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Identification of the sex pheromone of the pink grass worm, *Tmetolophota atristriga,* reveals two different taxa

Ashraf M. El-Sayed[*] and Lee-Anne M. Manning

The New Zealand Institute for Plant and Food Research Limited, Canterbury Research Centre, Lincoln, 8152, New Zealand

[*]Author for correspondence email: *ashraf.el-sayed@plantandfood.co.nz*

Abstract: The pink grass worm, *Tmetolophota atristriga* (Walker), is a New Zealand native species. It is abundant throughout the North and South Islands and is a pest which defoliates pasture. In this study, the sex pheromone of this species was investigated. Analysis of the extract of the female sex pheromone gland by GC/EAD, GC/MS and chemical derivatization identified six compounds: two monounsaturated compounds, (Z11)-hexadecenal (Z11-16:Ald) and (Z11)-hexadecenyl acetate (Z11-16:Ac), three saturated compounds, hexadecanal (16:Ald), hexadecyl acetate (16:Ac), octadecan-1-ol (18:OH), and a triene hydrocarbon, (3Z,6Z,9Z)-tricosatriene (Z3Z6Z9-23:Hy). Several field trapping experiments were conducted testing various pheromone blend combinations of the six identified compounds. Results suggested that the two different taxa of *T. atristriga* respond differently to the female sex pheromone compounds. The first taxon responds equally to the two-component and other blends including the six-component blend. The second taxon responds only to the six-component blend containing Z11-16:Ald, Z11-16:Ac, 16:Ac, 16:Ald, 18:Ald and Z3Z6Z9-23:Hy or a ternary blend containing Z11-16:Ald, Z11-16:Ac and Z3Z6Z9-23:Hy. In experiments testing various doses (0.1, 1, and 10 mg) of Z11-16:Ald and Z11-16:Ac in a binary blend or six-component blend, the 1 mg dose of these two compounds was the optimum dose for male attraction in both taxa. This pheromone identification is the second of any New Zealand Noctuidae species and suggests similarities with some Australian native Noctuidae species. In addition, this study reports the first occurrence of Z3Z6Z9-23:Hy in the sex pheromone blend of any Noctuidae species.

Keywords: New Zealand**,** Noctuidae, *Tmetolophota atristriga*, sex pheromone, monitoring, pasture pest.

Introduction

The pink grass worm, *Tmetolophota atristriga* (Walker) (Lepidoptera: Noctuidae), is a New Zealand native species. It is abundant throughout the North and South Islands and is considered a minor pest, defoliating pastures in New Zealand. With changes in farming practices along with climate change, the pest status of insects can rapidly change. Sex pheromones are being used for monitoring and control of insect pests and provide many advantages over the use of pesticides, being species-specific, non-toxic and leaving almost no residue. The identification of the sex pheromone for *T. atristriga* will enable the development of lures to identify the spread of this pest and offers a potential means to monitor pest populations reaching an action threshold. As such, the *T. atristriga* sex pheromone could be a key tool in the future for the control of sudden or sporadic outbreaks of this pest.

Surprisingly, New Zealand has a relatively small number of Noctuidae, with about 140 known species being endemic to the country and occurring nowhere else (Dugdale 1989). In New Zealand, the sex pheromone has been identified for only one other noctuid species, *Graphania mutans* (Walker) (Frérot and Foster 1991). Two distinct taxa within *G. mutans* were found. Females from an Auckland population produced (Z)-9-tetradecenol (Z9-14:OH), (Z)-9-tetradecenyl acetate (Z9-14:OAc), (Z)-7-tetradecenol (Z7-14:OH) and (Z)-7-tetradecenyl acetate (Z7-14:OAc), while females from a Canterbury population produced these four compounds plus (Z)-9-tetradecenal (Z9-14:Ald). Male responses from each population were specific to the pheromone blend produced by females of the same population.

This work was undertaken to identify the sex pheromone of *T. atristriga* and to develop a lure for monitoring and possible control of this pest. In addition, the identification of the sex pheromone of a second noctuid species will help to understand the biology, ecology and behaviour of this pest as well as shed light on the evolution and speciation of Noctuidae species in New Zealand. Here, we report the conclusive identification of the pheromone blends of *T. atristriga* and the evaluation of their biological activity in field bioassays in Canterbury, New Zealand.

Materials and Methods

Insects

As no moths of this species are laboratory-reared in New Zealand, and little is known about the life cycle, adult moths were field-collected by light trapping in Canterbury during the summer months of 2013. All Noctuidae moths were housed individually once caught, before being transported back to the laboratory for identification of *T. atristriga* species. Female and male *T. atristriga* from light trapping samples were kept individually and maintained at a natural summer light and temperature regime.

Pheromone gland extraction

The sex pheromone glands of calling females (five females) were removed during the first 2 h of the scotophase and extracted in 20 μL of hexane (Merck Ltd, Darmstadt, Germany) contained within a liquid-nitrogen-cooled 0.5-mL conical vial (Wheaton, Millville, NJ, USA) for 5–10 min. After all glands had been excised, the vial and its contents were brought to room temperature, and the liquid phase was transferred to a 1.1-mL conical glass vial (Alltech, Deerfield, IL, USA) for storage in the −80°C freezer before analysis. Females were collected from two locations in Canterbury (Lincoln and Little River) and analyzed separately.

Chemicals

All compounds used as authentic standards in the chromatographic analysis or the field trapping experiments were >98% chemically pure and >99.5% isomerically pure by gas chromatography (GC analysis) and were stored at −80 °C until used. (Z)-11-hexadecenal (Z11-16:Ald), (Z)-11-hexadecenyl acetate (Z11-16:Ac), hexadecanal (16:Ald), hexadecyl acetate (16:Ac) and octadecanal (18:Ald) were purchased from Plant Research International,

Wageningen, The Netherlands. (3Z,6Z,9Z)-tricosa- 3,6,9-triene (Z3Z6Z9-23:Hy) was synthesized according to the method described by Gibb et al. (2007).

Gas chromatography/electroantennogram detector (GC/EAD)

Coupled GC/EAD analysis of pheromone gland extracts was conducted on a Varian 3800 GC equipped with a flame ionization detector (FID) and a splitless injector. The column effluent was split 1:1 between the FID and EAD apparatus. Antennal depolarization was detected using a high-resistance EAD Probe (Signal Interface Box, Type ID-02) and Intelligent Data Acquisition Controller (Type IDAC-02) (Syntech, Hilversum, The Netherlands). Antennae from 2 to 3-day-old males collected from Lincoln and Little River were excised at the base and attached to the silver electrodes housed in saline-filled glass electrodes using a micromanipulator (Narishige, Tokyo, Japan) to facilitate electrical connection. Up to five antennal preparations from each location were tested with different female extracts from the same location for GC/EAD analyses. A 30 m × 0.25 mm internal diameter (ID) × 0.25 µm VF5-MS capillary column (Factor Four, Varian Inc.) and a Y splitter (Alltech, Deerfield, IL) were used for the analysis. The oven temperature was programmed to increase from 80°C (held for 1 min) to 240°C at 10 °C/min. Helium was used as the carrier gas.

Gas chromatography/mass spectrometry (GC/MS) analysis

The gland extracts and the synthetic chemicals were analysed on a Saturn 2200 GC/MS (Varian Walnut Creek, CA, USA) using an ionization voltage of 70 eV and a mass range of 30–650 *m/z*, equipped with two different capillary columns: a non-polar 30 m × 0.25 mm ID × 0.5 µm VF5-MS capillary column (Factor four, Varian Inc., USA) and a polar 30 m × 0.25 mm ID × 0.5 µm VF23-MS capillary column (Factor Four, Varian Inc.). In both the columns, the injection was splitless and the oven was programmed to increase from 80°C (held for 1 min) to 240 °C at 10 °C/min and then held for 13 min. Compounds were identified by

111 comparing the retention time and mass spectra with those of synthetic compounds on two
112 different capillary columns.

113 **Dimethyldisulfide derivatizations (DMDS)**

114 We followed the procedure described by Buser et al. (1983) and Leonhardt and DeVilbiss
115 (1985). Approximately 50 μL DMDS and 5 μL iodine solution (60 mg of I_2 in 1 mL of diethyl
116 ether) were added to 20 female equivalents in a 1.8-mL glass vial, sealed with a Teflon-lined
117 cap, and held at 40°C for 15 h. The reaction was quenched with 50 μL of 5% aqueous sodium
118 thiosulphate, and the organic layer was dried with anhydrous sodium sulphate and transferred
119 to a clean 1.5-mL tapered-bottom vial, and blown down with a stream of argon to
120 approximately 10 μL. A 1-μL aliquot [ca. two female equivalents (FE)] was immediately
121 analysed by GC/MS.

122 **Field Trapping Experiments**

123 In all field trials, green unitrap bucket traps (International Pheromone Systems Ltd., Cheshire,
124 UK) were suspended 1 – 1.5 m above the pasture, in a random block design, with a minimum
125 of 20 m between each trap and 20 m between each replicate. Each treatment was randomly
126 assigned to a trapping station within each trapping row. Each trap contained a 2-cm killing
127 strip of dog flea collar (Bayer, Germany), which contained 5% Diazinon insecticide as an
128 active ingredient. All the blends of the synthetic compounds were applied to the large ‘wells’
129 of red rubber septa (West Pharmaceutical Services, Kearney, NE, USA) which were diluted in
130 150μL of n-hexane GR (Merck Ltd, New Zealand). The solvent was allowed to evaporate in a
131 fume hood and the septa were stored in heat-sealed foil bags at −20°C until use. Pheromone
132 impregnated septa were placed in the top compartment of the trap. In all field trials, five
133 replicates for each treatment were tested. Traps were checked weekly in all trials, and moths
134 were returned to the laboratory to be identified using a binocular microscope and the
135 reference key by Bejakovic and Dugdale (1997).

Testing individual compounds (Trial 1) The relative attractiveness of the individual EAD active compounds (i.e. Z11-16:Ald; Z11-16:Ac, 16Ald, 16:Ac,18:OH and Z3Z6Z9-23:Hy) found in the sex pheromone gland were field-tested in an organic apple orchard near Lincoln, Canterbury, New Zealand. The orchard had a thick mixed pasture understorey. Field-testing was conducted during three weeks in January 2014. Compound loading was 1 mg per septa. Traps baited with a blank lure were used as controls.

Testing binary blends with different ratios of Z11-16:Ald and Z11-16:Ac The relative attractiveness of three binary blends containing various ratios of Z11-16:Ald and Z11-16:Ac were field-tested in the same organic apple orchard used in Trial 1, during three weeks in February 2014. The ratios of Z11-16:Ald and Z11-16:Ac in the binary blends were 75:25, 50:50 and 25:75. In all binary blends, the total loading of the two compounds was 1mg. Traps baited with a blank lure were used as controls.

Dose-response experiment of binary blend The effect of three doses (i.e. 0.1, 1, 10 mg) of the optimum binary blend obtained in Trial 1 was investigated for the attraction of male *T. atristriga* in the same organic apple orchard. The trial was deployed for three weeks in April 2014. Traps with a blank lure were used as controls.

Testing the minor compounds identified in the sex pheromone gland In a subsequent experiment, four pheromone blends were tested to investigate the synergistic effect of the minor compounds: 1) a two-component blend containing Z11-16:Ald andZ11-16:Ac at a ratio of 0.25:0.75 mg; 2) a five-component blend containing Z11-16:Ald, Z11-16:Ac,16:Ald, 16:Ac and 18:OH at a ratio of 0.25:0.75 0.05:0.05:0.05 mg; 3) a three-component blend containing Z11-16:Ald, Z11-16:Ac and Z3Z6Z9-23:Hy at a ratio of 0.25:0.75:0.05 mg; 4) a six-component blend containing Z11-16:Ald, Z11-16:Ac,16:Ald, 16:Ac, 18:OH, and 3Z6Z9-23:Hy at a ratio of 0.25:0.75:0.05:0.05:0.05 mg. Traps with a blank lure were used as controls. This trial was tested for three weeks from January to February 2015 in two locations:

1) the same apple orchard as used in Trial 1; and 2) in a mixed fruit orchard, containing cherries, apricots, peaches, plums and nectarines in Little River, Canterbury, New Zealand. The distance between the two sites is about 50 km. The experimental design and protocol were identical to the above experiments.

Dose-response experiment of six-component blend The effect of the three doses (i.e. 0.1, 1, 10 mg) of the optimum six-component blend was investigated for the attraction of male *T. atristriga* in the mixed fruit orchard in Little River, Canterbury, New Zealand. The trial was deployed for three weeks from February to March 2015. Traps with a blank lure were used as controls.

Data analysis

The variance of the mean captures obtained with each treatment was stabilized using the $\sqrt{(x+1)}$ transformation. The significance of the treatment effects in the field trapping experiments were tested using ANOVA (SAS Institute Inc. 1998). Significantly different means were identified using Fisher's Protected Least Significant Difference.

Results

GC/EAD analysis Analysis of the female sex pheromone gland extracts by GC/EAD revealed that six compounds consistently elicited EAD responses from male moth antennae (Fig. 1). The GC/EAD profile from the Lincoln population was quite similar to the Little River population. These compounds were later identified as Z11-16:Ald (1), 16:Ald (2), Z11-16:Ac (3), 16:Ac (4), 18:OH (5), Z3Z6Z9-23Hy (6). Both Z11-16:Ac and Z11-16Ald elicited the strongest EAD responses, while the other four compounds elicited similar EAD responses.

Chemical identification. The mass spectrometric data of the EAD active compounds suggest the compounds are a mixture of saturated and unsaturated aldehyde, acetate and hydrocarbon compounds. Comparison of the retention times of the EAD active compounds

185 with synthetic compounds on a non-polar column enabled the tentative identification of six
186 EAD active compounds as follows: (Z)-11-hexadecenal (Z11-16:Ald), hexadecanal (16:Ald),
187 (Z)-11-hexadecenyl acetate (Z11-16:Ac), hexadecyl acetate (16:Ac), octadecanol (18:OH)
188 and (3Z,6Z,9Z)-tricosatriene (Z3Z6Z9-23:Hy). GC/MS analysis of DMDS-derivatized extract
189 showed an adduct with a molecular ion at *m/z* 332 (13%), the diagnostic ions at *m/z* 117
190 (63%, $C_6H_{13}S^+$) and m/z 215 (100% $C_{12}H_{23}OS^+$), indicating the addition of DMDS to a
191 double bond at position 11 for Z11-16:Ald. In addition, the DMDS-derivatized extract
192 showed another adduct with a molecular ion at *m/z* 376 (11%), and the diagnostic ions at *m/z*
193 117 (57%, $C_6H_{13}S^+$) and m/z 259 (100% $C_{14}H_{27}OS^+$), indicating the addition of DMDS to a
194 double bond at position 11 for Z11-16:Ac. The geometry of the double bond in the two
195 unsaturated compounds was confirmed by the chemical analysis of both *E* and *Z* isomers of
196 both compounds. The mass spectrum data of compound 6 were very similar to the mass
197 spectrum data provided in Millar (2000) and El-Sayed et al (2013). Further confirmation of
198 the identity of the compounds present in the gland extracts was confirmed by comparing
199 authentic standards with the gland extract on a polar capillary column. The chemical
200 composition of the sex pheromone gland from females collected from Lincoln was similar to
201 females collected from Little River.

202 ***Testing individual compounds*** When the six EAD active compounds identified in the
203 sex pheromone gland were tested individually at a 1-mg loading, none of these compounds
204 alone attracted male *T. atristriga*.

205 ***Testing binary blend with various ratios of Z11-16:Ald and Z11-16:Ac*** Changing the
206 ratio of Z11-16:Ald and Z11-16:Ac in the binary blend significantly affected the number of *T.*
207 *atristriga* caught in traps (Treatment, $F_{1,8} = 5.7$, $P < 0.04$) (Figure 2). A significantly higher
208 number of male *T. atristriga* were caught in traps baited with the binary blend at the ratio of
209 0.25:0.75 mg than traps bated with the blend containing a 0.5:0.5 mg ratio; no males were
210 caught in traps baited with the binary blend at a ratio of 0.75:0.25 mg (Figure 2).

Dose-response experiment of binary blend The amount of the binary blend loaded onto red rubber septa significantly affected the number of *T. atristriga* captured (Treatment, $F_{2,12}$ = 28.9, $P < 0.01$) (Figure 3). Increasing the dose from 0.1 to l mg resulted in a significant increase in the number of males caught ($P < 0.01$). Furthermore, increasing the dose to 10 mg resulted in a significant reduction in the number of males caught, compared with the 1-mg dose (Figure 3).

Testing the minor compounds identified in the sex pheromone gland In Lincoln Canterbury, the addition of the minor components in various combinations to the binary blend did not result in any significant increase in the number of males caught (Treatment, $F_{3,16} = 0.23$, $P = 0.87$) (Figure 4). In contrast, in Little River, no males were caught in traps baited with the binary blend alone (Figure 4). Males were only caught in a binary blend combined with either Z3Z6Z9-23Hy or 16:Ald, 16:Ac and 18:OH or all of the four minor components. The highest catch was obtained in traps baited with the binary blend combined with 16:Ald, 16:Ac, 18:OH and Z3Z6Z9-23Hy (Treatment, $F_{1,8} = 6.6$, $P < 0.05$) (Figure 4).

Dose-response experiment of six-component blend The amount of the six-component blend loaded onto red rubber septa significantly affected the number of *T. atristriga* captured (Treatment, $F_{3,16} = 4.7$, $P < 0.01$) (Figure 5). Increasing the dose from 0.1 to l mg resulted in a significant increase in the number of males caught ($P<0.0001$). Furthermore, increasing the dose to 10 mg resulted in a significant reduction in the number of males caught, compared with the 1-mg dose (Figure 5).

Discussion

The sex pheromone gland of female, *T. atristriga* contained at least six candidate pheromone compounds that elicited EAD responses from male antennae. None of these compounds was attractive when tested alone. Only a binary blend of Z11-16:Ac and Z11-16Ald at a ratio of 75:25 was attractive to males in one location (Lincoln), while this blend was not attractive

when tested in another location (Little River) just 50 km away. Interestingly, the minor compounds (16:Ald, 16:Ac, 18:OH and Z3Z6Z9-23:Hy) did not enhance male attraction in Lincoln, while in Little River at least Z3Z6Z9-23:Hy was critical for male attraction and the addition of the remaining compounds (16:Ald, 16:Ac, and 18:OH) significantly enhanced male attraction. These results suggest that there are two populations of *T. atristriga* in Canterbury with different response profiles to female sex pheromone. In the Lincoln population, males responded to a binary blend of Z11-16:Ac and Z11-16Ald at a 75:25 ratio with no synergistic effect of the minor compounds. Meanwhile, in the Little River population, males showed a more conservative response to the female sex pheromone blend and they responded only to a complex pheromone blend including the same binary blend plus the other four minor compounds. Analysis of the pheromone gland with GC/MS and GC/EAD indicated similar pheromone gland contents and male EAD response profiles. Therefore, it is unlikely that these two populations are two distinctly different strains because the females of each population produce the same pheromone blend and therefore we anticipate there will be no reproduction barrier between these two taxa based on the sex pheromone.

Of the four minor components, Z3Z6Z9-23:Hy was essential for male attraction in the Little River population. This is evident because males were caught only in traps baited with a binary blend containing Z3Z6Z9-23:Hy. Similarly, male *Conogethes pluto* (Butler) respond only to a multicomponent pheromone blend that contains Z3Z6Z9-23:Hy (El-Sayed et al. 2013). So far, Z3Z6Z9-23:Hy has been reported in Crambid, Arctiid and Geometridae species (El-Sayed 2021). This result indicates that this polyunsaturated hydrocarbon plays a role in the sexual communication system of *T. atristriga*, and is the first report of this compound in any Noctuid species. This finding has added to the growing list of lepidopterous species that have been found to use aliphatic aldehyde, alcohol, acetate, and polyunsaturated hydrocarbon components in their pheromone blends. The variation in male response between the two locations could be due to males in the Little River population being under strong selection pressure to recognize compatible mates due to partial overlap in chemical composition with

other moth species in that location, which is not the case in Lincoln's population. Interestingly, this intraspecific variation in sex pheromone has evolved even though the two populations are only 50 km apart. In oblique banded leafrollers *Choristoneura rosaceana* (Walker) there is no difference in the response of males to female sex pheromone between populations found in Eastern and Western North America, which are separated by thousands of kilometres (El-Sayed et al. 2001). This might indicate that distance between populations is less important than selection pressure within a geographical area.

In the New Zealand ecosystem, the reported intraspecific variations in sex pheromone systems are accounted for by variation in the content of the sex pheromone gland. For example, New Zealand greenheaded leafroller, *Planotortrix excessana* (Walker), populations collected from Auckland and Christchurch were found to use a mixture of (Z)-8-tetradecenyl acetate and tetradecyl acetate. A population from the mid-North Island was found to use two completely different monounsaturated acetates, (Z)-5- and (Z)-7-tetradecenyl acetate (Z5-14:Ac and Z7-14:Ac) (Galbreath et al. 1985). In a field cage experiment, males of the two populations (Christchurch and mid-North Island) were attracted only to pheromone extracts from females of their own population (Foster et al. 1989), which suggests that these two populations are sibling species. Further examination of the populations that use Z5-14:Ac and Z7-14:Ac found two populations use two different ratios of these compounds at 3:97 to 71:29 with a small number of females that overlap in both ratios (Foster et al. 1989). However, males from both populations mated with tethered females from both populations, suggesting a cross attraction exist between the two populations. In contrast, two distinct taxa within *G. mutans* were found: females from an Auckland population produced Z9-14:OH, Z9-14:Ac, Z7-14:OH and Z7-14:Ac, while females from a Canterbury population produced these four compounds plus Z9-14:Ald. Male responses from each population were specific to the pheromone blend produced by females of the same population (Frérot and Foster 1991). In the common forest looper, *Pseudocoremia suavis* (Lepidoptera: Geometridae), three compounds are produced by females that include two major compounds, (Z6)-cis-9,10-

epoxynonadec-6-ene and (Z3,Z6)- cis-9,10-epoxynonadeca-3,6-diene, and one minor compound, (Z3,Z6)-cis-9,10-epoxyhenicosa-3,6-diene (Gibb et al. 2006). Field testing of these compounds in several locations in the South Island revealed two distinct taxa, where males of the first taxon responded to (6Z)-cis-9,10-epoxynonadec-6-ene, and males in the second taxon were attracted to lures containing (Z3,Z6)-cis-9,10-epoxynonadeca-3,6-diene (Gibb et al. 2006). Since the authors did not analyze sex pheromone gland content of females from different locations, it is not clear if this intraspecific variation is based on the difference in the pheromone blend produced by females paralleled with a tuned male response, or is just a variation in male response. Therefore the case described in our study might be the first example that demonstrates intraspecific variation in the sex pheromone system within a given species based on a variation in male response to sex pheromone and not on variation in the content of the sex pheromone gland. The geographical isolation of the New Zealand ecosystem from the rest of the world provides a great opportunity to shed light on intraspecific and interspecific variation in sex pheromone between species and its role in the evolution of new species.

Of the trapping systems tested for the capture of *T. atristriga*, green bucket traps baited with 1 mg of either the binary blend of Z11-16:Ac and Z11-16:Ald at a ratio of 75:25 or the six component blend containing the same binary blend with other for minor compounds were efficient for monitoring the *T. atristriga* population in the two locations. The inclusion of Bayer dog flea collar killing strip, which contained 5% Diazinon insecticide, was appropriate for long-term studies. However, it may be possible to further refine the trapping system because *T. atristriga* males were caught in reasonable numbers in bucket traps without an insecticidal strip. Such traps would be useful for population suppression through mass trapping (El-Sayed et al. 2006) or lure and kill (El-Sayed et al. 2009).

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Figure legends

Figure 1. Coupled Gas Chromatography/Electroantennogram Detector (GC/EAD) responses of the antennae of male *T. atristriga* to female gland extract. Chromatographic column and conditions: a non-polar VF5-MS capillary column was used for the analysis, the oven temperature was programmed to increase from 80°C (held for 1 min) to 240°C at 10°C/min. 1) Z11-16:Ald, 2) 16:Ald, 3) Z11-16:Ac, 4) 16:Ac, 5)18:OH, 6) Z3Z6Z9-23:Hy.

Figure 2. Mean catch ± SEM of *T. atristriga* in traps baited with binary blends containing different ratios of Z11-16:Ald and Z11-16:Ac. Different letters on columns indicate significant differences ($P < 0.05$).

Figure 3. Mean catch ± SEM of *T. atristriga* in traps baited with three doses of the binary blend containing Z11-16:Ald and Z11-16:Ac at a ratio of 25:75. Different letters on columns indicate significant differences ($P < 0.05$).

Figure 4. Mean catch ± SEM of *T. atristriga* in traps baited with four blends of the six candidate pheromone compounds found in the female sex pheromone gland. The trial was conducted in two locations: Lincoln (top) and Little River (bottom). Different letters on columns indicate significant differences ($P < 0.05$).

Figure 5. Mean catch ± SEM of *T. atristriga* in traps baited with three doses of the six-component blend containing Z11-16:Ald, Z11-16:Ac, 16:Ald, 16:Ac, 18:OH and Z3Z6Z9-23:Hy. Different letters on columns indicate significant differences ($P < 0.05$).

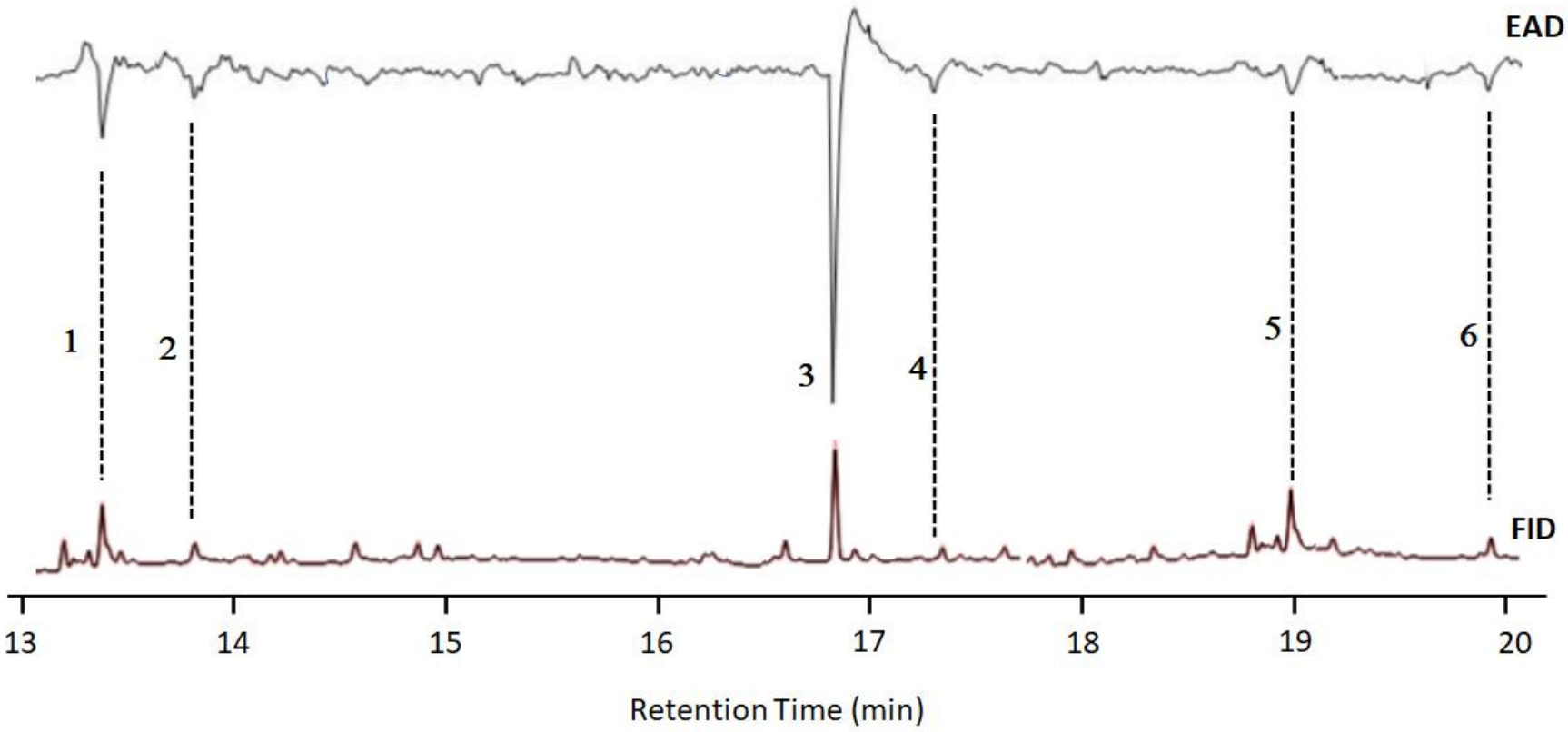

Fig. 1

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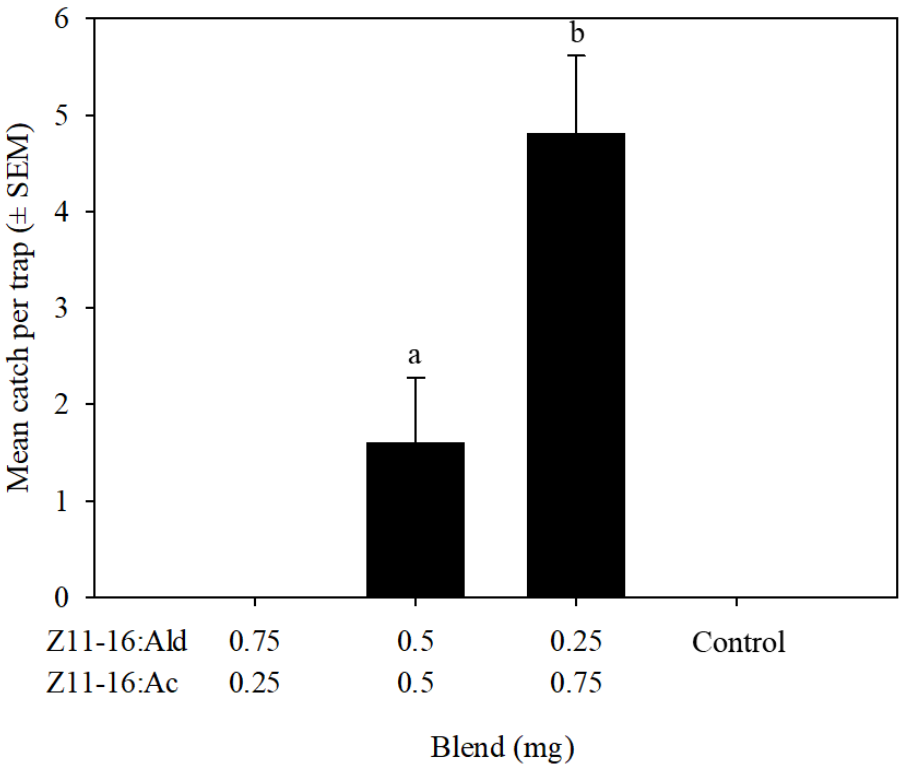

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400 Fig 2

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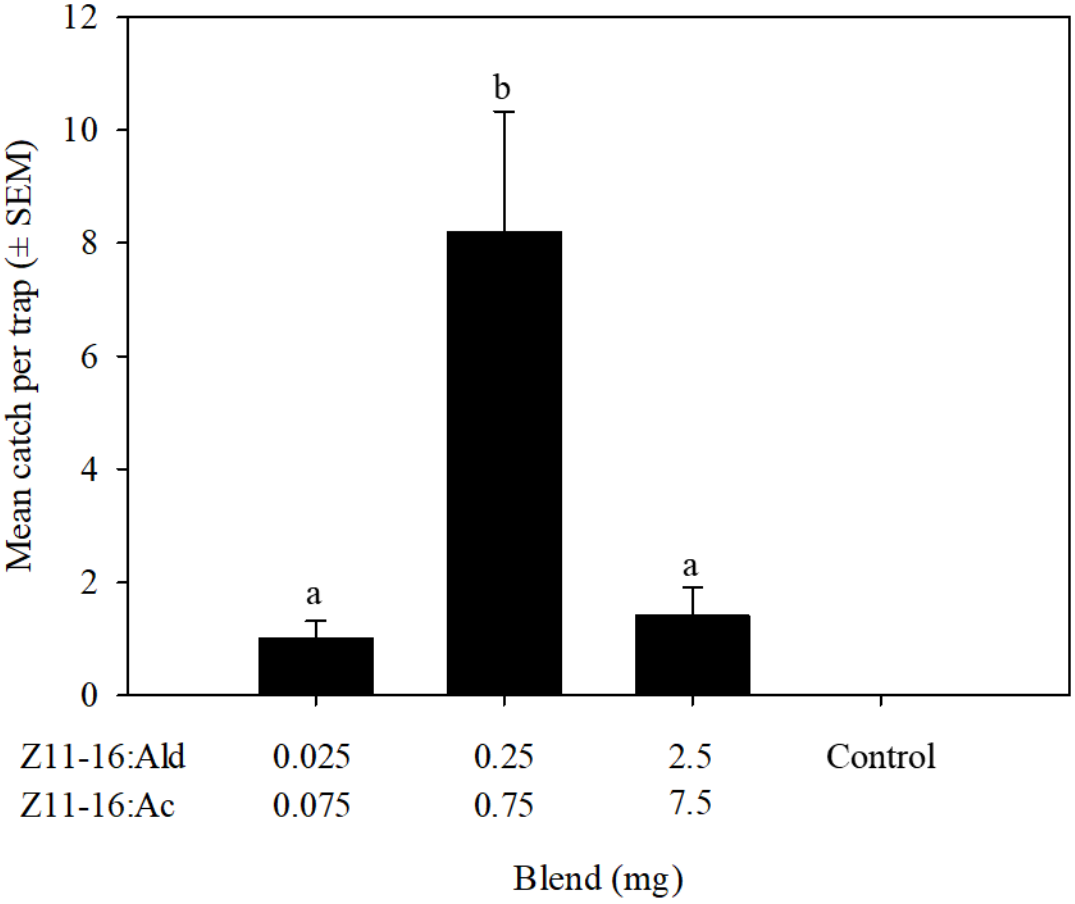

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404 Fig. 3

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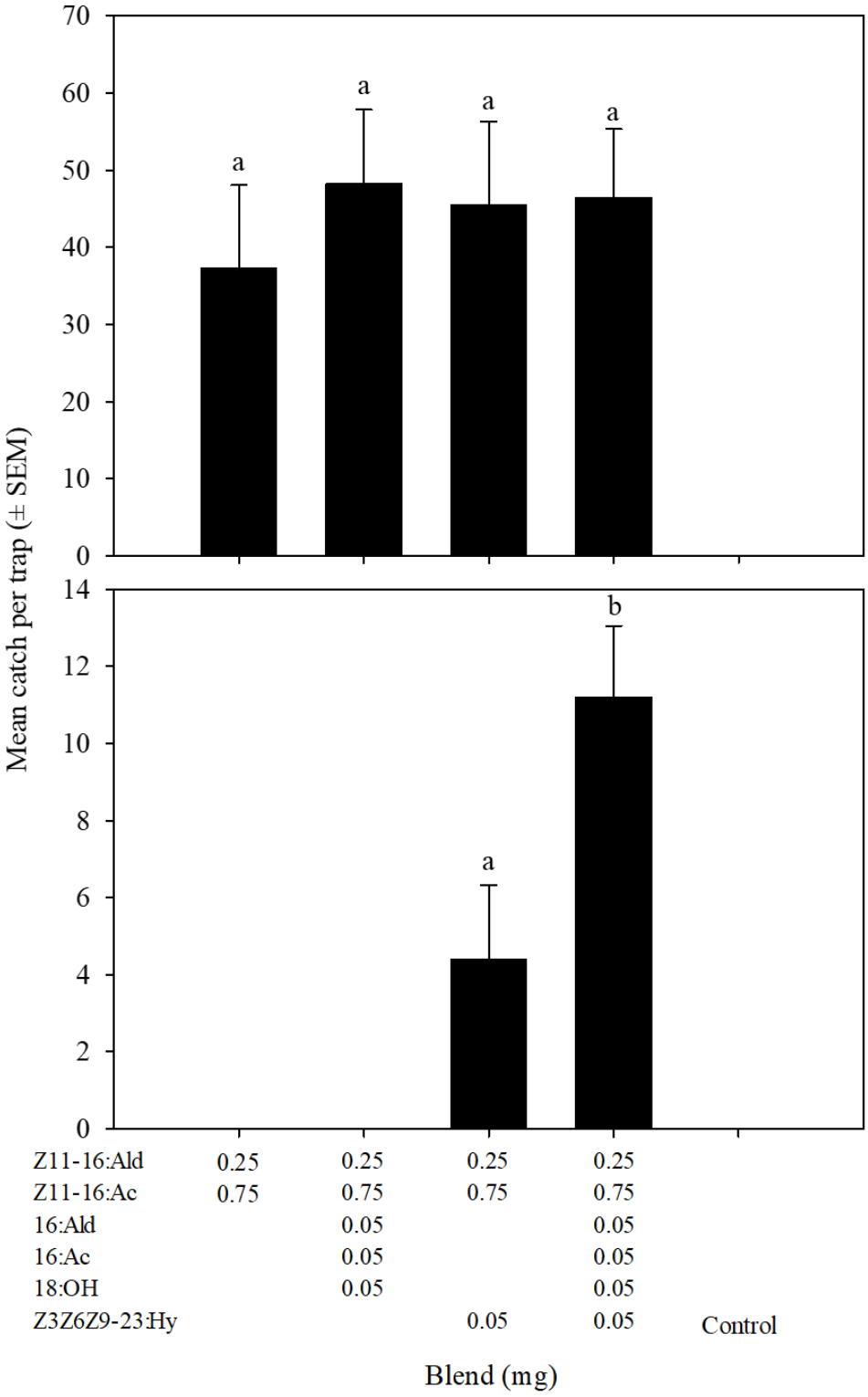

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408 Fig. 4

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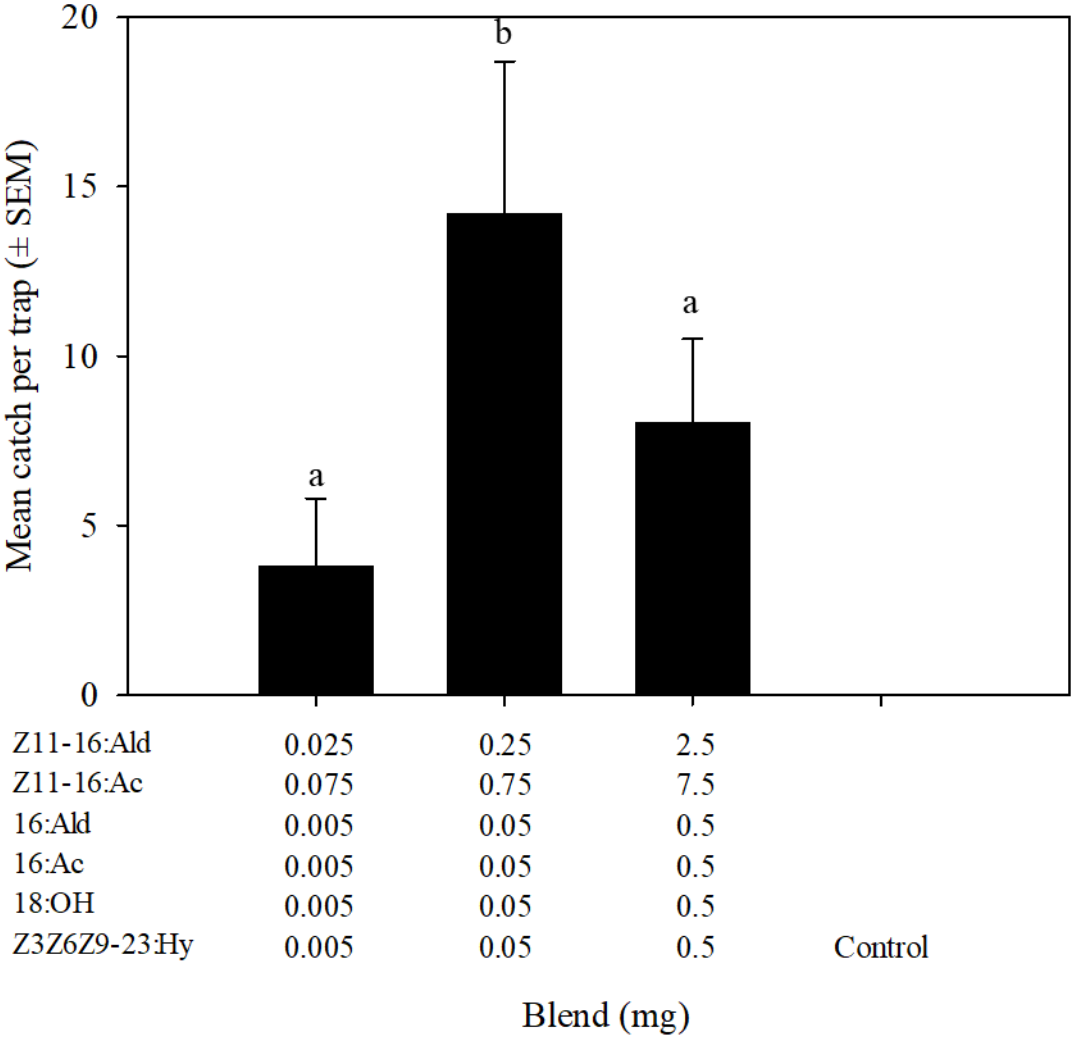

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412 Fig. 5