.gu.se/en/catalogue/study/2022-248

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How to cite this article: Svensson, G. P., Anderbrant, O., Öberg, E., Jirle, E. V., Hellqvist, S., & Löfstedt, C. (2023). Identification of (*E*)- and (*Z*)-11-tetradecenyl acetate as sex pheromone components of the currant pest *Euhyponomeutoides albithoracellus*. *Journal of Applied Entomology*, *147*, 313–319. https://doi.org/10.1111/jen.13115