



# Food plant odor perception in three sympatric alpine grasshopper species (Orthoptera: Acrididae: Catantopinae) in Aotearoa New Zealand

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## Abstract

The alpine grasshoppers *Sigaüs nivalis*, *Sigaüs australis* and *Sigaüs nitidus* are sympatric in the central mountains of South Island, Aotearoa New Zealand. These grasshoppers feed on a range of alpine plants but show preference towards dicots over monocots. Because herbivorous insects often use smell and taste to locate and recognize food plants it was expected that these grasshoppers would show sensitivity to their favorite foods and potential sensitivity to nonhost plants. Here, we determined feeding preference in captivity allowing each of these three sympatric grasshoppers the same choice of six native alpine plant species. We analyzed the chemical compositions of the plants used in these experiments using gas-chromatograph coupled with mass-spectrometry (GC-MS) and then recorded olfactory responses in the grasshoppers to plant-derived smells (with synthetic compounds) using electroantennogram (EAG). The grasshoppers were able to distinguish between the potential food plants and ate the shrub *Coriaria sarmentosa* but not the grass *Chionochloa pallens*, however, the chemicals we detected in the six plant species were very similar. High sensitivity to fatty acid derived aldehydes (decanal, (*E,Z*)-2,6-nonadienal, hexanal) and a 6-carbon alcohol ((*Z*)-2-hexen-1-ol) compared to terpenoids ( $\alpha$ -phellandrene,  $\beta$ -myrcene,  $\beta$ -ocimene, eucalyptol, (*S*)-(-)-limonene, (1*S*)-(-)- $\alpha$ -pinene) or an aromatic compound (2-phenylethanol) was recorded in the antennae of all three grasshopper species and no species- or sex-specific sensitivity to particular compounds was observed. As aldehydes and alcohols are emitted upon plant damage, it is possible that these generalist grasshoppers are sensitive to the smells of damaged plants rather than species-specific plant smells.

**Keywords** Acrididae · Electroantennogram · Generalist · Plant smells · Sympatry

## Introduction

Plant-derived chemicals, which herbivores use to recognize their food (Nakano et al. 2022) vary greatly among plant taxa. Compounds commonly found in plants that are implicated in herbivore response include green leaf volatiles (GLVs) as 6-carbon alcohols, aldehydes or esters, terpenoids derived

from 5-carbon isoprene units, and aromatic compounds that contain at least one benzene ring (Zhou and Jander 2022). Insects detect these chemicals by chemo-sensilla (Nakano et al. 2022, 2023) distributed on the sensory organs such as antennae and mouthparts.

Insect olfactory response to potential food plants requires identification of chemical compounds produced by the plants. A combination of gas chromatography coupled with mass spectrometry (GC-MS) and electrophysiological tools such as gas chromatography coupled with electroantennographic detection (GC-EAD) can reveal insect olfactory responses (Chen et al. 2004; Chen and Kang 2000; Seenivasagan et al. 2009; Twidle et al. 2015, 2022). This approach makes it possible to identify active compounds by comparing antennal responses of individual insects to a mixture of synthetic compounds or plant extracts (Liu et al. 2021) and evaluate olfactory sensitivity by exposing insects to

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individual compounds at different concentrations (Chen et al. 2004; Seenivasagan et al. 2009).

Electrophysiological studies show that grasshoppers respond to a variety of plant-derived smells (Blust and Hopkins 1987a, b; Chen et al. 2004; Chen and Kang 2000; Kang and Hopkins 2004; Njagi and Torto 1996) although variance in sensitivity could indicate positive or negative associations. Some herbivorous insects have been found to display feeding preference by high olfactory sensitivity to particular plant chemicals. For example, the specialist grasshopper *Hypochlora alba* feeds primarily on sage *Artemisia ludoviciana* and shows significantly higher electrophysiological response to terpenoids derived from this plant than does the generalist grasshopper *Melanoplus sanguinipes* (Blust and Hopkins 1987b). The abundance and type of antennal sensilla is correlated with olfactory sensitivity as higher electrophysiological responses are observed in grasshoppers with more olfactory sensilla on their antennae (Blust and Hopkins 1987b; Chen and Kang 2000; Kang and Hopkins 2004).

Sympatric species of New Zealand alpine grasshoppers, *Sigaus nivalis* (Hutton 1897), *Sigaus nitidus* (Hutton 1897) and *Sigaus australis* (Hutton 1897) (Trewick et al. 2023; Fig. 1), feed on a variety of plant species including rushes, grasses, dicot herbs, shrubs, ferns and mosses, but have preference towards dicots over monocots (Nakano 2024; Watson 1970). The abundance of different morphological types of antennal sensilla in these grasshoppers are similar, but male *S. australis* have significantly more olfactory sensilla than conspecific females (Nakano et al. 2023; Fig. 1). Here, we explored food choice in captivity, giving

wild-caught adult grasshoppers suitable native monocot and dicot plants to eat and observed food selection. We analyzed the chemical composition of these food plants using GC-MS and recorded electrophysiological responses of each *Sigaus* species to the smells of potential food plants. Due to similarity in the abundance and types of sensilla found in these three species of *Sigaus* (Nakano et al. 2023) and observed similarity in their diets (Nakano 2024; Watson 1970), we expected similar olfactory responses to the smells of food plants. We also expected *S. australis* males to respond more strongly than conspecific females due to the higher abundance of sensilla on their antenna (Nakano et al. 2023).

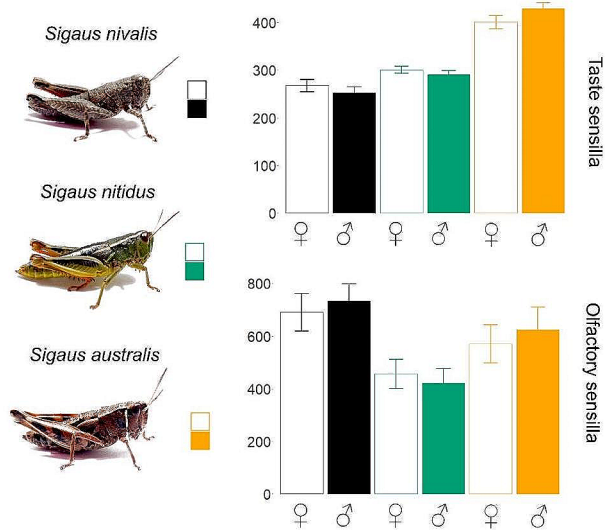
## Materials and methods

### Insects

Adult grasshoppers and plants were collected at Foggy Peak in the Torlesse Range (−43.294107, 171.744770), and Mount Hutt skifield in the Mount Hutt Range (−43.5118, 171.5492), Canterbury, New Zealand in February 2023 with authority from the New Zealand Department of Conservation (authorization number: 97397-FLO) and ski area operators. Plants and live insect specimens were transported to the laboratory (Plant & Food Research, Lincoln, Canterbury, New Zealand) and kept at ambient temperature with natural light, for food plant choice tests and electrophysiological analysis. Plants were cut at the stem or root in the field and transported with a wet paper towel wrapped at the stem or root in clean plastic bags at 4 °C until used in choice tests.

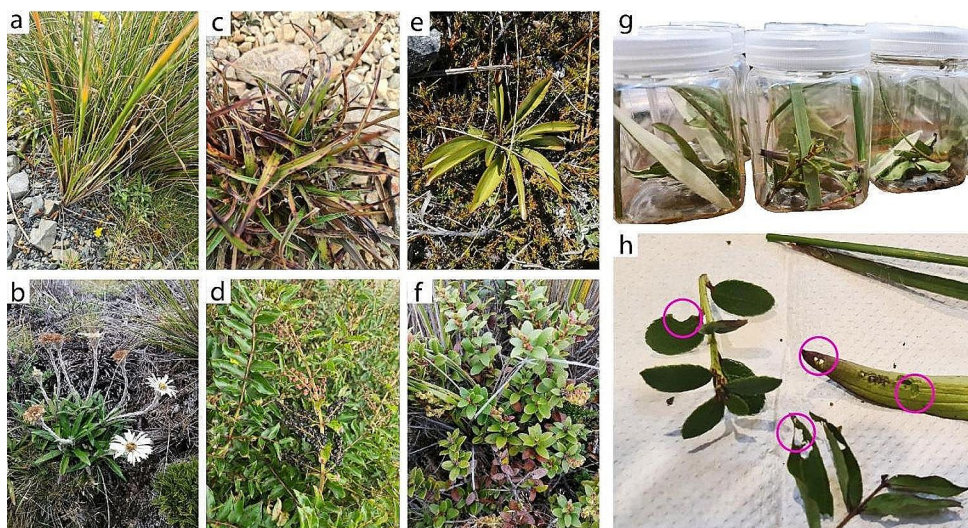
### Food plant choice test

Each grasshopper was housed in a 6 × 8 × 5 cm plastic container with a mesh top and provisioned with leaves from six native plant species: tussock grass *Chionochloa pallens*, rush *Luzula rufa*, the dicot herbs *Gentianella corymbifera* and *Celmisia spectabilis*, and the shrubs *Coriaria sarmentosa* and *Gaultheria crassa* (Fig. 2). These plant species were selected because they are known components of the diet of New Zealand alpine grasshoppers (Nakano 2024; Watson 1970) or are naturally common in their environment; they represent the spectrum of available foliar types. Grasshoppers were held without food for the night prior to each 6-hour daytime trial (09:00–15:00), which was run in ambient light and temperature. After each feeding trial, the presence/absence (1/0) of feeding sign(s) (Fig. 2h) on each plant species was recorded. Each grasshopper was used once and between nine and 14 trials were performed for each sex of each species (total number = 69).



**Fig. 1** Number of taste and olfactory sensilla found on the middle to distal antenna segments of sympatric New Zealand alpine grasshoppers. *Sigaus nivalis* (black), *Sigaus nitidus* (green) and *Sigaus australis* (orange). Data derived from Nakano et al. 2023

**Fig. 2** Feeding trials with New Zealand *Sigaus* grasshoppers used a choice of six plant species: *Chionochloa pallens* (a), *Celmisia spectabilis* (b), *Luzula rufa* (c), *Coriaria sarmentosa* (d), *Gentianella corymbifera* (e), and *Gaultheria crassa* (f) in individual containers (g). Examples of feeding sign observed after feeding trial (h)



**Table 1** Supplier and purity of 11 compounds used in electroantennographic analysis of New Zealand *Sigaus* grasshoppers

Chemicals	Supplier	Purity
<i>Terpenoids</i>		
(1 <i>S</i> )-(-)- $\alpha$ -Pinene	Fluka	97%
( <i>S</i> )-(-)-Limonene	Sigma Aldrich	96%
$\alpha$ -Phellandrene	Merck-Schuchardt	85%
Eucalyptol	Fluka	99%
$\beta$ -Myrcene	Sigma Aldrich	90%
$\beta$ -Ocimene	Sigma Aldrich	90%
<i>Fatty acid-derived alcohol and aldehydes</i>		
( <i>Z</i> )-2-Hexen-1-ol	Bedoukian	95%
Hexanal	Sigma Aldrich	98%
( <i>E, Z</i> )-2,6-Nonadienal	Sigma Aldrich	96%
Decanal	SAFC	98%
<i>Aromatic compound</i>		
2-Phenylethanol	Sigma Aldrich	99%

### Chemical collection and analysis from alpine plants

Volatile compounds released by plants can be collected from plant head-space but this was only possible for one of the six plant species (Table S1) due to adverse alpine conditions during this study. Therefore, compounds were extracted from plant tissue using a standard solvent method. For chemical extraction, 1 g of freshly cut leaf tissue from each *Chionochloa pallens*, *Luzula rufa*, *Gentianella corymbifera*, *Celmisia spectabilis*, *Coriaria sarmentosa* and *Gaultheria crassa* was submerged in cool 20 mL of 95% hexane ( $n=4$ ) at Foggy Peak 2023 and stored at 4 °C fridge for 24 h. Preliminary trials showed this timeframe resulted in a broad range of compounds being captured (Table S2). Plant chemicals extracted in the same way in March 2022 were used for preliminary GC-EAD analysis to identify candidate olfactory-active compounds. Samples were analyzed using a gas chromatograph – mass spectrometer (GCMS-QP2010,

Shimadzu Corporation, Kyoto, Japan) with a 30 m x 0.32 mm DB-5 capillary column. Each hexane extract was injected in a split mode, with the temperature steps programmed for 3 min at 50 °C then incrementally increased to 95 °C at 5 °C/min, 145 °C at 15 °C/min, and finally 200 °C at 10 °C/min (23.83 min total). Compounds were identified by comparing retention times and mass spectra to those in the NIST (National Institute of Standards and Technology) Library 2005. Some of the plant compounds we identified are highly volatile (e.g. terpenoids, green leaf volatiles) and are known to be released from other plant species (Effah et al. 2020a, b; Pare and Tumlinson 1999). While the compounds were all sufficiently volatile to be separated on a gas chromatography column their pattern of natural release from intact or damaged leaves of these plants is not known.

### Electroantennographic bioassays

To identify olfactory-active compounds and measure sensitivity in *Sigaus* grasshoppers we used a gas chromatograph coupled with electroantennographic detection (GC-EAD) equipped with a DB-5 column (30 m x 0.25 mm ID, J & W Scientific, Folsom, CA, USA). Grasshoppers were maintained in captivity for up to two weeks before the experiment. In preparation, grasshopper antenna was abscised at the scape or pedicel immediately before the measurement and then fixed between two glass capillary electrodes by placing the base and tip of the antenna into electrically conductive gel (Signagel®, Parker Laboratories) (Fig. S1a).

Preliminary trials used hexane leaf extracts of *Chionochloa pallens*, *Gentianella corymbifera*, *Celmisia sarmentosa* and *Gaultheria crassa* to identify olfactory-active compounds derived from grasshopper's food plants (Fig S2). Active compounds from plant extracts were identified by calculating the Kovats retention index on the comparison

of their retention times with reference C7 to C21 alkanes (Supelco) on GC-MS and GC-EAD.

Electroantennogram (EAG) responses of grasshoppers to 11 compounds (Table 1) were recorded. The six terpenoids were selected as we observed some of them in a head-space collection of above ground foliage of three *Celmisia* species from Canterbury (Broken River Ski Area; Table S1), others were used as they are known to be genuine volatiles of other plant species (Effah et al. 2020a, b). Odor stimulus cartridges were prepared by loading 10  $\mu$ l of each synthetic compound to a piece of filter paper inside a glass Pasteur pipette (Sigma-Aldrich; Fig. S1b). Solvents were allowed to evaporate from the filter paper for 10 s under a fume hood before being inserted into the Pasteur pipette. These pipettes were used to deliver test stimuli with 0.1 s of ‘puffs’ by a stimulus controller (CS-55, Syntech) into a continuous flow of humidified and charcoal-filtered air stream (600 mL/min) flowing over the antennal preparation. The antennal responses to each of the 11 volatile compounds were measured at dosages of 0.1 mg/mL, 1 mg/mL, 10 mg/mL and 100 mg/mL in hexane.  $\beta$ -Caryophyllene (90% purity, Sigma Aldrich) was used as a positive control at a concentration of 10 mg/mL in hexane as this compound elicited stable, consistent responses from the grasshopper antennae. EAG responses to stimuli were recorded sequentially from lower to higher doses and the order of exposure to 11 compounds at each dose was randomized for each specimen. Hexane and positive controls were used at the beginning of each dose to ensure no deterioration in the sensitivity of antennae between the doses. An interval of 30 seconds was given between successive stimulations, as preliminary assays showed this was sufficient for the antenna to recover olfactory sensitivity. Preliminary observations showed that the quantity of (1S)-(-)- $\alpha$ -pinene released from Pasteur pipette cartridge decreased more rapidly than other test compounds, so test pipettes were refreshed with newly treated filter paper after five puffs for (1S)-(-)- $\alpha$ -pinene and after ten puffs for other compounds.

## Statistical analysis

As insects used in this study were adults of unknown age and mating status, which might influence their olfactory sensitivity (Gadenne et al. 2016; Onagbola and Fadamiro 2011), normalized responses were used for the analysis. These were calculated by dividing the absolute EAG response (mV) to a given test compound with the EAG response (mV) to the positive control  $\beta$ -caryophyllene at 10 mg/mL. This positive control was used to normalize instead of a hexane control as it gave a consistent response in insects. Statistical analyses were performed in the R statistics environment (R Core Team 2023) using the software platform

RStudio 4.0.3 (Boston, MA, USA) with graphics generated using RStudio 4.0.3 and Inkscape 1.2.2. The statistical normality of data was tested by Shapiro-Wilk test. To test for significant differences in normalized EAG responses among 11 test stimuli among grasshopper species and sexes, data were treated to Analysis of variance (ANOVA) followed by Tukey’s Honest Significant Test.

## Results

### Feeding choice test

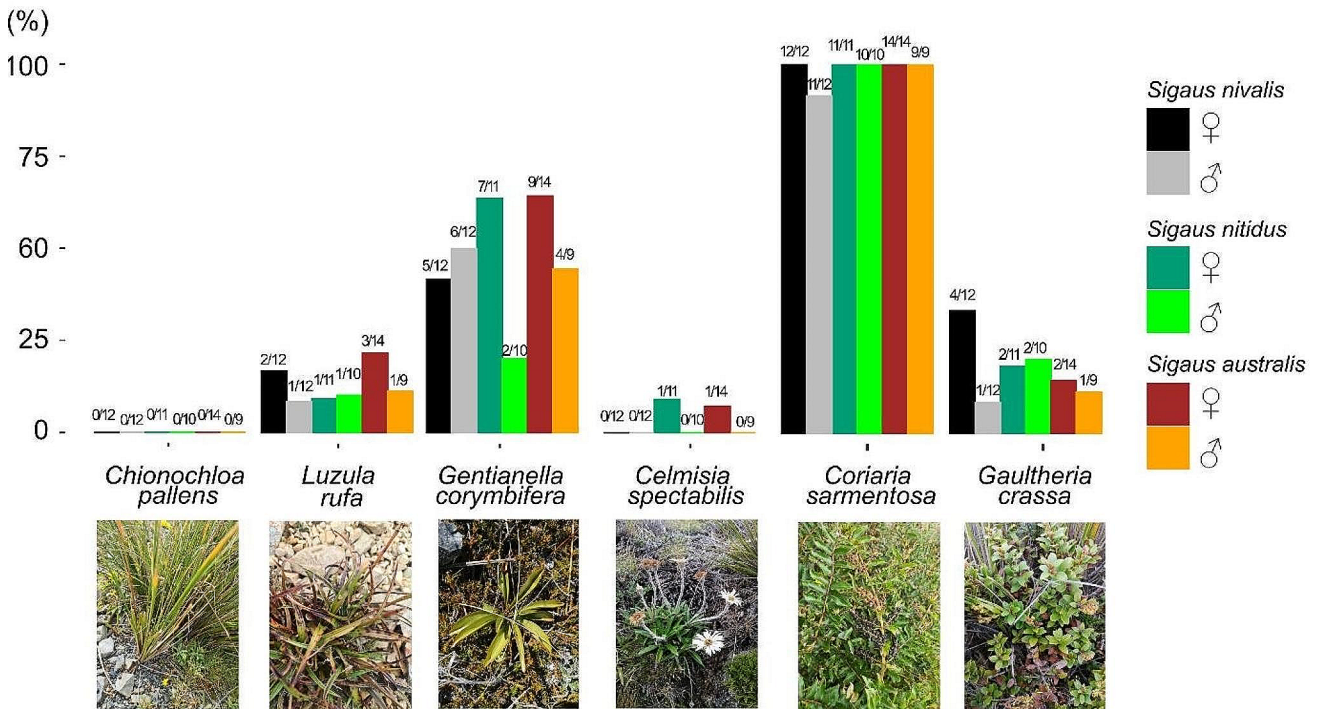
Adult alpine grasshoppers showed clear feeding preferences when given a choice of six plant species in captive trials. The shrub *Coriaria sarmentosa* was highly favored by all individuals except one *S. nivalis* male (Fig. 3). This contrasted with the rejection by all grasshoppers of the tussock grass *Chionochloa pallens*. The dicot herb *Gentianella corymbifera* was eaten by 20–60% of grasshoppers and the rush *Luzula rufa* and the shrub *Gaultheria crassa* were eaten by a few individuals. No difference was detected in the food choices of the *Sigaus* grasshopper species or sexes.

Eighteen volatile compounds were detected from the leaf extracts of *Chionochloa pallens*, *Luzula rufa*, *Gentianella corymbifera*, *Celmisia spectabilis*, *Coriaria sarmentosa* and *Gaultheria crassa* (Table 2). This consisted of six terpenoids, 11 fatty acid derived compounds, and one ketone. Four compounds were detected in all six plant species;  $\alpha$ -pinene, limonene, hexanal and (*E,Z*)-2,6-nonadienal. Three plant species had unique chemicals detected (*C. pallens*, *G. corymbifera*, and *G. crassa*) but each species had a distinct combination of compounds. Five compounds identified from hexane extracts were used for further EAG analysis (Table 2).

The preliminary GC-EAD analysis using leaf extracts indicated that the grasshoppers responded consistently to the compounds with the same retention times, regardless of the plant species used (Fig. S2). Six compounds were identified to be fatty acid-derived aldehydes and alcohols: (*Z*)-2-hexen-1-ol, hexanal, nonanal, (*Z,Z*)-3,6-nonadienal, (*E,Z*)-2,6-nonadienal, and 2-hexyl-1-octanol.

### Electroantennogram (EAG)

All individuals responded to (*E,Z*)-2,6-nonadienal at the lowest concentration (0.1 mg/mL) but other compounds at this concentration did not elicit responses significantly stronger than the solvent hexane on its own (Fig. S3). Thus, among our sample grasshoppers were sensitive only to (*E,Z*)-2,6-nonadienal at 0.1 mg/mL. All grasshoppers responded to every test stimulus when exposed to the



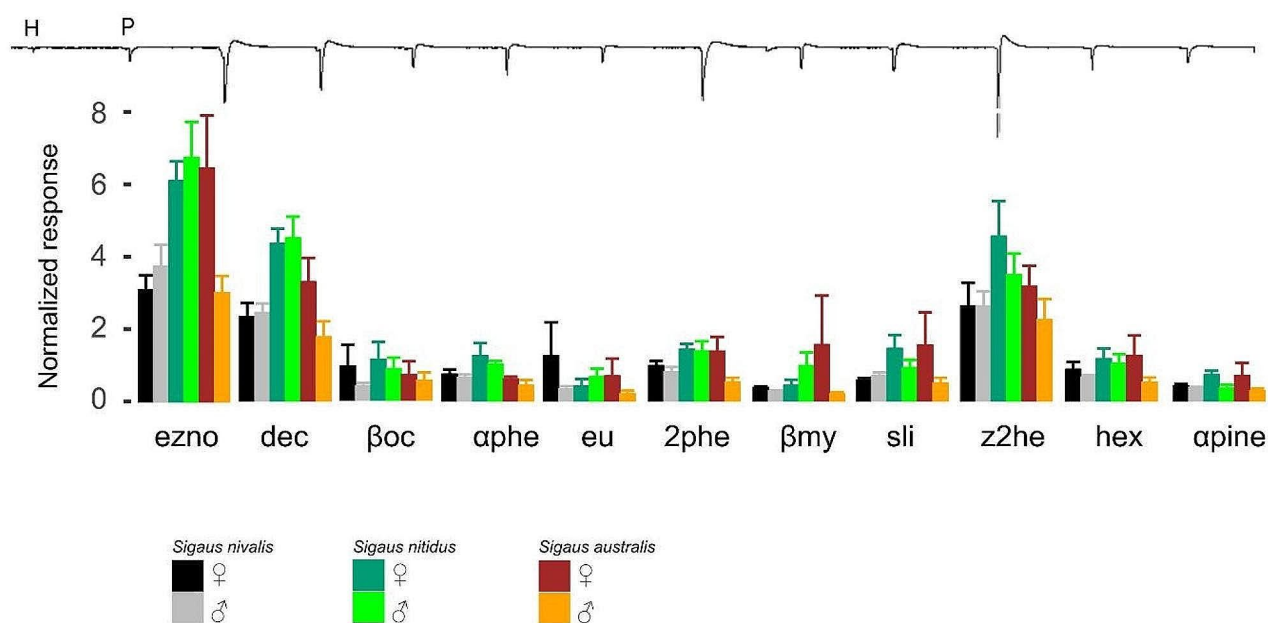
**Fig. 3** Proportion (%) of grasshopper individuals ( $n=9-14$ ) that fed during captive trials on each of six plant species: tussock grass *Chionochloa pallens*, rush *Luzula rufa*, dicot herbs *Gentianella corymbifera* and *Celmisia spectabilis*, and shrubs *Coriaria sarmentosa* and *Gaultheria crassa*. Numbers above each bar = (number of individuals fed on plant) / (sample size)

**Table 2** Chemical compounds found in leaf extracts of alpine plants in Foggy Peak, New Zealand. Compounds in bold are those used for electroantennogram (EAG) analysis

Chemicals	<i>Chionochloa pallens</i>	<i>Luzula rufa</i>	<i>Gentianella corymbifera</i>	<i>Celmisia spectabilis</i>	<i>Coriaria sarmentosa</i>	<i>Gaultheria crassa</i>
<i>Terpenoids</i>						
<b><math>\alpha</math>-Pinene</b>	+	+	+	+	+	+
<b>Limonene</b>	+	+	+	+	+	+
<b>Eucalyptol</b>			+			
<i>cis</i> -Geraniol			+		+	
Phytol						+
Copaene						+
<i>Fatty acid derivatives</i>						
<b>Hexanal</b>	+	+	+	+	+	+
( <i>E</i> )-3-Hexen-1-ol	+		+	+	+	+
( <i>Z</i> )-3-Hexen-1-ol	+		+	+	+	+
<b>(<i>Z</i>)-2-Hexen-1-ol</b>	+	+			+	+
<i>cis</i> -3-Hexenyl acetate	+	+				
4,8-Dimethyl-1-nonanol			+	+		
( <i>Z,Z</i> )-3,6-Nonadienal	+	+	+			
Nonanal	+			+	+	+
2-Propyl-1-heptanol	+					
<b>(<i>E,Z</i>)-2,6-Nonadienal</b>	+	+	+	+	+	+
2-Hexyl-1-octanol	+		+		+	+
<i>Ketone</i>						
2-Heptanone				+	+	+

**Table 3** Ranking of normalized electroantennogram (EAG) responses from three species of New Zealand *Sigaus* grasshopper to 11 test stimuli at concentrations 10 mg/mL. Emphasis indicate chemical classes: fatty acid-derived compounds=italics, aromatics=**bold**, terpenoids=none. Abbreviations ezno = (*E,Z*)-2,6-nonadienal, dec=decanal,  $\beta$ oc= $\beta$ -ocimene,  $\alpha$ phe= $\alpha$ -phellandrene, eu=eucalyptol, 2phe=2-phenylethanol,  $\beta$ my= $\beta$ -myrcene, sli = (*S*)-(-)-limonene, z2he = (*Z*)-2-hexen-1-ol, hex=hexanal,  $\alpha$ pine = (1*S*)-(-)- $\alpha$ -pinene

Rank	<i>Sigaus nivalis</i>		<i>Sigaus nitidus</i>		<i>Sigaus australis</i>	
	F	M	F	M	F	M
1	<i>ezno</i>	<i>ezno</i>	<i>ezno</i>	<i>ezno</i>	<i>ezno</i>	<i>ezno</i>
2	<i>dec</i>	<i>z2he</i>	<i>z2he</i>	<i>dec</i>	<i>dec</i>	<i>z2he</i>
3	<i>z2he</i>	<i>dec</i>	<i>dec</i>	<i>z2he</i>	<i>z2he</i>	<i>dec</i>
4	<b>2phe</b>	<b>2phe</b>	sli	<b>2phe</b>	$\beta$ my	$\beta$ oc
5	sli	sli	<b>2phe</b>	<i>hex</i>	sli	<b>2phe</b>
6	$\alpha$ phe	<i>hex</i>	<i>hex</i>	$\alpha$ phe	<b>2phe</b>	<i>hex</i>
7	<i>hex</i>	$\alpha$ phe	$\alpha$ phe	$\beta$ my	<i>hex</i>	sli
8	$\alpha$ pine	$\beta$ oc	$\beta$ oc	sli	$\beta$ oc	$\alpha$ phe
9	$\beta$ my	$\alpha$ pine	$\alpha$ pine	$\beta$ oc	$\alpha$ pine	$\alpha$ pine
10	eu	eu	$\beta$ my	eu	eu	$\beta$ my
11	$\beta$ oc	$\beta$ my	eu	$\alpha$ pine	$\alpha$ phe	eu



**Fig. 4** Electroantennogram (EAG) recordings and normalized responses (absolute EAG in relation to positive control) of New Zealand *Sigaus* grasshoppers to 11 plant volatiles at concentrations of 10 mg/mL. Example trace (top) shows the first two responses (left side) are to hexane control (H) and positive control ( $\beta$ -caryophyllene; P) at 10 mg/

mL of hexane. Error bars are standard error of the mean (SEM). Abbreviations ezno = (*E,Z*)-2,6-nonadienal, dec=decanal,  $\beta$ oc= $\beta$ -ocimene,  $\alpha$ phe= $\alpha$ -phellandrene, eu=eucalyptol, 2phe=2-phenylethanol,  $\beta$ my= $\beta$ -myrcene, sli = (*S*)-(-)-limonene, z2he = (*Z*)-2-hexen-1-ol, hex=hexanal,  $\alpha$ pine = (1*S*)-(-)- $\alpha$ -pinene

highest experimental concentration (100 mg/mL) but some individuals did not show response to hexanal and 2-phenylethanol at 1 mg/mL, and terpenoids at 1 mg/mL and 10 mg/mL (i.e., did not show a stronger response than to the hexane control).

Table 3 displays the ranking of normalized EAG responses among 11 test stimuli at a concentration of 10 mg/mL. Grasshoppers generally exhibited the strongest responses to fatty acid derived aldehydes and alcohols ((*E,Z*)-2,6-nonadienal, decanal, (*Z*)-2-hexen-1-ol and hexanal); all grasshoppers

showed more than twice or thrice the average normalized responses to aldehydes and alcohols compared to most of the terpenoids at 10 mg/mL (Fig. 4). This was followed by 2-phenylethanol (aromatic), and then terpenoids (Table 3). This ranking of responses was similar at the concentrations of 1 mg/mL and 100 mg/mL (Table S3 & 4).

Significant differences in normalized responses were observed to some test compounds at different concentrations. *Sigaus nivalis* females showed significantly higher responses to (*E,Z*)-2,6-nonadienal at 0.1 mg/mL than *S.*

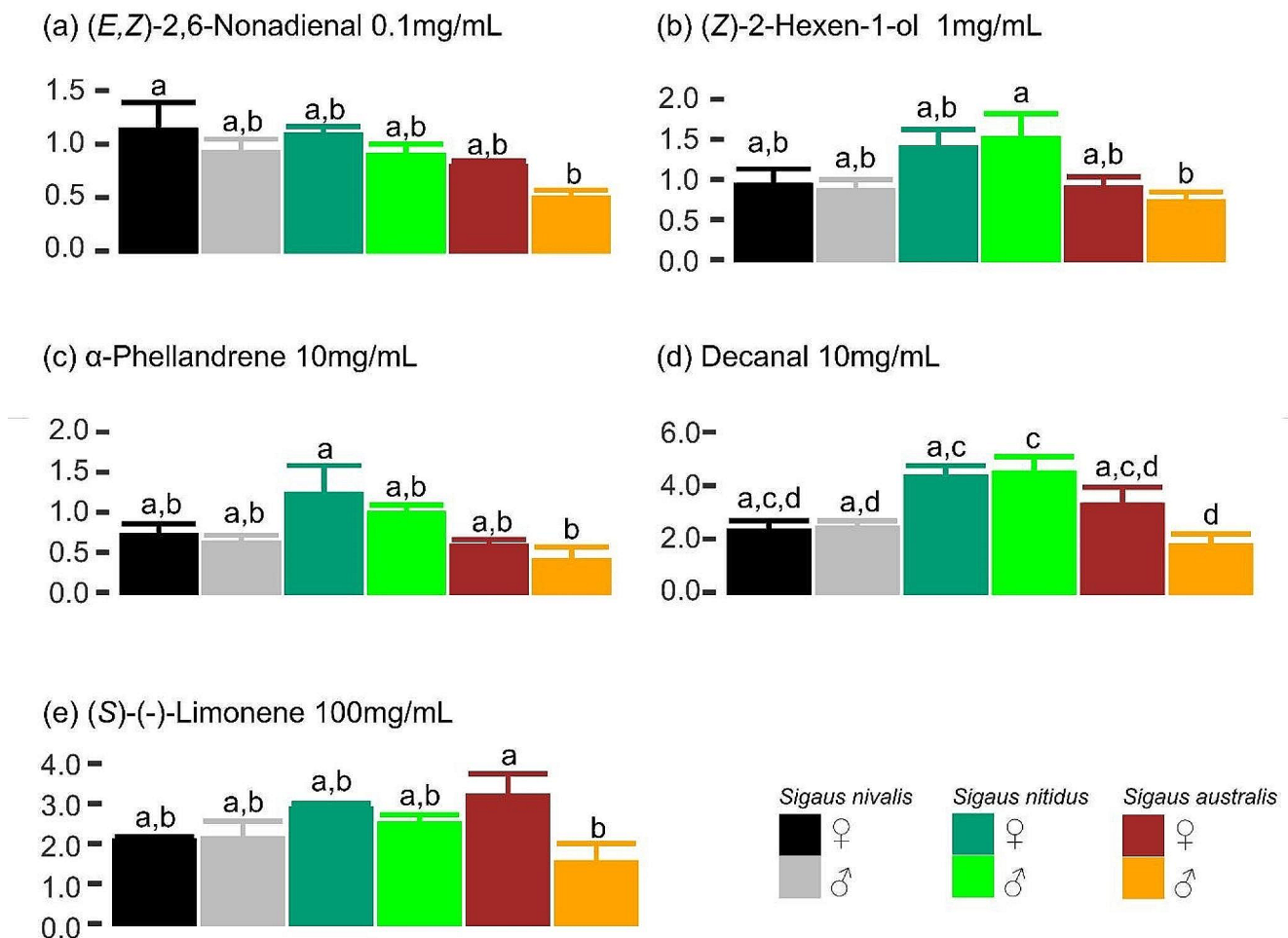
*australis* males (Fig. 5a). *Sigaus nitidus* males showed significantly higher responses to (*Z*)-2-hexen-1-ol at 1 mg/mL (Fig. 5b) and to decanal at 10 mg/mL (Fig. 5d) than *S. australis* males. *Sigaus nitidus* females showed significantly higher responses to  $\alpha$ -phellandrene and decanal at 10 mg/mL than *S. australis* males (Fig. 5c, e).

Between sexes, significantly higher responses to (*S*)-(-)-limonene at 100 mg/mL were observed in *S. australis* females than conspecific males (Fig. 5e). However, no significant sexual differences were observed in normalized EAG responses of *S. nivalis* or *S. nitidus* to any of the compounds tested.

*Sigaus* grasshopper EAG responses increased in a dose-dependent manner along the tested concentration range (0.1 mg/mL–100 mg/mL) (Fig. 6). A decrease or no change in response to decanal and (*E,Z*)-2,6-nonadienal was observed in male and female *S. nitidus* when the concentration increased from 10 mg/mL to 100 mg/mL (Fig. 6I, J).

## Discussion

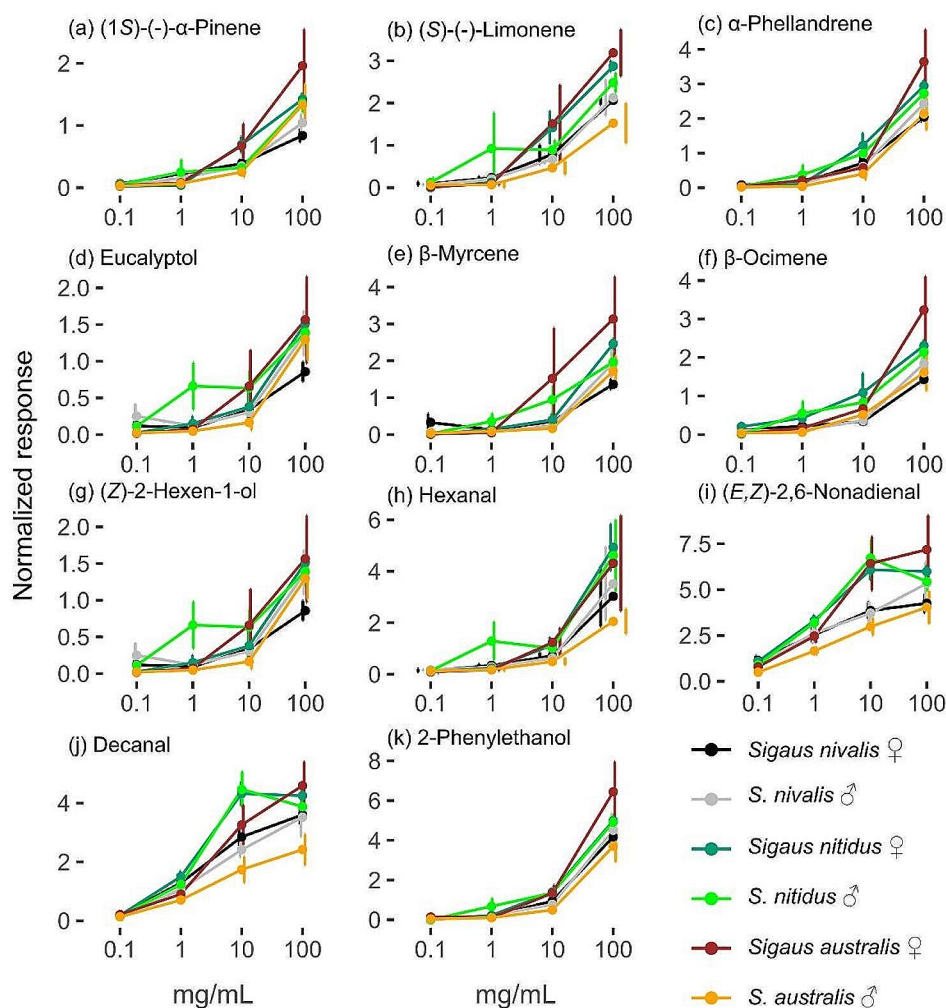
Insects display olfactory responses to chemicals in their food plants (Biasazin et al. 2019; Chen et al. 2004; Chen and Kang 2000; Kang and Hopkins 2004; Njagi and Torto 1996; Twidle et al. 2015, 2022). Sympatric New Zealand *Sigaus* grasshoppers, *S. nivalis*, *S. nitidus*, *S. australis* are all generalists, known to feed on more than 100 plant species but they have a preference for dicots over monocots (Nakano 2024; Watson 1970). As New Zealand grasshoppers have olfactory sensilla on their antennae (Nakano et al. 2023), sensitivity to plant smells is predicted for these species to successfully locate and recognize food. This study explored feeding preferences of grasshoppers using six native alpine plant species and for the first time recorded electrophysiological responses of alpine grasshopper antennae to the chemicals found in these plants.



**Fig. 5** Normalized electroantennogram (EAG) responses (absolute EAG in relation to positive control) of male and female New Zealand *Sigaus* grasshopper species to five synthetic compounds. Lowercase letters on bars indicate significant differences in responses between

species or sexes revealed from the analysis of variance followed by a pair-wise post hoc Tukey honest significant test. Error bars are standard error of the mean (SEM)

**Fig. 6** Normalized electroantennogram (EAG) responses (absolute EAG in relation to positive control) of male and female New Zealand *Sigaus* grasshoppers to each of 11 volatile compounds at four dose ranges (0.1 mg/mL, 1 mg/mL, 10 mg/mL and 100 mg/mL). Error bars are standard error of the mean (SEM)



## Food choice

When given a choice, all three New Zealand *Sigaus* grasshopper species ate the shrub *Coriaria sarmentosa* but none ate the tussock grass *Chionochloa pallens*. We know all three grasshoppers share the same habitat where these plants are common so the feeding trials revealed a clear ability by the insects to distinguish among potential food species. However, no species- or sex-specific preference towards particular plants was observed in captivity, with all individuals eating *C. sarmentosa* (mountain tutu). A particular plant species may be targeted as a food for many different reasons but most involve trade-offs between nutrition and plant defenses (Carlson and Agrawal 2023). *Coriaria* species produce the toxin tutin which is particularly poisonous to mammals (Bascand 1976) but not insects, and it is possible that an evolutionary response to limit mammalian herbivory exposes this plant to insect damage. In addition, plants that provide potential for insect protection via sequestration of plant secondary metabolites might be attractive

(McLellan et al. 2021). New Zealand alpine grasshoppers were also observed to eat the fruits of *C. sarmentosa* and the availability of these throughout the year may increase the attractiveness of this species.

## Food plant and active compounds

From the six plant species used in feeding trials, 17 volatile compounds belonging to terpenoids, alcohols, aldehydes, and one ketone were detected. Electroantennogram (EAG) analysis showed that New Zealand *Sigaus* grasshoppers are highly sensitive to fatty acid-derived aldehydes and alcohols. Olfactory responses to six compounds belonging to these chemical classes (*Z*-2-hexen-1-ol, hexanal, nonanal, (*Z,Z*)-3,6-nonadienal, (*E,Z*)-2,6-nonadienal, and 2-hexyl-1-octanol), and several other volatile compounds were detected from all grasshopper antennae. (*E,Z*)-2,6-Nonadienal was detected even at the lowest concentration. EAG responses to fatty acid-derived compounds were two or three times higher than most terpenoids at the higher test



concentrations (10 mg/mL and 100 mg/mL). Higher sensitivity to alcohols and aldehydes than to terpenoids has also been observed in generalist grasshoppers elsewhere including adult *Oedaleus decorus asiaticus* and *Angaracris barabensis* (Chen et al. 2004) and *Schistocerca gregaria* nymphs (Njagi and Torto 1996) regardless of dicot or monocot feeding habits. On the other hand, the specialist grasshopper *Hypochlora alba* responded to terpenoids found in their host plant *Artemisia ludoviciana* even at very low concentration (0.1 ng/μl: 1/1000 of the lowest concentration used in the present study) (Blust and Hopkins 1987b). Indeed, most of the compounds found in *A. ludoviciana* elicited significantly higher responses from *H. alba* than the generalist grasshopper *Melanoplus sanguinipes*. *Melanoplus sanguinipes* showed a stronger EAG response to geraniol which is the compound that occurs more commonly in many plants (and also detected in the present study) compared to other terpenoid compounds found in *A. ludoviciana*. Thus, generalist and specialist grasshopper species may have very different olfactory sensitivity.

Fatty acid derivatives are emitted immediately after plants are damaged (Ameje et al. 2018; Dicke and Baldwin 2010), and the hexane extraction method used in the present study involved damaging plant tissues. Behavioral observations using a Y-maze olfactometer showed smells from damaged plants were significantly more attractive to the polyphagous grasshopper *S. gregaria* (Njagi and Torto 1996) and *M. sanguinipes* (Hopkins and Young 1990; Kang and Hopkins 2004) than smells of undamaged plants. It is yet to be determined whether these aldehydes and alcohols act as attractants or repellents in New Zealand *Sigaus* grasshoppers, so this needs further behavioral studies.

### Between species and sexes

Most of the New Zealand alpine grasshoppers showed, as expected, enhanced sensitivity to test stimuli as the dose increased. The exception was that the response of *S. nitidus* to decanal and (*E,Z*)-2,6-nonadienal remained unchanged or even decreased when the concentration of these compounds increased from 10 mg/mL to 100 mg/mL. The reason behind this is unknown but it is unlikely that decreased sensitivity to these compounds is associated with decreased sensitivity of the antenna as the sensitivity was ensured with exposure to controls. It is possible that the maximum responses to these compounds are reached at concentrations around 10 mg/mL in *S. nitidus*.

High sensitivity to external stimuli is considered to be related to a higher abundance of sensilla (Nakano et al. 2022), and both higher abundance of sensilla and stronger olfactory response was observed in male *O. decorus asiaticus* (Chen et al. 2004) and *H. alba* (Blust and Hopkins

1987b) than conspecific female grasshoppers. In contrast, *Sigaus australis* males were less sensitive to test stimuli (after normalisation) than females even though they have higher abundance of sensilla (Nakano et al. 2023), presumably due to their high response to the positive control β-caryophyllene. It is also possible that *S. australis* males have specific olfactory sensitivity to compounds not yet explored, that differs from conspecific female sensitivity.

Although the alpine grasshoppers showed a preference for specific plant species, the chemicals found in the six plant species examined were similar and it is not yet known how these grasshoppers recognize their favorite food. Each plant did have a distinct combination of compounds and complex blends of compounds rather than individual chemicals may enable food detection. Taste can also be involved in the food selection of the grasshoppers, as seen in other grasshoppers including *H. alba*, *M. sanguinipes* (Blust and Hopkins 1987a), *Chorthippus* species (Picaud et al. 2003), *Boettix argentatus*, *Ligurotettix coquilletti*, and *Cibolacris parviceps* (Chapman et al. 1988). Macronutrients are important factors in determining food, and one study showed time spent palping on filter paper loaded with sucrose and fructose was significantly different among four *Chorthippus* grasshopper species (Picaud et al. 2003). Structural aspects of plants (i.e., size, toughness: Clissold 2007; Krenn 2019; Patterson 1983, 1984) as well as visual cues including colors (Picaud et al. 2002) and branching patterns (Picaud et al. 2003) of host plants can also be important factors in discriminating host plants in grasshoppers. Altogether, further studies of chemical, structural and visual cues involved in the food plant selection of New Zealand *Sigaus* grasshoppers are required.

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**Data availability** The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

**Code Availability** (software application or custom code) Inkscape 1.0, R Studio 4.0.3.

## Declarations

**Competing interests** Not applicable.

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