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Pheromone stereochemical specificity in the biology of the bean beetle *Acanthoscelides obtectus*

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Abstract

1. The dried bean beetle, *Acanthoscelides obtectus*, is a serious pest of legume crops, particularly *Phaseolus vulgaris* beans, and its management is challenging due to the beetle's cryptic larval stage. The major male-produced pheromone of *A. obtectus* is methyl (*E*,*R*)-2,4,5-tetradecatrienoate, crucial for female attraction, with the (*S*)-enantiomer emitted in smaller amounts (*R*:*S* ca. 9:1). Despite its identification half a century ago, it is still not commercially available for bruchid surveillance due to production and stability issues, with gaps in our knowledge relating to the bioactivity of the stereoisomers.
2. We thus aim to clarify the behavioural specificity of the (*R*)- and (*S*)-enantiomers of methyl (*E*)-2,4,5-tetradecatrienoate, along with two commercially available isomers (methyl myristoleate and methyl myristate).
3. Electrophysiological (EAG) assays with unmated females show that stereochemically pure methyl (*E*,*R*)-2,4,5-tetradecatrienoate is EAG-active alone or when part of the natural 9:1 or the racemic 1:1 blends, whereas the stereochemically pure (*S*)-enantiomer is inactive. Moreover, the structural analogues do not elicit significant EAG responses.
4. Unmated females give positive behavioural responses in the olfactometer only to methyl (*E*,*R*)-2,4,5-tetradecatrienoate, but not to its antipode or the structural analogues. Furthermore, stereochemically pure methyl (*E*,*R*)-2,4,5-tetradecatrienoate elicits the same level of behavioural activity as the 9:1 blend. Curiously, the presence of the (*S*)-enantiomer in equal proportions synergizes female preference for stereochemically pure (*R*).
5. These findings provide a better understanding of the pheromone biology of *A. obtectus* and create a platform for the development of pheromone trap-based surveillance with racemic methyl (*E*)-2,4,5-tetradecatrienoate.

KEYWORDS
Acanthoscelides obtectus, beetle, chiral, enantiomer, pest management, pheromone

INTRODUCTION

Larvae of the dried bean beetle, *Acanthoscelides obtectus* Say (Coleoptera: Chrysomelidae), are major storage pests of legume crops, particularly *Phaseolus vulgaris* L. beans (Fabaceae) (Imura, 1990). Originating in the Neotropics, the beetle has now spread worldwide, and infestations are observed globally (Alvarez et al., 2005). The pest has multiple generations per year and can reproduce both in the field and granaries, resulting in yield losses and a reduction in the quality of stored seeds (Paul et al., 2010).

Detecting and controlling infestations in storage facilities or warehouses can be challenging due to the cryptic lifestyle of the larvae, which are difficult to detect visually. The first instars bore into bean seeds, feeding and developing internally until they mature into adult beetles that emerge through circular exit holes (Njoroge et al., 2017). Greater attention is being directed towards environmentally benign integrated pest management practices, that is, a combination of chemical, cultural, biological and mechanical approaches, the efficacy of which can be increased with the development of sensitive beetle surveillance (Vuts et al., 2024). For this, the use of semiochemicals, particularly pheromones, can be a feasible option.

The major male-produced volatile sex pheromone component of *A. obtectus* was previously identified as methyl (*E*,*R*)-2,4,5-tetradecatrienoate, the presence of which is crucial for female attraction, although methyl (*E*,*S*)-2,4,5-tetradecatrienoate is also emitted in small quantities (*R*:*S* ca. 9:1) (Vuts, Francke, et al., 2015). Furthermore, host shift leads to an increase in the production of methyl (*E*,*R*)-2,4,5-tetradecatrienoate within a single generation (Vuts et al., 2018). However, using methyl (*E*)-2,4,5-tetradecatrienoate for detection and monitoring poses several challenges. Firstly, the behavioural specificity of its stereoisomers is unknown. This is crucial because the activity of optical isomers is defined by their molecular configuration (Mori, 2007; Pickett et al., 2013; Sims et al., 2022; Tumlinson et al., 1977). For example, in the scarab beetle *Oryctes rhinoceros*, the pheromone (*R*)-ethyl 4-methyloctanoate is significantly more attractive than its inactive antipode and as attractive as the racemic mixture (Hallett et al., 1995). The weevil *Rhynchophorus palmarum* responds more strongly to (*S*)- and racemic (2*E*)-6-methyl-2-hepten-4-ol than to the corresponding (*R*)-enantiomer (Oehlschlager et al., 1992). Curiously, the naturally occurring contact sex pheromone of the cockroach *Blatella germanica* is less effective in eliciting courtship responses in males compared to other stereoisomers that do not occur naturally (Eliyahu et al., 2004). There are also instances where insects do not differentiate between enantiomers, such as the case of termites and the queen signal 2-methyl-1-butanol (Yamamoto et al., 2012). Therefore, an investigation into the relationship between stereochemistry and bioactivity is crucial before the practical utilization of the *A. obtectus* main pheromone component.

Another issue with the use of methyl (*E*,*R*)-2,4,5-tetradecatrienoate is that its synthesis is complex, costly, low-yielding and the product is unstable (Mori, 2012). This may be overcome by comparing its biological activity with that of structural analogues, which may be cheaper, more stable and more easily synthesized. Here, using a combination of electrophysiological and behavioural assays with unmated *A. obtectus* females, we assess the bioactivity of the two optical isomers of methyl (*E*)-2,4,5-tetradecatrienoate, as well as two commercially available isomers. Our aims were to 1) clarify the bioactivity of the optical isomers of methyl (*E*)-2,4,5-tetradecatrienoate individually, as well as in the naturally emitted 9:1 *R*:*S* and racemic 1:1 blends, and 2) determine if cheaper structural analogues (methyl myristoleate and methyl myristate) were feasible to be used as attractants for field deployment.

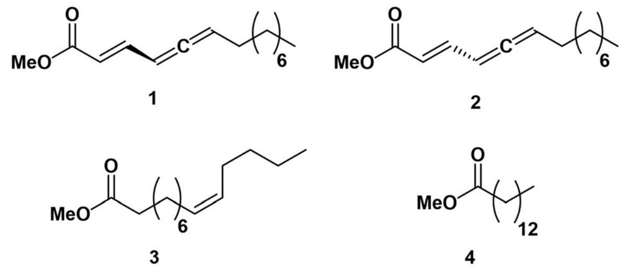

FIGURE 1 Structure of methyl (*E*,*R*)-2,4,5-tetradecatrienoate (**1**), methyl (*E*,*S*)-2,4,5-tetradecatrienoate (**2**), the mono-unsaturated methyl myristoleate (**3**) and the saturated analogue methyl myristate (4).

MATERIALS AND METHODS

Insects

Acanthoscelides obtectus beetles were reared on dry *Phaseolus vulgaris* L. (Fabaceae) 'Cannellini' beans (Waitrose Limited, UK) in a controlled environment room (20°C temperature, 60% relative humidity and a 16:8 h light: dark photoperiod). Individual beans were placed in separate wells of a plastic Eppendorf rack and covered with a transparent acetate sheet until adult beetles emerged. The wells were inspected every day, and upon the emergence of a single adult from each bean, the sex of the beetle was identified under a microscope (ZEISS, Germany), based on morphological characteristics (Nahdy, 1994). Male and female beetles were immediately separated to ensure female beetles remain unmated for electrophysiological and behavioural tests. If more than one adult beetle was found in a single well, those individuals were excluded from the experiments. This precaution was taken due to the observed weak response of mated females to the pheromone (Vuts et al., 2024). Moist cotton wool was provided to the beetles as a source of water.

Chemicals

Enantiomerically pure methyl (*E*,*R*)- and methyl (*E*,*S*)-2,4,5-tetradecatrienoate (purity: 99% for both **1** and **2** respectively, Figure 1) were synthesized as previously described (Mori, 2012). Briefly, nonanal was treated with lithium trimethylsilylacetylide, and the resulting propargyl alcohol was chemoenzymatically resolved with immobilized lipase PS. Each resolved propargyl alcohol was subjected to ortho ester Claisen rearrangement to generate the allene moiety, with retention of chirality. Conversion of the resulting ester group to the final compound methyl (*E*,*R*)- and methyl (*E*,*S*)-2,4,5-tetradecatrienoate was achieved through reduction, tosylation, cyanation, hydrolysis, phenylselenation and oxidation. Methyl myristoleate (**3**, purity: ≥98.5%) and methyl myristate (**4**, purity: 99%) were obtained from Sigma-Aldrich®, Merck Life Science UK Limited (Figure 1). Diethyl ether (Thermofisher Scientific) was redistilled to obtain high-purity solvent for creating dilutions of test compounds.

Sample analysis

Samples of compounds 1–4 were analysed on an Agilent 7890A GC (Agilent Technologies, USA), equipped with a cool-on-column injector, flame ionization detector (FID) and an HP-1 capillary GC column (50 m length × 0.32 mm inner diameter (ID) × 0.52 μm film thickness). The oven temperature was maintained at 30°C for 0.5 min and increased at 5°C/min to 150°C, where it was held for 0.1 min before increasing at 10°C/min to 230°C, and then held for 25 min. Retention index (=Kováts [KI] index) values were calculated using a series of C7-C22 alkanes, giving methyl (*E*)-2,4,5-tetradecatrienoate =1806, methyl myristoleate = 1694, methyl myristate = 1709 (Figures S2 and S3).

The methyl (*E*)-2,4,5-tetradecatrienoate enantiomers were analysed by enantioselective GC to confirm no degradation of optical purity had taken place during storage, for which an Agilent 6890 N gas chromatograph equipped with a cool-on-column injector, an FID and a 50 m × 0.32 mm ID × 0.52 μm film thickness SUPELCO® BetaDEX™ (Sigma-Aldrich, Gillingham, UK) 120 fused silica capillary column was used. The oven temperature was maintained at 30°C for 1 min and then programmed at 4°C/min to 180°C, where it was held for 0.1 min, then at 10°C/min to 230°C and held for 16 min. The mass spectra of the synthetic methyl (*E*,*R*)- and methyl (*E*,*S*)-2,4,5-tetradecatrienoate samples were compared with published data (Mori, 2012), for which an Agilent GC-triple quad (7010B GC/TQ, source temperature 220°C) coupled with an Agilent GC (8890 GC) fitted with an HP-1 capillary column (50 m × 0.32 mm ID, 0.52 μm film thickness) was used. Injection was via a cool-on-column injector. The oven temperature was maintained at 30°C for 0.1 min and increased at 5°C/min to 150°C, where it was held for 0.1 min, then at 10°C/min to 230°C and held for 26 min. The separation of synthetic methyl (*E*,*R*)- and methyl (*E*,*S*)-2,4,5-tetradecatrienoate by chiral GC is demonstrated in Figure S1 and spectral data are presented in the Supporting Information (Figures S2 and S3).

Electroantennography (EAG)

Approximately 10-day-old unmated female beetles were used to study the electrophysiological response of antennae to different doses of compounds. EAG was performed as described in Wadhams (1990), with amendments. An antenna was carefully excised from the live beetle's head and suspended between two electrodes made from Ag-AgCl borosilicate glass filled with Ringer solution (without glucose) and connected to silver wire (0.37 mm diam., Biochrom Ltd., Cambridge, UK). The base of the antenna was connected to a grounded electrode. A glass tube positioned approximately 5 mm away from the antennal preparation was connected to a stimulus controller (CS-02; Ockenfels Syntech GmbH, Kirchzarten, Germany) and facilitated a continuous flow of charcoal-purified and humidified air towards the antenna at a rate of 10 mL/min. The signal was passed through a high-impedance amplifier (UN-06, Syntech) and recorded using the Syntech EAG software package EAG v1.0 (6/1993). The absolute negative amplitude changes in response to the stimuli were recorded in mV and normalized against the ether controls (=100%), resulting in test stimuli being expressed as percentages (Birkett et al., 2006).

a. EAG trial 1: Serial dilutions of 0.1, 1, 10 and 100 ng/μL were prepared for methyl (*E*,*R*)- and methyl (*E*,*S*)-2,4,5-tetradecatrienoate, methyl myristoleate and methyl myristate in redistilled diethyl ether. Approximately 10 μL of each standard was applied to a filter paper strip (20 mm length, 1–2 mm width; Whatman, Little Chalfont, UK), achieving an ascending test dose range of 1 to 1000 ng (due to the limited availability of methyl (*E*,*R*)- and methyl (*E*,*S*)-2,4,5-tetradecatrienoate, this was the highest test dose). The solvent was allowed to evaporate for 20 s, after which the filter paper strip was placed into a glass Pasteur pipette and test compounds delivered to the antennae for 2 s by puffing 500 mL/min of air, using a stimulus controller. Filter paper strips with 10 μL of diethyl ether served as controls after 20 s of application of solvent and were recorded at the beginning and end of each replicate. Seven biological replicates (one replicate = one antenna from an individual beetle) were carried out, and the order in which the compounds were tested was completely randomized.
b. EAG trial 2: The *A. obtectus* pheromone is reported to be 93–94% methyl (*E*,*R*)- and 7%–6% methyl (*E*,*S*)-2,4,5-tetradecatrienoate (Mori, 2012; Vuts, Francke, et al., 2015); we thus tested (i) the 9:1 (*R*):(*S*) (1000 ng in total) blend as its approximation, (ii) the 1:1 (2000 ng) racemic mixture as an easier synthesis target, (iii) stereochemically pure methyl (*E*,*R*)-2,4,5-tetradecatrienoate (1000 ng) and (iv) stereochemically pure methyl (*E*,*S*)-2,4,5-tetradecatrienoate (1000 ng). Although EAG is generally not suitable for testing mixtures of compounds because of the different vapour pressures of components affecting the number of molecules that stimulate the antenna (Andersson et al., 2012; Roelofs, 1977), the enantiomeric mixtures of methyl (*E*)-2,4,5-tetradecatrienoate did not pose such a problem due to the identical physico-chemical properties of enantiomers. Ten biological replicates were performed as above.

As the normalized EAG data did not follow a normal distribution, the differences in antennal response as a factor of various compounds were compared using the Kruskal–Wallis test within each dose ($\alpha = 0.05$). The EAG response was considered independent of dosage, since sufficient time (ca. 40 s) was allowed for the antennae to return to the arbitrary zero baseline after each stimulation (Roelofs, 1977). Dunn's multiple post hoc test with Bonferroni corrections was carried out to compare the difference between compounds at doses where significant differences were indicated by the Kruskal–Wallis test. All statistical analyses were performed using the statistical software R (version 4.2.3, R Development Core Team, 2023). The R packages used were FSA (Ogle, 2025), ggplot2 (Wickham, 2016) and gridextra (Auguie, 2017) and are available in https://CRAN.R-project.org/package.

Behavioural choice assays

The behavioural responses of unmated female beetles aged 10 days were studied using a Perspex four-arm olfactometer (Pettersson, 1970). The olfactometer comprised a slightly larger middle circular Perspex piece held together between two smaller Perspex pieces with plastic nuts and bolts. The middle piece had four equidistant lateral holes, while the top piece had a central hole, and the lower piece was lined with Whatman filter paper. Glass arms were attached to the lateral holes of the centre piece of the olfactometer (Ukeh et al., 2010; Vuts, Francke, et al., 2015). The centre hole was connected to a flow meter and vacuum pump using a Teflon tube. Test compounds were applied on a piece of filter paper (30 × 5 mm) and placed in the glass arms. The vacuum pump pulled air through the central opening, consequently drawing the signal through each of the four arms at a rate of 75 mL/min/arm, which was later vented from the room. A single beetle was introduced through the central hole and was given 2 min to acclimatize. The experiment was run for 16 min, and every 4 min, the olfactometer was rotated by 90° to control for any directional bias. The assay was conducted in a dark controlled environment room with a temperature of 20°C and 60% relative humidity, equipped with an extraction fan. A single light source was provided by two 18 W/35 white fluorescent light bulbs screened with red acetate fitted approximately 40 cm above the olfactometer. To eliminate visual stimuli, the olfactometer was placed in the centre of a black-walled box with an observation opening at the front. Before each bioassay, the Perspex pieces were washed with an aqueous solution of Teepol (Teepol commercial, UK), 80% ethanol and distilled water, then air-dried. The glass arms were washed with Teepol in an aqueous solution, distilled water and acetone, then dried in an oven at 150°C for 2 h. Each assay was replicated 10 times. The dose level was set at 1000 ng based on Vuts, Powers, et al. (2015), who reported that the total amount of methyl (*E*,*R*)-2,4,5-tetradecatrienoate produced per male was approximately 1000 ng.

a. Choice assay 1 to 4: Each of methyl (*E*,*R*)- and methyl (*E*,*S*)-2,4,5-tetradecatrienoate, methyl myristoleate and methyl myristate (1000 ng dose) versus 10 μL of solvent (diethyl ether) in the three other arms.
b. Choice assay 5: Methyl (*E*,*R*)-2,4,5-tetradecatrienoate (1000 ng dose) versus 1:1 enantiomeric mixture (2000 ng dose) versus 20 μL of solvent (diethyl ether) in two opposing arms.
c. Choice assay 6: Methyl (*E*,*R*)-2,4,5-tetradecatrienoate (2000 ng dose) versus 1:1 enantiomeric mixture (2000 ng dose) versus 20 μL of solvent (diethyl ether) in two opposing arms.
d. Choice assay 7: Methyl (*E*,*R*)-2,4,5-tetradecatrienoate (1000 ng dose) versus 9:1 (*R*):(*S*) enantiomeric mixture (1000 ng dose) versus 10 μL of solvent (diethyl ether) in two opposing arms.

The olfactometer was divided into five regions, corresponding to each of the four arms and the centre neutral zone. The total time spent by the beetle in each region of the olfactometer was recorded using software (OLFA, Udine, Italy). Residual maximum likelihood (REML) method was used to fit a generalized linear mixed model to the square root-transformed data in Genstat (2022, 22nd edition, VSN International Ltd., Hemel Hempstead, UK). The fixed model included the main effect of treatment, while the random model controlled for the design structure of the olfactometer (areas within them) and replicate runs as split plots. Based on the F-test, statistical significance was assessed at 5% ($\alpha = 0.05$).

RESULTS

Electrophysiological assays

In trial 1, there was no significant difference in the EAG responses of unmated female *A. obtectus* across the tested compounds at doses ranging from 1 to 100 ng ($H_4 = 5.23$, $p = 0.16$; $H_4 = 3.69$, $p = 0.29$ and $H_4 = 2.21$, $p = 0.53$ at 1 ng, 10 ng and 100 ng respectively), although a significant response was observed at a dose of 1000 ng ($H_4 = 13.86$, $p = 0.003$). Post hoc analysis revealed that the EAG response to methyl (*E*,*R*)-2,4,5-tetradecatrienoate at 1000 ng was significantly greater than that to methyl myristoleate ($Z = -3.51$, $P_{adj} = 0.003$) and methyl myristate ($Z = -2.83$, $P_{adj} = 0.028$). Contrastingly, methyl (*E*,*S*)-2,4,5-tetradecatrienoate elicited an intermediate response, with no statistical difference compared to methyl (*E*,*R*)-2,4,5-tetradecatrienoate ($Z = 2.18$, $P_{adj} = 0.18$), methyl myristate ($Z = -0.65$, $P_{adj} = 1$) and methyl myristoleate ($Z = -1.33$, $P_{adj} = 1$) at a 1000 ng dose (Figure 2).

In trial 2, both methyl (*E*,*R*)-2,4,5-tetradecatrienoate (at a dose of 1000 ng) and the 1:1 racemic mixture (at a dose of 2000 ng) induced significantly greater antennal responses compared to methyl (*E*,*S*)-2,4,5-tetradecatrienoate at a dose of 1000 ng ($Z = 2.92$, $P_{adj} = 0.02$, and $Z = 2.65$, $P_{adj} = 0.048$, respectively) (Figure 3). Antennal response to the 9:1 (*R*):(*S*) mixture was intermediary; no significant difference was observed compared to methyl (*E*,*R*)-2,4,5-tetradecatrienoate ($Z = 1.25$, $P_{adj} = 1.00$), methyl (*E*,*S*)-2,4,5-tetradecatrienoate ($Z = 1.66$, $P_{adj} = 0.57$) and the 1:1 racemic mixture ($Z = 0.98$, $P_{adj} = 1.00$) (Figure 3).

Behavioural assays (olfactometry)

In choice assay 1, unmated female beetles spent significantly more time in the treatment arm containing 1000 ng of methyl (*E*,*R*)-2,4,5-tetradecatrienoate compared to the control arms containing diethyl ether (choice assay 1, Table 1). Contrastingly, there was no significant difference in the amount of total time spent in the arm containing 1000 ng of methyl (*E*,*S*)-2,4,5-tetradecatrienoate compared to

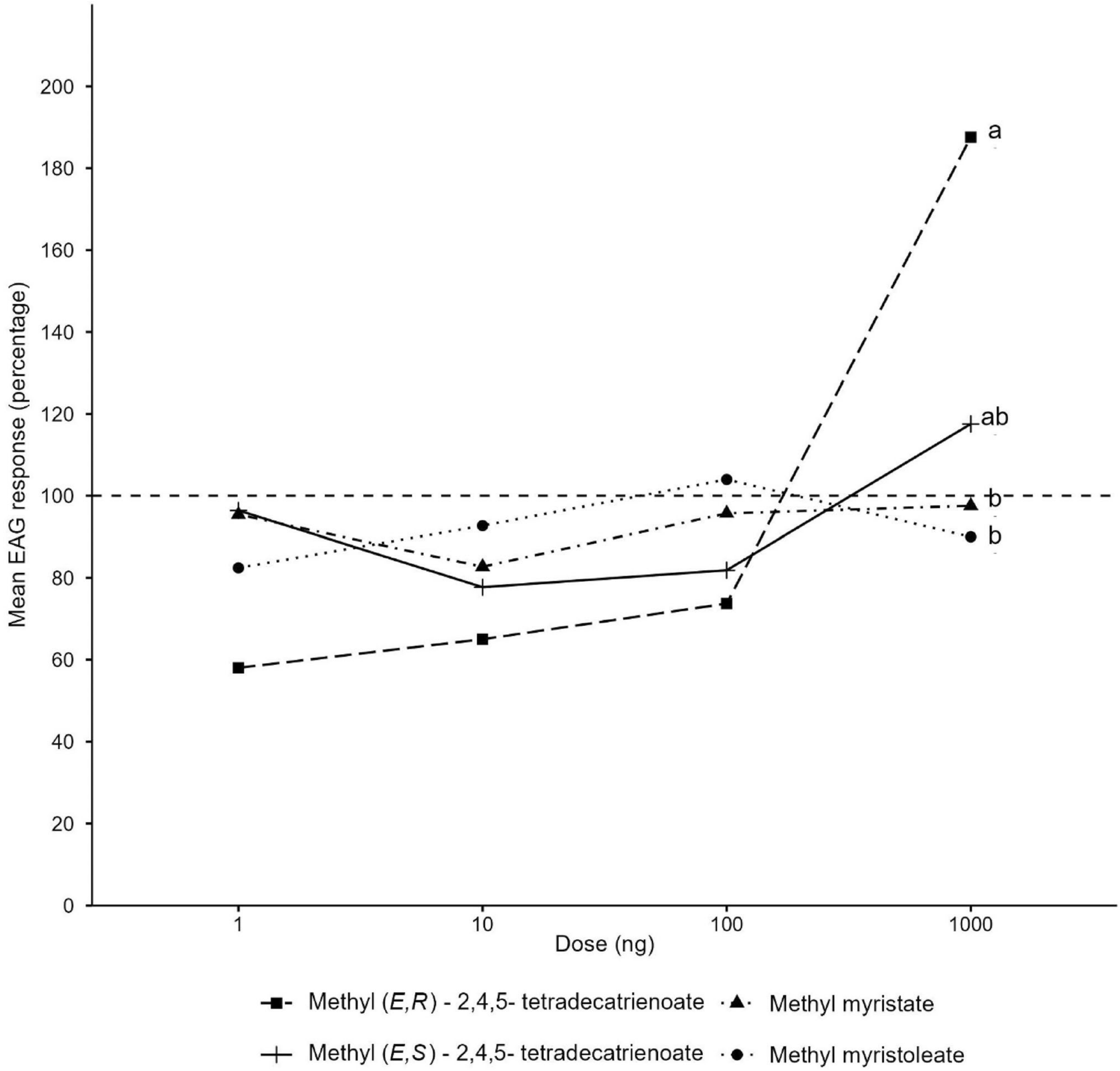

FIGURE 2 Normalized EAG responses of antennae of unmated *Acanthoscelides obtectus* females to enantiomers of methyl (*E*)-2,4,5-tetradecatrienoate and mono-unsaturated and saturated synthetic analogues. Same letters are not significantly different ($p = 0.05$). Dashed line: ether control. (Mean EAG response ± SE values are given in Table S1, p_{unadj} and p_{adj} values for post hoc Dunn's test are given in Table S2).

the control arms (choice assay 2, Table 1). Similarly, there was no significant preference towards either of the synthetic analogues tested in choice assay 3 (methyl myristoleate) and choice assay 4 (methyl myristate, Table 1).

In choice assay 5, the beetles exhibited a significant preference for the 1:1 mixture of enantiomers at a 2000 ng dose compared to methyl (*E*,*R*)-2,4,5-tetradecatrienoate at a 1000 ng dose ($p = 0.006$). When the dose of methyl (*E*,*R*)-2,4,5-tetradecatrienoate was doubled (2000 ng), there was no significant difference in behavioural preference between them ($p = 0.4611$; choice assay 6 in Table 1). Moreover, the beetles demonstrated a similar preference for stereochemically pure methyl (*E*,*R*)-2,4,5-tetradecatrienoate (1000 ng) as for the 9:1 (*R*):(*S*) enantiomeric mixture (900:100 ng) ($p = 0.2852$; choice assay 7 in Table 1).

DISCUSSION

Our findings demonstrate that stereochemically pure methyl (*E*,*R*)-2,4,5-tetradecatrienoate elicits the same level of behavioural activity from female beetles as the natural 9:1 *R*:*S* pheromone blend, but the (*S*)-enantiomer is inactive. Interestingly, increasing the ratio of the (*S*)-enantiomer to equal proportions to the (*R*)-enantiomer further enhances female preference. These findings support and

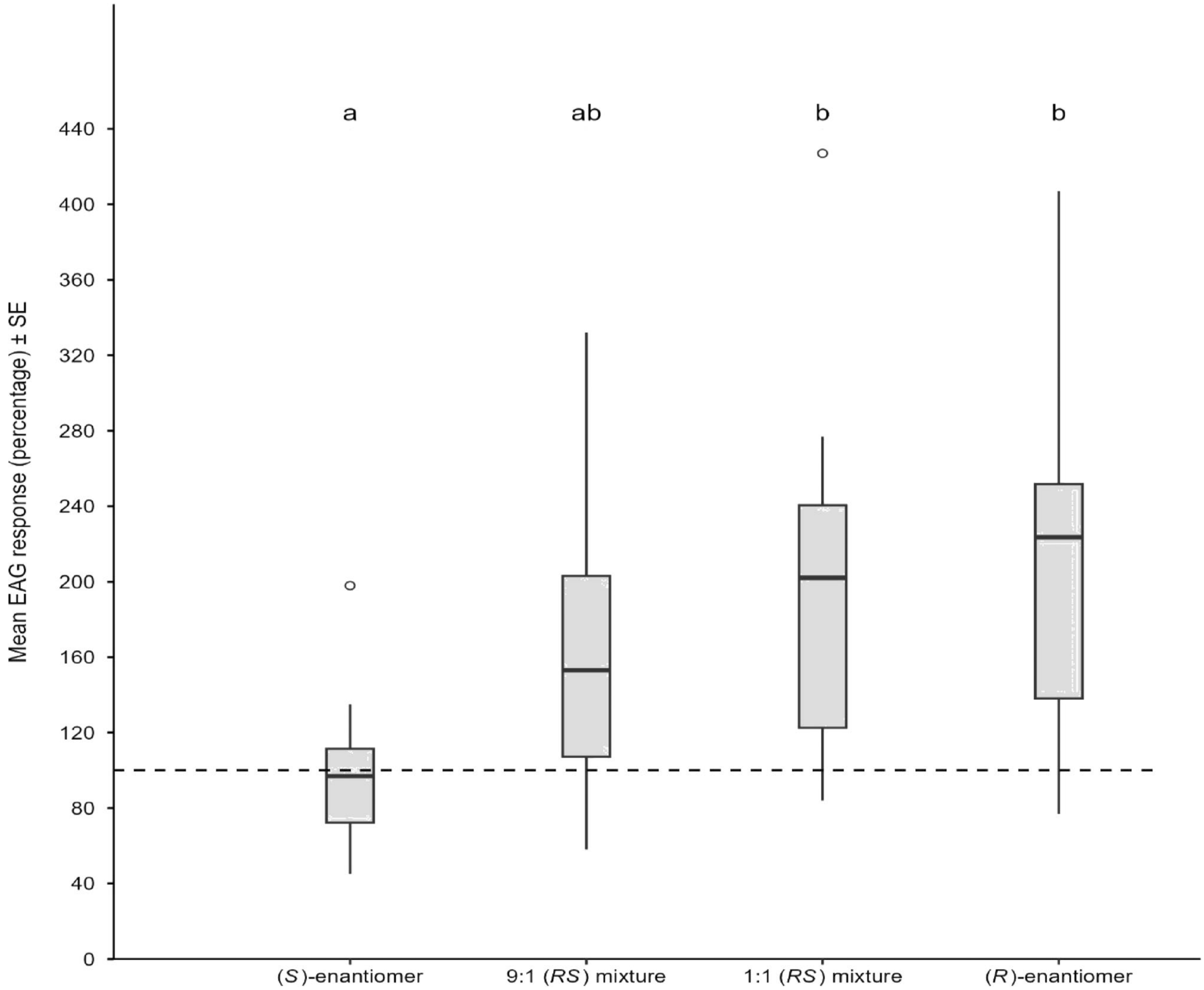

FIGURE 3 Normalized EAG responses of antennae of unmated *Acanthoscelides obtectus* females to enantiomers of methyl (*E*)-2,4,5-tetradecatrienoate and their different mixtures. Box plots with the same letter are not significantly different ($p = 0.05$). Dashed line: ether control. Kruskal–Wallis test: $H_4 = 10.45$, $p = 0.02$. (P_{unadj} and P_{adj} values for post hoc Dunn's test are given in Table S2).

extend existing literature indicating that female *A. obtectus* is predominantly attracted to the (*R*)-enantiomer (Vuts et al., 2015a, Mori, 2012) and suggest a synergistic interaction with its antipode. Adding the otherwise inactive (*S*)-enantiomer at proportions of up to 10% does not affect the behavioural activity of stereochemically pure methyl (*E*,*R*)-2,4,5-tetradecatrienoate. However, when present in equal amounts, a significant increase in bioactivity is observed. We did not explore the specific proportion to (*R*) at which the (*S*)-enantiomer transitions from a synergist to an inactive state, but our results imply this lies between 50% and 100%. We did not perform a choice test between the 9:1 and the 1:1 blends either, which were compared to stereochemically pure methyl (*E*,*R*)-2,4,5-tetradecatrienoate instead (choice assay 6 and 7), not differing significantly from the (*R*) enantiomer. In *Lymantria dispar*, the inactive enantiomer inhibits or drastically reduces the response of the bioactive enantiomer when its concentration is higher than that of the bioactive enantiomer (Vité et al., 1976). The reason is due to different homologous odour-binding proteins (OBPs) in the antennae, where LdisOBP1 preferentially binds (−)-disparlure, while LdisOBP2 shows a preference for (+)-disparlure (Miller et al., 1977; Plettner et al., 2000). Other possible explanations for such a trend are that the enantiomers may bind to OBPs at distinct sites with varying levels of affinities (Sims et al., 2022) or exhibit different interactions with olfactory receptors (Saïd et al., 2003). Additionally, conformational changes in OBPs (Gomez-Diaz et al., 2013; Pesenti et al., 2008; Zhang et al., 2017) might enhance binding when both enantiomers are present (Gomez-Diaz et al., 2013, Pesenti et al., 2008, Zhang et al., 2017). For example, *Anomala osakana* and *Popillia japonica* utilize different enantiomers of japonilure despite possessing identical OBPs in their activated sensilla (Wojtasek et al., 1998). Another probable option is that enantiomeric information can be transferred via common receptor cells, as well as chiral-specific receptors (Payne et al., 1982).

The fact that the male *A. obtectus* pheromone composition is altered within a single generation following host shifts and the

TABLE 1 Behavioural responses of unmated *Acanthoscelides obtectus* females in four-arm olfactometer bioassays to enantiomers of methyl (*E*)-2,4,5-tetradecatrienoate, their different mixtures, as well as mono-unsaturated and saturated synthetic analogues.

Choice assay	Treatment	Mean time spent ± SE	Significance*
1	(a) Methyl (*E*,*R*)-2,4,5-tetradecatrienoate	9.646 ± 0.923	<0.001*
	(b) Control	0.665 ± 0.162	
2	(a) Methyl (*E*,*S*)-2,4,5-tetradecatrienoate	2.876 ± 0.768	0.597
	(b) Control	2.165 ± 0.364	
3	(a) Methyl myristoleate	0.199 ± 0.108	0.203
	(b) Control	2.202 ± 0.408	
4	(a) Methyl myristate	1.566 ± 0.362	0.805
	(b) Control	1.764 ± 0.250	
5	(a) 1:1 racemic mixture (2000 ng)	3.576 ± 1.115	b
	(b) Methyl (*E*,*R*)-2,4,5-tetradecatrienoate (1000 ng)	0.977 ± 0.716	a
	(c) Control	0.086 ± 0.054	a
6	(a) 1:1 racemic mixture (2000 ng)	3.248 ± 1.045	b
	(b) Methyl (*E*,*R*)-2,4,5-tetradecatrienoate (2000 ng)	1.927 ± 0.440	ab
	(c) Control	1.156 ± 0.306	a
7	(a) 9:1 *R*/*S* mixture (1000 ng)	1.989 ± 0.729	ab
	(b) Methyl (*E*,*R*)-2,4,5-tetradecatrienoate (1000 ng)	4.21 ± 1.535	b
	(c) Control	0.754 ± 0.218	a

*At $\alpha = 0.05$, Same letters are not significantly different ($\alpha = 0.05$).

female responds plastically (Vuts et al., 2018) indicates that rapid epigenetic mechanisms at the olfactory periphery or changes in the central nervous system control transgenerational shifts in pheromone perception and information processing in females. Whilst the *A. obtectus* pheromone has been extensively studied due to its pest status, little attention has been given to related species. In the Mexican Altiplano, both *A. obtectus* and its sister species *A. obvelatus* feed on *Phaseolus vulgaris*, although the pheromone components of *A. obvelatus* have not yet been characterized (Alvarez et al., 2006). Thus, we emphasize the need for research at the molecular and sensillar level to elucidate how enantio-selectivity is determined in the antennae and to explore the evolutionary driving forces by studying non-pest and sibling species.

In conclusion, our study highlights the specificity and complexity of the *A. obtectus* pheromone system, providing insights that can enhance pheromone-based pest management strategies. Understanding these mechanisms at the enantiomeric level is crucial for developing effective and targeted pest control solutions, potentially reducing the impact of *A. obtectus* on stored grain products worldwide. To this end, the sensitivity of female *A. obtectus* to the racemic mixture of the male pheromone component has the promise to be utilized in designing effective pheromone traps for monitoring beetle populations.

AUTHOR CONTRIBUTIONS

Anusha Mohan-Kumar: Conceptualization; data curation; formal analysis; investigation; methodology; writing – original draft. **Gareth Thomas:** Conceptualization; investigation; methodology; writing – review and editing. **John C. Caulfield:** Conceptualization; writing – review and editing. **David M. Withall:** Conceptualization; writing – review and editing. **József Vuts:** Conceptualization; funding acquisition; investigation; methodology; writing – review and editing.

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CONFLICT OF INTEREST STATEMENT

We declare that we have no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available at https://doi.org/10.23637/wmvf0e2t, [Mohan-Kumar, et al 2025].

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SUPPORTING INFORMATION

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

DATA S1. Supporting Information.

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