Bulletin of Entomological Research

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New insight into the behaviour modifying activity of two natural sesquiterpenoids farnesol and nerolidol towards *Myzus persicae* (Sulzer) (Homoptera: Aphididae)

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Research Paper

Cite this article: Wróblewska-Kurdyk A, Dancewicz K, Gliszczyńska A, Gabryś B (2020). New insight into the behaviour modifying activity of two natural sesquiterpenoids farnesol and nerolidol towards *Myzus persicae* (Sulzer) (Homoptera: Aphididae). *Bulletin of Entomological Research* **110**, 249–258. https://doi.org/10.1017/S0007485319000609

Received: 14 March 2019
Revised: 1 June 2019
Accepted: 31 July 2019
First published online: 27 September 2019

Key words:
Antifeedant activity; aphid probing; EPG

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Abstract

The effect of structurally related sesquiterpenoids (E,E)-farnesol and *cis*-nerolidol on the host-plant selection behaviour of the peach potato aphid *Myzus persicae* (Sulz.) was evaluated using electrical penetration graph (EPG) technique. No repellent effects of (E,E)-farnesol and (Z)-nerolidol to *M. persicae* were found but aphid probing activities on (E,E)-farnesol- and *cis*-nerolidol-treated plants were restrained. During non-phloem phases of probing, neither (E,E)-farnesol nor (Z)-nerolidol affected the cell puncture activity. On (E,E)-farnesol-treated plants, the total duration of phloem phase, the mean duration of individual sustained ingestion periods were significantly lower, and the proportion of phloem salivation was higher than on control plants. On (Z)-nerolidol-treated plants, the occurrence of the first phloem phase was delayed, and the frequency of the phloem phase was lower than on control plants. The freely moving aphids were reluctant to remain on (E,E)-farnesol- and (Z)-nerolidol-treated leaves for at least 24 h after exposure. (E,E)-Farnesol and (Z)-nerolidol show complementary deterrent properties, (E,E)-farnesol showing ingestive and post-ingestive activities and nerolidol showing pre-ingestive, ingestive, and post-ingestive deterrent activities.

Introduction

The challenge of present-day agriculture is to maximize the food production for a rapidly growing human population, which requires not only the enhancement of natural productivity of crop plants but also the protection against adverse abiotic and biotic factors. It is estimated that herbivorous arthropods alone are responsible for destroying close to one-fifth of the world's total crop production annually, which translates into the loss of more than $470 billion per year (Culliney, 2014). Aphids (Hemiptera: Sternorrhyncha: Aphididae) damage crops directly by removing nutrients from sieve elements and act as very efficient vectors of plant diseases (Blackman and Eastop, 2017). A study in Australia demonstrated that the aphid feeding and virus injuries in cereals, oilseed and pulse crops resulted in potential economic costs of $241 and $482 million year^{-1}, respectively (Valenzuela and Hoffman, 2015). The peach potato aphid *Myzus persicae* (Sulz.) (Hemiptera: Aphididae), a cosmopolitan and highly polyphagous species, can infest plants of over 40 different families, including many economically important ones worldwide, and is able to transmit more than 100 virus diseases (Margaritopoulos *et al.*, 2009; Blackman and Eastop, 2017). Crop losses due to insect pests have been reported to be greater under modern than under traditional agricultural practices, which has been attributed to an over-reliance on synthetic chemicals for pest control (Culliney, 2014). The growing concern about the use of existing commercial synthetic insecticides is mainly due to their broad-spectrum toxicity towards non-target organisms, especially the beneficial arthropods, and the potential threat to humans and environment, which includes extensive groundwater contamination (Campos *et al.*, 2019). The worldwide damage due to the use of pesticides reaches $100 billion annually (Koul *et al.*, 2008). At the same time, the effectiveness of pesticides is affected by the evolution of resistant pathogens, weeds, and insect pests (Hawkins *et al.*, 2019). *M. persicae* has evolved at least seven independent mechanisms of resistance to several classes of insecticides, which makes this species extremely difficult to control (Bass *et al.*, 2014). As a result, many research groups focus their attention on substances of natural origin, mainly plant secondary metabolites, as insect control agents (Gerard *et al.*, 1993; Martinez and Van Emden, 1999; Wróblewska-Kurdyk *et al.*, 2015; Jackowski *et al.*, 2017). Plant secondary metabolites are basic components of natural plant resistance against herbivores and the qualitative and quantitative variation in the content of these allelochemicals can make the plants less or more suitable for

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aphid feeding (Gabryś and Pawluk, 1999; Schliephake, 2010; Kordan *et al.*, 2012; Philippi *et al.*, 2015; Phuong *et al.*, 2015). The exogenous application of xenobiotics may alter aphid response to otherwise acceptable host plants, which has been shown in studies on aphid antifeedants of different chemical groups, terpenoids, quassinoids, flavonoids, and cyanogenic glycosides (Polonsky *et al.*, 1989; Gutierrez *et al.*, 1997; Halarewicz and Gabryś, 2012; Goławska *et al.*, 2014; Gabryś *et al.*, 2015; Stompor *et al.*, 2015). In our previous studies, we demonstrated that the application of pulegone, limonene, camphene, β-ionone and other monoterpenoids to the plants caused various disturbances in aphid behaviour, including a decrease in probing activities and a general failure to reach sieve elements and uptake the phloem sap by *M. persicae* (Dancewicz *et al.*, 2008, 2016). In nature, mono- and sesquiterpenoids, the main components of plant essential oils, are mediators in ecological interactions, especially in plant-insect relationships, as kairomones for pollinators, predators, and parasitoids and/or repellents and antifeedants to herbivores (Abbas *et al.*, 2017). Two natural sesquiterpene alcohols, farnesol and its structural analogue nerolidol (fig. 1) occur in the essential oils of plants that belong to many plant families, including Cactaceae, Fabaceae, Oleaceae, Orchidaceae, Primulaceae, and Solanaceae (Knudsen *et al.*, 2006). Farnesol is the component of the aggregation pheromone of the spined citrus bug, *Biprorulus bibax* Breddin (Hemiptera: Pentatomidae) (James *et al.*, 1996), the marking substance of the Scandinavian bumble-bee, *Bombus pratorum* (L.) (Hymenoptera: Apidae) (Bergman and Bergstrom, 1997) and *B.* (*Diversobombus*) *diversus* Smith (Kubo and Ono, 2010), a component of recruitment pheromone in bumblebee *B. terrestris* (L.) (Strube-Bloss *et al.*, 2015), and acts as a repellent to the corn leaf aphid, *Rhopalosiphum maidis* (Fitch) (Hemiptera: Aphididae) (Halbert *et al.*, 2009). At the same time, these lower terpenoids act as repellents and/or insecticides towards specific pests without harmful side effects to humans and animals (Isman, 2000; Koul *et al.*, 2008; Cantrell *et al.*, 2012; Mossa, 2016; Hawkins *et al.*, 2019). Nerolidol reduces the fecundity and survival of Mexican mite *Tetranychus mexicanus* (McGregor) (Acari: Tetranychidae) (Amaral *et al.*, 2017), is a repellent to winged and wingless *M. persicae* and ladybird *Coccinella septempunctata* (L.) (Coleoptera: Coccinellidae) (Vucetic *et al.*, 2014; Dahlin *et al.*, 2015). In addition, nerolidol is an attractant to the predators of predatory mites in *Arabidopsis thaliana* (L.) Heynh. (Brassicaceae) (Abbas *et al.*, 2017), and is released from plants following the injury by larvae of *Spodoptera exigua* (Hübner) (Lepidoptera: Noctuidae).

Considering the wide and varied spectrum of farnesol and nerolidol activities towards insects, we decided to evaluate in detail the potential deterrent properties of these sesquiterpenoids towards *M. persicae*. In our previous study concerning *M. persicae*, we found that the freely moving aphids were less likely to settle on whole plant leaves treated with (E,E)-farnesol. Specifically, the application of this terpenoid caused the avoidance of treated leaves by the peach potato aphid for at least 24 h after exposure (Dancewicz *et al.*, 2010). Similar results involving leaf discs were reported by Gutierrez *et al.* (1997). The effect of nerolidol on the host plant selection behaviour of *M. persicae* has not been previously reported.

The aim of the present research was to investigate the potential repellent and feeding deterrent activities of the two structurally related sesquiterpenoids farnesol and nerolidol towards *M. persicae*. The behavioural background of the repellent and deterrent effects of farnesol and nerolidol were studied by direct monitoring aphid initial responses and aphid stylet penetration in plant tissues using the electrical penetration graph (EPG) technique, respectively. EPG technique makes possible the localization of the deterrent activity of natural as well as artificially applied chemicals within particular plant tissues and the association of this activity to particular phases of aphid probing (Gabryś *et al.*, 2015; Dancewicz *et al.*, 2016; Paprocka *et al.*, 2018). We were especially interested what phases of aphid probing in plant tissues were, if at all, the most strongly affected by the application of farnesol and nerolidol, how the slight differences in the compounds' structures affected the biological activity, and what consequences these aspects might have on plant infestation by aphids.

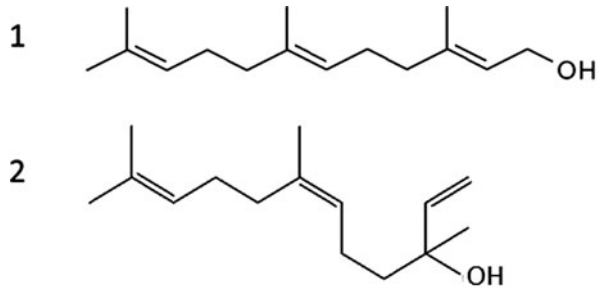

Figure 1. Structures of (E,E)-farnesol (**1**) and (Z)-nerolidol (**2**).

Materials and methods

Chemicals

The sesquiterpenoids used in the experiments were (E,E)-farnesol (**1**) and a racemic mixture of (Z)-nerolidol (**2**) purchased from Sigma-Aldrich as *trans, trans*-farnesol and *cis*-nerolidol, respectively (fig. 1.).

Plant and aphid cultures

The peach potato aphid, kept as a multiclonal colony on Chinese cabbage *Brassica rapa* subsp. *pekinensis* (Lour.) Hanelt., was reared in the laboratory at 20°C, 65% r.h., and L16:D8 photoperiod in a growing chamber Sanyo MLR-351H (Sanyo Electronics Co. Ltd.). One- to seven day old adult apterous females of *M. persicae* and three-week old plants with four to five fully developed leaves were used for all experiments. All experiments were carried out under the same conditions of temperature, relative humidity (r.h.), and photoperiod as those used for the rearing of plants and aphids. All bioassays started at 10:00–11:00 h MEST (Middle European Summer Time). Aphids show distinct diurnal feeding activity under long-day conditions, with peak activity during daytime, independently of host plants (Joschinski *et al.*, 2016; Beer *et al.*, 2017).

Application of sesquiterpenoids

Each compound was dissolved in 70% ethanol to obtain a 0.1% solution (Polonsky *et al.*, 1989). All compounds were applied on the adaxial and abaxial leaf surfaces by immersing a leaf in the ethanolic solution of a given compound for 30 s. Control leaves of similar size were immersed in 70% ethanol that was used as a solvent for the studied sesquiterpenoids. There is no effect of ethanol application on aphid probing behaviour and plant condition (Halarewicz-Pacan *et al.*, 2003).Treated and control leaves were allowed to dry for 1 h before the start of the

https://doi.org/10.1017/S0007485319000609 Published online by Cambridge University Press

experiment to permit evaporation of the solvent (Gabryś *et al.*, 2015). Each plant and aphid was used only once.

Aphid behaviour

Aphid initial responses (no choice-test)

Aphid behaviour during initial contact with the studied compound was assessed by direct monitoring of the freely moving aphids on a treated leaf in a no-choice test. Considering the volatility of the sesquiterpenoids studied, this bioassay allowed to separate aphid responses to olfactory cues from responses to gustatory cues. The results are supposed to show whether a given compound is active at plant surface or within plant tissues. In this experiment, an aphid was placed in a Petri dish containing a freshly prepared leaf and the observation of aphid movements was started immediately. The experiment was carried out for 15 min and replicated 10 times for each compound and control, one newly selected aphid per replication. The following parameters were determined based on the data obtained in this experiment: total time spent by an aphid on the leaf, total probing time, total non-probing time, time to the first probe, number of probes, and mean duration of a probe. The time spent on the leaf and the duration of probing were recorded based on the relationship between antennal and body movements and penetration of the stylets as described by Hardie *et al.* (1992) who associated stylet penetration with the position of antennae parallel to the abdomen and the cessation of body movements.

Aphid responses during probing in plant tissues (no choice-test)

The probing behaviour of *M. persicae* was monitored using the electrical penetration graph (EPG) technique that is frequently employed in insect–plant relationship studies considering insects with sucking-piercing mouthparts and their responses to xenobiotics (Mayoral *et al.*, 1996). In this experimental set-up, aphid and plant are made parts of an electric circuit, which is completed when the aphid inserts its stylets into the plant. Weak voltage is supplied in the circuit, and all changing electric properties are recorded as EPG waveforms that can be correlated with aphid activities and stylet position in plant tissues (figs 2 and 3) (Pettersson *et al.*, 2017). In the present study, an aphid was attached to a golden wire electrode (1.5–2.0 cm long, 0.18 µm diam.) with water-based conductive silver paint (EPG-Systems, Dillenburg 126703 CJ Wageningen, The Netherlands) and starved for 1 h prior to the experiment. Probing behaviour of 15 apterous females per studied compound was monitored for 8 h continuously with four-channel DC EPG recording equipment. Each aphid was given access to a freshly prepared leaf of an intact plant. Signals were saved on the computer and analysed using the stylet + software provided by W. F. Tjallingii (http://www.epgsystems.eu). The following aphid behaviours were distinguished: non-probing (waveform 'np'–aphid stylets outside the plant), penetration of non-phloem tissues (pathway phase 'C' and derailed stylet movements 'F'), phloem phase salivation into sieve elements (waveform 'E1'), phloem phase ingestion of phloem sap (waveform 'E2'), and xylem phase (ingestion of xylem sap, waveform 'G') (fig. 2a). Waveforms F and G occurred rarely, therefore were analysed with waveform C as non-phloem phase probing activities. Waveform patterns that were not terminated before the end of the experimental period (8 h) (i.e., were artificially short due to the end of the 8 h recording) were included in the calculations. Additionally, the frequency and duration of cell punctures during pathway probing in non-phloem tissues were analysed. These cell punctures serve as an opportunity to collect samples of cytoplasm for gustatory purposes during host plant suitability assessment by aphids (Pettersson *et al.*, 2017). Accidentally, during these cell punctures, transmission of non-persistent and semi-persistent plant viruses may take place. The cell punctures, manifested in EPG recordings as potential drops ('pd') are divided into 'short' pds (pd-S) and 'long' pds (pd-L), which differ mainly in the number of pulses within the subphase II-3 that is associated with virus acquisition (fig. 2b) (Martin *et al.*, 1997). The cell punctures pd-S have 0-2 pulses in subphase II-3, while pd-L punctures – more than three pulses (Chen *et al.*, 1997; Martin *et al.*, 1997; Moreno *et al.*, 2012). The parameters derived from EPGs were analysed according to their frequency and duration in configuration related to activities in non-phloem and phloem tissues (figs 2 and 3).

Aphid settling success (choice-test)

Aphids settle on a plant only when they accept it as a food source (Harrewijn, 1990). Therefore, the number of aphids that settle and feed on a given substrate is a good indicator of its suitability. This bioassay allows studying aphid host preferences under semi-natural conditions. Aphids are given free choice between control and treated leaves. In the present study, aphids were placed in the Petri dish along the line that divided the arena into two halves so that aphids could choose between treated (on one half of a Petri dish) and control leaves (on the other half of the dish). Aphids that settled, i.e. they did not move and the position of their antennae indicated feeding (Hardie *et al.*, 1992) on each leaf were counted at 1, 2, and 24 h intervals after access to the leaves (8 replicates, 20 viviparous apterous females/replicate). Aphids that did not settle on any of the leaves were not included in calculations.

Statistical analysis

Parameters describing aphid initial responses to farnesol and nerolidol-treated plants (no choice-test) were statistically analysed using Mann–Whitney *U*-test at $P < 0.05$ in relation to control. EPG parameters describing aphid probing behaviour (no choice-test) were calculated manually and individually for every aphid using the EPG analysis Excel worksheet created especially for this study. Subsequently, the mean and standard errors were determined. The data were analysed by the Mann–Whitney *U*-test at $P < 0.05$ in relation to control. The results on aphid settling success (choice test) were statistically analysed using Student *t*-test: the number of aphids on control leaves was compared to the number of aphids on the test leaf for each compound/time interval separately. If aphids showed a clear preference for the leaf treated with the tested compound ($P < 0.05$), the compound was described as having attractant properties. If aphids settled mainly on the control leaf ($P < 0.05$), the compound tested in the respective choice-test was designated a deterrent. Student *t*-test was used for analysis of the choice-test at $P = 0.05$.

All statistical calculations were performed using STATISTICA (data analysis software system), version 12, http://www.statsoft.com.

Results

Aphid initial responses (no choice-test)

Aphid responses during initial contact with plants treated with sesquiterpenoids differed depending on the compound applied.

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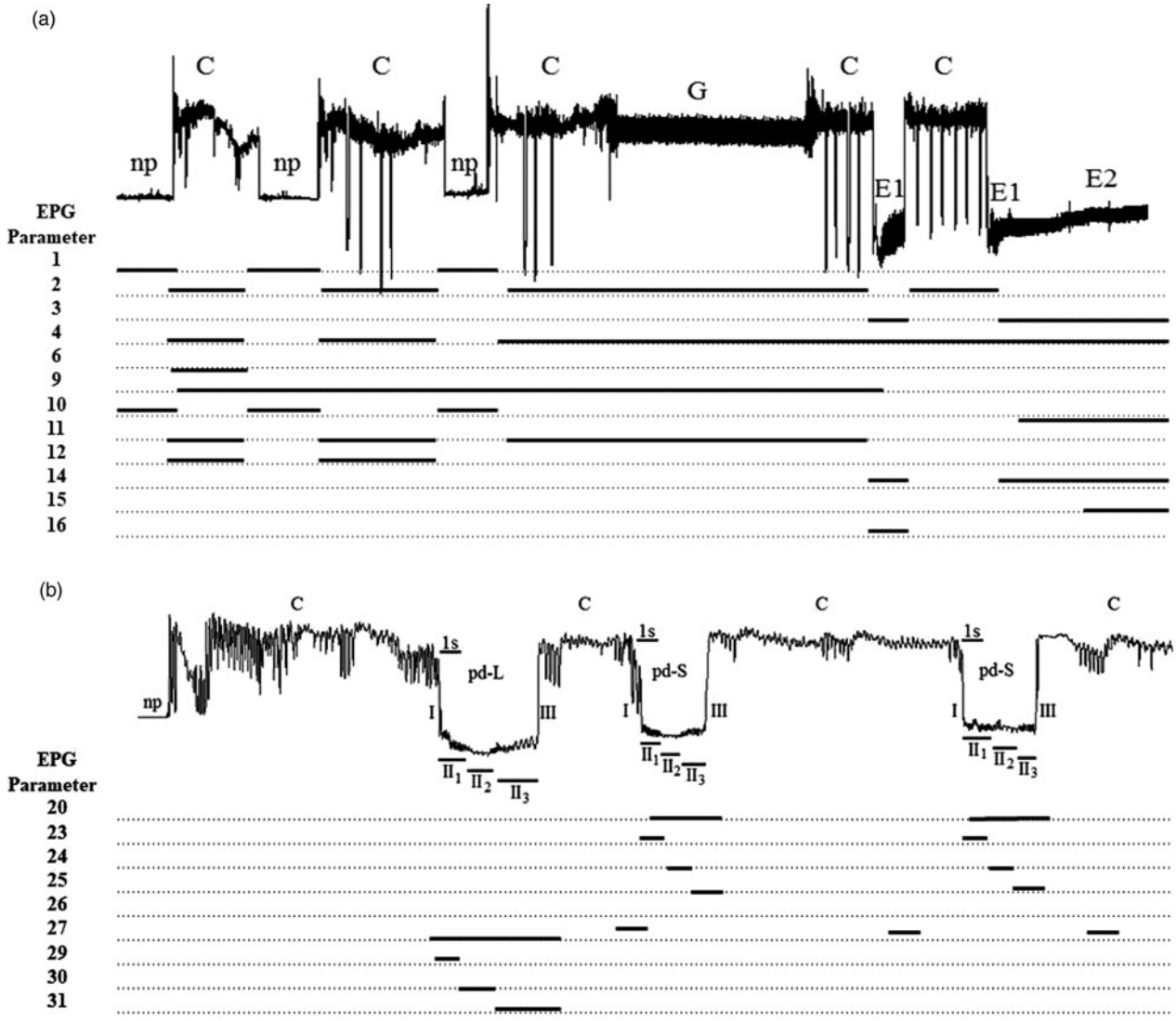

Figure 2. (a) Graphical presentation of EPG parameters related to general aphid probing behaviour. (b) Graphical presentation of EPG parameters related to exploratory cell punctures ('pd'). Numbers represent parameters included in table 2 and table 3. np – non probing, C – pathway activities; G – xylem phase; E1 – phloem phase salivation; E2 – phloem phase ingestion; pd-S – 'short' potential drops; pd-L – 'long' potential drops; I – potential drop subphase I; II_1 – potential drop subphase II-1; II_2 – potential drop subphase II-2; II_3 potential drop subphase II-3; III – potential drop subphase III. Drawing based on EPG recordings from the present work.

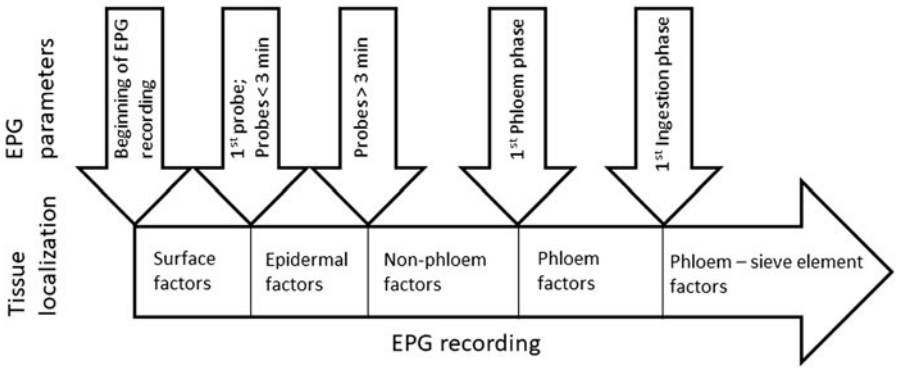

Figure 3. Interpretation of key phases in aphid probing and plant factors affecting aphid behaviour, as visualized in EPG recordings.

Aphids on farnesol-treated plants spent the whole time of the 15 min experiment on the treated leaves and the main activity was probing (80% of experimental time), which was more than on control leaves (table 1). However, the number and the mean duration of probes were similar to those on control plants. On nerolidol-treated plants, aphids spent 30% of experimental time

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Table 1. Initial responses of *M. persicae* on control and (E,E)-farnesol (**1**) and (Z)-nerolidol (**2**) treated plants

EPG parameter	Control	Farnesol	Nerolidol
Time spent on the leaf (s)	900.0 ± 0.0	900.0 ± 0.0	6988 ± 333.5
Total non-probing time (s)	371.1 ± 214.3	192.1 ± 134.6*	213.1 ± 197.0*
Total probing time (s)	528.9 ± 214.3	707.9 ± 134.6*	485.7 ± 347.3
Time to first probe (s)	36.5 ± 64.7	18.0 ± 21.0	108.4 ± 279.1
Number of probes	6.7 ± 4.3	5.3 ± 1.4	5.1 ± 3.0
Mean probing time (s)	164.5 ± 201.3	141.4 ± 44.4	116.3 ± 131.9

*Significant difference in relation to control.

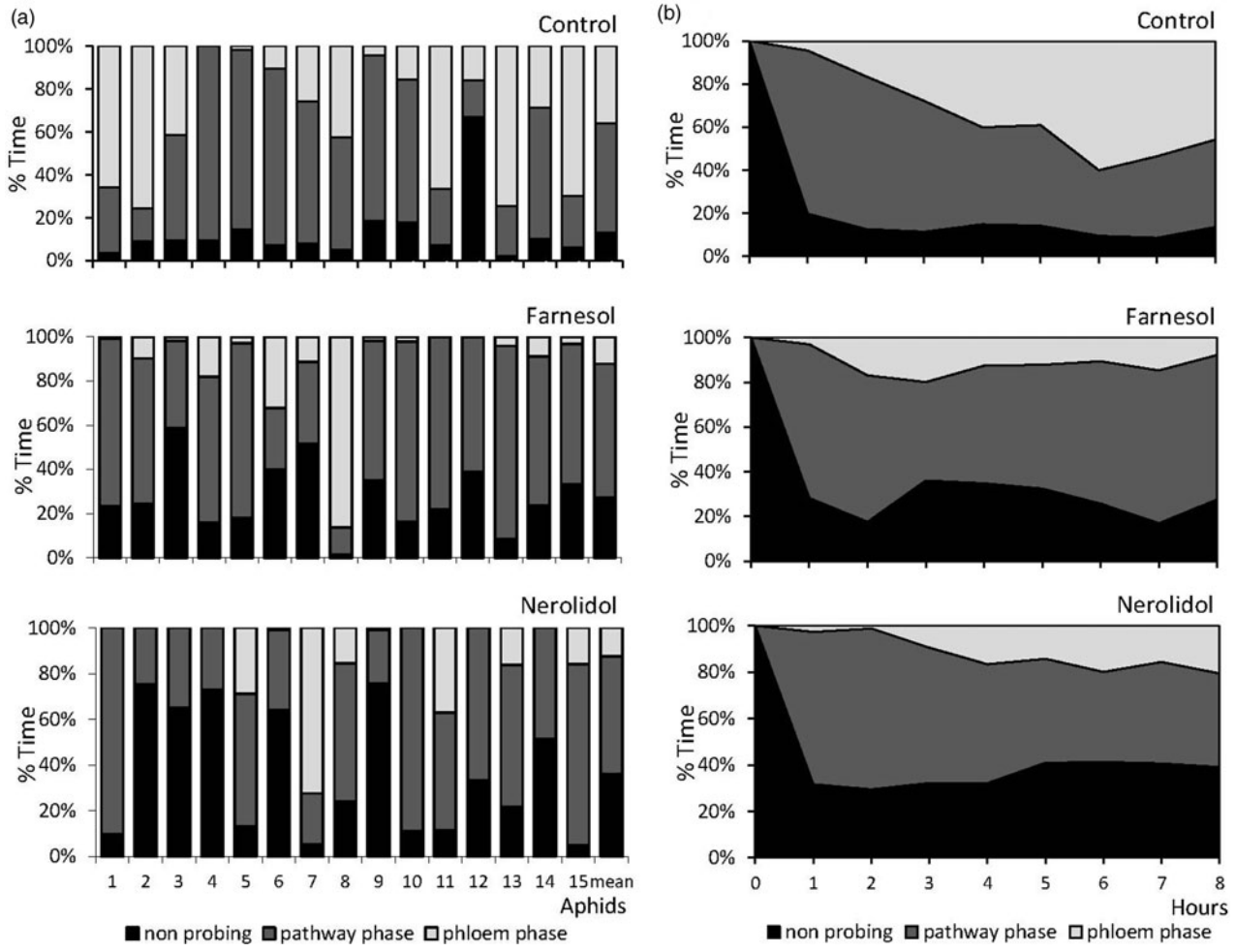

Figure 4. (a) Individual variation in probing behaviour of *M. persicae* on control and (E,E)-farnesol (**1**) and (Z)-nerolidol (**2**) treated plants. (b) Sequential changes in *M. persicae* probing behaviour on control and (E,E)-farnesol (**1**) and (Z)-nerolidol (**2**) treated plants; non probing (aphid stylets outside the plant), pathway phase (probing in non-phloem tissues; waveforms A, B, C, F, and G); phloem phase (waveforms E1 and E2).

wandering out of the treated leaves. However, the non-probing time during aphid presence on the leaves was shorter than on control leaves. The number and the mean duration of probes were similar to those on control plants. The time to the first probe varied from 18.0 ± 21.0 s on farnesol-treated leaves to 1.6 ± 4.6 min on nerolidol-treated plants, which was twice as short and three times as long as on control leaves, respectively (table 1).

Aphid responses during probing in plant tissues (no choice-test)

General aspects

Despite individual variation within experimental groups (fig. 4a), certain trends in aphid behaviour could be observed. Irrespective of the substance that was applied to the aphid host plant, all

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Table 2. Probing behaviour (EPG parameters) of *M. persicae* on control and (E,E)-farnesol (**1**) and (Z)-nerolidol (**2**) treated plants[a]

No.	EPG parameter	Compounds		
		Control	Farnesol	Nerolidol
	General aspects of aphid probing behaviour	*n* = 15	*n* = 15	*n* = 15
1	Total duration of non-probing[1] (h)	1.0 ± 1.3	2.2 ± 1.2*	2.9 ± 2.2*
2	Total duration of probing in non-phloem tissues[2] (h)	4.1 ± 2.1	4.8 ± 1.7	4.1 ± 1.9
3	Total duration of phloem phase (h)	2.9 ± 2.3	1.0 ± 1.8*	1.0 ± 1.6*
4	Total number of probes	24.7 ± 9.8	27.2 ± 13.3	28.7 ± 16.3
5	Mean duration of a probe (min)	21.7 ± 17.2	28.8 ± 58.0	17.9 ± 18.1
6	Duration of first probe (min)	1.8 ± 4.3	41.8 ± 49.8*	4.3 ± 5.9
7	Proportion of aphids reaching phloem phase	0.9 ± 0.3	0.9 ± 0.4	0.5 ± 0.5
8	Proportion of phloem phase in total probing[3]	0.4 ± 0.3	0.2 ± 0.2	0.1 ± 0.2
	Aphid behaviour before the first phloem phase	*n* = 14	*n* = 13	*n* = 8
9	Time from first probe to first phloem phase (h)	2.4 ± 2.1	3.0 ± 2.5	5.0 ± 3.1*
10	Total duration of non-probing before first phloem phase[b] (h)	0.4 ± 0.3	0.7 ± 0.6	1.1 ± 1.7
11	Total duration of probing in non-phloem tissues before first phloem phase[b] (h)	1.6 ± 1.3	1.7 ± 1.4	1.7 ± 1.4
12	Number of probes	13.2 ± 9.6	7.2 ± 8.1	11.6 ± 14.0
13	Number of probes <3 min.	8.0 ± 6.5	3.1 ± 4.9	7.4 ± 12.0
	Aphid behaviour during phloem phase	*n* = 14	*n* = 13	*n* = 8
14	Number of phloem phases	4.7 ± 3.0	3.3 ± 3.2	2.1 ± 2.3*
15	Number of sustained sap ingestion phases[4]	2.1 ± 1.7	0.5 ± 0.7*	1.1 ± 1.5
16	Duration of first phloem phase[b] (h)	0.6 ± 1.5	0.4 ± 0.7	0.2 ± 0.2
17	Mean duration of sap ingestion[b] (h)	0.9 ± 1.4	0.4 ± 0.9*	0.5 ± 0.5
18	Proportion of salivation in phloem phase[5]	0.1 ± 0.1	0.3 ± 0.4	0.1 ± 0.3

Numbers in the first column refer to graphical presentation of parameters in fig. 2.
[a]Values are means ± SD; *n* = number of replications;*significant difference in relation to control, ($P < 0.05$, Mann–Whitney *U* test).
[b]Only aphids that showed a phloem phase were used for analysis; [1]C + G + E + F; [2]C + G + F; [3]E1 + E2/C + G + E + F; [4]E2>10 min; [5]E1/E1 + E2.

aphids started probing immediately after being given access to the plants at the beginning of the experiment. In the course of time, the proportion of time committed to individual phases of probing varied among aphids, depending on the compound applied. Non-probing and pathway activities predominated during the first hour of the experiment in all aphids on all plants (fig. 4b). Later, on control plants, the proportion of non-probing phase decreased while the proportion of non-phloem and phloem probing phases in aphid activities increased, and at the end of the 8 h experiment, phloem phase was the main aphid activity on these plants (fig. 4b). On farnesol- and nerolidol-treated plants, the main activity was probing in non-phloem tissues and the proportion of non-probing remained relatively high throughout the experiment as compared to control, especially on farnesol-treated plants (fig. 4b).

Generally, on control plants, the main activity of aphids was probing, 87% of the 8 h experiment, which was significantly longer than on farnesol- and nerolidol-treated plants (table 2). The total duration of probing in non-phloem tissues was similar in all aphids but the total duration of phloem phase was three times as short on farnesol- and nerolidol-treated plants as on control. The phloem phase occupied 40% of aphid probing on control plants, while on farnesol- and nerolidol-treated plants it was 20 and 10%, respectively (table 2). The mean number and the mean duration of probes were similar on all plants, and ranged from 24.7 ± 9.8 min on control to 28.7 ± 16.3 min on nerolidol-treated plants and from 17.9 ± 18.1 min on nerolidol-treated to 28.8 ± 58.8 min on farnesol-treated plants, respectively. On control plants, phloem phase occurred in 8% of probes and 93% of aphids reached phloem phase, on farnesol-treated plants phloem phase occurred in 7% of probes and 87% of aphids reached phloem phase and on nerolidol-treated plants, phloem phase occurred in 4% of probes and 53% of aphids reached phloem phase (table 2). Among the three aphid populations studied, the frequencies (number of events per aphid) of phloem phases and sustained sap ingestion phases were the highest on control plants. On nerolidol- and farnesol-treated plants, the frequencies of phloem phases were 1.4 and 2.2 times lower than on control, respectively. Likewise, the frequencies of sustained sap ingestion phases were 4.2 and 1.9 lower on nerolidol- and farnesol-treated plants than on control, respectively. Time to reach the first phloem phase from the first probe, i.e., the first insertion of stylets into plant tissues from the start of the experiment, was the shortest on control (2.4 ± 2.1 h) and the longest on nerolidol- treated plants (5.0 ± 3.1 h) (table 2). On control plants, the phloem phase was usually divided into 4.7 ± 3.0 bouts, 2.1 ± 1.7 of which included sustained ingestion, while on farnesol- and nerolidol-treated plants, these values were 3.3 and 0.5, and 2.1

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Table 3. Exploratory cell punctures (pd = potential drops) in non-phloem tissues by *M. persicae* on control and (E,E)-farnesol (**1**) and (Z)-nerolidol (**2**) treated plants [a]

No.	EPG parameter	Compounds		
		Control	Farnesol	Nerolidol
19	Proportion of probes with potential drops[1] (%)	0.80 ± 0.11	0.71 ± 0.26	0.73 ± 0.21
	Short potential drops (pd-S)			
20	Total number of pd-S	126.7 ± 64.8	133.3 ± 88.5	108.3 ± 65.7
21	Mean number of pd-S in one probe	7.0 ± 2.8	8.9 ± 7.0	6.3 ± 4.6
22	Mean duration of pd-S (s)	5.1 ± 1.6	4.4 ± 0.6	4.6 ± 1.1
23	Total duration of subphase II-1 of pd-S (s)	301.1 ± 229.0	239.8 ± 158.2	208.9 ± 156.7
24	Total duration of subphase II-2 of pd-S (s)	146.4 ± 72.1	128.1 ± 79.1	116.7 ± 70.1
25	Total duration of subphase II-3 of pd-S (s)	209.4 ± 128.2	209.5 ± 144.6	150.7 ± 104.9
	Long potential drops (pd-L)			
26	Total number of pd-L	6.3 ± 4.2	8.9 ± 8.8	6.5 ± 6.0
27	Mean number of pd-L in one probe	0.3 ± 0.2	0.5 ± 0.4	0.3 ± 0.1
28	Mean duration of pd-L (s)	6.4 ± 2.2	5.7 ± 1.8	5.8 ± 0.9
29	Total duration of subphase II-1 of pd-L (s)	10.7 ± 7.4	16.0 ± 16.1	9.4 ± 8.5
30	Total duration of subphase II-2 of pd-L (s)	7.1 ± 4.9	9.5 ± 10.0	7.0 ± 6.3
31	Total duration of subphase II-3 of pd-L (s)	26.9 ± 23.1	26.7 ± 25.0	19.5 ± 15.5

[a]Values are means ± SD; $n = 15$; no significant differences in relation to control (Mann–Whitney U test); [1]pd-S and pd-L.

and 1.1, respectively, which was significantly lower (nerolidol) than on control (table 2).

Activities in non-phloem tissues before the first phloem phase

On control plants, probing activities, divided into 13.2 ± 9.6 probes on average, predominated during the period before the first phloem phase (80% of aphid activities during that period) and 62% of these probes were shorter than 3 min. On farnesol-treated plants, probing activities occupied 71% of that period duration. There were 7.2 ± 8.1 probes per aphid on average and 43% of these probes were shorter than 3 min (table 2). On nerolidol-treated plants, probing activities occupied 61% of that period duration. There were 11.6 ± 14.0 probes per aphid on average and 41% of these probes were shorter than 3 min (table 2).

Activities in sieve elements

The duration of the first phloem phase ranged from 0.2 ± 0.2 h on nerolidol-treated plants to 0.6 ± 1.5 h on control. The average duration of the sap ingestion phase was highest on control (0.9 ± 1.4 h), and the lowest on farnesol-treated plants (0.4 ± 0.9 h), which was significantly lower than on control. Salivation occupied 6% of the phloem phase on control and 21% and 12% on farnesol- and nerolidol-treated plants, respectively (table 2).

Exploratory cell punctures

Exploratory cell punctures (cell punctures for gustatory purposes) occurred in 71–80% of probes and the majority of these punctures were 'short' (pd-S) cell punctures (94%) on all plants, irrespective of treatment (table 3). The frequency of pd-S (number of pd-S per probe) was similar on all plants and ranged from 6.3 ± 4.6 on nerolidol-treated plants to 7.0 ± 2.8 on control plants. The 'long' cell punctures (pd-L) occurred rarely but the frequency of pd-L was also similar on all plants and ranged from 0.3 ± 0.1 on nerolidol-treated plants to 0.5 ± 0.4 per probe on farnesol-treated plants. The average duration of pd-S was 4.4 ± 0.6 to 5.1 ± 1.6 s while the duration of pd-L ranged from 5.7 ± 1.8 to 6.4 ± 2.2 s. In pd-S, the longest subphase was II-1, and amounted to 45% (control), 44% (nerolidol) and 41% (farnesol) time of the total duration of these punctures. In pd-L, the longest subphase was II-3 and amounted to 60% (control), 51% (farnesol), and 54% (nerolidol) of the total duration of these punctures (table 3). No significant differences in parameters related to exploratory cell punctures in aphids on sesquiterpenoid-treated plants in relation to control were detected (table 3).

Aphid settling success (choice-test)

The potency and durability of deterrent effects on *M. persicae* settling activity after exposure to farnesol and nerolidol were very high. The deterrent effects of farnesol and nerolidol were manifested as soon as 1 h after aphids were confronted with the treated leaves and lasted at least until the end of the experiment, which was 24 h. Aphids settled mainly on control leaves. On control leaves there were three to four times more aphids than on farnesol-treated leaves and 3–9 fold times more than nerolidol-treated leaves, depending on the time after exposure (table 4).

Discussion

Direct monitoring of aphids that could freely explore and choose between non-treated and treated host plant leaves revealed that neither farnesol nor nerolidol affected the propensity to probe in *M. persicae*. Aphids have started the first probe almost immediately after having access to the leaves and the number of probes, the mean duration of a probe, and overall probing time during the 15 initial minutes of aphid contact with the plant material was similar irrespective of treatment. These results excluded the repellent activity of the studied sesquiterpenoids towards *M. persicae*,

https://doi.org/10.1017/S0007485319000609 Published online by Cambridge University Press

Table 4. Settling of *M. persicae* on control and (E,E)-farnesol (**1**) and (Z)-nerolidol (**2**) treated plants

Compounds		Mean number of aphids		
		1 h	2 h	24 h
Farnesol	Treated	5.3 ± 1.3	4.5 ± 1.0	3.3 ± 0.7
	Untreated	12.8 ± 1.5	11.1 ± 1.2	14.6 ± 1.8
	P	0.0023	0.0008	0.0000
Nerolidol	Treated	2.8 ± 0.5	1.5 ± 0.4	3.9 ± 0.9
	Untreated	11.6 ± 0.7	13.6 ± 1.4	12.1 ± 1.8
	P	0.0000	0.0000	0.0009

Numbers represent the mean number of aphids that settled on the treated and untreated (control) leaves ±SD; number of replications $n = 8$, and 20 aphids per replication; Student *t*-test at $P = 0.05$ was used to compare the number of aphids on treated and control leaves at each time point, separately.

which may be explained by the relatively low volatility of C_{15} terpenoids. However, sesquiterpenoids very often exhibit irritant activity (Gonzalez-Coloma *et al.*, 2010; Jiang *et al.*, 2016) and this quality may be responsible for alterations in aphid behaviour during probing discovered in the present study. The attachment to the electrode in the EPG experiment limited aphid movements to a small portion of a leaf for 8 h, which resulted in a longer exposure to farnesol and nerolidol. Significant changes in aphid probing activities on sesquiterpenoid-treated leaves in relation to control were revealed. The parameters describing aphid behaviour during probing and feeding, such as total time of probing, proportion of phloem patterns E1 (salivation) and E2 (ingestion), number of probes, *etc.*, are good indicators of plant suitability or interference of probing by chemical or physical factors in individual plant tissues (Mayoral *et al.*, 1996). The interpretation of the results of EPG recordings may indicate the tissue localization of deterrent (or attractant) factors and, consequently, may expose the physiological effects of allelochemicals in aphids (van Helden and Tjallingii, 1993). For example, long penetration time in non-phloem tissues as compared to total penetration time, high number of short vs long probes before the first phloem phase, relatively long time to 1st phloem phase within a probe, and a failure in finding sieve elements may be interpreted as pre-ingestive effects of antifeedants that restrain aphid probing at the level of non-phloem tissues. Likewise, the short total and mean durations of phloem sap ingestion and high proportion of salivation during penetration of phloem vessels may point to the ingestive mode of feeding deterrence, which has been found in aphid-non host plant and aphid-poor host plant interactions (Mayoral *et al.*, 1996; Gabryś and Pawluk, 1999). On suitable host plants, the sap ingestion periods may last for many hours with no interruption and the lower is the proportion of salivation during phloem phase the more suitable is the host plant (Gabryś and Pawluk, 1999; Alvarez *et al.*, 2006; Tjallingii, 2006). A high proportion of salivation during phloem phase is often interpreted as the activity associated with the detoxification of xenobiotics in the phloem sap (Miles, 1999). Extended sieve element salivation (E1 waveform in the electrical penetration graph) is a characteristic phenomenon during early sieve element punctures, particularly in resistant plants (Mayoral *et al.*, 1996; Wilkinson and Douglas, 1998; Ramírez and Niemeyer, 1999; Kordan *et al.*, 2019). Aphids tend to initiate a probe into any substrate irrespective of its structure or chemical composition and even in the presence of deterrents (Powell *et al.*, 1999). In the present study, the total time of probing on nerolidol and farnesol-treated leaves was 73 and 83% of the total probing time on control, respectively, and this was mainly due to the significant decrease in the duration of the phloem phase. On farnesol and nerolidol-treated leaves, the phloem phase was 34% of that activity on control leaves. The total duration of probing activities in non-phloem tissues was not affected by the application of farnesol and nerolidol. The number and the mean duration of probes before the first phloem phase were similar on all plants. The proportion of short (<3 min) epidermal probes was comparable on all plants. The internal structure of probes was also analogous: the number of 'short' and 'long' exploratory cell punctures and the proportion of subphases within these punctures did not differ among the treatments. However, the proportion of aphids that reached the phloem phase was lower and the first phloem phase was significantly delayed on nerolidol-treated plants as compared to control, which suggests a negative influence of nerolidol on aphids during the pre-ingestive (pre-phloem) phase of probing. The number of phloem phases and sustained phloem sap ingestion phases were lower on nerolidol and farnesol-treated plants than on control, respectively. Additionally, on farnesol-treated plants, the proportion of salivation during the phloem phase was three-fold higher than on control plants, which suggests a negative impact of farnesol on aphids during the ingestive (phloem), phase of probing.

Complementary to EPG experiments, was the choice test on aphid settling performed in this study. This test reveals aphid preferences during at least 24 h after exposure to allelochemicals and indicates the possible postingestive activity of an allelochemical, provided the aphids are able to feed upon phloem sap of the treated plants (Chapman and de Boer, 1995). The EPG experiments demonstrated that tethered aphids reached phloem vessels and consumed sap but the freely moving aphids were reluctant to remain on farnesol- and nerolidol-treated leaves for at least 24 h after exposure. It may be concluded then, that farnesol and nerolidol show complementary deterrent properties. Farnesol is not a repellent to *M. persicae* and it does not restrain aphid inclination to probing but it is a feeding deterrent with ingestive and postingestive activities. Specifically, it causes a decrease in the duration of sustained feeding, an increase in phloem salivation, and the avoidance of treated plants by the freely moving aphids. Nerolidol, although not repellent either, shows a pre-ingestive and ingestive deterrent activities, i.e., within non-phloem and phloem tissues. It causes the delay and reduction in the occurrence of phloem phase in tethered aphids and the avoidance of treated plants by the freely moving aphids. Nevertheless, although the effects of farnesol appear in more advanced phases of probing than those of nerolidol, the deterrent activity of farnesol becomes stronger than nerolidol over the course of time.

https://doi.org/10.1017/S0007485319000609 Published online by Cambridge University Press

The findings described in the present study further document the importance of the structure of the molecule in the expression of biological activity (Halarewicz-Pacan *et al*., 2003; Dancewicz *et al*., 2008; Gabryś *et al*., 2015; Stompor *et al*., 2015). We observed that the position of the hydroxyl group and conformation of the double bond in the chain of the sesquiterpenoids studied determine differences in their biological activity towards aphids. The primary hydroxyl group and (E) or *trans* conformation of both double bonds seem to be crucial structural elements responsible for significant decrease of the total duration of phloem phase and the mean duration of individual sustained ingestion periods what has been reported for (E,E)-farnesol.

The detection of aphid probing and feeding deterrent potential of farnesol and nerolidol in the present study opens a variety of options for future practical applications, from the implementation in push-pull strategies (Pickett *et al*., 2014) to the genetical engineering of plants for pest control purposes (Aharoni *et al*., 2006). The majority of essential oil chemicals are relatively non-toxic to mammals and fish in toxicological tests and the rapid volatilization of pesticides derived from plant essential oils makes them less harmful to the environment than synthetic pesticides (Isman and Machial, 2006; Koul *et al*., 2008). The behaviour-modifying substances may repel aphids or deter their probing and feeding, which may finally cause the rejection of a plant, or affect aphid development, fecundity, and longevity, and finally, the collapse. The best-known antifeedants come from natural sources, and the most widespread ones have an isoprenoid structure (Klein Gebbinck *et al*., 2002). The sesquiterpenoid polygodial was successfully applied in the field against bird cherry-oat aphid *Rhopalosiphum padi* giving similar results to those obtained with cypermethrin (Pickett *et al*., 1997). Farnesol possesses antifeedant and neurotoxic properties towards the migratory locusts *Locusta migratoria* (L.) (Orthoptera: Acridiidae) and has been proposed for use as a biocide admixture in bait traps in the integrated pest management programs against the locusts (Awad and Ghazawy, 2016). A mixture of farnesol isomers and (*E*, *E*)-α-farnesene causes high mortality among nymphs of the black bean aphid, *Aphis fabae* Scopoli and *M. persicae* when topically applied (Harrewijn *et al*., 2001). Both sesquiterpenoids used in the present study, farnesol and nerolidol, may be considered as factors that may reduce crop losses caused by *M. persicae* infestation and feeding but not as means for prevention of virus transmission. The success rate in reaching phloem vessels and the duration of feeding activities were limited on farnesol- and nerolidol-treated plants, which explains the avoidance of treated plants by freely moving aphids. However, neither compound limited the peach potato aphid probing activity in non-phloem tissues, as the number and the duration of cell punctures associated with virus transmission were not reduced.

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